

# Role of Short-Term Estradiol Supplementation in Symptomatic Postmenopausal COVID-19 Females: A Randomized Controlled Trial

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**ABSTRACT** **Background:** Protective role of estrogen in COVID-19 was speculated once the epidemiological studies reported increased susceptibility of estrogen-deficient population – males and postmenopausal females to severe disease category and involvement of angiotensin-converting enzyme 2 receptors and renin–angiotensin–aldosterone system in pathophysiology. **Materials & Methods:** An open-label randomized controlled trial was planned to assess the efficacy of short-course oral estradiol in preventing the clinical progression to severe disease and reduce case-fatality rate and the hospital stay duration in estrogen-deficient postmenopausal women. The intervention group ( $n = 40$ ) received 2 mg per day of estradiol valerate per orally for 7 days along with the standard care, while the control group ( $n = 40$ ) received only the standard care. **Results:** A significant difference was observed in the rate of reverse transcriptase–polymerase chain reaction negativization in the intervention versus control group at day 5 and day 7 of admission (42.5% vs. 15%,  $P = 0.007$ ; 72.5% versus 50%,  $P = -0.026$ ). No significant difference was noted in the duration of hospitalization ( $P = 0.213$ ). A significant decrease was noted in the mean values of inflammatory biomarkers – D-dimer, lactate dehydrogenase, and C-reactive protein on day 5 in the intervention group. Interleukin-6 also showed a declining trend on day 5 in the intervention group, while a rising trend was noted in the control arm. Only one case (2.5%) in the intervention group while seven in the control group (17.5%) progressed to the moderate category; however, the difference was not statistically significant ( $P = 0.057$ ). **Conclusion:** Oral estradiol in postmenopausal females can be a novel and efficient option for managing nonsevere COVID-19 infection.

**KEYWORDS:** COVID-19, immunomodulation, menopause, natural estrogen-deficient group, oral estradiol, viral clearance time

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## INTRODUCTION

Ever since the reporting of the first case of COVID-19 in Wuhan China, understanding regarding the pathophysiology of the disease has evolved rapidly. The key event in the initiation of the human infection process with severe acute respiratory syndrome coronavirus-2 (SARS COV-2) is the interaction of spiked glycoprotein on the virus surface with the angiotensin-converting enzyme-2 (ACE-2) receptors expressed in various human organs, which stimulate an immune response.<sup>[1]</sup> Pro-inflammatory cytokines released, result in multi-organ damage which is suppressed by the high endogenous estrogen

levels in females.<sup>[2]</sup> Epidemiological studies have also clearly shown the increased affection, morbidity, and fatality in men with COVID-19 compared to women.<sup>[3,4]</sup> Italian National Institute of Health reported 70% mortality in men and similar trends were seen in the USA and China, with the case-fatality ratio being 2.4 times higher among men than women.

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[5-7] However, this sex difference was not noted in postmenopausal women with age-matched men suggesting the possible immune-modulatory role of female sex hormones.<sup>[8]</sup> Estrogen downregulates the expression of ACE-2 receptors and has an established role in regulating the renin–angiotensin system.<sup>[9]</sup>

Observing the role of estrogen as an anti-inflammatory, antiviral, and immune-modulating agent, we speculated supplementing a short course of oral estradiol in management of postmenopausal women (estrogen deficient) having COVID-19 infection, with a perspective that it can be tried in males (another estrogen-deficient group) for avoiding the cytokine storm and other complications. Moreover, no drugs to date have been approved for the treatment of this disease.

## MATERIALS AND METHODS

An open-label single-center randomized controlled trial (RCT) was conducted on COVID-19-infected postmenopausal women admitted at the Government Institute of Medical Sciences, Greater Noida, India, from September 10, 2020, to December 31, 2020 (CTRI/2020/09/027622) after obtaining ethical approval (GIMS/IEC/HR/2020/20), to evaluate the role of short-term oral estradiol therapy. The underlying hypothesis was that short-term oral estradiol can reduce hospital stay by improving prognosis and reducing morbidity in COVID-19 postmenopausal women.

### Inclusion/exclusion criteria

Symptomatic postmenopausal women with reverse transcriptase–polymerase chain reaction (RT-PCR)-confirmed COVID-19 disease with the mild and moderate disease on admission (WHO criteria)<sup>[10]</sup> were included in the study, while women above 70 years, women already on estrogen hormone therapy, steroids, and antiepileptic drugs, women with postmenopausal genital bleeding (menstrual complaints) and breast and genital malignancies, and women with high risk for thromboembolism based on coagulation markers, preexisting liver impairment, heart disease, and previous history of the thromboembolic event were excluded from the study.

### Sample size

The observed average hospital stay duration in our institute for COVID-19 cases was 9.13 days + 2.59 days. Assuming that the intervention (estradiol) will reduce the average hospital stay duration by 20% i.e., to 7.30 days, considering 80% power, allowing for 5% type I error, and using the formula, the sample size calculated was 63.1 (rounded off to 64); however, anticipating 10% patients loss to follow-up, we need to add seven patients more; therefore, the total sample size would be 71.

Hence, minimum of 35.5 (rounding off to 36) patients in each arm would be included.

Eligible postmenopausal women were enrolled after written informed consent and randomized into two groups via computer-generated randomization: Intervention group received tablet estradiol valerate 2 mg per day orally for 7 days along with the standard care (as per the clinical guidelines by MoHFW, GOI),<sup>[11]</sup> while the control group received standard care only.

At admission, the demographic and clinical profiles were recorded. Baseline blood investigations were done on day 1(admission) and repeated on day 5, along with the inflammatory markers (C-reactive protein [CRP], procalcitonin, ferritin, interleukin [IL]-6, lactate dehydrogenase [LDH]) and coagulation markers. Nasopharyngeal and oropharyngeal RT-PCR test was repeated on day 5 and day 7 of admission. Women were monitored for the course of the disease. They were discharged only after negative RT-PCR and symptomatic improvement (maintaining SPO2 > 94% on room air with the declining trend of all inflammatory biomarkers), and were followed weekly via telephone till 4 weeks for the persistence or development of new symptoms and readmission if any. Clinical and biochemical outcomes of both groups were compared. The primary outcome measures included the total hospital stay duration and viral clearance time while clinical progression of the disease; admission to intensive care unit (ICU); requirement of oxygen supplementation, ventilatory support and special therapies like IL-6 inhibitors or plasma therapy; change in hematological, biochemical and inflammatory parameters; severe adverse events and mortality were considered as the secondary outcome measures.

### Statistical analysis

Continuous variables were presented as mean with standard deviation and statistical testing was done using independent *t*-test or as median with range and statistical testing was done using Mann–Whitney test. Categorical variables were expressed as numbers and percentages. The Chi-square test and Fisher’s exact test are used for testing statistical significance. *P* value of 0.05 is considered significant. Regression analysis was done to get adjusted estimates. Statistical analysis was done using SPSS 21.00 software (IBM SPSS Statistics for Windows version 21.0. Armonk, NY: IBM Corp).

## RESULTS

A total of 99 eligible postmenopausal enrolled cases were randomized into two groups using a computer-generated random numbers table: 49 in the intervention group and 50 in the control group. Women opting for home isolation and who discontinued estradiol during hospital

stay were dropped out. The CONSORT flow diagram of the study is shown in Figure 1.

Intervention and control group patients were found statistically comparable to the mean age, duration of menopause, comorbidities, presenting symptoms, and clinical severity of the disease. The common comorbidities noted were diabetes mellitus and hypertension. Fever was the most common presenting symptom followed by the cough. At the time of admission, 85% of women in the intervention group and 75% in the control group had mild disease ( $P$  value = 0.264). Baseline characteristics of the two groups are described in Table 1.

### Primary outcome measures

A significant difference was observed in the rate of RT-PCR negativization in the intervention versus control group at day 5 and day 7 of admission (42.5% vs. 15%,  $P = 0.007$ ; 72.5% vs. 50%,  $P = 0.026$ ). The average hospital stay duration was 7.77 and 8.77 days in the intervention and control groups, respectively; however, the difference was not statistically significant ( $P = 0.213$ ) [Table 2].

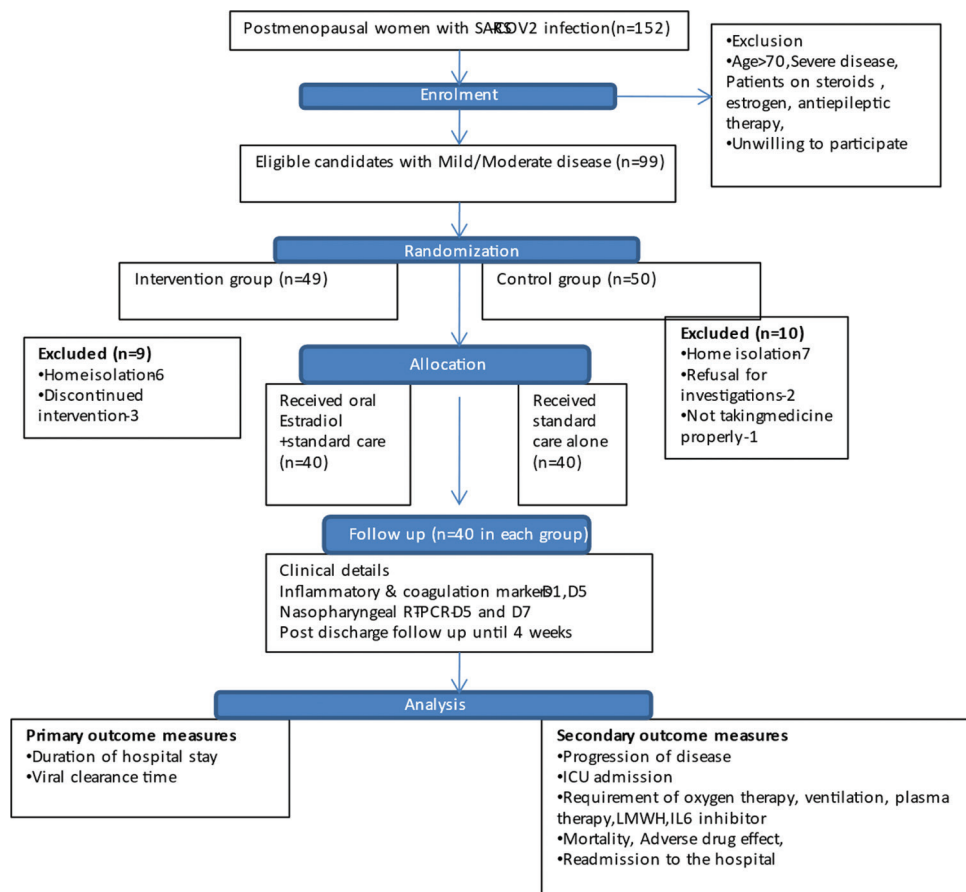
### Secondary outcome measures

Only one case (2.5%) with mild disease in the intervention group while seven cases (17.5%) in the control group

**Table 1: Baseline characteristics of the two groups**

Parameters	Intervention group (n=40), n (%)	Control group (n=40), n (%)
Age (years), mean±SD	61.1±8.71	62.425±9.84
Menopause duration (years), mean±SD	12.02±6.54	13.42±8.25
Comorbidities		
Hypertension	12 (30)	16 (40)
Diabetes mellitus	15 (37.5)	12 (30)
Heart disease	0	1 (2.5)
Thyroid disorder	8 (20)	6 (15)
Tuberculosis	1 (2.5)	1 (2.5)
Symptoms		
Fever	29 (72.5)	26 (65)
Cough	24 (60)	21 (52.5)
Sore throat	7 (17.5)	12 (30)
Shortness of breath	8 (20)	10 (25)
Gastrointestinal symptoms	3 (7.5)	2 (5)
Headache	3 (7.5)	2 (5)
Myalgia	6 (15)	8 (20)
Loss of taste	1 (2.5)	0
Disease severity		
Mild	34 (85)	30 (75)
Moderate	6 (15)	10 (25)

SD: Standard deviation



**Figure 1: The CONSORT flow diagram of the trial**

**Table 2: Primary and secondary outcomes measures in two groups**

Parameters	Intervention group (n=40), n (%)	Control group (n=40), n (%)	P
Primary outcome measures			
Viral clearance/RT-PCR negativization (days)			
5	17 (42.5)	6 (15)	0.007 (Chi-square test)
7	29 (72.5)	20 (50)	0.026 (Chi-square test)
Duration of hospital stay (days)			
Mean±SD	7.77±3.43	8.77±3.67	0.213 (t-test)
<7	12	11	0.96 ( $\chi^2$ )
7-9	19	20	
>9	9	9	
Secondary outcome measures			
Clinical progression			
Moderate disease	1 (2.5)	7 (17.5)	0.057 (Fisher's exact test)
Severe disease	0	1 (2.5)	>0.995 (Fisher's exact test)
ICU admission	3 (7.5)	5 (12.5)	>0.995 (Fisher's exact test)
Requirement of			
Oxygen therapy	4 (10)	5 (12.5)	>0.995 (Fisher's exact test)
Ventilator support	0	1 (2.5)	>0.995 (Fisher's exact test)
IL-6 inhibitor	0	1 (2.5)	>0.995s (Fisher's exact test)
LMWH	18 (45)	23 (57.5)	0.263 ( $\chi^2$ )
Plasma therapy	0	4 (10)	0.116 (Fisher's exact test)
Mortality/adverse event	0	0	-

IL-6: Interleukin-6, SD: Standard deviation, LMWH: Low-molecular weight heparin, ICU: Intensive care unit, RT-PCR: Reverse transcriptase–polymerase chain reaction

progressed to moderate disease; however, the difference was not statistically significant ( $P = 0.057$ ). None of the women in the intervention group progressed to severe disease. No significant difference was noted with regard to ICU admission (7.5% vs. 12.5%,  $P > 0.995$ ) and requirement of oxygen therapy (10% vs. 12.5%,  $P > 0.995$ ) in two groups.

In our study, the percentage of women requiring anticoagulant therapy (LMWH) was less in the intervention arm compared to the control arm (45% vs. 57.5%) though the difference was again statistically not significant ( $P = 0.263$ ). In the control group, one woman required ventilatory support, one received IL-6 inhibitor (Tocilizumab), and four (10%) received convalescent plasma, while none in the intervention group required these specialized treatments. There was no mortality or adverse events in either group [Table 2].

The mean values of all biomarkers at admission were statistically comparable between the two groups except NLR ( $P = 0.045$ ) and IL-6 ( $P = 0.001$ ). Significant changes were noted in the mean values of D-dimer, LDH, and CRP on day 5 in the intervention group as compared to the control group. Definitive rise (more than double) in mean D-dimer levels was observed in the control group from admission to day 5 (0.87–

2.16 mg/dl), while in the intervention arm, D-dimer values remained almost the same and the difference in the two groups was statistically significant (1.26 vs. 2.16,  $P = 0.035$ ). Significant difference was also noted in mean serum LDH and CRP values at day 5 between the two groups ([317.4 vs. 407.3,  $P = 0.002$ ], [9.46 vs. 17.05,  $P = 0.028$ ], respectively) [Table 3].

IL-6 showed a declining trend in mean values on day 5 in the intervention arm, while the reverse (rise) was noted in the control arm compared to admission values. However, the difference in the levels of IL-6 at day 5 between the two groups was not significant ( $P = 0.632$ ). Neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), fibrinogen, prothrombin time (PT), activated partial thromboplastin time (aPTT), ferritin, and procalcitonin values did not show any significant difference at day 5 among the two groups. Abnormal X-ray patterns were observed in 55% and 60% of women in intervention and control groups, respectively, at admission and no significant improvement in X-ray scoring was recorded on day 5 among the two groups [Table 3].

On follow up, the persistence of symptoms was reported in 11 (27.5%) and 8 (20%) women in the intervention and the control group, respectively, with the most

**Table 3: Laboratory parameters in the two groups**

Investigations	Mean±SD		P
	Intervention group (n=40)	Control group (n=40)	
NLR (days)			
1	2.94±2.2	4.25±3.43	0.045
5	4.017±3.40	3.73±2.503	0.67
PLR (days)			
1	162.03±185.44	176.23±128.51	0.69
5	198.03±230.31	192.74±176.47	0.91
D-dimer (normal range: 0-0.5 mg/l) (days)			
1	1.29±1.65	0.86±0.87	0.15
5	1.26±1.39	2.16±2.15	0.035
Fibrinogen (normal range: 200-400 mg/dl) (days)			
1	502.78±157.30	449.24±193.53	0.18
5	511.32±170.00	479.94±188.55	0.45
PT (days)			
1	13.24±1.22	13.36±1.3322	0.67
5	13.57±2.37	13.39±1.03	0.67
APTT (days)			
1	25.53±5.18	24.58±4.62	0.38
5	24.12±4.80	24.39±3.18	0.77
Procalcitonin (normal range: <0.05 ng/ml) (days)			
1	0.03±0.03	0.05±0.11	0.22
5	0.038±0.05	0.022±0.024	0.08
Serum ferritin (normal range: 10-291 ng/ml) (days)			
1	146.31±143.033	185.31±204.034	0.33
5	169.77±137.91	194.19±169.05	0.489
IL-6 (normal range: <17 pg/ml) (days)			
1	35.84±27.083	17.75±19.750	0.001
5	21.90±38.58	25.85±34.22	0.632
LDH (normal range: 140-280 U/L) (days)			
1	320.53±82.58	331.52±127.86	0.665
5	317.37±76.88	407.30±146.58	0.002
CRP (normal range: 0-6 mg/L) (days)			
1	12.06±16.18	11.32±14.84	0.834
5	9.46±12.4	17.048±17.012	0.028
Abnormal chest X-Ray (days), n (%)			
1	22 (55)	24 (60)	0.651 ( $\chi^2$ )
5	19 (47.5)	15 (37.5)	0.539 ( $\chi^2$ )

NLR: Neutrophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio, PT: Prothrombin time, APTT: Activated partial thromboplastin time, IL-6: Interleukin-6, LDH: Lactate dehydrogenase, CRP: C-reactive protein, SD: Standard deviation

common symptoms being myalgia and weakness, the difference being statistically nonsignificant ( $P = 0.432$ ). No new symptoms developed and no readmission was reported due to COVID-19-related complications in either group.

We applied stepwise linear regression to analyse the effect of independent variables like age, menopause duration; day -1 levels of inflammatory parameters (NLR, PLR, Fibrinogen, D-dimer, IL-6, Procalcitonin, CRP, Ferritin, and LDH); and comorbidities on the hospital stay duration. The probability to include a variable in the equation was kept at 0.05, while for exclusion, it was kept at 0.10. Only procalcitonin levels on day 1 were

found to be positively associated with the duration of hospital stay with an adjusted  $P$  value of 0.023 (95% CI: 1.583–20.407). We applied backward stepwise logistic regression to check if any baseline variables explained the RT-PCR negativization at day 5 of admission such as age, comorbidity, menopause duration, hemoglobin (Hb), TLC, creatinine levels, uric acid levels, PT, fibrinogen, D-dimer, IL6, CRP, ferritin, procalcitonin, X-ray, estrogen therapy, plasma therapy, remdesivir, tocilizumab, at day 1 of admission. The adjusted analysis suggests that raised CRP on the day of admission is associated with higher odds of RT-PCR test being positive on day 5 with adjusted  $P$  value = 0.03 (95% CI 1.014–1.302). On the other hand, the estrogen therapy group had lower odds

of having RT-PCR test positive on day 5 after admission, which was statistically significant  $P = 0.007$  (95% CI 0.017–0.53) [Table 4].

## DISCUSSION

Depletion of ovarian steroids at menopause is known to affect the innate and adaptive immune response predisposing them to increased morbidity and mortality from infectious diseases.<sup>[12]</sup> It has been now established that ACE-2 receptors and renin–angiotensin–aldosterone system regulated by estrogen play a key role in SARS COV-2 pathophysiology [Supplementary Figure].<sup>[13]</sup> COVID-19 infection triggers vigorous immune response activating inflammatory processes and coagulation cascade.<sup>[11]</sup> Exaggerated release of inflammatory cytokines (IL-2, IL-6, TNF- $\alpha$ ) induces vasoconstriction, endothelial dysfunction, and even thrombosis and is responsible for the development of ARDS or multi-organ failure.<sup>[14-16]</sup>

No clinical trial report was available in the literature related to the role of estrogen in COVID-19 disease at the time of writing this manuscript. Two trials using estrogen patch recruiting nonsevere COVID-19 patients (males >18 years, females >55 years) are underway in Mexico (ClinicalTrials.gov, NCT04539626) and USA (ClinicalTrials.gov, NCT04359329) with results pending. We decided to go ahead with oral 2 mg estradiol valerate per day due to easy availability and affordability. After oral administration, sustained blood levels of >40 pg/mL estradiol have been reported to be achieved within 2 h of oral intake [Supplementary Figure].<sup>[17]</sup>

Similar to other studies, fever and cough remain the most common presentation in our study as well and diabetes and hypertension are the most common comorbidities.

Significantly short viral clearance time was observed in the intervention group at day 5 (42.5% vs. 15%), which can be explained based on the downregulating effect of estradiol on ACE-2 receptors of pneumocytes thus reducing adherence and internalization of the viral genome. 17  $\beta$ -estradiol also acts through its intracellular receptor signaling pathway, triggering a cascade interfering with virus assembly, maturation, and/or release. This has been proved in an *in vitro* model exposing Huh7 (Human Hepatoma) cells infected with JFH1 virus to 17  $\beta$ -estradiol.<sup>[18]</sup> Ovariectomized female and male mice model supplemented with estrogen has also been shown to repress the transcription of HBV genes by upregulating ER $\alpha$  receptors.<sup>[19]</sup> Early viral clearance in our study indirectly suggests that estrogen has a positive effect in reducing infectivity.

The study by Spagnuolo *et al.*, evaluating the effect of low-dose corticosteroid in moderate-to-severe COVID-19 disease, reported a similar time of viral clearance in steroid and nonsteroid users ( $P = 0.985$ ) with older age and severe disease having a negative association.<sup>[20]</sup> A retrospective study by Arabi *et al.* in critically ill patients with MERS-COV revealed that corticosteroid therapy delayed the viral clearance (adjusted hazard ratio [HR], 0.35; 95% CI, 0.17–0.72;  $P$  value = 0.005).<sup>[21]</sup> However, in this study, the dose and time of initiation of steroids were widely variable. With all literature reports on viral clearance, it can be interpreted that starting steroids early in mild-to-moderate disease and estrogen in the deficient group (postmenopausal or males) is going to speed up the viral clearance.

We observed no significant difference in length of hospital stay in the two groups ( $P = 0.213$ ). Association of hospital stay duration with the severity of disease and not with menopausal status or comorbidities has been suggested by Neha *et al.*<sup>[22]</sup> and Liu *et al.*<sup>[23]</sup>

**Table 4: Association of variables with primary outcome measures**

	B	SE	Significance	Exp (B)	95% CI
Linear stepwise regression: Association of day 1 variables with hospital stay duration					
Constant	7.655	0.481	<0.001		6.694-8.616
Procalcitonin	10.995	4.710	0.023	0.282	1.583-20.407
Binary logistic regression – Backward stepwise: Association of day 1 variables with RTPCR negativization					
TLC	0	0	0.021	1	0.999-1
CRP	0.139	0.064	0.03	1.149	1.014-1.302
Ferritin	-0.005	0.003	0.058	0.995	0.989-1
Estrogen	-2.353	0.877	0.007	0.095	0.017-0.53
Plasma therapy	25.612	15334.882	0.999	1.32788E+11	0
Constant	4.973	1.642	0.002	144.449	

CI: Confidence interval, SE: Standard error, RT-PCR: Reverse transcriptase–polymerase chain reaction, TLC: Total leukocyte count, CRP: C-reactive protein

based on multivariate analysis, which contradicts the reports of Ding *et al.*<sup>[24]</sup> who reported menopause as an independent risk factor for COVID-19-related hospitalization. Spagnuolo *et al* reported a longer length of hospitalization in steroid-users compared to non-users (20 versus 14 days;  $p < 0.001$ ) and attributed this to higher degree of baseline respiratory impairment in steroid users.<sup>[20]</sup> In our study, enrolling nonsevere cases may probably be the reason for the shorter length of hospital stay in both the groups as compared to the overall average in the institute (9.13 days).

Progression of disease in more cases in the control group compared to the intervention group (8 versus 1) indirectly suggests estrogen's beneficial effect, although we did not measure the serum estradiol levels. The only case requiring ventilator (control group) had elevated procalcitonin levels at admission reflecting its predictive value for disease severity. Ding *et al.*'s study supports these where estrogen levels were shown to be negatively correlated with the severity of COVID-19 infection (adjusted HR 0.304 [95% CI, 0.092–1.001],  $P = 0.05$ ).<sup>[24]</sup>

We also noted a significant reduction in inflammatory biomarkers – D-dimer, LDH, and CRP in the intervention group over the 5 days. Reports have shown the association of elevated D-dimer levels and independent association of LDH with severity and poor outcome; a similar association was observed in our study where women with escalating D-dimer and LDH levels required ICU care, ventilatory support, and special therapies.<sup>[25,26]</sup>

IL-6, a major marker of COVID-19 cytokine storm, has been positively correlated with the severity of COVID-19 disease<sup>[14]</sup> and negatively correlated with estradiol levels ( $P = 0.048$ ) in menopausal COVID-19 women.<sup>[24]</sup> Mean levels of IL-6 have shown a declining trend in the intervention group in our study. The same anti-inflammatory effect of estradiol has been noted in female mice model infected with influenza-A virus receiving estradiol, showing 10-fold reduction in cytokine levels.<sup>[27]</sup>

The rising trend of NLR, PLR, ferritin, fibrinogen, and coagulation parameters has been reported to be associated with the increasing severity of COVID-19 disease.<sup>[28]</sup> No difference was observed in our study over 5 days of estrogen therapy, which may be due to the inclusion of more mild cases (80%).

In our study, there was no evident improvement in chest X-ray findings in the estrogen therapy group, but the definite symptomatic clinical improvement was there; this can be explained by the fact that the X-ray findings take time to develop as well as regress.

The literature reports contradictory results with estrogen. Channappanawar *et al.* demonstrated a higher mortality rate due to COVID-19 in female mice administered with estrogen receptor antagonists compared to normal counterparts and male mice, indirectly proving the beneficial role of estrogen. They also observed severe lung affection in gonadectomized female mice.<sup>[2]</sup> The study by Lee *et al.* failed to show any beneficial effect of menopausal hormone therapy (MHT) on COVID-19 postmenopausal women.<sup>[29]</sup> Similarly, Bonaccorsi *et al.* could not find any evidence that women on MHT have a lower risk than untreated women.<sup>[30]</sup> Due to scarcity of available evidence regarding the continuation of MHT containing estradiol in COVID-19 patients and the concerns of associated thromboembolic risk, Spanish menopause society has released a consensus suggesting withdrawal of systemic MHT or replacement with transdermal MHT, if required along with the addition of LMWH.<sup>[31]</sup> Contrary to this Seeland *et al* in a retrospective study including 16,891 COVID-19 women above 50 years of age reported reduced fatality (OR 0.33, 95% CI [0.18, 0.62] and Hazard ratio 0.29, 95% CI [0.11,0.76]) and increased survival probability at 180 days among estradiol users compared to nonusers (96.7% versus 84.9%). They concluded that, MHT itself had no adverse effects, hence refuting the concerns related to MHT continuation.<sup>[32]</sup> We also did not find increased morbidity, adverse effects, or mortality with short-course estrogen usage.

### Strength and limitations of the study

Ours is the first randomized clinical trial to address the role of oral estradiol in COVID-19 postmenopausal women with prospective postdischarge follow-up till four weeks; the rest are retrospective comparative studies. The limitations include relatively small sample size, open-label trial, and results not compared with serum estrogen levels.

### CONCLUSION

Estradiol has an established antiviral, anti-inflammatory, and immunomodulatory action in animal models. The epidemiological studies on COVID-19 have also suggested its possible protective role. Our study, being a RCT, has further added robust evidence in this direction opening the new area of research in prevailing pandemic inviting large RCT with perspective to find an effective therapy in an already immune-compromised estrogen-deficient group.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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## SUPPLEMENTARY FIGURE LEGEND

Pharmacodynamics (own work) and pharmacokinetics of estradiol<sup>[17]</sup> (source credit: [https://commons.wikimedia.org/wiki/File:Estradiol\\_levels\\_after\\_a\\_single\\_dose\\_and\\_with\\_continuous\\_administration\\_of\\_oral\\_estradiol\\_or\\_oral\\_estradiol\\_valerate\\_in\\_women.png](https://commons.wikimedia.org/wiki/File:Estradiol_levels_after_a_single_dose_and_with_continuous_administration_of_oral_estradiol_or_oral_estradiol_valerate_in_women.png)). NO, Nitric oxide; RAAS, Renin-angiotensin-aldosterone System; EV, Estradiol valerate

