

COVID-19 and Menstrual Status: Is Menopause an Independent Risk Factor for SARS Cov-2?

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ABSTRACT

Background: COVID-19 has shown a definite association with gender, a predilection for males in terms of morbidity and mortality. The indirect evidence of the protective effect of estrogen has been shown by Channappanavar, in the animal model and Ding T. in a multihospital study from China, suggesting menopause as independent risk factor and estrogen is negatively correlated with severity. **Objective:** Study the clinical profile and outcomes in premenopausal and menopausal. Covid-19-infected women and analyzed the effect of menstrual status on the outcome. **Materials and Methods:** A retrospective cohort study conducted on 147 mild and moderate category COVID-19 females admitted between May and August 2020 using hospital records and telephonic follow-up. Two groups formed based on menstrual status: group-1 (premenopausal/estrogenic) and Group-2 (menopausal/hypoestrogenic). Hospital stay duration was considered as primary, while the category of disease on admission, clinical course, the requirement of oxygen, and mortality and residual symptoms were taken as a secondary outcome to compare the groups. **Results:** Overall Group-1 had significantly more of mild disease, while Group-2 had moderate cases (39 [76.5%] vs. 14 [14.6%] $P < 0.01$). Menopausal group has significantly more requirement of oxygen (32 [62.7%] vs. 20 [20.8%]), ventilation (14 [27.5%] vs. 1 [1%]) progression-to-severe disease (23.5% vs. 7.3%) and prolonged hospital stay ($[14.1 \pm 8.9$ vs. 8.6 ± 3.9 days] $P < 0.01$). However, multivariate logistic regression failed to show a significant association between hospital stay and progression with menopause. Ferritin and residual symptoms found significantly higher in menopausal. **Conclusions:** No definite association was found between menopause and COVID-19 outcome with hospital stay duration or disease progression in our study.

KEYWORDS: COVID-19, estrogen, menopause, neutrophils/lymphocyte ratio, residual symptoms, World Health Organization Covid-19 category

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INTRODUCTION

The outbreak of COVID-19 in China in December 2019 has progressed into a pandemic affecting health infrastructure and economies worldwide.^[1] Since then, rigorous research is being carried out to understand its characteristics and outcomes. One of the interesting facts observed about COVID-19 is its definite association with gender in terms of morbidity and mortality. The preponderance of males over females being affected by Severe Acute Respiratory Syndrome– Corona

Virus-2 (SARS-Cov-2) has been observed by many studies.^[2,3] In China, it was observed that 60% of patients affected by COVID-19 were men.^[4] The severity as well as case fatality rate was reported higher in men as compared to women (2.8% vs. 1.7%).^[2,5] The reason for this can be drawn from the fact that 17 beta-estradiol

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downregulates expressions of angiotensin-converting enzyme-2 (ACE-2) mRNA in epithelial cells that are the primary entry point for the virus inside the host cell as been established by other studies.^[5,6]

The indirect evidence of the protective effect of estrogen has been shown by Channappanavar *et al.* in the mice model, demonstrating that female mice administered with estrogen receptor antagonist have 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.^[7]

It is a known fact that pretreatment of human macrophages with the estrogen reduces tumor necrosis factor- α expression through attenuation of nuclear factor-kappa-b (NFk-b) activation. Estrogen also attenuates monocyte-macrophage recruitment by downregulating chemokine ligand 2 expression among inflammation and dampening toll-like receptors 4-mediated NFk-b activation.^[8] In a multicentric study from Wuhan by Ding *et al.*, it was observed that estrogen and anti-Mullerian hormone (AMH) were inversely linked to the severity of COVID-19 with menopause accounting as an independent prognostic variable.^[9] However, robust clinical studies are lacking to suggest the true effect of menopause on its presentation and outcomes; hence, this retrospective study was planned to investigate whether menopausal status really affects the COVID-19 disease outcome in women.

MATERIALS AND METHODS

A retrospective cohort study was conducted on COVID-19-infected females admitted between May 2020 and August 2020 at tertiary care dedicated COVID hospital. The data were collected using hospital admission records and telephonic follow-up of eligible women by defined inclusion and exclusion criteria. A COVID-19 confirmed case was defined as a positive result on real-time reverse-transcriptase-polymerase-chain reaction (RT-PCR) assay of nasopharyngeal swab specimens. Permission from the institutional ethics committee was taken to conduct the study.

Inclusion criteria

- RTPCR confirmed COVID-19 female patients
- Female 18–70 years of age group
- Women having controlled diabetes/hypertension (HTN)/thyroid or other chronic diseases
- Having mild-to-moderate category disease at the time of admission in both groups.

Exclusion criteria

- Pregnant and lactating women
- Asymptomatic women
- Patient with severe category COVID-19 disease at admission
- Currently receiving estrogen-based hormonal therapy
- Having abnormal genital bleeding episodes in the past 3 months
- Subjects already on any cancer therapy
- Women on steroids
- Patients having uncontrolled HTN/diabetes mellitus (DM) or other medical diseases.

Eligible women not giving consent on the telephonic interview, language barrier, and those with incomplete investigation records were also excluded. The overall recruitment pattern is defined in Figure 1.

Data retrieved from hospital medical records included history and examination (general and systemic), COVID-19 disease category (mild/moderate/severe) according to the World Health Organization manual COVID-19.^[10] Enrolled women were divided into two groups based on menstrual status: group-1 (premenopausal/reproductive age) and Group-2 (menopausal). Premenopausal included those having regular cycles and menopausal having minimum 1 year of amenorrhea.

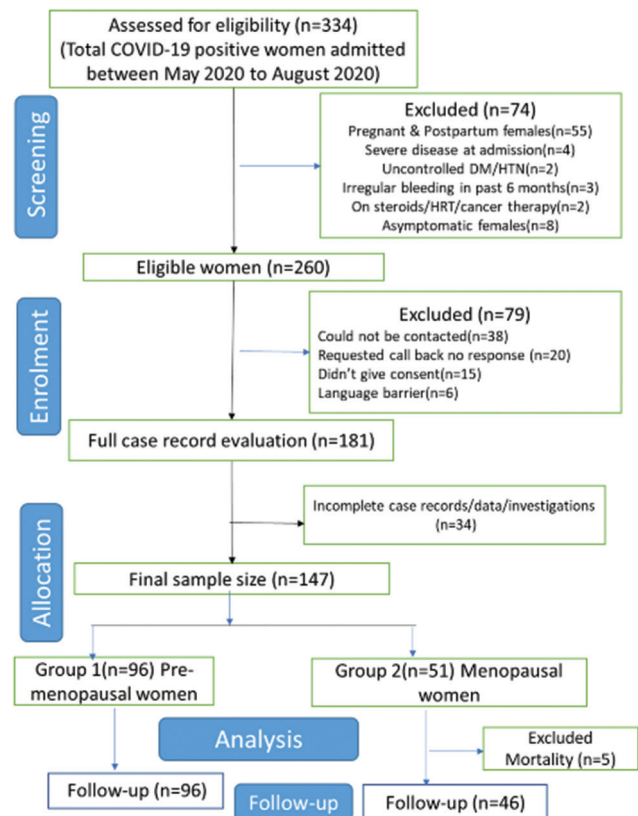


Figure 1: Recruitment chart

Following investigation profile of enrolled cases were noted; hemogram, liver function tests, renal function tests, electrolytes, prothrombin time, activated partial thrombin time, fibrinogen, inflammatory markers such as C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH) and d-dimer, chest X-ray, electrocardiogram, and arterial blood gas analysis. Clinical course, biochemical characteristics of the cases over the hospital stay duration, and date of discharge were notified to assess the following outcomes in two of the groups for comparison.

Primary outcomes measure – Hospital stay duration.

Secondary outcome: (1) Change in disease category: progression-to-severe disease. (2) The requirement of oxygen by a simple mask, reservoir mask, high flow nasal cannula (HFNC), noninvasive ventilation (NIV), or mechanical ventilation (3) Mortality.

The discharge criteria were two negative results on RT-PCR assay of nasopharyngeal swab specimens and the patient was advised home quarantine for 14 days. According to the institute's protocol, all discharged patients were followed up to 8–12 weeks by telemedicine consultation.

In the follow-up, the telephonic interview covered verbal consent, the status of health, gynecological complaints, residual symptoms, and any change in the menstrual pattern since COVID-19 infection in premenopausal women. The correlation of menstrual status with different variables was analyzed using statistical tests.

Statistical analysis

Continuous variables were expressed as the means and standard deviations. Categorical variables were summarized as the counts and percentages in each category. A two-tailed *t*-test was applied to continuous variables. Chi-square tests/Fisher's exact tests were used for categorical variables as appropriate. The univariate logistic regression analysis was done to see the association of length of hospital stay and disease severity with menstruation, age, obesity, comorbidities, oxygen/ventilator requirement, hemoglobin, and neutrophil-to-lymphocyte ratio (NLR). Then, the variables which came out to be significant were put in multivariate logistic regression analysis to see the independent effect of each variable on length of hospital stay and disease severity. All statistical analyses were carried out at 5% level of significance, and $P < 0.05$ was considered significant.

RESULTS

The data of a total of 147 COVID-19-positive females were analyzed of which 96 patients belonged to

Group-1 (premenopausal), while 51 belonged to Group-2 (menopause) suggesting estrogenic and hypoestrogenic clinical states, respectively.

While evaluating baseline and clinical characteristics, it was found that patients in Group-1 had a mean age of 30.1 ± 8.5 compared to 55.9 ± 10.7 years, and lower body mass index (BMI) (24.5 ± 3.4 vs 29 ± 3.2 kg/m², $P < 0.01$) as compared to Group-2. Overall Group-1 had significantly more number of mild disease cases, while Group-2 had moderate disease (39 [76.5%] vs. 14 [14.6%] $P < 0.01$) and the presence of comorbidities such as DM, HTN, chronic obstructive pulmonary disease (COPD), and cardiac disorders. The thyroid disorders were equally prevalent between both the groups.

Evaluating the clinical course of the disease, it was observed that in Group-1, seven (7.3%) cases progressed toward severe disease, while it was 12 (23.5%) in Group-2 making it statistically significant [Table 1]. The patients in Group-2 had significantly more requirement of oxygen (20 [20.8%] vs. 32 [62.7%], $P < 0.01$) and ventilator (1 [1%] vs. 14 [27.5%], $P < 0.01$). The Group-2 women had prolonged hospital stay also (14.1 ± 8.9 vs. 8.6 ± 3.9 days) $P < 0.01$.

There was no mortality in the premenopausal Group-1, while there were five (9.8%) deaths in the menopausal Group-2. All these five patients had moderate disease at the time of admission, and they progressed rapidly to a critical stage. They all required O₂ supplementation since admission and later required respiratory support in the form of HFNC/NIV/mechanical ventilation. They were managed as per institutes' protocol with low molecular weight heparin 60 IU subcutaneously twice daily, methylprednisolone, and high-end antibiotics as required. Among these five menopausal women, two were diagnosed cases of COPD, one had valvular heart disease, another was elderly diabetic and one progressed to acute respiratory distress syndrome despite having no comorbidity.

Biochemical parameters

The total leukocyte count (TLC) (7800 ± 264.6 vs. 5850 ± 264.6) and neutrophils/lymphocyte ratio (5.2 ± 5.1 vs. 2.6 ± 2.3) was significantly higher ($P < 0.01$) in Group-2 as compared to Group-1. No significant difference was found in mean values of inflammatory markers (CRP, LDH, ferritin, D-dimer) in the two groups; however, in a subset analysis where we compared inflammatory markers between the moderate disease cases in two groups, only ferritin was found significantly higher in the menopausal group [Table 2].

Table 1: Clinico-demographic characteristics

Parameters	Group 1 (n=96), n (%)	Group 2 (n=51), n (%)	P
Demographic parameters			
Age (years)±SD	30.1±8.5	55.9±10.7	<0.01
Place of residence			
Rural	17 (63.0%)	10 (37.0%)	>0.05#
Urban	79 (65.8%)	41 (34.2%)	
BMI (kg/m ²)	24.5±3.4	29±3.2	<0.01
Comorbidities			
DM	7 (7.3)	18 (35.3)	<0.01
HTN	6 (6.3)	26 (51)	<0.01
COPD	0	4 (7.8)	<0.01
Cardiac disorders	0	3 (5.9)	<0.01
Thyroid disorders	3 (3.1)	3 (5.9)	>0.05#
Clinical course during hospital stay			
Oxygen requirement	20 (20.8)	32 (62.7)	<0.01
NIV/mechanical ventilator	1 (1)	14 (27.5)	<0.01
Progression toward severe disease	7 (7.3)	12 (23.5)	<0.01
Mortality	0 (0)	5 (9.8)	<0.01
Hospital stay duration	8.6±3.9	14.1±8.9	<0.01

#Nonsignificant. SD: Standard deviation, BMI: Body mass index, NIV: Noninvasive ventilation, HTN: Hypertension, COPD: Chronic obstructive pulmonary disease, DM: Diabetes mellitus

Table 2: Biochemical parameters

Parameters	Group-1 (n=96), mean/median±SD	Group 2 (n=51), mean/median±SD	P
Hb (g/dl)	11.77±1.76086	11.5±2.31974	>0.05
TLC	5850±264.6	7800±264.6	<0.01
N/L ratio	2.6±2.3	5.2±5.1	<0.01
Platelet count	1.85±0.9294	1.72±0.599	>0.05
CRP (mg/ml)	15.3±54.5	17.6±26.1	>0.05
LDH (U/l)	364.0±338.2	609.6±773.8	>0.05
D-Dimer (µ/ml)	1795.2±3029.8	2968.6±3125.3	>0.05
Serum ferritin (ng/ml)	90.1±122.0	127.2±95.4	>0.05

Subset analysis of moderate disease cases

Parameters	Group-1 (n=14), mean±SD	Group 2 (n=39), mean±SD	P
CRP (mg/ml)	13.9±56.4	20.2±25.4	>0.05
LDH (U/l)	364.7±492.2	558.56±452.5	>0.05
D-Dimer (µ/ml)	1952.7±3262.9	2507.1±2860.9	>0.05
Serum ferritin (ng/ml)	70.4±87.5	165.3±147.4	0.002

TLC: Total leukocyte count, CRP: C-reactive protein, LDH: Lactate dehydrogenase, SD: Standard deviation, Hb: Hemoglobin

Symptomatology in COVID-19 female patients

Fever and cough were the most common symptoms observed in both groups. The presence of cough, breathlessness, and chest pain were significantly more in Group-2 as compared to Group-1 (72.5% vs. 41.7%; 70.6% vs. 5.2%; 5.9% vs. 0%, $P < 0.01$), respectively. The rest of the symptoms except the loss of taste were more common in Group-2, although statistically not significant. On telemedicine follow-up, 11 women persistently experienced weakness in Group-2 as compared to only four in Group-1 and five women experienced occasional breathlessness compared to none in Group-1; the difference being statistically significant. Overall, 12.5% and 52.2% of patients

had residual symptoms in Group-1 and Group-2, respectively. Among Group-1, 12 women experienced oligomenorrhea (infrequent menstrual bleeding), two had menorrhagia (heavy menstrual bleeding), two hypomenorrhea (shortened menstrual bleeding), one each experienced hypo-oligomenorrhea and polymenorrhagia after recovery [Table 3].

The length of hospital stay was categorized into a binomial categorical variable by taking the median number of days (9 days) as the cut off. Univariate logistic regression statistical analysis depicted that menopause status, age, disease progression, presence of any comorbidities, and N/L ratio (cut off = 3.1) were

Table 3: Symptomatology of enrolled cases

Symptoms	Group 1 (n=96), n (%)	Group 2 (n=51), n (%)	P
At admission[#]			
Fever	69 (71.9)	43 (84.3)	>0.05
Cough	40 (41.7)	37 (72.5)	<0.01
Anosmia	10 (10.4)	7 (13.7)	>0.05
Rash	1 (1)	0	>0.05
Sore throat	17 (17.7)	14 (27.5)	>0.05
Myalgia	8 (8.3)	9 (17.6)	>0.05
GI symptoms	7 (7.3)	7 (13.7)	>0.05
Sudden onset breathlessness	5 (5.2)	36 (70.6)	<0.01
Loss of taste	2 (2.1)	0	>0.05
Chest pain	0	3 (5.9)	<0.01
Weakness	2 (2.1)	2 (3.9)	>0.05
Symptoms	Group 1 (n=96) 12 (12.5%)	Group 2*(n=46) 24 (52.2%)	P
Residual symptoms at 8-12 weeks follow-up			
Cough	3 (3.1)	2 (4.3)	>0.05
Weakness	4 (4.1)	11 (23.9)	<0.01
Breathlessness	0	5 (10.8)	<0.01
Anosmia	3 (3.1)	2 (4.3)	>0.05
Myalgia	0	2 (4.3)	>0.05
Chest pain	2 (2.1)	2 (4.3)	>0.05
Menstrual pattern changes	18 (18.75)	Not applicable	-
Oligomenorrhea	12 (66.6)		
Hypomenorrhea	2 (11.1)		
Oligohypomenorhoa	1 (5.55)		
Menorrhagia	2 (11.1)		
Polymenorrhagia	1 (5.55)		

[#]Symptoms were overlapping among patients. *5 menopausal women were excluded from the analysis (mortality cases). GI: Gastrointestinal

significantly associated with the hospital stay duration. On putting these variables in a multivariate logistic regression model, the severity of the disease of the study participants was noted to be actually predicting the length of hospital stay. Patients with progressive disease were four times more likely to have a hospital stay of >9 days than those with mild and moderate disease [Table 4].

On univariate logistic regression, menopause status, age, duration of hospital stay, presence of any comorbid conditions, and NLR of the study participants were observed to be significantly associated with the progression of the disease. After using a multivariate logistic regression model, it was noted that only the duration of hospital stay of the study participant actually found associated ($P < 0.05$) with the severity of disease (AOR = 4.684, confidence interval = 1.155–18.995) [Table 5].

On subgroup analysis, we found more number of affected cases in the late menopause group (38/51; 74.5%) and this group showed more progressive disease (10/12), the requirement of ventilatory support (12/14), and mortality (4/5), although the results were not found statistically significant [Table 6].

DISCUSSION

Clinical and biochemical data of a total of 147 women, 51 menopausal and 96 premenopausal, respectively, were collected and 8–12 weeks follow-up information collected telephonically and compared to assess the association of menopausal status and other variables with hospital stay and disease progression. The difference in COVID-19 disease characteristics and afflictions has been notified globally between two genders and the basic difference between male and female physiology is menstruation and sex hormone estrogen.^[1,11,12] In this study, we tried to find out the impact of menopausal status (hypoestrogenic population) on COVID-19 outcome by comparing clinical profiles with premenopausal normal menstruating women (eu-estrogenic) as the hormonal milieu of both groups is different.

Our study delivered a few interesting findings. Most cases in the menopausal group were in the moderate category at admission (76%) compared to only 14% in the premenopausal group. The menopausal women in our study were overweight (significantly higher BMI) as compared to reproductive-aged females, same as reported by Atapattu.^[13]

Table 4: Multivariate logistic regression for predictors of length of hospital stay

Variables	Crude's OR	95% CI		P	Adjusted OR	95% CI		P
		Lower limit	Upper limit			Lower limit	Upper limit	
Menopause								
Yes	3.410	1.679	6.927	0.001*	5.344	0.949	30.090	0.057
No		Reference				Reference		
Age group								
<25	0.217	0.063	0.749	0.045*	0.673	0.261	1.738	0.660
25-40	0.202	0.063	0.672		0.351	0.056	2.189	
41-60	0.362	0.109	1.207		0.426	0.042	4.372	
>60		Reference				Reference		
Progression of disease								
Severe	6.685	2.094	21.335	0.001*	4.935	1.327	18.352	0.017*
Mild/mod		Reference				Reference		
Co-morbidities								
Yes	2.192	1.100	4.369	0.026*	1.115	0.391	3.185	0.838
No		Reference				Reference		
Hemoglobin								
<11	1.746	0.852	3.579	0.128	-	-	-	-
≥11		Reference			-	-	-	-
NLR								
≤3.1	3.015	1.447	6.283	0.003*	1.974	0.862	4.520	0.198
>3.1		Reference				Reference		

*Statistically significant. CI: Confidence interval, NLR: Neutrophils/lymphocyte ratio, OR: Odds ratio, CI: Confidence interval

Table 5: Multivariate logistic regression for predictors of severity of disease

Variables	Crude's OR	95% CI		P	Adjusted OR	95% CI		P
		Lower limit	Upper limit			Lower limit	Upper limit	
Menopause								
Yes	3.912	1.432	10.690	0.008*	2.878	0.618	13.403	0.262
No		Reference				Reference		
Age groups								
<25	0.016	0.003	0.095	0.015*	0.164	0.012	2.195	0.186
25-40	0.157	0.044	0.564		0.142	0.012	1.645	
41-60	0.148	0.039	0.561		0.494	0.086	2.847	
>60		Reference				Reference		
Hospital stay days								
≤9		Reference		0.001*		Reference		0.031*
>9	6.685	2.094	21.335		4.684	1.155	18.995	
Co-morbidities								
Yes	6.632	2.231	19.709	0.001*	3.465	0.653	18.392	0.145
No								
Hemoglobin								
<11	3.199	1.196	8.555	0.021*	2.384	0.716	7.932	0.157
≥11		Reference				Reference		
NLR								
≤3.1	7.078	2.476	20.231	<0.001*	3.086	0.837	11.374	0.091
>3.1		Reference				Reference		

*Statistically significant. CI: Confidence interval, NLR: Neutrophils/lymphocyte ratio

This all might be explained by a combination of advanced age, estrogen depletion, sedentary lifestyle, and higher comorbidities in the menopausal Group-2.

The menopausal women had significantly prolonged hospital stay; almost double 14.1 +8.9 days compared

to Group-1 8.6 +3.9 days ($P < 0.01$), progressive disease, and increased requirement of oxygen support. In this study, both groups varied not only in menstrual status (estrogen), but other variables such as age, BMI, comorbidities, along with disease severity at the time of admission. All these variables have its own

Table 6: Age wise comparison of outcome among menopausal study participants

Parameters	Age groups			P
	Early menopause	Late menopause		
	<50 (n=13), n (%)	50-60 (n=21), n (%)	>60 (n=17), n (%)	
Oxygen requirement, 32/51 (62.7%)				
Yes (n=32)	7 (21.9)	14 (43.8)	11 (34.4)	0.294
No (n=19)	6 (31.6)	7 (36.8)	6 (31.6)	
Ventilator requirement, 14/51 (27.5%)				
No (n=37)	11 (29.7)	16 (43.2)	10 (27.0)	0.82
Yes (n=14)	2 (14.3)	5 (35.7)	7 (50.0)	
Progression to disease severity, 12/51 (23.5%)				
No (n=39)	11 (28.2)	13 (33.3)	15 (38.5)	0.424
Yes (n=12)	2 (16.7)	8 (66.7)	2 (16.7)	
Mortality, 5/51 (9.8%)				
Yes (n=5)	1 (20.0)	1 (20.0)	3 (60.0)	0.503
No (n=46)	12 (26.1)	20 (43.5)	14 (30.4)	
Hospital stay mean, 14.1±8.9 days				
≤9	7 (35.0)	8 (40.0)	5 (25.0)	0.127
>9	6 (19.4)	13 (41.9)	12 (38.7)	

effect on hospital stay duration; therefore, multivariate analysis was tried to overcome such variable bias. Multivariate regression clearly outlined no significant association between menopausal status with hospital stay, controlling age, disease progression, comorbidity, and BMI, while on univariate analysis, it was found significantly associated. Contrary to this Ding *et al.* from China, has shown menopause as an independent risk factor for the increased hospital stay and for determining the disease severity. They measured female hormones also in 78 women and concluded that estradiol and AMH are negatively correlated with disease severity.^[9] We report a significant association between hospital stay and severe disease and vice versa as per multivariate analysis, which has been experienced by Liu *et al.* too.^[14]

In the present study, the mortality rate of postmenopausal women was found higher (five vs. nil; progressing from moderate disease), but the number is too small to comment on significance truly. Cagnacci and Xholli calculated the difference in COVID-19 mortality among men and women according to age and they found favorable prognosis in women which declined after 50–59 years of age. This study points toward the importance of the hormone milieu in women. The hormone, especially estrogen produced by ovaries play a key role in inflammation, immunity, and the expression of ACE receptor, which gets lost after menopause.^[11] However, Bonaccorsi *et al.*, in another study, stated that menopause *per se* does not increase the severity of COVID-19 and endorsed that there are no data to suggest to start or withdraw menopause hormone therapy to protect against it.^[15] Another study

by Lee *et al.* reported nonassociation of gender with mortality and the role of menopausal hormone therapy in improving clinical outcomes.^[16] The drawback of our study is that we did not assess the estrogen levels and considered estrogenic status clinically based on the presence of regular monthly menstrual cycles in premenopausal Group-1.

Similar to our findings, UK COVID Symptoms Study also concluded that menopause is not linked to hospitalization days, respiratory support, and disease severity significantly as compared to premenopausal women and they attributed poor prognosis in menopausal women to epigenetic age acceleration. They also inferred that combined oral contraceptive intake had a protective effect among premenopausal women, whereas menopausal hormone therapy failed to show any beneficial effects on menopausal women. Since this study is still in preprint, the findings have to be taken with pinch of salt, but at the same time, they cannot be ignored due to its large sample size of 152,637 women.^[17]

Our study did not find any definite association between menopausal status and disease outcome, as stated by others.^[9,18] The reason could be our relatively modest sample size and nonavailability of the value of female hormones. However, we tried to negate that effect by excluding the women with irregular cycles having fluctuating hormone levels. One possible reason may be the good recovery rate of COVID-19 in India.^[19] The poorer prognosis apparent in COVID-19 in menopausal females might be due to higher age and associate comorbidities.^[17] The true answer to this question can only be answered by larger future prospective studies or randomized control trials.

Early menopause, i.e., stage + 1 STRAW +10 last approximately 5–8 years after the final menstrual period, while late menopause, i.e., stage +2 STRAW starts after than when somatic razing (senescence) add over and peripheral fat-based estrone production also is lost. Although clinically apparent, we did not find the statistically significant difference in disease outcome in early versus late menopausal cases or even in >60 years cases in this study. This may be due to a small sample of menopausal group cases. Another hypothesis suggests although the immune-protective effect of estrogen is lost at the same time, the ACE-2 expression is also decreased with age, which reduces the predilection for COVID-19.

Biochemical parameters

The TLC, as well as NLR, was observed to be significantly higher in menopausal women (Group-2). Both can be linked to the increased severity of disease in menopausal women, as observed in other studies.^[18] Liu *et al.* observed NLR as an independent predictor for predicting critical illness in COVID-19. They found that patients having NLR >3.13 were predicted to develop critical illness and should be considered for early admission to the intensive care unit (ICU).^[20] In a retrospective analysis by Ciccullo *et al.*, also concludes that NLR is useful for early screening for critical illnesses in COVID-19. According to them, NLR <3 was predictive of clinical improvement, while a value >4 was predictive of admission to ICU.^[21] Based on these, we also calculated an independent association of NLR with severity adjusting for other factors and did not found such association. We suggest further prospective studies assessing different cut-off values of NLR should be carried out to establish an association between NLR and severe disease.

The other inflammatory markers such as CRP, LDH, D-dimer, and ferritin were observed to be raised in both the groups above the normal values. Mean values of all inflammatory markers found to be much raised in Group-2 (menopausal) compared to Group-1, but no statistical significance could be noted except in subset analysis where ferritin was raised significantly in menopausal patients having the moderate disease [Table 2]. This could again be due to the modest sample size in our study. High values can be due to more number of moderate category disease cases, 76% in Group-2 versus 14.6% in Group-1, but it points out that the immune system is optimally working to cause rise in inflammatory markers in the old menopausal group. Other studies also have found a definite association between disease severity and raised inflammatory markers.^[22-25] This could again be due to the modest sample size in our study.

As per the present study among premenopausal COVID-19 cases, approximately 85% had mild disease, while there were significantly more moderate category cases (76%) in the postmenopausal group. Yu *et al.* found that though asymptomatic/mild infection could happen at any age, while patients <45 years had a higher chance of being asymptomatic/mild.^[26] Fever and cough were the two most common presenting symptoms in both the groups, as observed by other studies.^[27,28] We also found that cough, breathlessness (sudden onset), and chest pain were significantly more in the menopausal Group-2. COVID symptom study by Costeira *et al.* showed more of anorexia, anoxia, hoarse voice, myalgia, and fever in the menopausal group.^[17]

After recovery at the time of telephonic follow-up, 12.5% of premenopausal women had residual symptoms (menstrual symptoms excluded) as compared to 52.2% in menopausal women in our study. Similarly, Tenforde *et al.* also found residual symptoms in 47% of patients above the age of 50 years.^[29] Regarding residual symptoms among two groups breathlessness and weakness were found to be significantly more in Group-2 (9.8% vs. 0%; 21.6% vs. 4.1%). It is really difficult to say the persistence of breathlessness is related to menopausal status (hypo-estrogenism) or due to older age or moderate disease status. The menstrual irregularities experienced by premenopausal women could be due to stress of COVID-19 infection or may be due to infection *per se* we could not find any studies on menstrual pattern post-COVID-19 infection to comment further.

The strengths of the present study

1. Strict inclusion and exclusion criteria: patients with irregular cycles and severe disease at the time of admission were excluded
2. Independent effect of menstrual status on COVID-19 clinical outcomes was calculated.

The limitations of the present study

1. Retrospective data
2. Modest sample size
3. Hormones levels were not measured.

CONCLUSIONS

In the earlier studies, estrogen is being given the credit of favorable prognosis among COVID-19 cases based on male preponderance, animal model studies using estrogen antagonists, and human studies measuring estrogen levels, respectively.^[7,9,11] Multi-variate logistic regression model suggested that menopausal status independently is not associated with hospital stay duration or progression to the severity in this study,

while stay duration and progressive disease were found independently associated with each other. Although estrogen being steroid theoretically have the immune and inflammation-modulating activity, the pro-coagulant effect cannot be ignored.^[30] Therefore, prospective randomized trials are actually required to truly comment on its role and efficacy in managing COVID-19 disease.

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Conflicts of interest

There are no conflicts of interest.

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