Systematic Review and Meta-Analysis (Original Article)

Safety and efficacy of lopinavir/ritonavir combination in COVID-19: A systematic review, meta-analysis, and meta-regression analysis

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Abstract:

BACKGROUND: Being protease inhibitors and owing to their efficacy in SARS-CoV, lopinavir + ritonavir (L/R) combination is being used in the management of COVID-19. In this systematic review and meta-analysis, we have evaluated the comparative safety and efficacy of L/R combination.

MATERIALS AND METHODS: Comparative, observational studies and controlled clinical trials comparing L/R combination to standard of care (SOC)/control or any other antiviral agent/combinations were included. A total of 10 databases were searched to identify 13 studies that fulfilled the predefined inclusion/exclusion criteria.

RESULTS: No discernible beneficial effect was seen in the L/R group in comparison to SOC/control in terms of “progression to more severe state” (4 studies, odds ratio [OR]: 1.446 [0.722–2.895]), “mortality” (3 studies, OR: 1.208 [0.563–2.592]), and “virological cure on days 7–10” (3 studies, OR: 0.777 [0.371–1.630]), while the L/R combination arm performed better than the SOC/control arm in terms of “duration of hospital stay” (3 studies, mean difference (MD): −1.466 [−2.403 to −0.529]) and “time to virological cure” (3 studies, MD: −3.272 [−6.090 to −0.454]). No difference in efficacy was found between L/R versus hydroxychloroquine (HCQ) and L/R versus arbidol. However, in a single randomized controlled trial (open label), chloroquine (CQ) performed better than L/R. The combination L/R with arbidol may be beneficial (in terms of virological clearance and radiological improvement); however, we need more dedicated studies. Single studies report efficacy of L/R + interferon (IFN, either alpha or 1-beta) combination. We need more studies to delineate the proper effect size. Regarding adverse effects, except occurrence of diarrhea (higher in the L/R group), safety was comparable to SOC.

CONCLUSION: In our study, no difference was seen between the L/R combination and the SOC arm in terms of “progression to more severe state,” “mortality,” and virological cure on days 7–10; however, some benefits in terms of “duration of hospital stay” and “time to virological cure” were seen. No significant difference in efficacy was seen when L/R was compared to arbidol and HCQ monotherapy. Except for the occurrence of diarrhea, which was higher in the L/R group, safety profile of L/R is comparable to SOC. Compared to L/R combination, CQ, L/R + arbidol, L/R + IFN-α, and L/R + IFN-1β showed better efficacy, but the external validity of these findings is limited by limited number of studies (1 study each).

Keywords:

COVID-19, lopinavir, meta-analysis, ritonavir

Abbreviations:

L/R: Lopinavir + Ritonavir, A: Arbidol, SOC: Standard of care, HCQ: Hydroxychloroquine, CQ: Chloroquine, IFN: Interferon, ADR: Adverse drug reaction, MD: Mean difference, OR: Odd ratio, COVID-19: Corona virus disease-19, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase

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Introduction

Human coronaviruses (a total of 7 strains) are frequently implicated in different human diseases. The common four strains of coronaviruses causing human diseases are 229E, NL63, OC43, and HKU1, of which the first two are *Alpha-coronavirus* and the latter two are *Beta-coronavirus*. The other three strains that are responsible for causing serious human disease are SARS-CoV (first reported in 2003), MERS-CoV (reported in 2012), and SARS-CoV-2 (Wuhan, China, in 2019), all three being *Beta-coronavirus* genera of *Orthocoronaviridae* subfamily.

The effectiveness of lopinavir–ritonavir (L/R) combination against SARS-CoV was demonstrated *in vitro* in 2004. Later, these findings were established in clinical studies using historical control (adjusted odds ratio [OR]: 0.076 [0.01–0.589]). The safety and efficacy of L/R combination in SARS-CoV and MERS-CoV are already reviewed.

Following the same line, L/R showed good binding affinity in *in silico* studies against SARS-CoV-2 main protease, which was validated by molecular dynamic (MD) simulation studies. No difference was observed in the binding pattern of L/R to SARS-CoV-2 main protease; however, some studies demonstrated a better binding profile of ritonavir than lopinavir. In *in vitro* settings, L/R inhibited SARS-CoV-2 replication. On the background of its clinical evidence of efficacy against “SARS-CoV” and “MERS-CoV,” L/R use was recommended as a treatment option for COVID-19, and many regulatory agencies recommended its use, especially during the initial few months of the disease outbreak. Since then, there came a trend of use of this combination in COVID-19, and few randomized controlled trails (RCTs) and observational studies came up evaluating the safety and efficacy of this combination with varied results. While many of the case series showed beneficial effect of L/R combination with regard to virological as well as clinical cure, many reports refuted its treatment efficacy. In this regard, we have conducted this systematic review, meta-analysis, and meta-regression analysis to evaluate the comparative safety as well as efficacy of L/R combination in the present scenario of “COVID-19 pandemic.”

Materials and Methods

Recommendations laid down by “Cochrane Community” and “Preferred Reporting Items for Systematic Reviews and Meta-Analysis” guidelines were followed while conducting this study.

Aim

The aim of this study is to evaluate the safety as well as efficacy of L/R combination therapy in the management of COVID-19.

Inclusion/exclusion criteria

Studies with the below-mentioned characteristics were included in this systematic review and meta-analysis.

1. Study design: Comparative, observational/interventional studies and RCTs, which compared L/R combination to either standard of care (SOC)/control or other standard drug/drug combinations, were included. Nonpeer-reviewed articles/studies/preprints were excluded
2. Participants: Laboratory-confirmed COVID-19 patients
3. Intervention/treatment: L/R
4. Control: Symptomatic treatment/SOC/other standard drug.

Exclusion criteria

We excluded case report and series as well as single-group observational studies in our study.

Objectives

1. Virological cure
2. Mortality
3. Clinical worsening/progression/deterioration of disease state during treatment
4. Safety/tolerability of the studied drug.

Comparisons

1. L/R versus conventional/SOC/control
2. L/R versus other antiviral drugs/drug combinations.

Definitions

- Virological cure: Nondetection of SARS-CoV-2 RNA in the respective biologic fluid
- Progression to severe disease: For our study purpose, “progression of disease” was defined as mild-to-moderate disease progressing to severe disease or in case of severe disease, progressing to critical state requiring mechanical ventilation (invasive or noninvasive), or a higher level of care was considered as “progression of the disease.”

Search strategy and study selection

A total of 10 literature databases were searched namely PubMed, Scopus, EMBASE, CINAHL PLUS, OVID, Web of Science, ScienceDirect, Wiley Online Library, CNKI, and Cochrane CENTRAL Library from interception to September 21, 2020, with appropriate keywords without language restriction. Articles in other languages were translated into English language using an online doc translator. The full text of the relevant articles was further screened using the predefined “inclusion/
exclusion criteria” by HK and SK. Any discrepancies were resolved by consulting with BW and PS.

**Data extraction**
Two authors namely PS and HK separately did the data extraction using “pretested data extraction forms.” “Online doc translator” was used to translate documents in other languages to English.[23]

**Risk of bias**
For RCT, risk of bias (ROB) was evaluated in accordance with the “Cochrane risk of bias tool for randomized control studies.”[22] For observational studies, “Newcastle Ottawa Scale” was used.[26] Three investigators PS, AB, and HK independently evaluated the ROB of all the included studies. For any discrepancy, the issue was solved after consulting BM.

**Assessment of heterogeneity and statistical analysis**
While estimating the point estimate, for “dichotomous data,” OR/risk ratio (RR) with 95% confidence interval (CI) was calculated as appropriate. For continuous data, mean difference (MD) with 95% CI was calculated. Heterogeneity was assessed using F and Chi² statistics.[25] In case of the presence of “significant heterogeneity” (>50%), a “random-effect model” was used, otherwise “fixed-effect model” was used to negotiate the effect.[27] “Review Manager (RevMan) 5.3” software (Cochrane community) was used for meta-analysis. [28] Interconversion of the data in different formats was conducted as per the standard methodology.[29]

**Exploration of heterogeneity: meta-regression analysis**
In case of high heterogeneity, we explored the possible cause of high heterogeneity using meta-regression analysis. Factors that we investigated were sex distribution, age, time from disease onset to treatment initiation, severity of disease, and use of corticosteroids (these factors are known to prolong virological shedding in COVID-19). Analysis was conducted using using SPSS (IBM, New-york) and Metafor[31] package in "R".

**Results**

**Details of included articles**
We searched 10 databases with appropriate keywords, and finally, 557 articles were screened with the help of title and abstract using the predefined inclusion–exclusion criteria, among which 40 articles were further screened using full text. A total of 13 studies satisfying predefined “inclusion/exclusion criteria” were included in the final analysis: five[32–36] were RCTs and rest eight studies[37–44] were comparative, observational studies. One of the studies was retracted (retractionwatch.org)[45] and hence was excluded [Figure 1 and Table 1].

**Risk of bias**
ROB of the included studies (8 observational and 5 RCTs) is shown in Supplementary Figure 1 and Supplementary Table 1.

**Overall safety and efficacy of lopinavir + ritonavir combination in SARS-CoV-2 (irrespective of disease severity)**

Lopinavir + ritonavir combination versus standard treatment/control
A total of six studies[32,33,37–40] evaluated the comparative effectiveness and safety of L/R combination versus SOC/control. The details of therapy in the standard treatment group are given in Table 1.

Lopinavir + ritonavir versus standard of care: Duration of hospital stay
A total of three studies[32,37,38] have reported the duration of hospital stay between L/R and SOC. Treatment with L/R was associated with significant decrease in the duration of hospital stay (MD: −1.466 [−2.403 to −0.529], F = 0%, fixed-effect model) [Figure 2].

Lopinavir + ritonavir versus standard of care: Mortality
Three studies[32,33,39] reported comparative mortality between L/R and SOC. No differences were seen among groups (OR: 1.208 [0.563–2.592], F = 26.42%) [Figure 3].
Lopinavir + ritonavir versus standard of care: Progression of disease to a more severe state

A total of four studies\textsuperscript{32,33,37,39} reported “progression of disease to a more severe state.” No difference was seen between the LR and SOC arm in terms of “progression of disease to a more severe state” (OR: 1.466 [0.722–2.895],  \(\hat{I}^2 = 43.6\%\)) [Figure 4]. Three studies\textsuperscript{32,33,39} reported “virological cure on days 7–10” post-therapy. No difference was noted between the two arms (OR: 0.777 [0.371–1.630],  \(\hat{I}^2 = 23.14\%\)) [Figure 5].

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### Table 1: Details of the included studies

| Study, author             | Design                      | Population                          | Intervention                                                                                       | Control          | Outcome                                                                 |
|---------------------------|-----------------------------|-------------------------------------|-----------------------------------------------------------------------------------------------------|------------------|-------------------------------------------------------------------------|
| Cao et al., 2020\textsuperscript{30} | RCT, open label             | RT-PCR-confirmed severe COVID-19     | L/R (400/100 mg) BD (n=99)                                                                        | SOC (n=100)      | Clinical improvement, day-28 mortality, hospital stay duration, virological improvement |
| Yan et al., 2020\textsuperscript{41} | Retrospective study         | RT-PCR confirmed COVID-19 (noncritically-ill) | L/R (400/100 mg) BD (n=78)                                                                        | SOC (n=42)       | Duration of viral shedding                                               |
| Shi et al., 2020\textsuperscript{38} | Observational study         | RT-PCR-confirmed, COVID-19 patients  | Symptomatic treatment, \(n=17\) Arbidol, \(n=30\) L/R=27 L/R + Arbidol: \(n=25\) IFN, \(n=41\) L/R + IFN: \(n=21\) IFN + daraunavir, \(n=23\) | Symptomatic treatment/SOC (\(n=17\)) | Pneumonia resolution, radiological cure, length of hospital stay          |
| Li et al\textsuperscript{33} | RCT, 3-arm                  | MM COVID-19                         | L/R (200/50 mg) BD (n=34) Arbidol (200 mg) TDS (n=35)                                             | Control=no antiviral (\(n=17\)) | Virological cure (day 7, 14), progression of disease (progression from MM to SC) |
| Lecronier et al., 2020\textsuperscript{39} | Retrospective, observational study | Clinically confirmed critically ill COVID-19 patients | 3-arm study L/R BD for 5 days, \(n=20\) HCQ: 200 mg, BD, \(n=38\) | SOC (\(n=22\)) | Requirement of treatment-escalation after initiation of therapy, mortality, safety, virological cure |
| Ye et al., 2020\textsuperscript{41} | Observational study         | RT-PCR-confirmed COVID-19 patients  | L/R (400/100 mg) BD or L/R (800/200 mg) OD + adjuvant therapy (\(n=42\)) | Adjuvant therapy only (\(n=5\)) | Changes of body temperature, blood routine and blood biochemistry |
| Zhu et al., 2020\textsuperscript{41} | Retrospective study         | RT-PCR-confirmed COVID-19 patients  | L/R (400/100 mg) BD (n=34) Arbidol (0.2 g TDS) (n=16)                                             | Viral load (CT value), safety only |
| Kim et al., 2020\textsuperscript{41} | Retrospective cohort study  | RT-PCR-confirmed COVID-19 patients  | L/R (400/100 mg) BD (n=31) HCQ 400 mg OD (n=34)                                                   | Virological cure, clinical improvement, and adverse events |
| Karolyi et al., 2020\textsuperscript{41} | Retrospective cohort study  | Clinically confirmed severe COVID-19 patients were included | L/R (400/100 mg) BD (n=67) HCQ (loading dose of 400 mg BD on 1st day, followed by 200 mg BD (n=20) | Requirement of ICU. Mortality, hospital stay duration, virological cure, and side effects |
| Huang et al., 2020\textsuperscript{41} | RCT                         | RT-PCR confirmed COVID-19 patients  | L/R (400/100 mg) BD (n=12) CQ 500 mg BD × 10 days (n=10)                                          | Virological cure, adverse event |
| Deng et al., 2020\textsuperscript{41} | Retrospective cohort study  | Laboratory-confirmed (RT-PCR) patients with COVID-19 | L/R (400/100 mg) BD + arbidol (200 mg × TDS) (n=16)                                               | Virological cure (day 7, 14), radiological improvement in chest CT |
| Huang et al., 2020\textsuperscript{33} | Open-label RCT              | Clinically confirmed MM COVID-19 patient | Total 3 intervention groups IFN-alpha + RBV (n=27), IFN alpha + L/R (n=28)                         | -                | Virological cure, mortality |
| Hung et al., 2020\textsuperscript{39} | Open-label RCT              | RT-PCR-confirmed COVID-19           | L/R + RBV + IFN-beta-1b (n=86)                                                                  | L/R (n=40)       | Virological cure, clinical improvement, hospital stay duration, mortality |

RCT=Randomized control trial; CQ=Chloroquine, Arb=Arbidol, L/R=Lopinavir+ritonavir, O=Oxelamivir, RBV=Ribavirin, MM=Mild to moderate, SC=Severe and critical, SOC=Standard of care, CT=Computed tomography, ICU=Intensive care unit, IFN=Interferon, RT-PCR=Reverse transcription polymerase chain reaction

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Lopinavir + ritonavir versus standard of care: Time to virological cure

Although there was no difference in terms of “virological cure on days 7–10,” when the treatment effect was measured in terms of “time to virological cure,” treatment with L/R was associated with a shorter “time to virological cure” (MD: −3.272 [−6.090 to −0.454]). However, heterogeneity among the included studies was high ($I^2 = 68.58\%$) [Figure 6].

Meta-regression analysis to evaluate the causes of high heterogeneity in “time to virological cure”

As heterogeneity was high among the included studies ($I^2 = 68.58\%$), we tried to explain the heterogeneity with the help of meta-regression. As male sex,$^{46}$ age,$^{37}$ time from disease onset to treatment initiation,$^{37}$ severity of disease, and use of corticosteroids$^{47}$ are already reported to prolong the viral shedding, we intended to evaluate the effect of these covariates on the overall result and their contribution to the heterogeneity. However, the number of studies was too less ($n = 3$) and data were not available for most of the covariates. Hence, as meta-regression was carried out with only variable “difference in percentage of male sex LR-SOC/control,” this variable could not explain the high heterogeneity among the included studies [Figure 7 and Table 2].
Lopinavir + ritonavir versus standard of care: Safety

L/R combination was associated with higher incidence of diarrhea (2 studies, OR: 11.44; 95% CI: 0.21–1.36, \(I^2 = 0\%\), fixed effect); however, no difference was seen in terms of other adverse effects, e.g., loss of appetite (2 studies, OR: 5.97; 95% CI: 0.71–49.96, \(I^2 = 0\%\), fixed effect), elevation of Alanine aminotransferase (ALT) (5 studies, OR: 0.53; 95% CI: 0.52–1.63, \(I^2 = 0\%\), fixed effect), elevation of Aspartate aminotransferase (AST) (5 studies, OR: 0.92; 95% CI: 0.29–1.04, \(I^2 = 0\%\), fixed effect), and occurrence of serious adverse effect (2 studies, OR: 0.55; 95% CI: 0.29–1.04, \(I^2 = 0\%\), fixed effect) [Supplementary Figure 2].

Other adverse effects that are reported in both the arms with no difference in between were occurrence of thrombocytopenia, prolonged QT, sleep disturbance, neutropenia, rash, leukopenia, lymphopenia, anemia, and abdominal discomfort. However, the incidence of nausea, vomiting, and increased bilirubin was higher in the L/R group as compared to the standard treatment group (single study[32]).

Lopinavir + ritonavir versus hydroxychloroquine: Efficacy and safety evaluation

A total of three studies[39,42,43] reported comparative efficacy and safety of L/R versus hydroxychloroquine (HCQ).

Lopinavir + ritonavir versus hydroxychloroquine: Virological cure

No difference was observed between the L/R and HCQ group in terms of number of patients showing “virological cure” (three studies, OR: 2.266 [0.964–5.329], \(F = 0\%\)) [Figure 8].

Lopinavir + ritonavir versus hydroxychloroquine: Time to virological cure:

In the study by Kim et al., 2020[42] “time to negative conversion” of viral RNA was shorter in the L/R arm (median 21 days) compared to the HCQ group (median 28 days) and the difference was statistically significant. However, no difference was noted by Karolyi et al., 2020[43].

Lopinavir + ritonavir versus hydroxychloroquine: Duration of hospital stay

Karolyi et al., 2020[43] did not find any difference between the L/R and HCQ arm in terms of length of hospital stay among the survivors.

Lopinavir + ritonavir versus hydroxychloroquine: Progression to severe disease during therapy

Karolyi et al., 2020[43] reported that 12.8% of the patients in the L/R arm required admission to intensive care unit (ICU), while only 20% of the patients in the HCQ arm required ICU admission. Again, 4.3% of patients in the L/R arm required mechanical ventilation, while 10% of patients in the HCQ arm required mechanical ventilation and there was no statistically significant difference between the two groups with regard to both of these parameters.

Similarly, in the study by Lecronier et al., 2020[39] also, no difference was observed between both the arms with regard to “requirement of ICU during therapy, “treatment escalation after day 1 until day 28,” and ventilator-free days at day 28.

Lopinavir + ritonavir versus hydroxychloroquine: Mortality

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**Table 2: Result of meta-regression (variable percentage difference in male sex lopinavir + ritonavir-standard of care)**

| Covariate                              | Coefficients | Lower bound | Upper bound | Standard error | \(P\) | Omnibus \(P\) |
|----------------------------------------|--------------|-------------|-------------|----------------|------|-------------|
| Intercept                              | -1.923       | -4.692      | 0.845       | 1.412          | 0.173| 0.153       |
| Percentage difference in male sex L/R-SOC | -0.074       | -0.176      | 0.028       | 0.052          | 0.153|             |

L/R-SOC=Lopinavir+ritonavir-standard of care
No difference was observed in the L/R versus HCQ group in terms of mortality (3 studies, OR: 0.701 [0.276–1.777], \( P = 0\% \)) [Figure 9].

Lopinavir + ritonavir versus hydroxychloroquine: Safety

Although the occurrence of any adverse events was higher in the L/R arm, there was no difference in terms of “occurrence of serious adverse events” and “occurrence of diarrhea.” Data are shown in Supplementary Figure 3. Other adverse effects noted in both the arms are increase in liver enzymes, thrombocytopenia, nausea, vomiting, loss of appetite, lymphopenia, neutropenia, skin rash, etc.

Lopinavir + ritonavir versus chloroquine

In one limited sample sized RCT,\(^{[34]}\) chloroquine (CQ) administration was associated with better virological cure and the percentage of patients becoming “SARS-CoV-2 negative” were slightly higher (day 7, 10, and 14). Again, by day 9 post-therapy, 60% of the patients in the CQ group achieved radiological lung clearance compared to 25% in the L/R group. Although vomiting, abdominal pain, nausea, diarrhea, rash, cough, and shortness of breath were reported, there were no serious adverse events and none of the patients required withdrawal and overall CQ was well tolerated.\(^{[34]}\)

Lopinavir + ritonavir versus arbidol: Efficacy and safety assessment

A total of three studies\(^{[33,41,48]}\) compared L/R combination to arbidol.

In terms of “virological cure on day 14,” no significant difference was seen between two groups and favors arbidol groups compared to L/R group (2 studies, OR: 0.19; 95% CI: 0.01–2.89, \( P = 66\% \), random effect) [Figure 10].

Lopinavir + ritonavir versus arbidol: Progression to severe disease

As documented by Li \textit{et al}., 2020,\(^{[49]}\) 8/34 (23.52%) in the L/R arm and 3/35 (8.57%) in the arbidol group progressed to severe/critical clinical status; however, this difference in proportions was not statistically significant. Two patients in the L/R arm required mechanical ventilation.

Comparative efficacy and safety of lopinavir + ritonavir + arbidol versus Arbidol monotherapy

In a single retrospective study\(^{[44]}\), the use of L/R + arbidol (A) combination therapy was associated with better virological clearance on day 7 (75% in
L/R + A group compared to 35% in the A group, \( P < 0.05 \) and on day 14 (94% in L/R + A group compared to 52.9% in the A group, \( P < 0.05 \)). Again, the number of patients showing radiologic improvement was more in the L/R + A group compared to the arbidol monotherapy group (69% vs. 29%, \( P < 0.05 \)).

**Efficacy of lopinavir + ritonavir + interferon-alpha combination therapy**

In a retrospective study by Zuo *et al.*, 2020,[50] after adjusting for multiple factors, they found that treatment with L/R + interferon (IFN)-alpha therapy was associated with a shorter duration of viral shedding (other treatment arms were L/R monotherapy and L/R + IFN-alpha + arbidol).

**Comparative efficacy of lopinavir + ritonavir + interferon-alpha, lopinavir + ritonavir + interferon-alpha + ribavirin, and ribavirin versus interferon-alpha**

A single randomized open-labeled prospective study,[35] has evaluated the comparative antiviral effectiveness of these three regimens. Although there was no difference in terms of efficacy between these three arms, the incidence of gastrointestinal adverse events was significantly higher when L/R and ribavirin were coadministered.

**Lopinavir + ritonavir + interferon-1-beta (triple antiviral therapy) versus lopinavir + ritonavir: comparative safety and efficacy**

A Phase 2 open-label RCT,[36] found that early triple antiviral therapy was superior to L/R alone in terms of shortening the “time to virological cure” and alleviating the clinical symptoms. Another important point in this study was that the median time from symptom onset to initiation of treatment was 5 days.

**Discussion**

At present, when mankind is in dire need of safe and effective therapeutics against COVID-19, every possible option is being tested in different evaluation platforms. Owing to its therapeutic efficacy in SARS-CoV, L/R was one of the first agents being tried against SARS-CoV-2. Both these drugs belong to the class “protease inhibitors,” which are active against human immune deficiency virus protease (HIV protease). Lopinavir is prescribed in combination with low-dose ritonavir,[51] in which ritonavir acts as a pharmacokinetic enhancer. Ritonavir inhibits CYP450-mediated metabolism of other protease inhibitors,[52] thus enabling better bioavailability, requirement of reduced dose, and decreased frequency of dosing of the second protease inhibitor. In addition, ritonavir also protects the second protease inhibitor from intestinal first-pass metabolism.[53] Another possible benefit of ritonavir boosting is the inhibition of p-glycoprotein and multidrug resistance protein-1 and 2-mediated efflux of protease inhibitors from the intestinal epithelial cells.[52] Ritonavir boosting also increases the therapeutically active unbound fraction of the second protease inhibitor by the saturation of protein binding sites.[53,54] Protease in SARS-CoV-2 plays an important role in the life cycle of the virus owing to its role in the cleavage of polyproteins and subsequent release of nonstructural proteins. Although L/R combination came out to be good binders against SARS-CoV-2 protease and showed inhibitory activity against SARS-CoV-2 activity in vitro, clinical efficacy is not established. In this meta-analysis, we are trying to find out an answer regarding the efficacy and safety of this combination and rationale of using this combination in COVID-19.

In our study, compared to SOC, significant lower “duration of hospital stay” was observed in L/R-treated group. Interestingly the direction of effect of all the three included trials was in the same direction. However, no significant difference in “progression to more severe state” and “mortality” was found in between the groups. Regarding “virological cure on days 7–10,” although no difference was found in L/R versus SOC, significant difference was found favoring L/R group in terms of “time to virological cure.” Regarding adverse effects, except the incidence of diarrhea which was higher in the L/R group, no difference was found in other adverse effects. However, few issues complicate the interpretation, e.g., treatment in the standard treatment arm varied among different studies and it ranged from the standard of care,[32] adjuvant/symptomatic treatment,[38,40] to no antiviral[49] and no L/R.[55] Again, the baseline viral RNA copies were higher in the study by Cao *et al.*, 2020[42] (4.4 ± 2 in the L/R group and 3.7 ± 2.1 in the standard care group). Another point of importance is that Yan *et al.*, 2020[55] found that the administration of L/R within 10 days of initiation of symptom was associated with a shortening of the duration of viral shedding. However, in the study by Cao *et al.*, 2020, the median time from onset of disease to randomization was 13 days (11–16 days). However, in the study by Li *et al.*, 2020[49] it was 3.5 days (2–6) in the L/R group and 5 days (2–8) in the control group. Ye *et al.*, 2020[40] and Shi *et al.*, 2020[56] did not mention time from onset of disease to time of the start of treatment. Yan *et al.*, 2020[55] mentioned old age as a potential risk factor for prolonged viral RNA shedding even after adjusting for other potential confounders (sex, comorbidity, and drug use). In this regard, the median age of the population in the study by Cao *et al.* was 58 years (interquartile range 49–68 years), in the study by Ye *et al.* age of the
study population ranged from 5-68 years (n=9 under 30 years and n=38 above 30 years and interestingly in this study, use of L/R showed clinical benefit), Shi et al. (mean age 48.7 ± 15 year), and Li et al. age 50 ± 15.4 year in L/R group and 44.3 year (range 27–62 years) in the control or standard treatment group. These issues can be addressed in further clinical trials.

While comparing L/R vs. HCQ, no significant difference was seen between two arms in terms of “virological cure,” but “shorter time to negative conversion of viral RNA” was found in the L/R arm (single study) although another study found no significant difference. Similarly, no added advantage was seen in terms of “duration of hospital stay” and “progression to severe disease” during treatment period and mortality. To summarize, no difference was observed in terms of efficacy between the L/R combination and HCQ therapy. On the other hand, CQ therapy was associated with better virological cure and percentage negative SARS-CoV-2 conversion as compared to L/R combination although reported by single RCT. No significant difference in efficacy was seen when L/R was compared to arbidol (monotherapy) in terms of virological cure on day 14 (2 studies) and “progressed to severe/critical clinical status.” Regarding adverse event, both arbidol monotherapy and L/R combination showed no significant difference (ALT elevation, nausea, diarrhea, and loss of appetite). No difference was observed in the occurrence of serious ADRs between the two arms. However, L/R + arbidol combination showed significant protection in terms of “virological clearance” and “radiological improvement.” However, data are from a single study. Although very little literature is there showing effectiveness of L/R + IFN-α and L/R + IFN-1β-based triple therapy regimen; however, there is only one study each against each of these comparisons. We need more data on it, whether it is the sole effect size of IFN or there is a synergistic effect.

Limitation
The limitations of the present study are that there was not even a single double-blinded high-quality RCT. Most of the study comparisons are based upon observational studies, which typically provide low quality of evidence. Again, in most of the studies, gap from symptom onset to initiation of treatment highly varies among the studies. Again, few comparisons comprised only one/very few studies.

Conclusion
Although no difference was found in terms of “mortality,” “progression of disease to a more severe state,” and “virological cure on days 7–10,” L/R therapy showed some benefit in terms of decreasing “time to virological clearance” and decreased the “duration of hospitalization.” Efficacy of HCQ and L/R is comparable. CQ may be better in terms of efficacy compared to L/R; however, findings are limited by single study. No difference is observed between safety and efficacy of L/R combination and arbidol monotherapy. The combination L/R with arbidol may be beneficial (in terms of virological clearance and radiological improvement); however, we need more dedicated studies. Single studies report efficacy of L/R + IFN (either alpha or 1-beta) combination. We need more studies to delineate the proper effect size. Another issue that complicates the situation is that, in most of the studies, treatment was initiated late, whereas early initiation of L/R may be associated with better outcomes. These issues need to be clarified in future trials. Again, most of the included studies are observational studies imparting a low level of evidence in the evidence-based grading process.

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Conflicts of interest
There are no conflicts of interest.

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### Supplementary Table 1: Risk of bias in observational studies (Newcastle Ottawa Scale)

| Study, author | Study design | S   | C   | O   | Q  |
|---------------|--------------|-----|-----|-----|----|
| Yan et al., 2020[37] | Retrospective study | *** | ** | *** | G  |
| Shi et al., 2020[38] | Observational study | ** | * | *** | F  |
| Lecronier et al., 2020[39] | Retrospective, observational study | *** | ** | *** | G  |
| Ye et al., 2020[40] | Observational study | **** | ** | *** | G  |
| Zhu et al., 2020[41] | Retrospective study | *** | ** | *** | G  |
| Kim et al., 2020[42] | Retrospective cohort study | *** | ** | *** | G  |
| Karolyi et al., 2020[43] | Retrospective cohort study | ** | * | *** | F  |

C=Comparability, S=Selection, O=Outcome, SQ=Study quality, G=Good, F=Fair, P=Poor
Supplementary Figure 2: Comparative safety between lopinavir + ritonavir versus standard of care
Supplementary Figure 3: Comparative adverse event profile of lopinavir + ritonavir versus hydroxychloroquine

Supplementary Figure 4: Comparative safety of lopinavir + ritonavir versus arbidol (Increase level of ALT)