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OPEN PEER REVIEW

Efficacy of N-Acetylcysteine in Children with Moderate COVID-19: A Placebo-Controlled Randomized Clinical Trial

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

4 comments · 16 Aug 2023 – 1 Sep 2023

Referee 1 Reviewer

MAJOR REVISION 16 Aug 2023

Dear Author
Thank you for your submission.
This article has important information on the effect of NAC on COVID19 patients.
There are some notes:
Abstract:
1- The total number of words should be 250.
2- Please rewrite the background part. The last sentence should be completed to explain how NAC is related to the treatment of COVID19.
3- Please give the most important findings of your study in the results part.
4- In the conclusion, please focus on your results. As you know, there was no significant difference in the mortality rate between the two groups.
Background:
1- A large number of references are related to the first two sentences of this part, which is not logical. Please check this.
2- Please give the results of Iranian studies in adults.
Material and Method:
1- Please omit the subtitles.
2- What were the mean, median, and SD of age in your cases?
3- How did you check the NAC dose in your cases? Was it based on their weight? Please give a reference.
4- If cases using corticosteroids were excluded from your study, how were some of them admitted to the ICU?
5- How were your two study groups matched based on the severity of their disease? As you know, the two groups should be equal in terms of the severity of their illness. What were the criteria for disease severity in your study?
6- In this sentence, "There was no significant difference in CRP levels at the beginning (P = 0.065) and at the end of the study (P = 0.659)." it seems there is no difference between the two groups based on CRP results, but the P value is significant in each group. Please give a table to show the differences in changes in variables between your two study groups and their P values because they are your important findings.
7- If there was no need for figures, please check this with your statistical advisor. It seems that a table such as table 4 comparing the variables could better show the important results.
Discussion:
1- Some of the variables, such as clinical findings and WBC count, that were studied in the results part are not discussed in the discussion part. Please check all the results to provide a good review of the literature.
2- Please explain why the results of other authors are similar to or different from your results.
3- What are the advantages and disadvantages of your study?
Conclusion:
1- Please check this part in the same way as the conclusion in the abstract.
Best Regards

Referee 2 Reviewer

MAJOR REVISION 30 Aug 2023

Thank you for your practical study.
On line 72, the inclusion criteria were a positive COVID-19 test and CXR. It would be better if you included cases with a positive PCR test and respiratory distress with a positive CXR.
You should define moderate COVID-19.
You should define the type of PCR used.
What was your placebo?
On line 109, it would be better to compare the RDS scores of the two groups instead of clinical signs.
Please write a practical conclusion.
Please discuss the importance of decreasing the hospitalization time in the discussion section.
The discussion needs to be rewritten.

Associate Editor

MAJOR REVISION 1 Sep 2023

Dear EIC,
This study analyzes the efficacy of N-acetylcysteine in children with moderate COVID-19.
It is a useful study but needs some revision. All comments should be addressed by the authors, and changes should be made based on the comments.

Editor-in-Chief

MAJOR REVISION 1 Sep 2023

Dear Author,
Thank you for submitting the article. It needs some revision. Please revise it based on the reviewers' and AE's comments.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
Dear Author
Thank you for your submission
This article has important information on NAC effect on COVID19 patients
There are some notes:
Abstract:
1- Total number of words should be 250

We revised it.

2- Please rewrite the background part. The last sentence should be completed on how NAC is related to treatment of COVID19

N-acetylcysteine above the mucolytic effect in lung disorders acts through numerous mechanisms such as enhancing the immune system, suppressing viral replication, and decreasing inflammation and these pharmacological activities of NAC make it a potential therapeutic agent for COVID-19.

3- Pls give the most important findings of your study in the result part.

All the investigated variables in both groups improved significantly at the end of the study. Analysis showed that the changing pattern of CRP and WBC in the NAC group compares to the placebo was not significant (p = 0.659 and 0.067 respectively). There was a significant improvement in oxygen saturation in the NAC group compared with the placebo group at the end of the study (P = 0.001). The length of hospital stay was significantly decrease in NAC group compares to the placebo (P-value = 0.001). In addition, the mortality rate in the intervention and placebo groups was 0.0% versus 7.4% (P-value = 0.491), respectively.

4- In conclusion please focus on your results . As you know there was no significant difference in two groups in mortality rate .

The results of this research supported the idea that NAC could be useful in reducing the duration of hospitalization and increasing oxygen saturation among COVID-19 children.

Background:
1- A high number of references are related to first two sentences of this part that is not logical. Pls check it

We revised it.

2- Please give the results of Iranian studies in adults

We revised it.

Material and Method:
1- Pls omit the subtitles

Corrected

2- What was the mean, Median and SD of ages in your cases?

It was noted in Table 1. Patients' characteristics in two groups at the beginning of the study

3- How did you checked the NAC dose in your cases? Was it based on their weight? Pls give reference.

Due to lack of data regarding NAC dose on covid-19 and due to NAC safety, we used it as a fixed-dose regimen same as adult patients.

4- If cases using corticosteroid were excluded from your study, how some of them were admitted to ICU?

ICU admission was one of our exclusion criteria, in below sentence: Six patients were excluded during the study due to unwillingness to use the drug or ICU admission, therefore, 52 patients completed the study and were analyzed.

Patients with severe diseases that need ICU care, immunosuppressed or receiving immunosuppressive therapy (equivalent daily dose of ≥20 mg prednisolone for >2 weeks), asthma, or history of bronchospasm as well as a known hypersensitivity reaction to NAC were excluded

5- How your two groups of study were matched based on the severity of their disease?

As you know the two group should be equal based on the severity of their illness. What was the criteria of disease severity in your study?

Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation measured by pulse oximetry (SpO_2) ≥94% on room air at sea level

6- In this sentence "There was no significant difference in CRP levels at the beginning (P = 0.065) and at the end of the study (P = 0.659)." it seems there is no difference in two groups based on CRP results but P value is significant in each group. Pls give a table to shows the differences in changes of variables in our two groups of study and their P values because they are you important findings .

It was noted in Table 2. Patients' characteristics changes in two groups.

7- If there was no need for figures pls check it with your statistical advisor.it seems if a table as table 4 compares the variables it can better show the important results.

Discussion:
1- Some of variables such as clinical finding and WBC number that were studied in the result part are not discussed in the discussion part . pls check all the result to give a good review of literature

This was a double-blind, placebo-controlled, randomized trial, where 58 children diagnosed with moderate COVID-19 were enrolled. At the baseline, there was no significant difference between the NAC and placebo groups in clinical signs and symptoms. At the end of the study, WBC and CRP were not significantly different between the groups (P=0.069 and 0.067 respectively). This finding was in concordance with Rahimi et al., (30) and in contrast to them, in a report of Ibrahim et al, the level of CRP was decreased (31).

Differences between the two studies can be explained by considering that CRP and WBC are non-specific for the evaluation of COVID-19 solely. Besides, it is possible that this dose (600mg/BD per oral route) is not strong enough for a positive response in non-selective inflammatory markers

2- Pls give the explanation of why the results of other authors are similar or dissimilar with you results.

We revised it.

3- What are advantages and disadvantages of your study?

Advantage: our clinical trial is the first study that evaluates the efficacy and safety of NAC in children with moderate COVID-19. Disadvantages: fixed dose regimen and short term follow up of the patients

Conclusion:
1- Pls check this part the same as this part in abstract.

We revised it.

Best Regards
Reviewer 2:
Thank you for your practical study

In line 72 inclusion criteria were positive Covid test and CXR. It would be better if you included cases with positive PCR and respiratory distress with positive CXR.

We include patients with positive PCR and respiratory distress with positive CXR.

You should define moderate COVID-19.

Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation measured by pulse oximetry (SpO_2) ≥94% on room air at sea level

You should define the kind of PCR that is used.

It was Reverse transcription **polymerase chain reaction**.

What was your placebo?

Placebo tablets have been prepared by pharmaceutical laboratory of Shahid Beheshti University of medical science.

In line 109 it is better you compare the RDS scores of two groups instead of clinical signs.

We revised it.

Please write a practical conclusion.

We revised it.

Please discuss the importance of decreasing the hospitalization time in the discussion part.

We revised it.

The discussion needs to be rewritten.

We revised it.

> Associate Editor's Comments:

Associate Editor 1:
Dear EIC,
This study analyze the Efficacy of N-acetylcysteine in Children with Moderate COVID-19.
it is a useful study but needs some revision. All comments should be reply by authors and changes should be done based on comments.

2 Revision 1

4 comments · 4 Nov 2023 – 5 Nov 2023

Referee 2 Reviewer

MAJOR REVISION 4 Nov 2023

We still see half of the references in the first paragraph.
What is the unit in this sentence? "The mean (SD)in the NAC group was 12.80 (7.48) and in the placebo group was 6.20 (2.85)".
You should exclude mild respiratory disease, ensure that the two groups are equal in terms of the severity of respiratory disease, or evaluate mild respiratory disease in the two groups.
What was the composition of your placebo?
What was the prescribed route of administration of NAC?
You should compare the two groups based on ICU admission.
Your study has not supported the statement that NAC could be useful in reducing the mortality rate, as you wrote in the conclusion.
The discussion section still needs more explanation.

Referee 1 Reviewer

MINOR REVISION 4 Nov 2023

Dear author
Thank you for the correction.
Results:
What were the age and weight ranges of the patients?
How did you calculate the NAC dose?
Were the two study groups the same in terms of disease severity and the presence of underlying disease?
There is no need to show the results in figures; numeric presentation in tables is adequate.
It is recommended to arrange all results in two main tables. Please don't show different tables with the same design.
Discussion
Please check all the findings in the results section and discuss them in this section.
Please give the advantages and disadvantages of your study.
Best regards

Associate Editor

MAJOR REVISION 5 Nov 2023

Dear EIC,
The great effort in improving the manuscript is highly appreciated. However, there is some room for further improvement that the authors should consider.

Editor-in-Chief

MAJOR REVISION 5 Nov 2023

Dear Author,
Your manuscript still needs revision. Please revise it carefully based on the reviewers' and AE's comments.

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
We still see half of the references in the first paragraph.
It was corrected.

What is the unit of this sentence?"The mean (SD)in the NAC group was 12.80 (7.48) and in the placebo group was 6.20 (2.85)".
It was corrected.

You should exclude mild respiratory disease or be equal based on the severity of respiratory disease or evaluate mild respiratory disease in two groups.

It was corrected based on last update of NIH covid 19 treatment guideline.
Inclusion criteria were ages 8 to 18 and definitive moderate COVID-19 infection confirmed by PCR and evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation measured by pulse oximetry (SpO_2) ≥94% on room air at sea level beside that pulmonary disease can progress rapidly and they should be closely monitored.
Patients with mild disease that do not have shortness of breath, dyspnea on exertion, or abnormal imaging or severe diseases that need ICU care, SpO_2 <94% on room air at sea level, PaO_2/FiO_2 <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%

What was the compound of your placebo?
It contains polyvinylpyrrolidone, sodium saccharin, sodium bicarbonate, lactose monohydrate . all component of NAC effervescent without active form of drug (NAC)

What was the route of the prescription of NAC?

we used drug orally.

You should compare two groups based on ICU admission.

Patients with severe disease that need ICU care are not enrolled in our study, after starting the intervention one patient in each group admitted to the ICU. It was equal in both control and intervention group (Figure. 1).

Your study has not supported that NAC could be useful in reducing the mortality rate as you wrote in the conclusion.

Our study showed that NAC can reduce time of hospitalization, serum creatinine level and improve Oxygen saturation. Although the mortality rate in the intervention group was lower than placebo groups (0 patients versus 2) (table 3) but it was not statically significant .

There are multiple possible justifications for this result. First, because in our study, a significant percentage of patients in both groups received corticosteroid, it is feasible that corticosteroid acts as a strong anti-inflammatory modality treatment in COVID-19 patients and NAC adds nothing

further to its effect. Second, the sample size of our study is small thus we are able to detect just large effects. Third, although other drugs used for the treatment of COVID-19 did not differ between the patients of the two groups, the allowed use of these medications may also impress the effects of NAC on clinical consequence of the study populations. Fourth, our study was a single-center research. Thus, based on the outcomes of a single-center study, it is unfeasible to make crucial conclusions about the efficacy of the NAC as adjunctive therapy in patients with COVID19, and larger, multicenter studies are still needed to recognize its potential efficiency in such patients.

The discussion part still needs explained more.
It was completed.

Reviewer 2:
Dear author
Thank you for correction
Results:

What was the age and weight range of patients?

Amount of age and weigh details were written in table 1 .

How did you calculate the NAC dose.?

Based on our knowledge, it was first article that used N-acetylcysteine in Children with COVID-19 and previous stududies showed that up to 2700 mg/day of NAC is safe in children. We used NAC with routine dosing, so further studies with different dosage are recommended.

Were the two group of study the same in disease severity and having underlying disease?
Both groups of the study were the same in disease severity (moderate disease based on last update of NIH treatment guideline) and there was no difference in underlying disease (*P-value*=0.8).
We summarized that in main article but there were 8 patients in NAC group with underlying diseases (4 with seizure, 1 with septic arthritis, 2 with autism and 1 with thyroid dysfunction) an 11 patients in placebo group (5 with seizure, 1 with septic arthritis, 2 with autism, 1 with diabetes, 1 renal impairment, and 1 Multisystem Inflammatory Syndrom)

There is no need to show the results in figures, Numeric presentation in tables are adequate.
It is recommended to arrange all results in two main table. Pls don't show different tables with the same design

Figure 2,3 and 4 was deleted.

Discussion
Pls check all the findings in result part and discuss it it in this part
It was corrected.

Pls give the advantages and disadvantages of your study
It was corrected.

Best regards

Associate Editor's Comments:
Associate Editor 1:
Dear EIC,
The great effort in improving the manuscript is highly appreciated. However, there are a few rooms for further improvement which the authors should consider.

EIC Decision:
Dear Author,
Your manuscript still needs revision. Please revise it based on Reviewers and AE comments accurately.

3 Revision 2

4 comments · 10 Jan 2024 – 19 Jan 2024

Referee 2 Reviewer

MINOR REVISION 10 Jan 2024

Thank you for your correction, but please:
Provide a reference for severity in line 76 and one for line 78.
Please explain what the placebo contains in line 79.
Cases excluded due to ICU admission should be counted as treatment failures.

Referee 1 Reviewer

MINOR REVISION 18 Jan 2024

dear author
Thank you for the corrections.
There are still a few points.
Abstract:
Is the pattern of change in CRP different from its level? It is stated that the pattern of change in CRP did not differ between the two groups, but the CRP level was significantly lower in the treatment group.
Method:
What was your purpose in describing your study as a pilot study?
How did you calculate the sample size, and what was the sampling method?
Discussion:
Please add other literature on variables that were not considered, for example, length of study, creatinine, and others.
Please describe the limitations of this study.
Best regards

Associate Editor

MINOR REVISION 19 Jan 2024

Dear AE,
Reviewers have not been satisfied with the revision.

Editor-in-Chief

MINOR REVISION 19 Jan 2024

Dear Author,
Your manuscript still needs some revision. Please revise it based on the reviewers' and AE's comments.

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
Thank you for your correction but please:

Write a reference of severity in line 76 and one for line 78.

Corrected

Please explain the component of placebo in line 79.

Corrected

Excluded cases due to ICU admission should be calculated as a treatment failure.

It was first clinical trial that evaluated NAC in Children with Moderate COVID-19 and we excluded severe covid patients who need ICU admission, beside that ICU patients, usually were administered other antioxidant drugs such as vitamin C, and or other covid-19 modalities, so it can interference our study results. We add this to consort flow chart of the study.

Reviewer 2:
dear author
thank you for correction
there are still a few points

Abstract:
Is changing pattern of CRP different from its level? it is declared that changing pattern of CRP was not different in two groups but CRP level was significantly lower in treatment group

At baseline amount of CRP between group was similar (12 versus 14 in NAC and placebo respectively), in both group CRP was significantly decreased (from 12 to 4 in NAC group and from 14 to 5 in placebo group), amount of decreasing in each group was significant but amount of CRP at end of study was not significantly different between two groups.

Method:
What was your aim to introduce your study as a pilot study?

It was the first clinical trial to evaluate the efficacy of adding NAC as an adjunctive treatment in Children with COVID-19 and there was not any clinical trial in children with pneumonia, so it was small study to test research protocols, data collection instruments, sample recruitment strategies, and other research techniques in preparation for a larger study.

How did you calculate the sample size and what was sampling method?

For pilot trial designed with 90% power and two-sided 5% significance, recommended minimum sample size per treatment arm of 25 for standardized effect sizes is logical, we conducted the study on 58 patients based on minimum sample size and estimated covid-19 referred cases to our center.

Discussion:
Pls add other literature review document about other variables that was not considered for example length of study and creatinine and other

In our study, NAC supplementation decreased the length of hospital stay. This finding is following other studies that showed the association between the NAC administration and the length of hospital stay. Bhattacharya et al. showed a correlation between NAC administration and decreased time of hospital stay and increased hospital discharge rate (33).

Another study by Rangel Mendez et al in COVID-19 has shown positive results of NAC as an adjuvant treatment in the COVID-19 patients and reduce time of hospital stay

In the current study NAC could significantly decrease amount of serum creatinine (P=0.001). and this result is consistent with previous studies demonstrated significant reduction in serum creatinine after NAC administration

Pls describe your limitation in this study.

There are multiple possible justifications base on limitation for this result. First, because in our study, a significant percentage of patients in both groups received corticosteroids, it is feasible that corticosteroids act as strong anti-inflammatory modality treatment in COVID-19 patients and NAC adds nothing further to its effect. Second, the sample size of our study is small thus we are able to detect just large effects. Third, although other drugs used for the treatment of COVID-19 did not differ between the patients of the two groups, the allowed use of these medications may also impress the effects of NAC on clinical consequence of the study populations. Fourth, more specific inflammatory parameters such as interleukin, total antioxidant capacity and tumor necrosis factor-α were not evaluated so correlation between effect of NAC in inflammatory parameters and clinical status of patients wereambiguous. and fifth, our study was single-center research. Thus, based on the outcomes of a single-center study, it is unfeasible to make crucial conclusions about the efficacy of the NAC as adjunctive therapy in patients with COVID19, and larger, multicenter studies are still needed to recognize its potential efficiency in such patients.

about the efficacy of the NAC as adjunctive therapy in patients with COVID19, and larger, multicenter studies are still needed to recognize its potential efficiency in such patients.

4 Revision 3

4 comments · 13 Feb 2024 – 3 Mar 2024

Referee 2 Reviewer

MINOR REVISION 13 Feb 2024

In line 23, "was" should be deleted.
In line 25, "was" should be changed to "were".
In the abstract and discussion sections, you reported 58 cases, but in the results section, you reported 52 cases.

Referee 1 Reviewer

ACCEPT 3 Mar 2024

Dear Author
The article is accepted. There are a few notes:
1- Was it a pilot study?
2- Did the placebo have any effect? What was its dose?
Best regards

Associate Editor

ACCEPT 3 Mar 2024

Dear EIC.
Previous errors have been corrected.

Editor-in-Chief

ACCEPT 3 Mar 2024

Dear Author,
I read the manuscript, and the authors corrected the requested items. The article could be accepted at this stage. But I want the authors to fix the three items mentioned by our reviewers in the galley proof.
Sincerely;