Journal of Pharmaceutical Research International

33(60B): 3122-3129, 2021; Article no.JPRI.81226
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Increasing False Negative RT-PCR Test in COVID-19 Patients Admitted at Tertiary Care Hospital: A Retrospective Cross-Sectional Study

Bhargavi Yadav [a≡*] and Alka Revankar [bφ]

[a] Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi (Meghe), Wardha, Maharashtra, India.
[b] Department of Physiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi (Meghe), Wardha, Maharashtra, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i60B34987

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/81226

Study Protocol

Received 20 November 2021
Accepted 26 December 2021
Published 27 December 2021

ABSTRACT

Background: COVID-19 Pandemic has caused a lot of havoc for the human race, as the urgent need to detect the virus early and fight back stronger, scientists came up with the test RT-PCR, which later on was declared as the gold standard. But lately, an exponential increase in false-negative rates was observed, which calls for an accuracy check of the test and the statistical analysis of the reasons leading false-positive results. With the advent of the COVID-19 Pandemic, RT-PCR testing has become the gold standard for laboratory diagnosis of SARS-CoV-2 infection. But the second wave of COVID-19 in India has reported a high number of false-negative RT-PCR results. This calls for the need to measure the accuracy of the test in diagnosing infection, especially considering rise in variant strains each with its own virulence and infectivity. Imaging methods like HRCT and biochemical tests such as CRP, D-Dimer are being used increasingly by doctors to support the serological diagnostic method of Covid and draft further treatment protocols
Objectives: To measure the false negative rates of RT-PCR in COVID-19 Patients. To find the epidemiology of the root cause.

[≡] *Medical Student;*
[φ] *Professor And Head of Department;*
**Corresponding author: E-mail: bhargaviyadav832@gmail.com;*

Methodology: For this Retrospective Cross-sectional study, sample size of 1998 with prevalence of 7894/million population and confidence interval of 95% we would be analysing patients of Datta Meghe Institute of Medical Sciences TCH admitted in the months of April to June 2021 with suspected COVID-19 Infection.
Results: Increasing cases of false negative RT-PCR reports would be found.
Conclusion: There is an urgent need for the reality check on efficacy of RT-PCR Test Reports.

Keywords: COVID-19; RT-PCR; false-negative; pandemic.

1. INTRODUCTION

With the advent of the COVID-19 pandemic, RT-PCR testing has become the gold standard for laboratory diagnosis of SARS-CoV-2 infection [1]. But the second wave of COVID-19 in India has reported a high number of false negative RT-PCR results. This calls for the need to measure the accuracy of the test in diagnosing infection, especially considering rise in variant strains each with their own virulence and infectivity. Imaging methods like HRCT and biochemical tests such as CRP, D-Dimer are being used increasingly by doctors to support the serological diagnostic method of Covid and draft further treatment protocols. Studies on False-negative rates have been ranging from 1-30% [2,3] So it becomes necessary to identify the etiological factors that are responsible for the apparent discrepancies in the RT-PCR results, so that we can take the correct measures to rectify the testing and sampling process. So this study is essential to untangle the reasons for Unexplained heterogeneity in the extent of bogus negative RT-PCR results [1,2].

The surprising rise in cases of COVID-19 can be faulted for the unexpected bogus adverse outcomes in the nation and a recently distinguished twofold mutant variation of SARS-CoV-2, which was recognized first in quite a while. Subsequently this all has unexpectedly made such a responsibility on clinics and outrageous emergency interest of oxygen chambers all through the country [2].

World Health Organization indicates that it is characterized into two classes a. variations of Interest b. variations of concern. Whereas the later one incorporates alpha, beta, gamma and delta. What's more, the first incorporate eta, lota, kappa and lambda. As per WHPs week by week epidemiological update on COVID-19,24th August 2021 the Alpha variation reach was to 192 Countries followed by beta in 141 and gamma to 86 demonstrating the expanded contagiousness of all various variations however in perception delta variation was tracked down comparative contagiousness among immunized and unvaccinated people [3].

Following a half year of the pinnacle of Covid sickness flood in India during the time-frame of September 2020 the spike of COVID-19 cases was begun to notice again in the First seven-day stretch of March 2021. What's more, as of August 2021, the COVID-19 positive all out patients came to approx. of 33,000,000 and counting with day by day tragic instances of 40,000 and passing cases climb of 437,370 with terrible 500 Deaths each day. This Second rush of COVID-19 brought request of approx 520,000,000 examples as of 27th August 2021. Furthermore, in these circumstances of such high vulnerabilities India with wild needed to battle second flood of SARS-Cov-19 with outrageous strange increment of every day cases [4,5].

2. PRINCIPLE OF RT-PCR

RT-PCR represents continuous converse record polymerase chain response, and it is an atomic based methodology for recognizing microbe hereditary material, most generally DNA. Since COVID-19 is a RNA infection, the RNA is opposite interpreted into DNA utilizing specific compounds, and the subsequent DNA is intensified into many duplicates [6].

Patients' nasopharyngeal or oropharyngeal swabs are safeguarded in explicit answers for wipe out undesirable parts like proteins and lipids. The remaining hereditary material combination is tried utilizing RT-PCR hardware. To enhance the DNA into around 35 billion duplicates [from each strand of RNA], the machine goes through 35 cycles [standard count]. The fluorescent colors are then delivered through DNA strands with marker names joined. The measure of color is determined by a PC and showed on the screen continuously [7]. The measure of fluorescent color in the example is observed after each cycle, and in the event that it

arrives at a specific edge, the infection's presence can be affirmed [4]. The number of cycles needed to arrive at that limit decides the seriousness of the ailment. A low number of cycles suggests a genuine contamination. The Centers for Disease Control and Prevention [CDC] indicated that adverse outcomes don't preclude COVID-19 contamination, and more examples are required [6].

Similarly, the presence of viral RNA isn't generally a dependable sign of a patient's clinical indications. The rate of illness in the human body essentially affects the quantity of bogus positive and bogus adverse outcomes. The more normal an illness is, the more regular bogus adverse outcomes are and the other way around. RT-PCR is viewed as the best quality level as far as test dependability for COVID-19 discovery as a result of its high particularity and affectability. Nonetheless, a predetermined number of creators have expressed that the affectability of RT-PCR can be just about as low as 38%, which is remarkably difficult to accept [5]. Serology tests for IgM and IgG have been accounted for from one side of the planet to the other. However they are underutilized because of their low particularity and affectability.

3. COLLAPSING HEALTHCARE AND EFFECT OF FALSE-NEGATIVE RESULTS

The crumbling of a country's medical services framework, organization, and existing clinical foundation have been the main contributing causes to expanding dreariness and passing because of COVID-19. The new medical care industry emergency has exacerbated bogus negative RT-PCR results, coming about in more misdiagnoses and an expanded danger of contamination transmission. During this time, the essential driver of bogus negative test results among COVID-19 patients can be credited in enormous part to unpractised lab laborers managing a seriously expanded responsibility [6]. Subsequently, crumbling medical care and bogus negative RT-PCR results have become part of a self-taking care of endless loop. A few variables have been related to bogus negative RT-PCR results, including pre-logical, insightful, and post-scientific elements. The expanded utilization of RT-PCR for mass testing has brought about a lack of qualified workers, increasing burnout rates among the medical care calling, which incorporates doctors and clinical specialists. The mix of expanded responsibility, hazard of transmission, and a lack of vital assets has impacted the medical services workforce's physical and emotional wellness . The extending build-up of medical care administrations and systems, which should likewise be considered, may deteriorate doctor and medical services labourer weakness later on. Unseemly example assortment, bringing about lacking viral burden for location, inappropriate transportation, ill-advised example dealing with, wasteful RNA extraction, and incompetent expulsion of enhancement inhibitors from examples are for the most part direct results of a gifted work deficiency. The first characteristics may likewise be affected by an absence of gear, material, bio-wellbeing naming, and device needed for effective RT-PCR testing due to congestion in medical care.

As the sickness propels, the variable viral burden in the material keeps on confounding the hour of test assortment, adding to false negative outcomes. As the SARS-CoV-2 RT-PCR is utilized in the asymptomatic populace, avoidable recurrent testing is turning out to be more boundless [7]. This places a strain on the production network, creating setbacks for the conveyance of results and, all the more fundamentally, a shortage of tests for the individuals who need them most. The expanded interest has put unjustifiable weight on the essential assembling units, confusing by and large coordination's and keeping the overall population from getting indicative testing supplies. Mutations and Associated False-Negative Results. The primary SARS-CoV-2 genome succession was uncovered on January 10, 2020, making ready for the improvement of supplementing RT-PCR tests. Considering that the synthetic compounds for the units were additionally produced at that point, the infection might have changed because of the great transmission cycles, representing a danger to the affectability of RT-PCR. As a rule, the SARS-CoV-2 infection is a shut sRNA infection with a high transformation rate. Human Covid, then again, have a RNA polymerase with 3 to 5 editing action, which permits them to repeat "high-devotion" thus have a moderate transformation rate. SARS-CoV-2 changes occur at a pace of two nucleotides every month, contrasted with four nucleotides for flu and eight nucleotides for HIV. In any case, have subordinate RNA altering and a high pace of individual to-individual transmission should be considered. As indicated by specialists in India

who are intently concentrating on the subsequent wave, bronchoalveolar lavage performed on RT-PCR negative patients with COVID-19 side effects created COVID-19 positive outcomes. As per a specialist expressed in a similar source, 15 to 20% of COVID-19 patients have the previously mentioned issue, which is causing specialists issues.

As indicated by another review, changes in the SARS-CoV-2 infection might have permitted it to stay away from RT-PCR testing, and the packs should be re-designed immediately. Albeit the side effects on chest figured tomography [CT] [ground-glass obscurity and dim patches] are plainly symptomatic of COVID-19, rehashed RT-PCR testing has turned up negative in specific cases. Despite the fact that it is believed that the leftover patients are getting bogus negative discoveries on the grounds that RT-PCR has an affectability of 70%. Notwithstanding, on the grounds that these occasions are so new, the chance of SARS-CoV-2 mutant[s] being to be faulted can't be precluded totally. As indicated by measurements given by the National Institute of Virology, India, approximately 61% of tests taken from the Indian territory of Maharashtra tried positive for the presence of the twofold freak strain B.1.617 [8]. As per the Global Times, eleven Chinese team individuals tried positive for a freak Indian strain of SARS-CoV-2. The turn of events and transformation of SARS-genomic CoV-2 might disable the affectability of RT-PCR symptomatic packs, as indicated by a few examinations. Since the demonstrative tests utilized in RT-PCR are so explicit, even a couple of transformations can bring about an extensive loss of affectability. SARS-CoV-2 changes are generally normal in the Nucleocapsid [N] quality objective, groundworks, and tests, which are habitually utilized for infection location all throughout the planet. An assortment of late articles and logical proof help the hypothesis that changes cause more noteworthy bogus negative RT-PCR results for SARS-CoV-2. The determination of a SARS-CoV-2 positive patient utilizing business demonstrative units was hindered by a SNP [Single Nucleotide Polymorphism] in the N quality, as per a review [9]. As per the discoveries of a review, seven of the 27 tests investigated showed changes or bungles in their groundwork/test restricting areas. A change in the N quality of SARS-CoV-2 was distinguished in three individuals in another examination, which blocked business test location [10].

As indicated by considers, RT-PCR affectability for SARS-CoV-2 was diminished because of a solitary change in the forward N quality preliminary restricting site, which is broad everywhere. As indicated by a review that genotyped 31,421 SARS-CoV-2 genomic separates, "all of the current COVID-19 symptomatic targets have gone through changes." One more review found that by far most of RT-PCR tests and groundworks contain intra-have single nucleotide varieties [iSNVs] and single nucleotide polymorphisms [SNPs] We presume that changes like these could build the danger of bogus negative RT-PCR results, even though they had no impact on track hybridization in the review. On the off chance that the mutational profile and information could be incorporated into the formation of PCR exhibits, higher affectability for preliminary and test hybridization might be accomplished [11-19]. This implies that utilizing various quality focuses in RT-PCR brings down the shot at misfortune attributable to viral changes.

4. OBJECTIVES

To measure the false-negative rate of RT-PCR test in clinically diagnosed COVID patients.

5. METHODOLOGY

Chart 1. Study protocol

Type of Study	Retrospective cross-sectional study
Study setting	A tertiary care setup at DMIMS
Study Population	Patients admitted to the covid care hospital due to suspected COVID-19 infection.
Sample Size	1998 patients according to the covid prevalence of 7894 per million population and a confidence interval of 95%
Study duration	Retrospective study of patients admitted in the months of April to June 2021

Important terms-

1. Suspected COVID Patients- patients with clinical symptoms of covid such as fever, cough or low Sp02, or tested positive by RAT
2. False-negative- patient with suspected infection and an initial negative result by RT-PCR test, with a positive result on a subsequent test.
3. True positive- patient with suspected infection and positive result by RT-PCR test
4. True negative- patient with suspected infection and negative result on initial and subsequent RT-PCR tests

6. DATA COLLECTION PROCEDURES & STUDY TOOLS

A patient record form will be filled based on the medical records chart of the covid patient.

The form will have the following sections-

1. Patient demographics- age, sex, occupation, address.
2. Personal history- comorbidities, pregnancy/lactation, addiction, COVID-19 vaccination status.
3. COVID-19 history-symptoms and spO2 at the time of hospitalization, date of admission,
4. Covid investigations- [RAT/RT-PCR] and results of test. Repeat RTPCR, HRCT, D-Dimer, CRP if done, what were the values
5. Patient outcome- death, discharge, transfer

Based on the information obtained from the 1998 sample population, the data will be updated on the SPSS software and statistical analysis will be done to measure the false negative rate of RT-PCR.

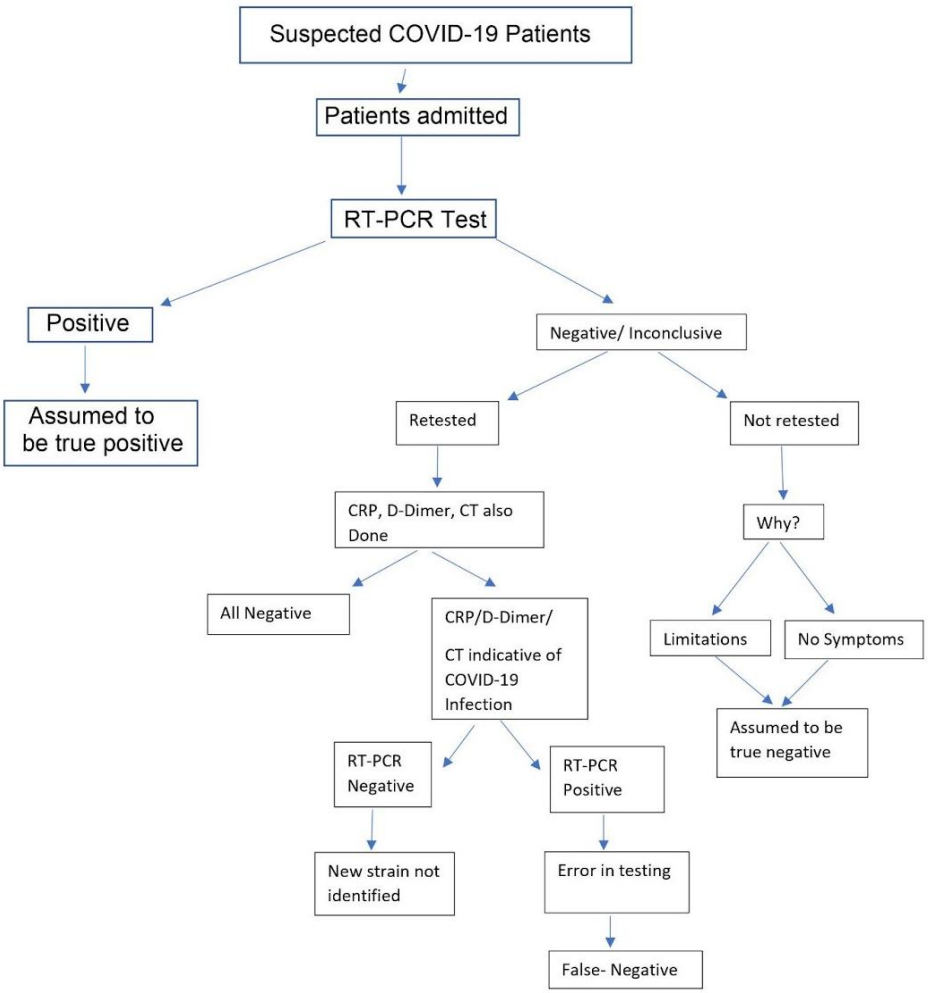

Fig. 1. Design of the study

Table 1. Clinical course and risk factors for mortality of adult inpatients with COVID-19

Lab Values Elevated in COVID-19	
CRP	>100 mg/L [normal range: <8.0 mg/L]
D-dimer	>1000 ng/mL [normal range: <500 ng/mL]

The accuracy +of the test can be determined, and a note can be made of the test's change in specificity and sensitivity since the first wave of COVID.

Additionally, a comparison of the patient demographics and personal history can be considered.

7. IMPLICATIONS

If the results of repeat RT-PCR turns out to be positive, it indicates that False negative results occurred due to a series of reasons including improper specimen collection, testing too early in the disease process, low analytic sensitivity, inappropriate specimen type, low viral load, or variability in viral shedding.

Suppose the results of repeat RT-PCR is negative/inclusive. In that case, that helps rule out sampling and testing error, and implies Mutations in the COVID-19 viral genome producing newer strains that can escape older gene targets in RT-PCR testing.

Our Study recommends active efforts to minimize human errors along with making the laboratory diagnostic methods more robust. Methods like dd-PCR which are more sensitive and specific can be used instead of RT-PCR as they can detect even lesser viral loads [10] and better primers and probes will have to be developed as variants arise [11]. A number of related studies on Covid-19 were reviewed [12-19].

By looking at the patient's personal history, we can have a look at the correlation of vaccination, addiction and comorbidities with the diagnostic results and disease outcome. The virus is prone to mutate and newer variants may escape neutralizing antibodies or reduce vaccine efficacy but if we keep evolving our diagnostic measures, we'll be able to limit spread and complications. This calls for the need to include on genome analysis of Covid virus for research and diagnostic purposes.

8. LIMITATIONS

- This methodology assumes the 100% specificity of RT-PCR and the tests used for clinical diagnosis of COVID.
- The patient selection bias due to the locality and the COVID center taken into consideration in our study.
- It is unfeasible to ascertain the absence of human error. However, it may be minimized with strong quality control measures and cautious processing.
- The area of sampling/swabbing [eg: throat, nasopharyngeal, rectal, etc] also affects the sensitivity of RT-PCR. Due to the complexities involved in obtaining other kinds of swabs, the study will mainly analyze throat and nasopharyngeal swabs only.
- Due to time and resources constraints, Genomic sequencing is not done to support the implications of presence of new strain of SARS-CoV-2.
- Apprehension of subjects to extensive testing would need to be countered. Proficient communication would be required from the team to achieve compliance and co-operation from the subjects.

9. CONCLUSION

There is a dire need of research on the efficacy of RT-PCR as the primary diagnostic test for COVID and we need to work

ETHICAL APPROVAL AND CONSENT

Ethical approval will be taken from the institutional ethics committee before the initiation of the study. Informed consent, wherever required, would be obtained duly from the patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Arnaout R, Lee RA, Lee GR, Callahan C, Yen CF, Smith KP, Arora R, Kirby JE. SARS-CoV2 testing: The limit of detection matters. bioRxiv [Preprint]. 2020;2020. 06.02.131144.
   DOI: 10.1101/2020.06.02.131144.
   PMID: 32577640
   PMCID: PMC7302192
2. Long DR, Gombar S, Hogan CA, Greninger AL, O'Reilly-Shah V, Bryson-Cahn C, et al. Occurrence and timing of subsequent SARS-CoV-2 RT-PCR positivity among initially negative patients. Clin Infect Dis. 2020.
3. Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, Zambrano-Achig P, del Campo R, Ciapponi A, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. medRxiv; 2020.
4. Kanji JN, Zelyas N, MacDonald C, et al. False-negative rate of COVID-19 PCR testing: A discordant testing analysis. Virol J. 2021;18:13.
5. Falzone L, Musso N, Gattuso G, Bongiorno D, Palermo CI, Scalia G, Libra M, Stefani S. Sensitivity assessment of droplet digital PCR for SARS-CoV-2 detection. International Journal of Molecular Medicine. 2020;46(3):957-64.
6. Suo T, Liu X, Feng J, Guo M, Hu W, Guo D, Ullah H, Yang Y, Zhang Q, Wang X, Sajid M. ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens. Emerging Microbes & Infections. 2020;9(1):1259-68.
7. Dandan Li, Jiawei Zhang, Jinming Li. Primer design for quantitative real-time PCR for the emerging Coronavirus SARS-CoV-2. Theranostics.
8. Kanji JN, Zelyas N, MacDonald C, et al. False-negative rate of COVID-19 PCR testing: A discordant testing analysis. Virol J. 2021;18:13.
9. Long DR, Gombar S, Hogan CA, Greninger AL, O'Reilly-Shah V, Bryson-Cahn C, et al. Occurrence and timing of subsequent SARS-CoV-2 RT-PCR positivity among initially negative patients. Clin Infect Dis.; 2020.
10. Lee RA, Lee GR, Callahan C, Yen CF, Smith KP, Arora R, Kirby JE. SARS-CoV2 testing: The limit of detection matters. bioRxiv [Preprint].
11. Zelyas N, MacDonald C, et al. False-negative rate of COVID-19 PCR testing: A discordant testing analysis. Clin Infect Dis.; 2020.
12. Acharya S, Shukla S, Acharya N. Gospels of a pandemic-A metaphysical commentary on the current COVID-19 crisis.
    Available:https://doi.org/10.7860/JCDR/2020/44627.13774
13. Arora D, Sharma M, Acharya S, Shukla S, Acharya N. India in "Flattening the Curve" of COVID-19 Pandemic-Triumphs and Challenges Thereof. Journal of Evolution of Medical and Dental Sciences. 2020;9(43):3252-6.
    Available:https://doi.org/10.14260/jemds/2020/713
14. Bawiskar N, Andhale A, Hulkoti V, Acharya S, Shukla S. Haematological manifestations of COVID-19 and emerging immunohaematological therapeutic strategies. Journal of Evolution of Medical and Dental Sciences. 2020;9(46):3489-95.
    Available:https://doi.org/10.14260/jemds/2020/763
15. Burhani TS, Naqvi WM. Telehealth--A boon in the time of COVID 19 outbreak. Journal of Evolution of Medical and Dental Sciences. 2020;9(29):2081- 5.
    Available:https://doi.org/10.14260/jemds/2020/454.
16. Butola LK, Ambad R, Kute PK, Jha RK, Shinde AD, DMIMS W. The pandemic of 21st century-COVID-19. Journal of Evolution of Medical and Dental Sciences. 2020;9(39):2913-9, 2913–18.
    Available:https://doi.org/10.14260/jemds/2020/637
17. Dasari V, Dasari K. Nutraceuticals to support immunity: COVID-19 pandemic-a wake-up call. Journal of Clinical & Diagnostic Research. 2020;14(7).
    Available:https://doi.org/10.7860/JCDR/2020/44898.13843
18. Dhok A, Butola LK, Anjankar A, Shinde AD, Kute PK, Jha RK. Role of vitamins and minerals in improving immunity during COVID-19 pandemic-a review. Journal of Evolution of Medical and Dental Sciences. 2020;9(32):2296-301.

Available:https://doi.org/10.14260/jemds/2020/497.

19. Gawai JP, Singh S, Taksande VD, Sebastian T, Kasturkar P, Ankar RS. Critical review on impact of COVID 19 and mental health. Available:https://doi.org/10.14260/jemds/2020/470

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/81226