Original Article

Assessment of clinical and virological outcomes of rural and urban populations: COVID-19

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Abstract

Objective: To assess the clinical and virological status in urban and rural populations. **Methods:** A cross-sectional study was conducted in a tertiary care hospital, Postgraduate Institute of Medical Sciences, Rohtak for a period of six months. Upper respiratory tract (URT) specimens including nasopharyngeal and oropharyngeal swabs were collected from the patients and their contacts and processed by RT-PCR technique for COVID-19 detection. Further, clinical and virological response in both the population were assessed and compared. **Results:** A total of 37,724 URT samples were tested, out of which 20,144 (53%) samples were from the rural population and 17,580 (47%) from the urban population. Out of the total samples from urban and rural population, COVID-19 positivity was 13.9% in urban population and 6.2% in rural population. Around 86% patients or contacts were asymptomatic in both the rural and urban population and rests were symptomatic 14%. Among the symptomatic patients, sore throat was seen as the most common presenting symptom (95-100%) followed by fever (80-83%), dry cough (55-61%), nasal discharge (18-23%), and breathlessness (3-5%) in both the rural and urban population. **Conclusion:** Our outcomes provide novel facts that the COVID-19 epidemic severely affected both rural and urban populations but with few differences. In our study, positivity rate in case of urban population was 13.9% as compared to 6.2% in rural population. There are two foremost facets that contributed variation in positivity in both the population. First, better immune response in rural population as compared to urban population which can be due to the fact that rural people in India are more exposed to various pathogens during their early lifetime thus, improving their immune status. Second, factor could be elevated population densities in urban areas which can contribute to increased infectiousness thus higher positivity rate. In addition, people living in urban population have to commute more for their work and are exposed to more people throughout the day thus, having more possibility to get infection of COVID-19 as compared to the rural population. To the best of our knowledge, there are no studies conducted on COVID-19, among rural population of Haryana. Hence, this study will allow us to fill the gap in knowledge about the variation in contagion spread and immune response in both rural and urban populations.

Keywords: Clinical, COVID-19, RT-PCR, virological seroprevalence

Introduction

Recently, World Health Organization (WHO) declared a pandemic of viral outbreak of novel coronavirus (COVID-19) caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[1]

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After initial cases in Wuhan, China, due to the rapid spread of the virus there has been hostile escalation in the number of emerging cases and mortality.[2,3] From the state of Kerala, India reported its first laboratory-confirmed case of SARS-CoV-2 in the later part of January 2020.[4] However, India has the highest number of COVID-19 cases, the fatality rate due to COVID-19 infection is lower than the global fatality rate.[4,5] Within India, metro cities like Delhi, Tamil Nadu, Andhra Pradesh, Karnataka,

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Kerala, Uttar Pradesh, and Maharashtra states have contribution of more than 65% cases.

Incongruity can be seen in corona cases among urban and rural areas. Urban areas are the chief centers of seroprevalence estimates and because of elevated population densities, are thought to be more susceptible to COVID-19. Densely populated urban cities have been more affected than rural areas.[6,7]

Although, rural area received reverse migration of millions of migrant workers exempt from lockdown. Besides, due to lockdown, many workers were deprived of their means of survival in cities and thus moved back to their villages. Such a drift elevated the prospect of spreading of the contagion to rural province, where community fitness infrastructure is still inadequate than that in cities.

Patients presented as; symptomatic or asymptomatic. In asymptomatic cases no symptoms was observed. Although, people with asymptomatic infection are less likely than those with symptoms to spread the disease, the risk is by no means nonexistent. In addition, it is quite imperative to self-isolate for the entire 10-day period from the last contact with COVID-19 positive patient.

Whereas, in case of symptomatic infection, people are more prone to infections. Symptoms include, respiratory symptoms, fever, cough, shortness of breath, breathing difficulties, and many more. The transmission of this virus in the first wave is largely maintained by asymptomatic categorized patients.[8,9]

In this article, chiefly, we describe the clinical and virological outcomes of COVID-19 patients in the rural and urban population, to gain knowledge about the spreading of contagion in the rural and urban populations. This study is relevant to the practice of primary care physicians. While, contagion spread to rural areas can affect the rural healthcare system, where primary health care (PHC) is most important. Besides, major problem in rural areas are fewer physicians and lack of access to healthcare facilities.[10] More resources to the rural areas should be given so that they can combat COVID-19 at grass root level.

Materials and Methods

Sample for this study was taken from tertiary care hospital Rohtak, Haryana, and nearby 17 villages. Data were collected from June 1 to November 30, 2020—a period of significant increase in the COVID-19 pandemic across the city and nearby region. Study cohort flowchart was made, respectively [Figure 1]. As the study involved only data collection of patients and samples were not collected in our laboratory. Hence, ethical clearance was not required in these studies.

For the analysis of clinical and virological response, upper respiratory tract (URT) specimens including nasopharyngeal and oropharyngeal swabs were collected from the designated urban and rural populations. The collected samples were then sent to VRDL, Department of Microbiology, Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana, maintaining the cold chain.

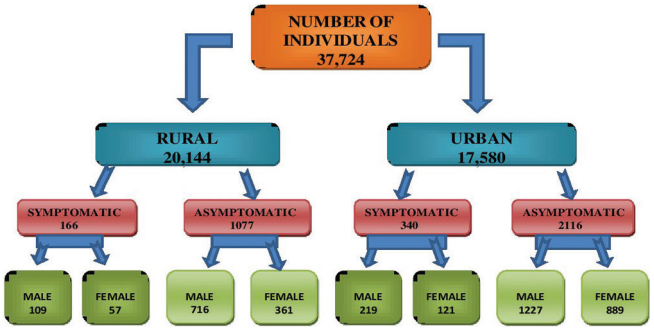

Figure 1: Study cohort flowchart. Children to adult patients from rural and urban province were taken with COVID-19 during the study period from June 1 to November 30

Total RNA was extracted using ICMR approved Genemag Viral DNA/RNA Purification kit (Genetix Biotech Asia Pvt. Ltd.). Taqman based qualitative multiplex Real-Time RT-PCR detection test was performed using Covidsure kit (Multiplex Real Time PCR, Trivitron Healthcare, India). One step RT-PCR was conducted by reverse transcriptase followed by Taq DNA polymerase. A total of 15 µl reaction mixture contains, 10 µl of 2X RT-PCR mix, 2 µl of primer probe mix, 3 µl of nuclease free water. Finally, 5 µl of total extracted RNA was added. Light sensitive primer probe mix containing a Taqman probe labeled with FAM dye specific to *ORF1ab* gene, probe labeled with HEX specific to E gene and probe labelled with ROX dye specific to housekeeping ribonuclease p subunit p30 (RPP30) gene. PCR Reverse transcription reaction was conducted at 46°C for 15 minutes followed by initial denaturation at 95°C for 10 sec, 58°C for 30 sec. FAM channel confirms the contagion, while, ROX channel confirms RNA extraction efficiency and conversion of RNA into cDNA. The threshold cycle (Ct) values ≤35 with appropriate sigmoidal graph considered positive for COVID-19. COVID-19 was confirmed by reverse transcription quantitative PCR (RT-qPCR) assays (CFX96 Real-Time System, Biorad Laboratories, Inc. CA. USA).

The clinical symptoms of patients who tested positive for COVID-19 were collected and the patients were categorized into two groups as asymptomatic and symptomatic, respectively.

Statistical methods

The data was collected and analysed using SPSS software in which one-way ANOVA was used, with a *P* value less than 0.05 to be statistically significant. Baseline data analysis was summarized using descriptive statistics including percentages and mean.

Clinical course and symptoms

Clinical course comprises symptom category during inception of illness (i.e., symptomatic, asymptomatic). According to a latest report, higher viral loads have been reported in symptomatic patients, generally noticed in the nasopharyngeal

and oropharyngeal, subsequent to the onset of their symptoms.[11] The peak point of viral loads was close to 5-6 days, subsequent to the inception of symptoms.[12]

Clinical features of COVID-19

Patients presenting with fever, cough, dyspnea, myalgia, arthralgia, chest pain, nasal congestion, runny nose, headache, sore throat, and diarrhea were considered as symptomatic patients in the present study[13-16] [Figure 2]. Asymptomatic category includes, those who comes in contact with others and persons coming from hotspot area.

Results

In our baseline model, we project that the crest of active cases occurs in the June and November month. Percentage of males as compared to females was highest in both cases whether it is symptomatic or asymptomatic.

Clinical outcomes of urban and rural area

A total of 37,724 samples were tested, out of which 20,144 (53%) samples were from the rural population and 17,580 (47%) were from the urban population. Moreover, out of total tested samples from urban and rural population, COVID-19 positive patients were (13.9%) from urban population and (6.2%) from rural population. Around 86% either patients or contacts were asymptomatic in both rural and urban population while, symptomatic patients were 14%.

Predominantly, patients with no signs of symptoms were categorized as asymptomatic; individuals who have symptoms of COVID-19 (e.g., sore throat, cough, fever, body ache, loss of taste and smell, headache, vomiting and diarrhea) were included in symptomatic category. Among the symptomatic patients, sore throat was seen as the most common presenting symptom (95-100%) followed by fever (80-83%), dry cough (55-61%), nasal discharge (18-23%), and breathlessness (3-5%) in both the rural and urban population [Table 1].

2-D pie chart [Figure 3] shows percentages of COVID symptomatic and asymptomatic cases in rural and urban region. Highest asymptomatic cases were from the urban (60%) whereas in rural region it is (27%). While, urban symptomatic cases were (9%) and rural cases were (4%) only.

Percentage of males was highest as compared to females in both cases whether it is symptomatic or asymptomatic. Asymptomatic patients from urban area were 33% male and 24% female whereas symptomatic male and female were 6% and 3%. In addition, asymptomatic patients from rural area were 19% male, 10% female whereas symptomatic male and female were 3% and 2%, respectively [Figure 4].

Figure 5 depicts highest percent positivity of rural province in the month of November whereas in urban province, September month shows highest percent positivity rate.

Table 1: Clinical profile of symptomatic patients

	Rural %	Urban %
Sore throat	94.8	100
Fever	82.8	80.2
Dry cough	61.1	55.2
Nasal discharge	18.3	23
Breathlessness	3.4	4.9

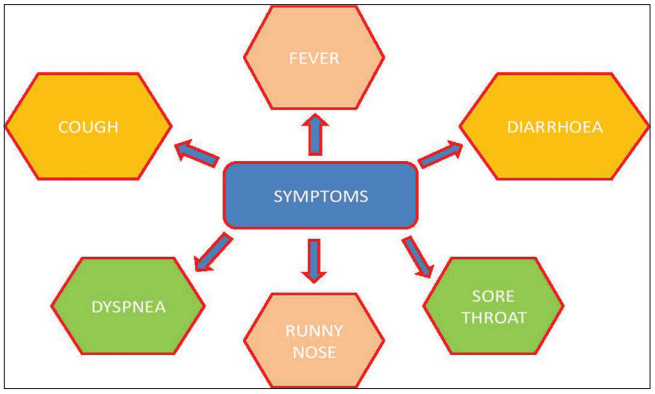

Figure 2: Common symptoms of symptomatic case

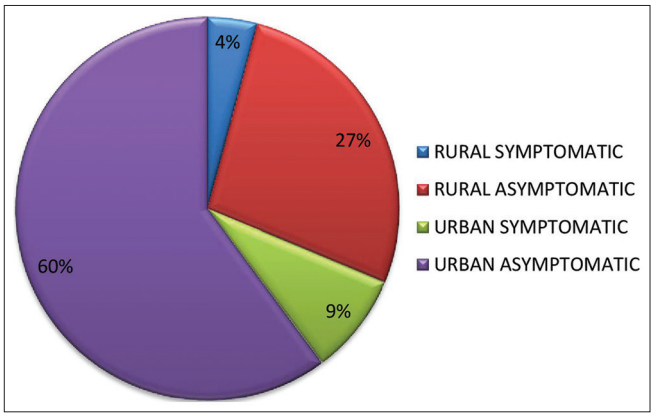

Figure 3: 2-D pie chart shows six months symptomatic-asymptomatic cases in rural-urban area

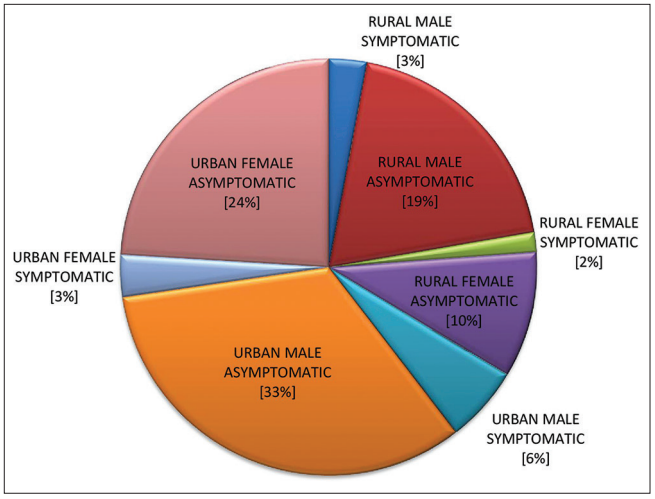

Figure 4: 2-D pie chart showing percentages of COVID symptomatic and asymptomatic male/female cases in rural-urban provinces

We observed a similar pattern in rural symptomatic male and female [Figure 6] and the maximum positivity rate was seen in the month of November. Six month data distribution shows crest of urban symptomatic male cases than urban symptomatic female in all the months with the maximum percent (65.39%) in November. However, urban asymptomatic female, rural asymptomatic male and female were less in the month of November. Consequently, rural asymptomatic patients were more during the month of September and October and declined in November, when we noticed an increase in rural symptomatic patients.

Our results shows symptomatic male and female were in the range of 0-20% whereas, asymptomatic male and female were in the range of 80-100%. Rural symptomatic male were more in the age group of 41-60, however, in urban population the age group was 21-40 years. Rural symptomatic female were more in age group of 1-20 years and urban symptomatic female present in 60 above age group. Furthermore, asymptomatic male and female were in extremes of age group both in rural and urban populations [Figure 7]. One-way ANOVA shows non- significant *P* value.

Urban symptomatic male and female percentage was highest in the November month as compared to rural symptomatic males and females [Table 2].

Virological outcomes of urban and rural area

Established virological procedures were applied for current diagnostic approach such as real-time PCR [RT-PCR].

Generally, urban province shows crest in both symptomatic as well as asymptomatic cases. Moreover, studies confirm that asymptomatic patients contributed dynamic role in transmission.

Mean Ct values depicts, COVID symptomatic cases were utmost in the 21-40 age groups in both rural and urban provinces. In addition, highest asymptomatic cases were in above 60 age groups in rural province whereas, in urban province highest was in 1-20 age groups. Lowest symptomatic cases in rural and urban province were in above 60 age groups. In addition, lowest asymptomatic cases in rural and urban province were in 41-60 age groups [Figure 8]. Moreover, mean Ct values in case of symptomatic and asymptomatic cases in rural region ranges from 20.2 to 23.5 and 22.6 to 23.1. Whereas, mean Ct values in urban region ranges from 21.6 to 24.2 and 20.2 to 24 in case of symptomatic and asymptomatic cases.

Table 2: Monthly distribution of data shows rural-urban symptomatic/asymptomatic cases in male and female

Months	RSM	RSF	RAM	RAF	USM	USF	UAM	UAF
June	27	15	80	46	0	0	128	68
July	5	3	43	32	0	0	241	158
August	10	6	94	42	0	0	413	205
September	3	2	194	64	6	6	483	278
October	3	1	144	68	24	17	138	110
November	61	30	161	109	189	98	100	70

* RSM – Rural Symptomatic Male, USM – Urban Symptomatic Male, RSF – Rural Symptomatic Female, USF – Urban Symptomatic Female, RAF – Rural Asymptomatic Female, UAM- Urban Asymptomatic Male, RAF – Rural Asymptomatic Female, UAF – Urban Asymptomatic Female

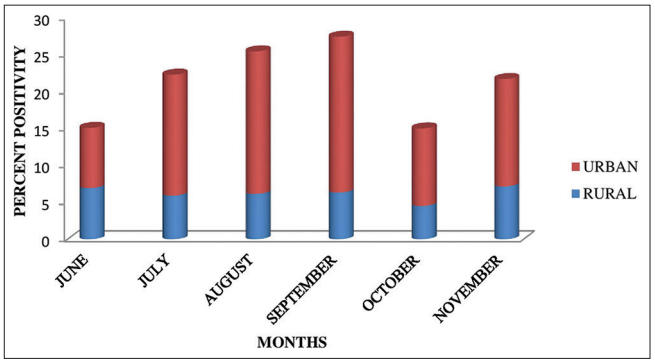

Figure 5: Bar graph showing percent positivity of rural/urban province

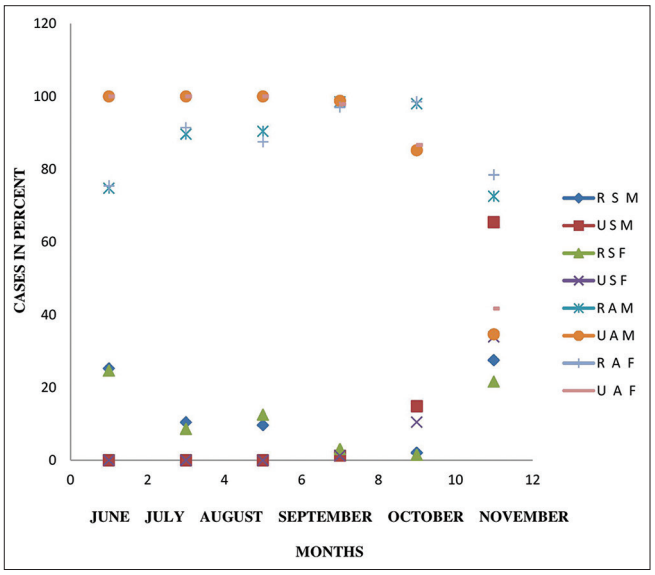

Figure 6: Scatter plot shows month wise data distribution of covid symptomatic and asymptomatic male/female cases in rural-urban provinces

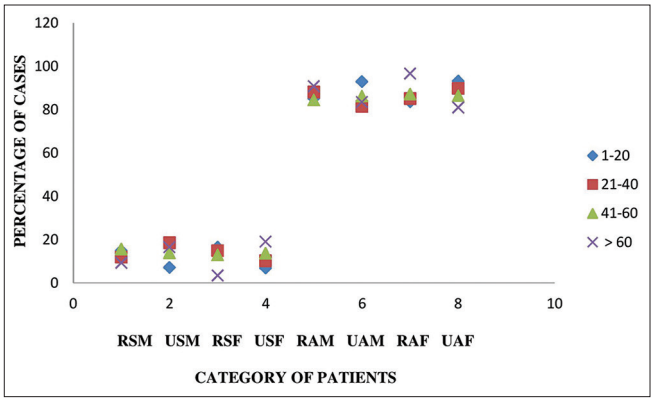

Figure 7: Scatter plot showing age wise percentage of covid symptomatic and asymptomatic male/female cases in rural-urban province

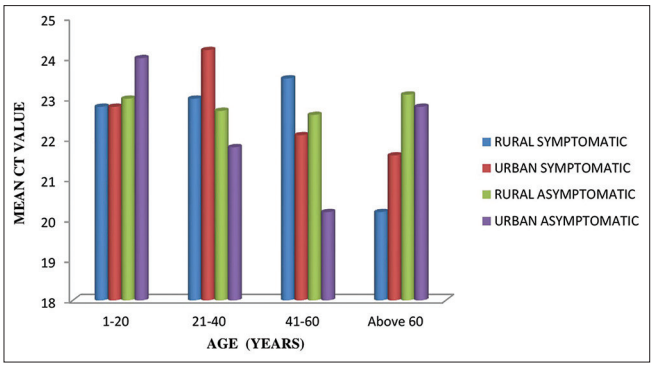

Figure 8: Mean CT value of urban/rural symptomatic and asymptomatic patients

Discussion

This study reveals the clinical and virological outcomes of COVID-19 prevalence rate in rural and urban province at a granular level. The increasing intensity of community spread was associated with increased urban COVID-19 prevalence rates. COVID-19 infection spread hastily from June to November month, affecting the population of rural and urban area.

Former studies have reported substantially higher prevalence rates in urban region as compared to rural region.[11,12] Urban community, on an average, had a substantially elevated prevalence of COVID-19. Earlier studies of Rajib Paul *et al.*,[17] describes the COVID-19 infectiousness spread was 98% in urban countries and 84% in rural countries in the United States.

According to Manoj Mohanan *et al.*,[18] the adjusted proportion of RT-PCR, in urban areas ranged from 4.0 to 10.5% and rural areas 1.5 to 7.7%. Urban areas are densely populated; hence thought to be more vulnerable but rural areas have millions of migrant workers, exempt from lockdown as agriculture was an essential sector. Rural areas are affected as much as urban areas. Our results are in streak with findings from other authors, but additionally identified few cases of COVID-19 infection in rural areas as well. In our study, positivity rate in case of urban population was 13.9% as compared to 6.2% in rural population.

A study conducted in Korea, shows asymptomatic patients were 25.8% and symptomatic patients were 74.2%.[19]

In consequence, previous studies and our observation say that contribution of asymptomatic cases were highly significant in community transmission.[20,21] Albeit, finding the concrete number of COVID-19 asymptomatic cases have been a substantial confront for all researchers. Asymptomatic patients are highly contagious,[22] and are reservoirs of contagions. Previous studies anticipated that further transmission could be stopped up by isolation of asymptomatic and mildly symptomatic COVID-19 patients,[23-25] otherwise any delays in diagnosis are increasingly measured in lives lost. Moreover, contagion deterrence and control measures are vital parts of the clinical supervision and could be opted for containment of COVID-19.

Besides, gender difference was also out looked by other recent studies. It is a global fact in case of male being more prone to mortality[26-31] or entail intensive care unit (ICU) admittance for covid epidemic. According to Hannah Peckham *et al.*,[31] male gender is allied with severe disease and high case fatality as measured by critical care admission. Studies conducted in Hong Kong and Singapore have showed an age –adjusted relative mortality, risk ratio of 1.62[32] and 3.10, respectively.[33]

In this study, the prevalence of COVID-19 symptomatic as well as asymptomatic cases was elevated among males in both urban and rural populations. Male sex prevalence in COVID-19 case reports might be discerning of sex differences in disease pervasiveness, harshness and immune response.[34,35] Similarly, sex and gender differences beside diverse comorbidities might also influence COVID-19 disease severity and outcomes of treatment.[36]

Immune response may be a robust factor behind the gender difference (e.g., $CD4^+$ T cells and types of interferon).[37,38] Females have elevated number of $CD4^+$ T cells and hence shows better adaptive immunity than males.[39] Moreover, in females production of type 1 interferon (IFN)[40] and Oestradiol, boosts up the necessary early response against COVID-19.[41] Moreover, one more plausible factor could be cultural and behavioral discrepancy[42,43] for gender difference.

Nevertheless, COVID-19 is highly contagious, as compared to severe acute respiratory syndrome coronavirus (SARS-CoV), 2003 and Middle East respiratory syndrome (MERS-CoV), 2012 cause of human epidemics. Besides, extensive measures are the recent requisite to lessen the transmission of COVID-19 to control the current outbreak. The adults and elderly people are severely affected due to COVID-19 epidemic, plausibly owing to a weak immune system that permits faster development of viral infection.

This study had some limitations in construing the results; the population enrolled was limited to a selected province. Follow-up of cases in our study was not feasible as the patients did not give their consent for further follow-up samples.

Conclusion

Our outcomes provide novel facts that the COVID-19 epidemic severely affected both rural and urban populations but with few differences. In our study, positivity rate in case of urban population was 13.9% as compared to 6.2% in rural population. There are two foremost facets that contributed variation in positivity in both the population. First, better immune response in rural population as compared to urban population which can be due to the fact that rural people in India are more exposed to various pathogens during their early lifetime thus, improving their immune status. Second, factor could be elevated population densities in urban areas which can contribute to increased infectiousness thus higher positivity rate. In addition, people living in urban population have to commute more for their work

and are exposed to more people throughout the day thus, having more possibility to get infection of COVID-19 as compared to the rural population.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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