Frontiers in Health InformaticsISSN-Online: 2676-7104 www.healthinformaticsjournal.com
2025; Vol 14: Issue 1 Open Access

Comparison Of Ct Values In Relation With Age And Gender

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Cite this paper as: Sneha Mohan, Parul Singhal, Kushal Singh, Pradeep kumar, Mukesh Manjhi and Dalip Kakru (2024), Comparison Of Ct Values In Relation With Age And Gender. *Frontiers in Health Informatics*, *14(1) 2165-2172*

Abstract: ***Introduction***

An acute respiratory infection is the most common health issue affecting all age groups and contributes to the world's disease burden. SARS COV-2 virus is a complex disease affected by multiple factors like inherited conditions, genetic factor, along with comorbidities and other associated risk factors. The comorbid conditions are the modifiable factors which along with the non-modifiable factors which are age and gender influences the outcome of the COVID 19 infection, prognosis, morbidity and mortality. The Cycle threshold (Ct value), value is inversely correlated with viral load. Assessing Ct value and comparing among different age groups and gender with SARS-CoV-2 infection is required to devise treatment and assess the transmission potential of that particular age group. Thus, understanding the viral kinetics of SARS-CoV-2 among different age groups is of vital importance. This study helps in determine the importance of Ct value as viral load assessment and its association with the age and gender of COVID-19 patients. thus helping out in monitoring the critically ill patients .

Methods and materials

The cross-sectional prospective study was conducted in BSL-2 virology Lab, Central laboratory in department of microbiology from April 2022 to May 2023. All the samples receive in the laboratory were then processed and analysed using RT-PCR in BSL-2 Virology lab. Ct values of all the COVID-19 positive samples were compared with different age groups and gender. Ct values were taken into consideration to correlate with the viral load. Ct value were categorised into high viral load (Ct <25), moderate viral load (Ct = 25-30) and low viral load (Ct>30). Age groups were also categorised into <18 years, 18-60 years and >60 years. The aim of

this study was to comparison of the viral load (Ct value) in relation to different age group and gender.

Results

Total 2836 patients were tested for SARS Cov-2 infection, out of which 285 (10.04%) patients were tested positive for the COVID infection. Most infected age group was found to be the middle age group with 79% positivity rate showing significantly high viral load. Among the gender , males were more infected with COVID -19 infection with 62.4%.

Conclusion

The study conducted shows high viral load in middle age group population. It also shows higher SARS CoV-2 infection among males. Most of the infected people are the healthcare workers and by providing the Ct value along with the test report will help in implicating various preventive measures, especially among healthcare workers..

INTRODUCTION

An acute respiratory infection is the most common health issue affecting all age groups and contributes to the world's disease burden.[1] Human coronaviruses (HCoVs) are also enveloped in single-stranded positive-sense RNA viruses among many other RNA viruses.[2] The first cluster of patients presenting with respiratory illness was reported in Wuhan, China in 2019 December who 2020. Most of the COVID 19 patients complained of mild symptoms while only 14% individual presented with severe acute respiratory symptoms and required hospitalization.[3] SARS COV-2 virus is a complex disease affected by multiple factors like inherited conditions, genetic factor, along with comorbidities and other associated risk factors. Age and sex are two important factors associated with risks and outcomes of COVID-19 disease. COVID-19 morbidity also seems to be affected by patient age and sex.[4] The standard molecular method for coronavirus disease 2019 (COVID-19) diagnosis is via real-time reverse transcription polymerase chain reaction (RT-PCR). Real-time RT-PCR cycle threshold (Ct) values represent the number of amplification cycles required for the target gene to exceed a threshold level. Ct values are therefore inversely related to viral load and can provide an indirect method of quantifying the copy number of viral RNA in the sample. Viral load determination in respiratory samples is difficult, and therefore some studies have correlated viral load to Ct value and have indicated that the lower the Ct value, the higher is the viral load or the infectiousness and severity of the disease.[5] SARS CoV-2 also known as COVID-19 disease is known for its high infectious nature. Commonly, in viral infection, RT-PCR uses Cycle threshold-value (Ct -value) for viral load analysis. The Cycle threshold (Ct value), value is inversely correlated with viral load. Many studies have used Ct-value as a marker for monitoring the disease severity in viral respiratory disease, but its use has reported varying results.[6] Therefore, assessing Ct value and comparing among different age groups and gender with SARS-CoV-2 infection is required to devise treatment and assess the transmission potential of that particular age group. Thus, understanding the viral kinetics of SARS-CoV-2 among different age groups is of vital importance. [7] There are very few studies that had showed the association between viral load Ct value with age and gender. This study helps in determine the importance of Ct value as viral load assessment and its association with the age and gender of COVID-19 patients. thus helping out in monitoring the critically ill patients .

MATERIALS AND METHODS

METHODS

The cross-sectional prospective study was conducted in BSL-2 virology Lab, Central laboratory in department of microbiology from April 2022 to May 2023**.** All samples (oropharyngeal and nasopharyngeal swabs) from the suspected COVID-19 cases were collected using VTM vials at sample collection area and immediately

transported to the laboratory. Under laboratory biosafety guidelines, swabs were then processed and analysed using RT-PCR in BSL-2 Virology lab. Viral RNA extraction was done within 2 hours of receiving the sample in the laboratory by either automated or manual procedure according to the manufacturer's instructions. The extracted RNA was subjected to amplification in the thermocycler using the amplification kit available. The RT-PCR was conducted with primers and probes targeting the RdRp and N genes and positive reference/control. The amplification curves were analysed on the system and results were considered positive when Cycle threshold value (Ct value) of both target genes was ≤ 35 and was considered negative when the Ct value of both the target genes was more than 35. The particular assay was considered valid only when the cycle threshold of the positive control was ≤ 35. Ct values of all the COVID-19 positive samples were compared with different age groups and gender. Ct values were taken into consideration to correlate with the viral load. Age groups were also categorised into ≤18 years (young), 18-60 years (middle age), and >60 years (older). Ct values were categorised into high with ct value >25 , moderate with Ct value >25-35 and low viral load with Ct value >35. Median Ct values of RdRp and N genes, i.e., confirmatory genes, were taken into consideration for comparison.

Internal control	RdRP gene	N gene	Conclusion
CT< 35	Has amplification curve CT<35	Has amplification curve CT<35	Positive
CT<35	No amplification curve	No amplification curve	Negative
CT<35	Has amplification curve CT<35	No amplification curve	Suspected : Retesting
CT<35	No amplification curve	Has amplification curve CT <35	Suspected : Retesting
CT<35	Has amplification curve CT>35	Has amplification curve CT >35	Suspected : Retesting
CT>35	-		Invalid: Suspected : Retesting

Ethical Considerations: The ethical approval was given by the Institutional Ethics Committee of SMS&R and Sharda Hospital, Sharda University with ref no. SU/SMS&R/76-A/2022/62.

RESULTS

Total 2836 patients were tested for SARS Cov-2 infection, out of which 285 (10.04%) patients were tested positive for the COVID infection. Ct values were evaluated among all 26 (9.1%) young, 226 (79.3%) middle age and 32 (11.2%) older age group. After comparing the viral load (Ct value) among all age groups, Middle age (62%) and older (62.5%) patients showed significantly ($p < 0.05$) high viral load (<25) as compared to young (54%) age group. (Table 1 and Fig 1)

Table 1: Distribution of Ct values in different Age Groups

Age Group	High Viral Load, n (%)	Moderate Viral Load, n (%)	Low viral load, n (%)
Young	14 (54)	9 (34.6)	4 (15.4)
Middle Age	140 (62)	82 (36.3)	4 (1.7)
Older	20 (62.5)	10 (31.2)	2 (6.2)

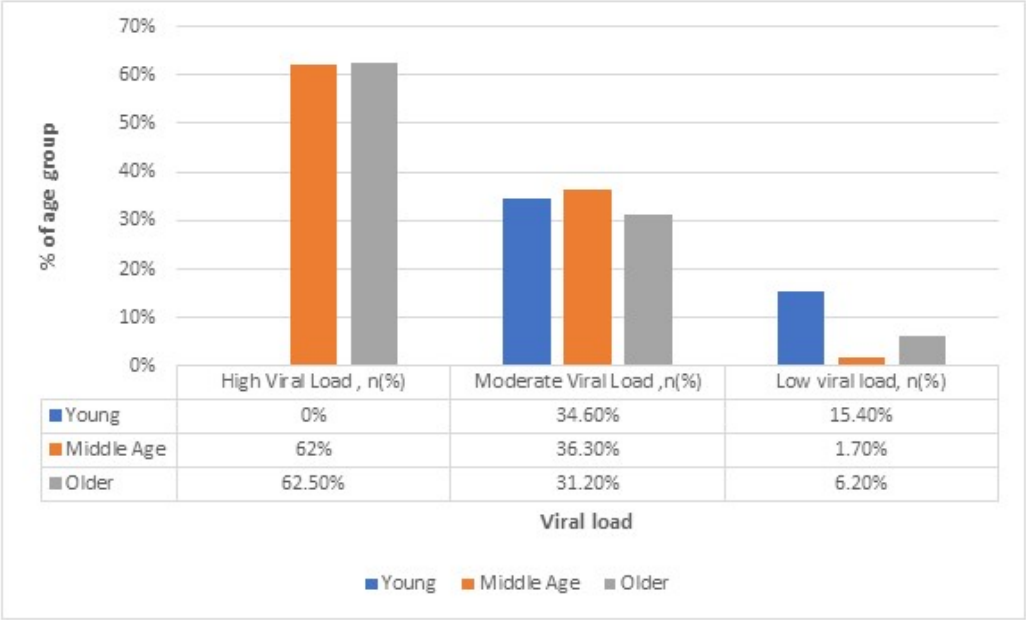

Figure 1: Distribution of Ct values in different Age Groups

Ct values were also evaluated among different gender, showing highest percentage of male patients (62.4%) followed by female (37.5%). The viral load (Ct value) were then compared among both the gender. The high viral load (61.2%) were observed among the male patients as compared to female with only 60.7% with no significant difference ($p>0.05$) .(Table 2 and Fig 2)

Table 2: Gender wise distribution of Ct values:

Gender	High Viral Load, n (%)	Moderate Viral Load, n (%)	Low viral load, n (%)
Male	109 (61.2)	63 (35.4)	6 (3.4)
Female	65 (60.7)	38 (35.5)	4 (3.7)

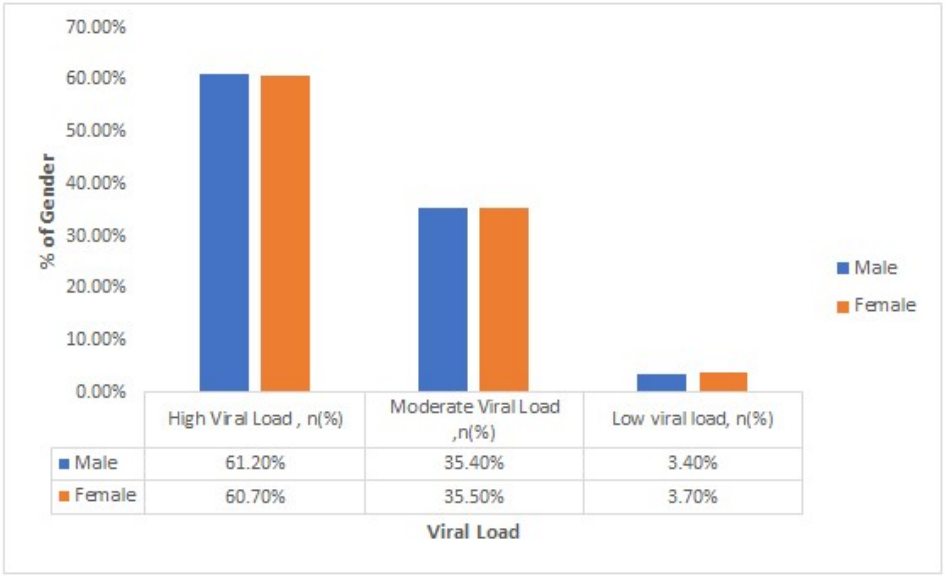

Figure 2: Gender wise distribution of Ct values:

When different age groups were compared in relation to both the gender, high viral load were observed in middle age (61.7%) and older (62.5%) age group patients in male category. Similarly in female patients, the high viral load was present in middle age (62.2%) and older (62.5%) age groups. It was also observed that in whole population, middle age patients (79.3%) were high in percentage as compared to other age groups. (Table 3 and Figure 3)

On overall analysis, it was observed , the middle age and older patients showed the high viral load percentage as compared to young population in the same viral load category and in both the gender .

Table 3: Age and gender distribution of Ct values:

Gender	Age Group	High Viral Load, n (%)	Moderate Viral Load, n (%)	Low viral load, n (%)
Male	Young	10 (55.5)	5 (27.7)	3 (16.6)
	Middle age	84 (61.7)	50 (36.7)	2 (1.4)
	Older	15 (62.5)	8 (33.3)	1 (4.1)
Female	Young	4 (44.4)	4 (44.4)	1(11.1)
	Middle Age	56 (62.2)	32 (35.5)	2 (2.2)
	Older	5 (62.5)	2 (25)	1 (12.5)

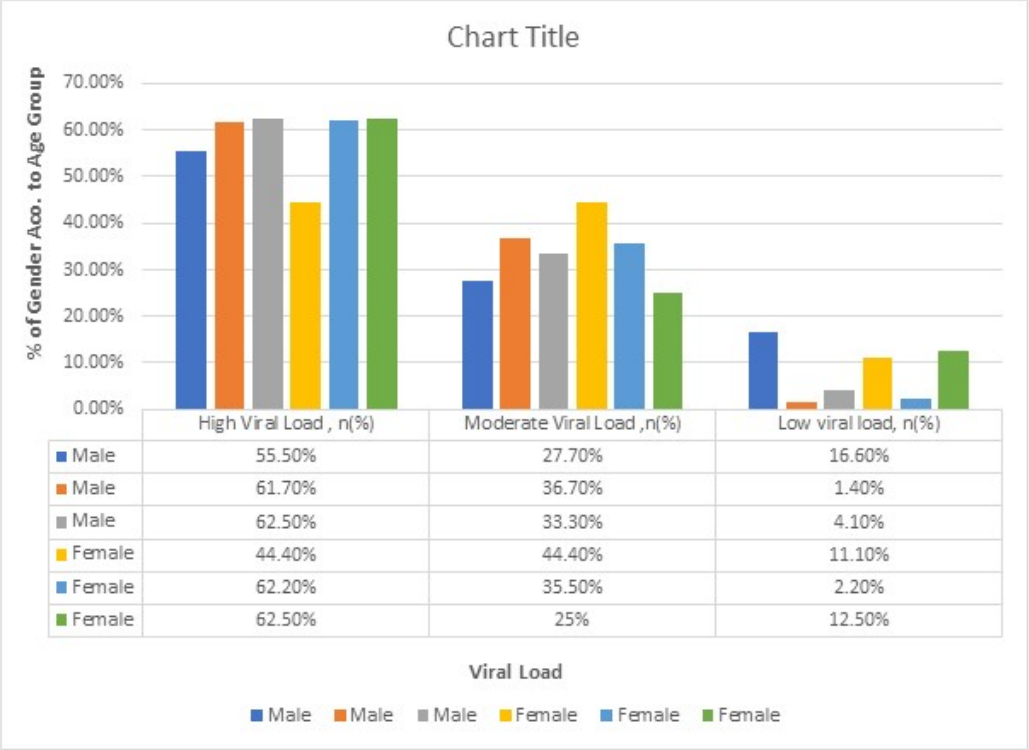

Figure 3: Age and gender distribution of Ct values:

DISCUSSION

SARS CoV-2 also known as COVID-19 disease is known for its high infectious nature. [8] Commonly, in viral infection, RT-PCR uses Cycle threshold-value (Ct -value) for viral load analysis. Ct value is the number of the PCR cycle at which the fluorescence signal crosses the threshold value, thus labelling a particular sample as positive or negative. Ct values are a surrogate marker of viral load and are inversely proportional to it. A lower Ct value signifies a higher viral load and vice versa.[7] VL refers to the quantitative numbers of viral particles

in the body fluid, which represents the infectivity levels.[9] Many studies have used Ct-value as a marker for monitoring the disease severity in viral respiratory disease, but its use has reported varying results.[6]

Ct values can be considered an indicator of virus transmissibility, with lower Ct values indicating a higher viral load and, in turn, increased viral shedding.[10] Age and sex are two important factors associated with risks and outcomes of COVID-19 disease . COVID-19 morbidity also seems to be affected by patient age and sex. It has been found that older age groups have more severe COVID-19 symptoms and higher fatality rates while children tend to have lower prevalence and milder symptoms than adults.[4]

Our study was conducted from 2022 – 2023, during which cases were seen to decrease and also the people were getting immune to the infection, which affected our total number of cases but still the positivity rate almost remained the same, as observed. Out of the total samples received in the laboratory for SARS CoV-2 testing by RT-PCR, 285 cases were confirmed positive for the COVID-9 disease with a positivity rate of 5.9%. there were very few studies that have reported a positivity rate. The study conducted in 2020 has reported 7% positivity rate in one month.[11] The studies done before were mostly carried out during the initial phase of pandemic when there was unawareness of the infection pathogenesis and its severity, which led further to panic and thus led to testing in large numbers.

In our study, viral load (assessed by Ct value) was compared in different age groups and between male and female. On analysis, viral load was found to be higher in middle age and older age group patients, which was in concordance with the results observed in the study done in 2022. [7] The study done by Kanta P et al also observed similar type of findings and reported to have more susceptibility to COVID 19 infection in older age group than young population.[9] Similarly the study done by Heald-Sargent T et al , 2020 reported to have HVL in elderly population.[12] The high viral load detection , as assessed by lower ct value in our study collaborates to severity of SARS CoV-2 infection in these two age groups , which might due to increased virus shedding seen in them. On the other hand, study done by Mahallawi WH et al. showed no such association between age groups and high viral load. This might be due to that there are other factors also that play an important part apart from viral load which contributes to the severity of the COVID 19 infection. World Health Organization has also reported that increased COVID-19 infectivity is seen among the elderly age group with underlying comorbid medical conditions than younger population with no comorbidities.[13]

This study also compared the viral load among both the gender. It was found that viral load was more in male than female. Similar findings were reported by the study done by Sadeghi, F. et al,2022, suggesting male to be more prone to COVID -19 infection due to their lifestyle differences such as smoking, whereas innate immunity is strong and presence of oestrogen hormone in woman plays a protective role in them.[14] The study done by Mahallawi WH et al. reported to have higher viral load among females than male.[4] While Kanta P et al . reported to have no association between different gender and high viral load.[9]

The relation of gender and its effects on viral load and immunological response to infectious disease is not surprising; it has been demonstrated in other diseases. This is thought to be related to a difference in immune response in which females develop a higher immune response to infectious agents, making them less susceptible to diseases.[15]

Our study also showed the comparison of Ct values in different age group population among both the gender. The high viral load was reported in middle age group and older age patients among both the genders. This shows that there is no difference of viral load seen among different gender and in both the gender elderly group of population id affected more than younger population.

CONCLUSION

The SARS CoV-2 infection was found to be associated more among make population and affecting the middle

age group and elder age group population. The limitation of this study is the small sample size that might be due to more awareness observed during the study period. Also, other factors like clinical severity and its progression was not studied. This study have looked into the viral load assessment using Ct values in different age groups and gender in northern sector of India.

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