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CORRELATION OF PROGNOSIS OF COVID POSITIVE PATIENTS BETWEEN REAL TIME RT-PCR AND CT-VALUES OF E. GENE, N.GENE, RDRP GENE

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ABSTRACT

INTRODUCTION: COVID-19 – Corona virus disease-19 – A respiratory illness caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), colloquially referred to as coronavirus. Real-time PCR is conducted to quantify the absolute amount of target sequence or to compare relative amount of a target sequence among samples. This technique monitors amplification of the target in real time via a target specific fluorescent signal emitted during amplification. During most real times PCR, a considerable amount of background fluorescence occurs. Inspite of this fact PCR fluorescent dyes and probes should be sequence specific. This CT value or cycle threshold value in a real time RT- PCR is defined as the number of cycles required for the fluorescent signal to cross the threshold i.e., exceeds background level). This is inversely proportional to the amount of target nuclei in the sample. i.e., lower the CT level the greater the amount of target nuclei in the sample.

AIM: To explore the association of the mutation pattern of covid-19 genetic variation with the severity and fatality of patients with covid-19 viral illness. Factors considered are Age, Sex, and Duration of hospital stay and outcome of patient.

MATERIALS AND METHODS: The study was a comparative, correlation, retrospective, lab values based study. All covid+ve patients tested at Peerless hospital, Kolkata by RT-PCR on admission. It is imperative to choose reference genes whose expression levels are not expected to change during our experiment. Common housekeeping genes include actin, alpha-tubuliun, gapdh and ubiquitin. it is wise to use at least two reference gene for one study may not be suitable for another. Total 370 participants were present in this study.

RESULT: In our study, 114(30.8%) COVID positive patients were in E GENE GR 11-20, 147(39.7%) COVID positive patients were in E GENE GR 21-30 and 109(29.5%) COVID positive patients were in E GENE GR 31-40. 163(44.1%) COVID positive patients were in N GENE GR 11-20, 132(35.7%) COVID positive patients were in N GENE GR 21-30 and 75(20.3%) COVID positive patients were in N GENE GR 31-40. 228(61.6%) COVID positive patients were in RdRp GENE GR 11-20, 112(30.3%) COVID positive patients were in RdRp GENE GR 21-30 and 30(8.1%) COVID positive patients were in RdRp GENE GR 31-40.

We found that, 30(8.1%) COVID positive patients died and 340 (91.9%) patients have survived.

CONCLUSION: The patients who died had significantly lower RT-PCR CT-values OF E. GENE, N.GENE, RdRp GENE than patients who survived. We concluded that the time of onset of symptoms were earlier for patients with lower CT value, than the other group who survived.

So we concluded after correlating CT-values of real time RT-PCR and E. GENE, N.GENE, RdRp GENE, that the patients with lower CT values had more severity and had poor prognosis than the other group with higher RT-PCR CT-values of E.GENE, N, GENE, RdRp GENE.

KEYWORDS

SARS-CoV-2, COVID-19, Real time RT-PCR, CT-values, E.GENE, N, GENE, RdRp GENE

INTRODUCTION

COVID-19: Corona virus disease-19 – A respiratory illness caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), colloquially referred to as coronavirus. The family of corona viruses incorporates not only SARS (-1 and -2) and MERS, but also milder forms (causing 15-20% of common colds).

DNA: Deoxyribonucleic acid – The molecule that human and most organisms use to store their genetic code. DNA contains the information that the cells use to make proteins. It's formed from two strands which bind together forming a helix shape.

Fluorescence: This is the light given off by certain molecules when they absorb energy.

RNA: Ribonucleic acid – In humans body RNA has numerous functions, but a main function is to copy sentences of DNA to make proteins. Some viruses use RNA rather than DNA to store their genetic code, including the virus that causes COVID-19.

This CT value or cycle threshold value in a real time RT- PCR is defined as the number of cycles required for the fluorescent signal to cross the threshold i.e., exceeds background level). This is inversely proportional to the amount of target nuclei in the sample.i.e., lower the CT level the greater the amount of target nuclei in the sample.

1. </29 are strong positive reactions with abundant target nuclei acid in sample.
2. 30-37-are positive reactions indicative of moderate amounts of target nucleic acid,
3. Above 38-40 are weak reactions indicative of minimal amounts of nucleic acid which could represent an infection state or environmental contamination.
4. Real-time reverse transcription polymerase chain reaction (RT-PCR) assay for SARS-CoV-2

Nasopharyngeal swab samples were collected and stored in special tubes with viral transport medium and then sent to the laboratory for SARS-CoV-2 RNA extraction and SARS-CoV-2 detection by real-time RT-PCR as previously described 71. A cycle threshold value (Ct-value) <37 was defined as a positive test result, while a Ct-value of 40 or more was defined as a negative test result. These diagnostic criteria were based on the recommendations of the National Institute for Viral Disease Control and Prevention (China) 72. A Ct-value of 37 to<40 required confirmation by retesting. The sampling time between the two test specimens was at least 24 h.

The severe acute respiratory syndrome coronavirus 2(sars-cov-2) was found as the viral pathogen that causes covid-19. The reference series of the full-genome of the sars-cov-2 was explored soon after the manifestation of the disease in Wuhan, China, sequencing of the viral genome was conducted from infected patients in other countries to compare the sequence with the reference sequence.

It is recommended that confirmatory testing is carried out for positive specimens; this should be done with a second gene target or a duplex test that has two gene targets in one reaction. Several of the viral mutations are associated with the receptor binding domain (RBD) of the spike surface glycoprotein. Since the RBD of the glycoprotein acts essential role in binding with receptors on the host cell, mutation may alter the virulence of the virus.

Based on the non-confirmatory conclusion of CT scan in COVID positive patients showing variation of changes, RT-PCR and the CYCLE THRESHOLD values are likely to be more specific in determining the severity of the disease.

AIMS AND OBJECTIVE

To explore the association of the genetic variation of cycle threshold values of RT-PCR in COVID positive patients. The mutation of variation is correlated with the severity and prognosis of patients with covid-19 viral illness.

MATERIALS AND METHODS

The study was a comparative, correlation, retrospective, lab values based study.

It is imperative to choose reference genes whose expression levels are not expected to change during our experiment. Common housekeeping genes include actin, alpha-tubuliun, gapdh and ubiquitin. It is wise to use at least two reference genes for one study may not be suitable for another.

The delta-delta Cq method makes a key assumption-that the amplification (PCR) efficiencies of our reference and target samples are almost 100% and within 5% of each other. Other normalization methods include the dealt-Cq method and the PFAFFL method.

To be certain that the variations in cq values are due to real biological changes and not technical issues, you will need to normalize your results. The most popular normalization method is known as "delta-delta Ct" or the LIVAK method. Here, you compare Cq values of the sample to the Cq values of your several reference (housekeeping) genes.

Inclusion Criteria

All covid+ve patients tested at Peerless hospital, Kolkata by RT-PCR on admission between April 2020-October 2020.

Exclusion Criteria

1. Patients having chronic immune-compromised state.
2. Patients tested by gene-expert
3. Patients having more than 3 comorbidities from the list below:

- **Hypertension,Diabetes Mellitus,Heart disease,Liver Disorder,Kidney Disorder.**

Sample Size

Sample size has been calculated with help of Epi Info (TM) 3.5.3. EPI INFO which is a trademark of the Centers for Disease Control and Prevention (CDC). For statistical analysis data were entered into a Microsoft excel spreadsheet and then analyzed by SPSS 27.0.and Graph Pad Prism version5. Data had been summarized as mean and standard deviation for numerical variables and count and percentages for categorical variables. Unpaired proportions were compared by Chi-square test or Fischer's exact test, asappropriate. Two-sample t-tests for a difference in mean involved independent samples or unpaired samples-value ≤ 0.05 was considered for statistically significant.

Sample Size Justification

One study56 found that the prevalence of SARS-CoV-2in India was46.7%. So, for this study p=0.467.

Thus, the number of patients required for this study was370.11 ~ 370 with power 87%.

The formula used for sample size calculation was as follows:-
n= 4pq / (L2)

Where, n= required sample size, p= 0.467 (as per the study byMonahan et al56),q = 1 – p,

L = Loss % (Loss of information)

Calculation:

Here p= 0.467,
q=1-p = 1-0.467=0.533,
4pq = 4 x 0.467 x 0.533 = 0.9956
L= 0.05180
L2= 0.00269
Loss of information percentage = 5.18%
n = 4pq / (L2) = 0.9956/0.00269 = 370.11 = 370

Study Group:

370participants were taken.

DISCUSSION

In our study, the speed of survivability [340(91.9%)] was beyond the fatality rate [30(8.1%)].

In our study out of 370 (100.0%) patients, most of the patients [152(41.1%)] were gift within the people of 51-60 years and 61-70 years [101(27.3%)]. Rest 27(7.3%) patients were ≤40 years recent, 78(21.1%) patients were 41-50 years recent and 12(3.2%) patients were 71-80 years recent. The mean Age (mean± s.d.) of patients was 55.8838 ± 8.5963years. 122(33.0%) patients were feminine and 248(67.0%) patients were male.

It was found that, the fatality rate was higher within the patients [12(40.0%)] mature cluster 61-70 years and 3(10.0%) patients died from the people of 41-50 years, 11(36.7%) patients died from the people of 51-60 years and 4(13.3%) patients had died from the people of71-80 years. Survivability rate was higher within the patients [141(41.5%)] mature cluster 51-60 years and conjointly 27(7.9%) patients had survived from the people people, 75(22.1%) patients survived from the people of 41-50 years, 89(26.2%) patients survived from the people of 61-70 years, 8(2.4%) patients survived from the people of 71-80 years.Association mature in cluster vs outcome was statistically vital (p=0.0023).

In our study, the mean Age (mean± s.d.) of dead patients was [61.5667 ± 7.6999 years] beyond the mean Age (mean± s.d.) of survived patients [55.3824 ± 8.5003 years] that was statistically vital (p<0.0001).

It was found that, 7(23.3%)Female patients and 23(76.7%) male patients died.115(33.8%) feminine patients and 225(66.2%) male patients survived.Association of Sex vs Outcome wasn't statistically vital (p=0.2413).

Cho H et al [1](2020) found that the limit of detection (LOD) for individual SARS-CoV-2 genes by Ct values with totally different concentrations of SPT templates and genomic RNAs from SARS-CoV-2 infected samples was firm.LODs with SPT templates were >10−15 (atto) M for RdRP, 10−12 (femto) to 10−13 (100 atto) M for E sequence, and 10−12 to 10−14 (10 atto) M for N sequence, severally. time period RT-PCR assay victimisation serially diluted genomic RNAs ready from SARS-CoV-2 virus infected cultures showed that picogram quantities of RNAs is resulted within the LOD. The sensitivity of RdRP and E sequences supported Ct values was but that of N gene with this platform.

We found that In E GENE GROUP number of patients were higher in E sequence cluster 21-30 [147(39.7%)] compared to different 2 E sequence teams that's E sequence GR 11-20 [114(30.8%)] and E sequence GR 31-40 [109(29.5%)]. The mean E sequence (mean± s.d.) of patients was twenty six.2895± 7.3003.

In N sequence cluster, most of the patients [163(44.1%)] were gift in N sequence GR 11-20, 132(35.7%) patients were in N sequence GR 21-30 and 75(20.3%) patients were in N sequence GR 31-40.The mean N sequence (mean± s.d.) of patients was twenty four.7097± 7.3700.

We conjointly found that in RdRp sequence GR, most of the patients [228(61.6%)] were gift in RdRp sequence GR 11-20, 112(30.3%) patients were in RdRp sequence GR 21-30 and 30(8.1%) patients were in RdRp sequence GR 31-40.The mean RdRp sequence (mean± s.d.) of patients was twenty one.4043± 5.4880.

Yu F et al [2](2020) found that in 95 samples that tested positive by each strategies, the cycle threshold (Ct) of RT-PCR was extremely related with the copy variety of ddPCR (ORF1ab sequence, R2 = 0.83; N gene, R2 = 0.87). Four (4/161) negative and forty one (41/67) single-gene positive samples tested by RT-PCR were positive in line with ddPCR with infectious agent hundreds starting from eleven.1 to 123.2 copies/test. The infectious agent load of metabolic process samples was then compared and also the average infectious agent load in mucus (17 429 ± 6920 copies/test) was found to be considerably beyond in throat swabs (2552 ± 1965 copies/test, P < .001) and nasal swabs (651 ± 501 copies/test, P < .001). moreover, the infectious agent hundreds within the early and progressive stages were considerably beyond that within the recovery stage (46 800 ± seventeen 272 vs 1252 ± 1027, P < .001) analyzed by mucus samples.

Loying P et al [3](2020) looked into the dynamics of ORF1ab and N sequence and located that N sequence need longer length of days with twelve.68 (S.D.±3.24) to become negative than ORF1ab with twelve.09 (S.D.±2.88) days and it differs considerably (p=0.012; p<0.05). The persistent of N sequence found in forty six patients out of a hundred and fifteen (39.65%) to the succeeding reading once three

days. They need conjointly looked into the mean variations within the between N and ORF1ab sequences each readings one by one and located that there have been no vital variations between the mean Ct worth of ORF1ab and N gene except within the day three ($p=0.015$; $p<0.05$). The persistent of quality of N sequence is considerably for additional length than ORF1ab.

In our study, fatality rate was solely found within the patients of E sequence GR 11-20 [30(100.0%)]. Survivability rate was found higher within the patients of E sequence GR 21-30 [147(43.2%)] then E sequence GR 31-40 [109(32.1%)] and at the moment E sequence GR 11-20 [84(24.7%)] severally that was statistically vital ($p<0.0001$). The mean E sequence (mean± s.d.) of survived patients was [27.2532± 6.8176] beyond the mean E sequence (mean± s.d.) of dead patients [15.3667± .6910]. Distinction of mean E sequence with each Outcome was statistically vital ($p<0.0001$).

We examined that the fatality rate was solely found within the patients of N sequence GR 11-20 [30(100.0%)]. Survivability rate was found higher within the patients of N sequence GR 11-20 [133(39.1%)] then N sequence GR 21-30 [132(38.8%)] and at the moment N sequence GR 31-40 [75(22.1%)] severally that was statistically vital ($p<0.0001$). The mean N sequence (mean± s.d.) of survived patients was [25.4941± 7.1737] beyond the mean.N sequence (mean± s.d.) of died patients [15.8200± .7783]. Distinction of mean N sequence with each Outcome was statistically vital ($p<0.0001$).

In our study, the mean Onset of Symptoms (mean± s.d.) of patients was nine.2676± 3.0277. The mean length of keep (mean± s.d.) of patients was eleven.5459± 2.5470 days. The mean Onset of Symptoms (mean± s.d.) of survived patients was [9.7235± 2.6949] over the mean Onset of Symptoms (mean± s.d.) of died patients [4.1000± 1.2959]. distinction of mean Onset of Symptoms with each Outcome was statistically vital ($p<0.0001$).

CONCLUSION

In our study, 114(30.8%) COVID positive patients were in E GENE GR 11-20, 147(39.7%) COVID positive patients were in E GENE GR 21-30 and 109(29.5%) COVID positive patients were in E GENE GR 31-40. 163(44.1%) COVID positive patients were in N GENE GR 11-20, 132(35.7%) COVID positive patients were in N GENE GR 21-30 and 75(20.3%) COVID positive patients were in N GENE GR 31-40. 228(61.6%) COVID positive patients were in RdRp GENE GR 11-20, 112(30.3%)COVID positive patients were in RdRp GENE GR 21-30 and 30(8.1%) COVID positive patients were in RdRp GENE GR 31-40.

We found that, 30(8.1%) COVID positive patients were Died and 340(91.9%) patients were Survived.

Poor outcome was observed in 61-70 years, 51-60 years, 71-80 and 41 – 50 years respectively which was statistically significant.

COVID positive patients with shorter duration Onset Of Symptoms was poor survival which was statistically significant.

COVID positive patients with lower E GENE level (11-20) was poor survival than higher E GENE level (21-30 and 31-40) which was statistically significant. The mean was low in death patients compared to alive patients.

Patients with lower N GENE level (11-20) was poor survival than higher N GENE level (21-30 and 31-40) which was statistically significant. The mean was significantly low in death patients compared to alive patients.

Poor outcome was found in patients with lower RdRp level (11-20) which was statistically significant. The mean was significantly low in dead patients compared to alive patients.

The patients who died had significantly lower CT-values OF E. GENE, N.GENE, RdRp GENE than patients who survived. The other parameters taken into account to reach this conclusion was age of patient, sex of patient, duration of stay in hospital, outcome of patient.

While correlating CT-values OF E. GENE, N.GENE, RdRp GENE with disease severity it was concluded that the prognosis and outcome of covid-19 disease is poor with lower CT values of E.GENE, N.GENE, RdRp GENE.

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