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Molecular Analysis of Covid-19 Patient Real Time PCR and their Medicational Clinical Trials

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Editorial

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Abstract

The corona name derived from their crown like spike proteins attach with cell receptors. It belongs to corona viradae family and nideo virales order, envelop virus, size range 65-125nm and positive single standard RNA between 26.4 to 31.7 kb and contain7096 amino acid. There are four subtypes that have been detected these are alpha, beta, gamma and delta. The 267 covid -19 blood and nasopharyngeal samples were collected from Multan region. RNA extraction from nasopharyngeal samples and run the PCR. The blood samples use for clinical tests, Lactate dehydrogenase, serum ferritin level, D-Dimer, TG, cholesterol, thyphoidot, HDL, lymphocyte count and CRP. The 127 (47.21%) out of 267 patients were covid-19 PCR positive and showed the amplification ORF1ab, E and N gene while140 individuals were covid-19 PCR negative and not showed the amplification of ORF1ab, E and N gene. The patients with negative Covid-19 PCR, the other analysis tests such as lactate dehydrogenase, HDL, ferritin, ESR,CBP, D. Dimer, Tg, cholesterol, CRP and CT scan. The patients affected covid-19 has higher values of D-Dimer, ESR, Neutrophils, LDH, CRP and ferritin level than normal ranges. But the values of the HDL, cholesterol and lymphocytes were decreased form the normal ranges. Drugs are mentioned in table #3 that is treating for covid 19- patients. These drugs are successful for Covid -19 treatments.

Keywords: COVID-19; ESR; Delta Viruses

Abbreviations: CRP: C Reactive Protein; LDH: Lactate Dehydrogenase; HDL: High Density Lipid; TG: Triglyceride; ESR: Erythrocyte Sedimentation Rate; CBP: Complete Blood Picture.

Introduction

The outbreak of novel SARS-CoV-2 was first described in the province of china, on 30 December the WHO declared emergency in the world based on growing case rate at Chinese and international airport [1]. The corona name derived from their crown like spike proteins attach with cell receptors [2]. It belongs to coronaviradae family and nideovirales order, envelop virus, size range 65-125nm and positive single standard RNA between 26.4 to 31.7 kb and contain 7096 amino acid [3]. There are four subtypes of the corona viruses that have been detected. the names of those subtypes are alpha, beta, gamma and delta from these four subtypes only alpha and beta are originated from mammals .alpha are particularly originated from bats. While Gamma and delta viruses subtypes are originated from pigs and birds. Among the 7 subtypes of coronavirus that infect human, the beta coronavirus cause dome serious illness.

N protein (nucleocapsid) is encoded by the four genes that play structural rules in viruses In case of beta coronaviruses (MAZHAR, MAHMOOD et al. 2021) there exist

spike protein (S), a small membrane protein(SM) and the glycoprotein (M) as well as an additional membrane protein. The genome of the SARS-COV-2 is 96% similar to the whole genome of bat corona virus [4].

Spike protein of viral attach with angiotensin-converting enzyme (ACE) receptors on The host cell membrane, virus enter the cell due to endocytosis, the virus enter into cytoplasm and release its RNA and hijack the machinery of the cell, replicate the RNA and N protein, and N proteins move towards endoplasmic reticulum to form spike proteins. Viral particle release from Golgi bodies to infect other cells. Meanwhile in some cases it leads to apoptosis and cell death the viral particle infect neighboring cells [5] (Figure 1).

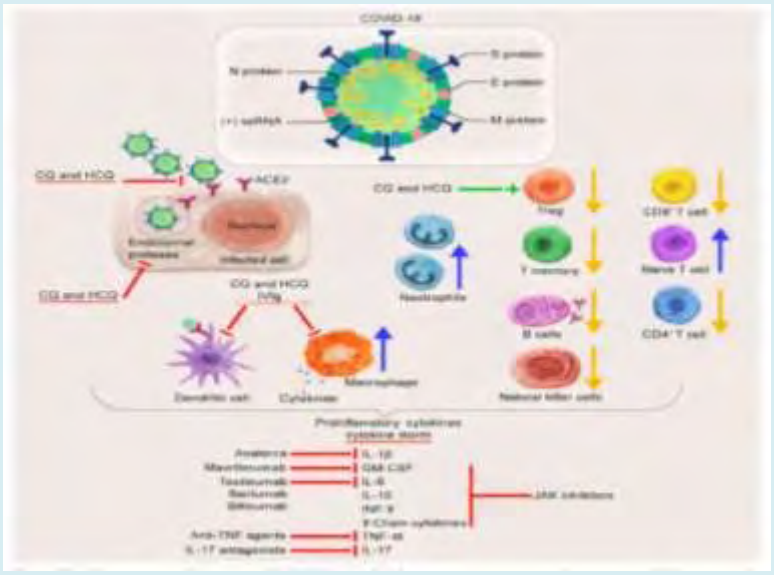


Figure 1: Attachment of the corona virus.

Attachment of the corona virus takes place through endocytosis After endocytosis it is also called as endosome The proteases proteins present in the endosome mediate the release of the viral genome virus binding with receptors and the virus endosome fusion is blocked by the use of chloroquine and hydroxychloroquine Hence these medicines proved to be effective for the treatment of the COVID-19 The production of cytokines in macrophages and the presentation of antigen in dendritic cells is inhibited by the use of CQ,HCQ and intravenous immunoglobulin. The count of neutrophils and leukocytes is increased in the case of COVID-19 while there is a decreased in the levels of total count of lumphocytesCD4+T cells, CD+8 T cells, regulatory T cells, memory T cells, natural killer cells and B cells {Soy, 2020 #223}. Activity of Treg is increased by the use of CQ and HCQ This is a beneficial effect of the CQ and HCQ The decrease in number of cells is indicated by the yellow arrow while the increase in number of cells is indicated by blue arrow as shown in Figure 1.

Methodology

Sampling

The 267 covid -19 samples were collected from Multan region. These biological samples we used nasopharyngeal swab in UTM. The RNA extracted by using high purified Viral RNA Kit (Roche) according to instruction of manufacturer. And it is based in capture of RNA using columns with silica filters.

RT- qPCR Analysis

For the validation of the selected RNA extraction procedure, RT-qPCR using Taqman probes and primers were used. Two viral targets were amplified: the nucleocapsid viral proteins N1 and N2. Primers and probe for N1 were N1-F: GACCCCAAAATCAGCGAAAT, N1-R: TCTGGTTACTGCCAGTTGAATCTG, and N1-probe: FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1. Primers and probe for N2 were N2-F: TTACAAACATTGGCCGCAAA, N2-R: GCGCGACATTCCGAAGAA, and N2-probe: FAMACAATTTGCCCCCAGCGCTTCAG-BHQ1. Primers and probe for RNase P were RP2-F: AGATTTGGACCTGCGAGCG, RP2-R: GAGCGGCTGTCTCCACAAGT, and RP2-probe: FAM–TTCTGACCTGAAGGCTCTGCGCG–BHQ1. RT-qPCR reaction was performed on Real time PCR Machine.

The test has been performed after extraction. After isolation, viral RNA is reverse transcribed into cDNA and amplified for specific detection of three genes (ORF1ab,

E and N gene) of virus in a single reaction. To check RNA extraction, PCR inhibition and sampling or application errors an internal control has been employed. Fluorescence detection is accomplished using FAM, HEX and Cy5 filters. For triple gene detection, CE and IVD marked kit is used on Real time PCR machine (Rotor gene Q) Ct values decide whether a result is COVID-19 positive or negative. To report a positive result, both viral targets N1 and N2 Ct must be lower than 40. To report a negative result both viral Ct value must be equal or higher than 40. If one of the viral targets Ct is lower than 40 and the other is Ct is equal and greater then 40, the result must be reported as undetermined. The RNase P target must be Ct equal and lower than35. We have performed some medicational trials to recover the COVID-19 patients on the basis of immune system booster.

Blood samples were collected in gel vial from covid patient to perform some other clinical test., Lactate dehydrogenase, serum ferritin level, D-Dimer, Tg, cholesterol, Thyphoidot, HDL, lymphocyte count and CRP.

Results

The swab sample of 267 patients belong to different areas of southern Punjab were used in molecular detection of ORF1ab, E and N gene. The mean age of patients was 49.0 ± 8.1while the minimum and maximum age at which the amplification of ORF 1ab, E and N gene was detect was 20 and 78 years, respectively (Table 1).

Sr. No	Patient Data	Patient Age
1	Minimum age	20
2	Maximum Age	78
Mean ±S.E		49.0 ± 8.1

Table 1: Minimum and maximum age of covid-19 patient collected from different areas of southern Punjab.

The age was categorized into 3 groups, 20-40, 41-60 and above 61 age formed 1st, 2nd and 3rd group. Number of patients in age groups 20-40, 41-60 and above 61 was, 19, 15 and 16, respectively. The no of patient was found in order of 19 >16 > 15 in 1st, 3rd and 2nd age groups, respectively. The highest no of covid patient (77) were found in 1st group while the lowest patients (19) were found in 2nd group. The 77 (58.33%) patients showed the amplification of ORF1ab, E and N while 55 patients (41.66%) showed no amplification ORF1ab, E and N gene were belong to the1st age group. The 19 (28.77%) patients showed amplification of ORF1ab, E and N gene while 47 patients (71.21%) showed no amplification ORF1ab, E and N gene were belong to the 2nd age group. The 31 (44.92%) patients showed amplification of ORF1ab, E and N while 38 patients (55.08%) showed no amplification of ORF1ab, E and N gene were belong to the 3rd age group. The 127 (47.21%) out of 267 patients were covid-19 PCR positive and showed the amplification ORF1ab, E and N gene while 140 individuals were covid-19 PCR negative and not showed the amplification of ORF1ab, E and N gene (Table 2).

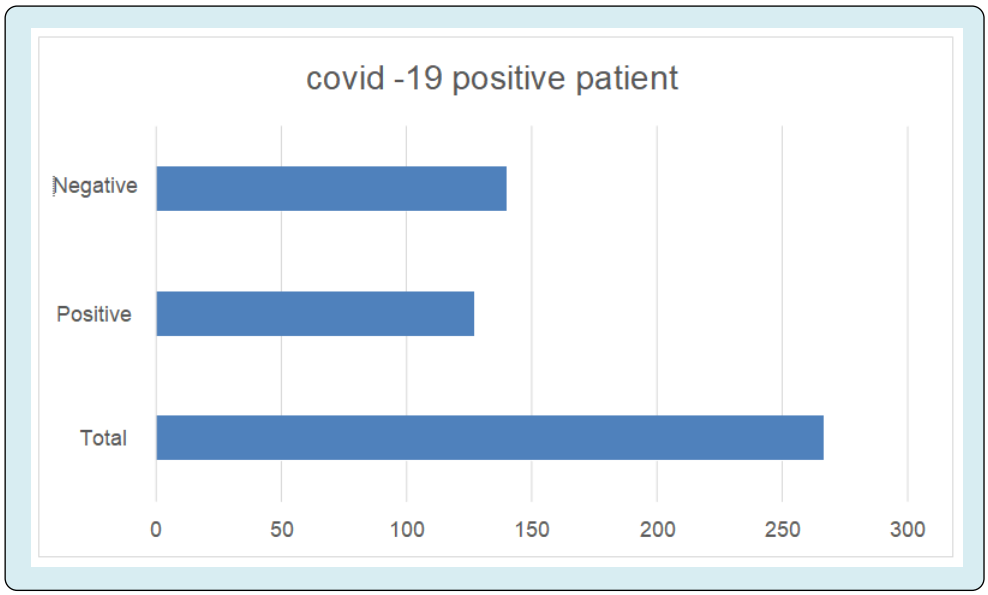
Age	Percentage (%)
20-40	**(n=132)**
ORF1ab, E and N gene	77 (58.39%)
Negative 55 (41.66%)	
41-60	**(n=66)**
ORF1ab, E and N gene	19 (28.77%)
Negative	47 (71.21%)
61< (above 61)	**(n=69)**
ORF1ab, E and N gene	31 (44.92%)
Negative	38 (55.08%)

Table 2: Distribution of ORF1ab, E and N gene in covid-19 patient by age group.

The maximum no of patients were observed in age 30 and 40, minimum no. of patients were found in both age 45 and 20. The results of paired t test was showed that the signification ($P<0.05$) (0.02) correlation was observed in age group and ORF1ab, E and N gene. The graph shows that covid 19-patients increases day by day and most common symptoms include Fever, Cough and Shortness of breath. some other symptoms may include Sore throat, Runny nose, Body aches, Headache, Chills, Fatigue, Gastrointestinal, diarrhea, nausea , Loss of smell and taste and sweating.

Covid -19 positive patient's blood tests are perform in which their MCV cells increases in mostly cases, serological test of Salmonella typhi IgG and IgM both are positive.

Some drugs were uses to treat poor covid patients. In which mostly are recovered and some patients that were lead to death we have found their blood glucose level becomes too high and cardiovascular disease.



Drugs	Dose
Azithromycin 500 mg	Two tab/ day
Panadol	3 tab / day
Ivermectin 6mg	Twice a day
Evion 400	One tab / day
Surbex z	One tab/ day
Folic acid	One tab/ day
Vitamin D injection	1 in week
Cac 1000 Plus	Twice a day

Table 3: Drugs trial treats for covid-19.

Conclusion

Mostly the identification of the Covid-19 is done by the PCR. But we cannot surely say the patients identified by the PCR analysis are affected with Covid-19. The patients with negative test may be affected with Covid-19. So, the other analysis tests such as lactate dehydrogenase, HDL, ferritin, D. Dimer, Tg, cholesterol, CRP and CT scan The patients effected with covid-19 have higher values of D-Dimer, LDH, CRP and ferritin level than normal ranges . After the treatment of two weeks of Covi-19 the test was again performed. The values of the Tg were significantly increased after two weak treatment. But the values of the HDL, cholesterol and lymphocytes were decreased form the normal ranges. In complete blood picture test the T-naive cells and neutrophils increases in mostly cases and their HB remains between 10-14 G/d. their ESR test remains higher in most cases that show acute infection in their lungs.

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