Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Detection of SARS-CoV-2 by using real-time PCR nasopharyngeal swabs in suspected patients and their clinical medication

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HDL (High density lipid)
TG (Triglyceride)
ESR (Erythrocyte sedimentation rate)
CBP (Complete blood picture)

ABSTRACT

Background: The corona name derived from their crown like spike proteins attach with cell receptors. It belongs to coronaviradae family and nideovirales order, envelop virus, size range 65–125 nm and positive single standard RNA between 26.4 and 31.7 kb and contain 7096 amino acid. There are four subtypes that have been detected these are alpha, beta, gamma and delta.

Methodology: 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.

Results: 127 (47.21%) out of 267 patients were covid-19 PCR positive and showed the amplification of ORF1ab, E, and N gene, while 140 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 effected covid-19 have higher values of D-Dimer, ESR, Neutrophils, LDH, CRP and ferritin level than normal ranges. However, the values of HDL, cholesterol and lymphocytes were decreased from the normal range.

1. Introduction

The outbreak of novel SARS-CoV-2 was first described in the province of China, on 30 December WHO declared emergency in the world based on the growing case rate at Chinese and international airport [1]. The corona name derived from their crown-like spike protein attach with cell receptors [2,3]. It belongs to coronaviradae family and nideovirales order, envelop virus, size range 65–125 nm and positive single standard RNA between 26.4 and 31.7 kb and contain 7096 amino acid [4]. There are four subtypes of corona virus 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 virus subtypes are originated from pigs and birds [5]. Among the 7 subtypes of coronavirus that infect humen, the beta coronavirus cause some serious illness.

N protein (nucleocapsid) is encoded by four genes that play structural rules in viruses. In the case of beta coronaviruses 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 [2,6].

Spike proteins of viral attached to angiotensin-converting enzyme

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(ACE) receptors on the host cell membrane, viruses 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 [2]. Viral particles release from Golgi bodies to infect other cells. Meanwhile, in some cases it leads to apoptosis and cell death, when the viral particles infect neighboring cells [7].

Attachment of the corona virus occurs through endocytosis after endocytosis, it is also called as endosome, proteases proteins present in the endosome mediate the release of the viral genome virus binding with receptors and the virus endosome fusion is blocked using 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 using 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 [8]. Activity of Treg is increased by the use of CQ and HCQ This is a beneficial effect of the CQ and HCQ. {Tufan, 2020 #803} The decrease in the number of cells is indicated by the yellow arrow while the increase in the number of cells is indicated by blue arrow as shown in Figs. 1 and 2.

2. Methodology

2.1. Clinical sample collection

Negative nasopharyngeal swab collected from March–June 2020 from 267 COVID -19 suspected patients. Their demographic and clinical information such as age, gender, symptoms were retrieved from database for the study. The severity of covid -19 was classified into three groups, 1) mild symptoms without pneumonia, 2) fever and respiratory symptoms with pneumonia, 3) respiratory distress, oxygen saturation below 93 at rest, ventilator, and admission to ICU hospital.

2.2. Sampling

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

2.3. 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: FAM-ACAATTTGCCCCCAGCGCTTCAG-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 genes) of the virus in a single reaction. To check RNA extraction, PCR inhibition, and sampling or application errors, an internal control has been employed. Fluorescence detection were accomplished using FAM, HEX, and Cy5 filters.

For triple gene detection, CE and IVD marked kit were used on Real time PCR machine (Rotor gene Q).

Ct values decide whether a result the 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 values 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 than 40, the result must be reported as undetermined. The RNase P target must be Ct equal and lower than 35.

We have performed some medicational trials to recover the COVID-19 patients on the basis of immune system boosters.

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

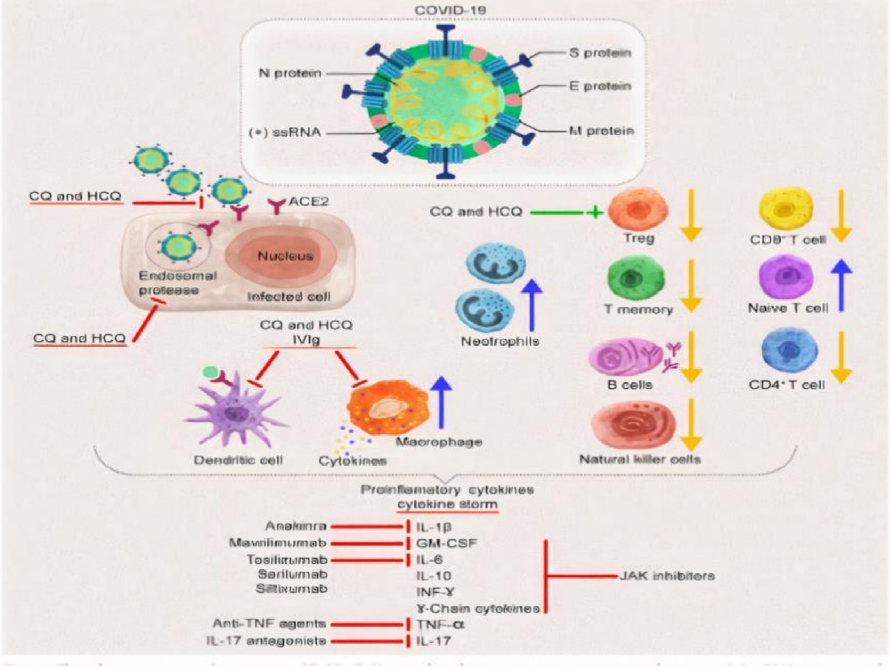

Fig. 1. The immune response and cytokines storm in COVID-19.

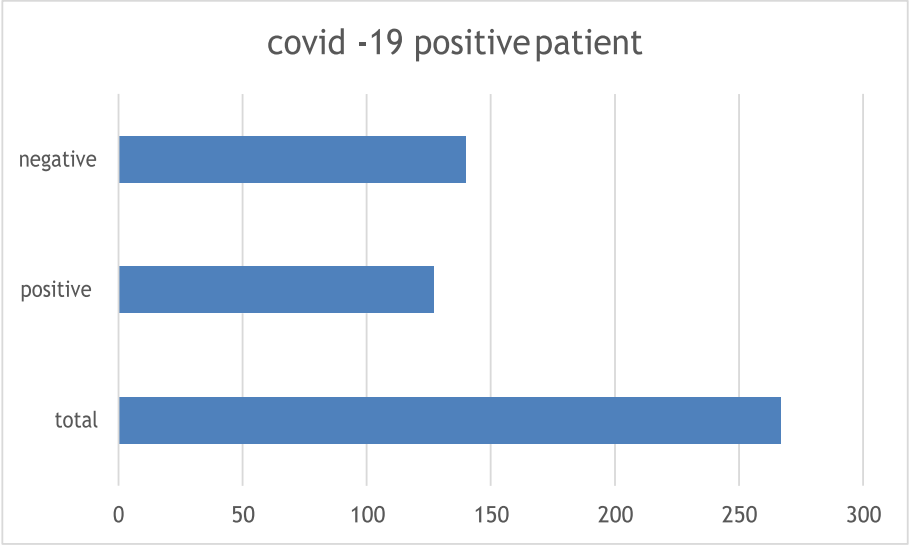

Fig. 2. Positive and negative cases covid-19 patient in Punjab.

CRP.

Continuous variables were represented as mean and standard deviation. Z -paired test and SPSS software were using for statistical analysis.

3. Results

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

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 were 19, 15, and 16, respectively. The number of the patient was found in the order of 19 > 16 > 15 in 1st, 3rd, and 2nd age groups, respectively. The highest number of covid patients (77) were found in 1st group while the lowest patients (19) were found in 2nd group. 77 (58.33%) patients showed amplification of ORF1ab, E, and N, while 55 patients (41.66%) showed no amplification of ORF1ab, E and N genes were belonging to the 1st age group. 19 (28.77%) patients showed amplification of ORF1ab, E, and N gene, while 47 patients (71.21%) showed no amplification ORF1ab, E and N genes were belonging to the 2nd age group. 31 (44.92%) patients showed amplification of ORF1ab, E, and N, while 38 patients (55.08%) showed no amplification of ORF1ab, E and N genes were belonging 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 genes (Table 2).

The maximum no of patients were observed in ages 30 and 40, minimum no. of patients were found in both ages 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 genes.

The graph shows that covid 19-patients increases day by day and the most common symptoms includes 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 performed in which their MCV cells increase in most cases, serological tests of *Salmonella typhi* IgG and IgM both are positive.

Some drugs were used to treat poor covid patients. In which mostly are recovered and some patients that were led to death, we have found their blood glucose level becomes too high and cardiovascular disease (Table 3).

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

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

Table 2
Distribution of ORF1ab, E, and N genes in covid-19 patients by age group.

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%)

4. Conclusion

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

Table 3
Drug trial treatment for covid-19.

Drugs	Dose
Azithromycin 500 mg	Two tab/day
Panadol	3 tab/day
Ivermectin 6 mg	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

Consent for publication

The written inform consent has been taken from the patients for this study.

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No funds

Availability of data and materials

The data associated with a paper is available, the data availability on demand through email contact of co-author.

Declaration of competing interest

All authors have no conflict of interest.

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