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Investigation of Some Inflammatory Parameters in Patients with COVID-19 Diagnosed by Nucleocapsid Gene (N) Via RT-PCR

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Abstract:

This study aimed to estimate immune molecules CD-4 , CD-79, TNF-B and IL-2 in patients diagnosedwith COVID-19, since nucleocapsids (N) gene is an essential in viral genome, it has been used for the detection of the virus by PCR technique. A total of (100) COVID-19 seropositive patients were screened for this study to know level of TNF-B & IL-2 in serum of patient and show expression of CD4 and CD79. Results showed that serum samples were analyzed for IL-2 & TNF-B by ELISA , showed highly significant increases ($p<0.05$) in serum level of COVID-19 patients as compared with healthy control groups , acute revealed high as well as , increases in serum level of TNF-B significantly($p<0.05$) ,while moderate cases moderate cases of disease express high increase in serum level of IL-2 significantly($p<0.05$).Activated markers study revealed high expression of CD79 & CD4 in COVID-19 patients as compared with healthy normal groups .

Introduction

In 1960, the principle instance of Covid was exhorted as cold. According to a Canadian report from 2001, approximately 500 patients were diagnosed with a flu-like framework.Polymerase chain reaction was used to set up 17-18 of them as infected with the Covid strain. Until 2002, crown infection was viewed as a nonfatal infection (1,2). The WHO called the new Covid-initiated pneumonia as Covid illness 2019 (COVID-19) on February 11, 2020, and it has rapidly spread in pestilence scale since it first appeared in Wuhan, China, in December 2019(3). Covids are infections with a single-abandoned RNA genome in the positive sense (26e32 kb) [4]. Four Covid genera (a, b, g, d) Human Covids (HCoVs) have been identified in the a Covid (HCoV-229E and NL63) and b Covid (MERS-CoV, SARS-CoV, HCoV-OC43 and HCoV-HKU1) genera. [5]. Covid-19 is the causative agent for the worldwide pandemic characterized by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Nucleocapsids is a helical protein important structural factor surrounded by the viral membrane of SARS-CoV-2 where the viral genome is encapsulated with. Because the infected cells produce high concentrations ofnucleocapsids protein of SARS-CoV-2 which enhancing the efficiency of viral RNA transcription, and viral replication, thus it's dependable in the diagnosis of the virus via PCR.

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The event of crucial CD8+ and CD4+ T reactions in tolerant blood experiences intense illness looks related with recovery .In think about, diminishing of a reaction appears to anticipate the finish of persistent infection. At that point, Ab - intervened decrease, reviewing was connected with persistent sickness, so approving the significant part that CD4+ act in the dispose of genuine disease(6) . Cytokines work as the particles of protection response that bring about various physiological jobs and change the guarded, provocative and fixing patient responses, and for the most part covered by mono and lymph cells. cytokines from T cells act basically in the host reaction. Invigorated T cells characterized into (2) subcategories delivering cytokines producing. T aide 1 cytokines, similar to IL-2 and IFN-ϑ, to prompt (CMI) reaction while T partner - 2 cytokines as IL-10 and IL-4 are worried about AMI. The two reactions have been uncovered to relate in a viral sickness () and the disparity among them incline toward HIR and discouraged change CMI, that is fundamental for resistance close to infections (7).

Materials &Methods

1- Patients

COVID (100) participants were enrolled in the study. Fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or low leukocyte counts, and radiographic evidence of pneumonia are all signs and symptoms of pneumonia were diagnosed by RT-PCR in 19 patients admitted to the public health laboratory.

2- Serum cytokine

Sizes of cytokines in the serum were done by ELISA test (R&D Systems). Absorbance was restrained in copies with a micro plate reader (Beckman Coulter). The last concentration was expressed in pg/ml.

3- Genomic DNA extraction

The study of genomic DNA was carried out using (MinigDNA Kit, Geneaid. USA). Where the extraction was carried out in accordance with company guidelines. Then, the extracted of DNA was put to the test with the help of a Nanodrop spectrophotometer and held at 20°C before using oligonucleotide primers and probes for precise detection of SARS-CoV-2 using Real-Time PCR for COVID-19 detection were chosen from SARS-CoV-2 genome's Open Reading Frame 1ab (ORF1ab) and nucleocapsid gene (N).ORF1ab gene (probe labeled with FAM) and N gene (probe labeled with VIC) primers/probes are packaged. The package also includes primers and a probe for the human RNase P gene (labeled with CY5) as an endogenous internal control for specimen integrity, nucleic acid separation, amplification, and detection. In a Real-time PCR instrument, RNA extracted and filtered from upper and lower respiratory tract specimens is reverse transcribed to cDNA and amplified using a one-step Master Blend. The probe has a reporter dye at the 5' end and a quenching dye at the 3' end. The reporter dye's fluorescent signals are absorbed by the quencher. During the PCR amplification phase, Probes hybridized to amplified templates are degraded by Taq DNA polymerase with 5'-3' exonuclease activity. separating the reporter dye from the quencher and creating fluorescent signals that expand with each cycle Based on the signal shift, The PCR instrument produces a real-time amplification curve for each optical channel and calculates

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cycle threshold (Ct) values (the point at which fluorescence is visible above background) that are interpreted by the operator to determine whether SARS-CoV-2 RNA is present or absent. And Then qPCR master mix was ready-made by using (AccuPower® SYBER Green qPCR PreMix kit. Bioneer. Korea). The freeze-dried pellet of (Taq DNA polymerase 1U, SYBER Green, dNTPs 250M, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) is included in the qPCR premix tube ,and the qPCR master mix reaction was prepared according to kit instructions in 20µl total volume, This was accomplished by adding 5 µl of filtered genomic DNA, µl of 10 pmole forward primer, and 1 µl of 10 pmole reverse primer to the qPCR premix tube, then deionizing qPCR water into 20 µl and briefly mixing with an Exispin vortex centrifuge (Bioneer. Korea). The reaction was carried out in a thermocycler (miniOpticon Real Time PCR, BioRad/USA) with the following thermocycler settings: initial denaturation temperature of 95°C for 3 min, followed by 30 cycles at 95°C for 30 s, annealing and extension 60°C for 40 cycles.

Statistical analysis

Statistical analysis was showed by using Chi-square ($\chi 2$) test to adjust the statistical vagaries amid assorted sets by spending an application statistical stand for social science (SPSS 19). The opportunity of ($P \leq 0.05$) was restrained to be statistically important.

Results and Discussion

1-Medical Remarks

Clinical sings in COVID 19 patients were, fever, nonproductive cough, dyspnea, myalgia, fatigue, normal or decreased leukocyte counts, and radiographic evidence of pneumonia, some patients showed intermediate and mild clinical sings asshown in table (1).

Table 1: Clinical sings in COVID 19 patients

NO.	Clinical signs	Number	Percentage%
1	acute	10	10
2	Intermediate	15	15
3	Mild	75	75

Our aftereffects of this examination showed that 10 (10%) of cases gave indications of fever, inefficient hack, dyspnea, myalgia, fatigue,while 15(15%) of cases showed middle of the road and 75(half) gentle infection independently. As per F-test the distinction in clinical sings were critical ($p<0.05$) . Side effects of intense period of COVID- 19 infection as in (7)

Presenting to a report distributed on 24 Jan 2020, Covid contaminated patient have a few shared sorts like fever, hack, and weakness while the runs and dyspnea were start to be as strange jaw. Several of them patient portrayed respective anomalies(8).

2- Detection of COVID 19 infection by nucleocapsids gene via RT-PCR

Using nucleocapsids (N) gene specific primers, gene upstream and downstream ends of specific primers were included within the kit. Results are shown in figure (1) below:-

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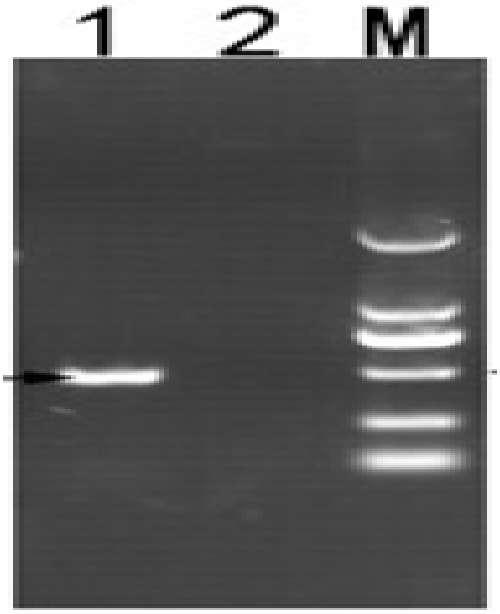

Figure 1: PCR amplification of nucleocapsids (N) geneLane M :DL2000 Lane 1：nucleocapsid (N) gene PCR product Lane 2：control

3-Consequences of IL-2 in COVID -19 patients

Serum of all patients with COVID- 19 and those with intense or middle sickness activity contain more significant level of IL-2 than solid benchmark group . IL-2 fixation was especially expanded in patients with moderate sickness (1330) and intense patients (950) correspondingly than gentle infection (440) as coordinated with control gatherings (70) .Nevertheless , assessment of contrast test uncovered that there was an incredible numerically considerable varieties between COVID -19 and sound benchmark groups ($p<0.05$). T-test ,showed that there was an expanded arithmetical considerable contrasts among transitional, intense sickness and gentle disease gathering ($p<0.05$) as table (2)

Table 2: Serum level of IL-2 among groups

Group	**NO.**	**Serum level of IL-2**		
		Mean	**Minimum**	**Maximum**
Mild	75	440	200	460
Acute COVID -19	10	950	500	980
Intermediate	15	1330	1200	1400
Control	10	70	66	78

Chemokines apply their organic action through connecting to certain cell surface receptors. A rare element of most prominent chemokine receptors is their extraordinary fascination for various ligands [9). Also to enrollment, IL-8 guides to animate the inspiration of neutrophils and monocytes [10]. Neutrophils offer the head course of protection as opposed to assaulting various microorganisms as infection. These cells release fiery cytokines, for example, IL-8,

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10 &12 ,make peevish oxygen species. IL-8 discharge impacts in a raised work of neutrophils into lung [11]. Moreover, the inception of I L - 8 can be expanded through connecting of the (TL R2/TL R3 and T LR7) cost like receptors that distinguish constituents of the infection multiprotein , twofold strand RNA and against viral composites of host individually[12].Moreover, the release of responsive O2-species from granulated cells perceived changing activity past, along these lines upsetting "I L – 8" appearance[13]. Lung I L - 8 is seen at less safeguarding grade at intense phase of COVID 19 contamination, while noticable ascents in blood serum and liver evaluation can be recognized patients with moderiate disease

4-level of TNF-B

Current study showed that all patients with C0VID-19 cover higher level of TNF-B than healthy control group , T NF-B concentration was improved particularly with acute COVID-19 patients ,Mild cases and moderate patients correspondingly .Analysis of variance among acute , moderate, and control people ($p<0.001$) .T- test exhibited that there was great statistical significant alteration among acute covid-19 , moderate and mild disease group ($p<0.001$)table(3)

Table(3) The level of TNF-B in patients and controls

Group	NO.	Serum level of TNF-B		
		Mean	Minimum	Maximum
mild	75	71	25	230
Acute	10	560	490	670
Moderate	15	18	19	16
Control	10	12	9	16

In acute agreeing infections, the response of the innate and adaptiveImmune system to COVID-19is capable and well-timed. Viral clearance involves the generation of a stout adaptive T cell retort inducing both a cytolyticTNF (entire superfamily individuals) has been related in not less than morphogenesis, irritation, angiogenesis, apoptosis, , attack, , multiplication , and metastasis. T NF - α is a focal cytokine to the of incendiary pathogenesis courses. The T N F - α favorable to fiery impact is encouraged by means of straight inception of other supportive of incendiary cytokines, The cytokine tempest will initiate a strong round by the safe framework to the body, cause ARDS and different organ disappointment, and finally lead to death in serious instances of SARS-CoV-2 infection(14)

5- CD-4 molecules in COVID-19 patients

Results shown that there was highly significant differences in mean of CD -4 expression among COVID-19 patients and healthy control groups ($p<0.005$) ,the cell surface CD4 was over expressed in acute COVID-19 compared to asymptomatic COVID-19 patients, mild cases and healthy control groups respectively the high expression seen in acute covid-19 disease.

6- Expression of CD79 in COVID-19 positive patients

The results demonstrated in table (5) shows there was high statistically significant difference in mean of CD79 expression among COVID-19 patients and healthy control

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groups ($p<0.005$),and the higher percentage of expression was found in acute patients , moderate disease followed by mild patients and control groups respectively .T –test results showed that there was high significant difference between acute patient ,moderate and mild disease groups($p<0.05$). To develop rid of COVID-19 is correlated with bubbly multi-vague C D 4+ and C D 8+ T cell responses ,while societies that movement chronic infection prospective to have fragile, slimly dedicated responses [15]. In COVID-19 infected people, C D8+ T C M cells occur in the verge are able of peculiar into E MC, that are enrolled to the body. CD8+ effector cells in the body were pledgee to have less useable adeptness, as substantiated by low IF N -yuntruth [16) The determination of liver pathogens is frequently attended thru frail "CD8+ T cell response " antigens subsequent[17,18]. We exasperated to conclude the pathogenic status of C D79 over comparing of its expression during infection , our results make it clear that robust up-regulation of both C D4&C D79 manage a tough mark that lymphocytes in peripheral blood of COVID-19 persons within formal of immune dysregulation. [19,20].

Table(4.) The level of CD54 in patients and controls

Group	NO.	Serum level of CD4		
		Mean	Minimum	Maximum
mild	75	10	4	12
Acute	10	14	10	16
moderate	10	5.5	4	7
Control	10	3	0.80	5.60

Table(5) The Concentration of CD79 in patients and controls

Group	NO.	Serum level of CD79		
		Mean	Minimum	Maximum
mild	75	9.2	6.00	13
Acute	10	40.5	20	42
moderate	10	15	11.	13.
Control	10	5	4	8

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