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Molecular Detection of SARS-CoV-2 by Real-Time RT-PCR and the Association of Immunological Markers (IL-4 and IL-6) with COVID-19 Patient Groups

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ABSTRACT

Objective: *Coronaviruses (CoVs) refer to one of the RNA virus infections that can appear as diseases affecting humans. They're enveloped viruses with massive single-strand positive-sense RNA genomes that can infect humans, animals, birds, bats, mice, and a number of other wild creatures' respiratory, gastrointestinal, hepatic, and central nervous systems.* ***Method:*** *Viral samples were collected from 90 patients through nasopharyngeal swabs. COVID-19 and non-COVID-19 serum patients' cytokines were identified by ELISA, while SARS-CoV-2 IL-4 and IL-6 were recognized using the chemiluminescence method.* ***Result:*** *The S gene is generally present in the early stages of infection, with only a few extended periods of contamination, whereas the E gene appears in the later stages of disease – one to several weeks after the onset of infection – while the N gene appears to a lesser extent. With 90% amino acid homology and fewer modifications over time, the N gene is more regulated and stable. The (S and E gene) heterozygote shows a higher rate than the others.* ***Novelty:*** *This study highlights distinct gene expression patterns of SARS-CoV-2 during different stages of infection, revealing that the N gene exhibits greater stability and regulatory consistency, which could be significant for diagnostic and molecular surveillance applications.*

INTRODUCTION

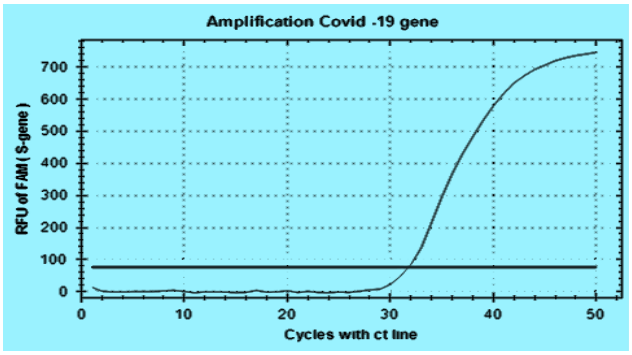
Coronaviruses (CoVs) family which RNA infections can sickness from people and animals. The term "Covid" was first applied to an illness that had spike-like projections. They're wrapped infections with massive single-strand positive sense RNA genomes that show effected humans, animals [1], [2]. Coronaviruses (CoVs) are RNA viruses that cause illness in humans and other vertebrates. The 229E, OC43, NL63, and HKU1 infections have all been linked to human sickness, however the 229E, OC43, NL63, and HKU1 infections induce symptoms cold as mode. SARS-CoV, SARS-CoV-2, which first occurred in Wuhan, China in December 2019, and Middle East Covids are RNA infections that have a single, abandoned positive-sense RNA genome ranging in size from 26 to 31 kilobases. [3]. The Covid genome is sequenced in the accompanying request: Finally, the envelope (E) - film (M) - nucleocapsid (N) chain receives the 3′UTR - poly (A) tail. It has two cross-over open understanding edges (ORFs) 1a and 1b, as well as other ORFs. 5′-pioneer UTR-then duplicate/transcriptase-spike (S)- It has two cross-over open understanding edges (ORFs) 1a and 1b, as well as other ORFs [4], [5].

RESEARCH METHOD

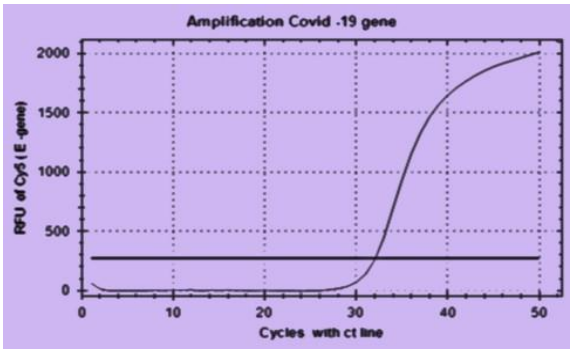
Human Interleukin 4 ELISA Kit was used to evaluate the presence of Human Interleukin 4 (also known as IL-4), and this unit is an Enzyme-Linked Immunosorbent Assay (ELISA) (ELISA). Human IL4 neutralizer has been pre- applied on the plate. The IL4 from the example is introduced to the wells and binds to the antibodies. Then, in the example, biotinylated Human IL4 Antibody is introduced and bonds to IL4. Streptavidin-HRP is then added, which binds to the biotinylated IL4 immune response. During the washing stage after hatching, unbound Streptavidin-HRP is removed by rinsing. The substrate pattern is then implemented, shading is calculated based on the amount of Human IL4 present.

RESULTS AND DISCUSSION

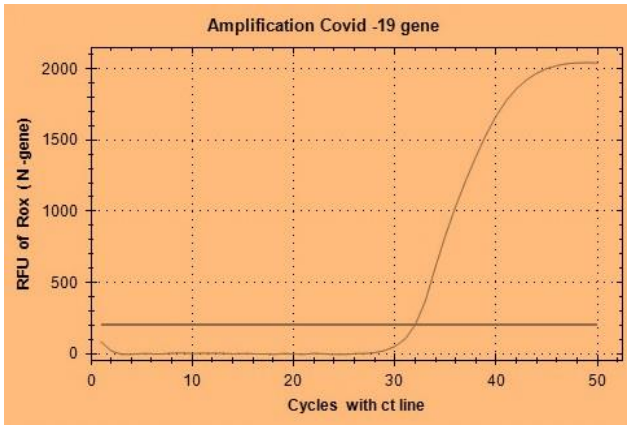
This indicated that the E-quality patients had crown disease. The intensification bend started at cycle 32 above CT and ended with an RFU convergence of 2000 units. This suggested that the patients' crown disease was in the E-quality. This research revealed a trend of intensification. It starts at cycle 32 above CT and progresses to 2100 Units of the N-quality stage, as illustrated in the diagram (1- C). The graphic depicts the internal control level at cycle no. 34 above CT with RFU up to 1000 Unit (1-D). Inward control was employed to analyze the substance consistency, intensification, and dendity of viral nucleic acids on a semi-quantitative basis.



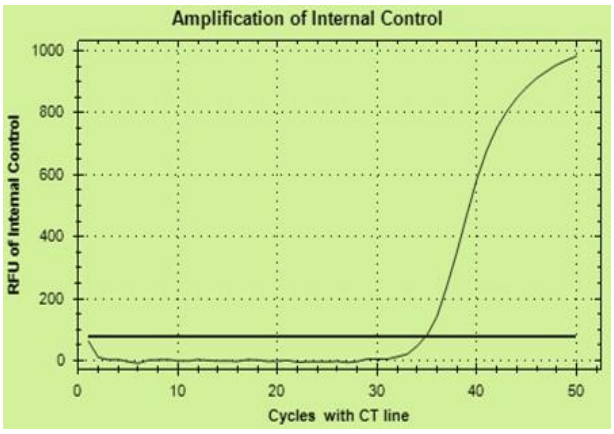
A. Curve of S – gene on FAM Channel



B. Curve of E – gene on Cy5 Channel



C. Curve of N – gene on Rox Channel



D. Curve of Internal control (IC) on Hex Channel

Figure 1. Internal monitor used to monitor Amplification of Covid-19 genes using real-time PCR.

In comparison to atomic (N-quality) divided into (circles), which has (43: 96 = 44.8 percent), heterozygote of (S and N qualities) divided into (triangle) has (4: 96= 4.2 percent), and spike (s) quality (divided into square) has (33: 96= 34.4 percent). Low S quality in comparison to N quality could indicate a long-term problem or that the patients have been exposed to pollution for at least fourteen days. With 90 percent amino corrosive homology and fewer changes over time, N quality is better preserved and stable (Marra et al., 2003, Drosten et al., 2003). Several Covid N proteins are immunogenic and generated in large quantities after infection [6].

The N protein serves as a signaling molecule [7], [8]. Enhancement and cytotoxicity Anti-infection IgG antibodies were found in greater concentrations in the serum of SARS patients [[9]. Hereditary succession of the first SARS-CoV virus strain from Wuhan, early discoveries by Cong Y. et al.; 2020 from hereditary grouping the presence of significant changes [10].

A. SARS-CoV-2 infected patients' particular rules are dispersed

Covids are a symbiotic category of RNA contaminations that cause disease in humans and animals. They are diseases with enormous single-strand positive sense RNA genomes that can infect humans, animals, birds, bats, mice, and other wild creatures' respiratory, gastrointestinal, hepatic, and tangible frameworks. Covids (CoVs) are an RNA contamination category that causes sickness in humans and other vertebrates [11].

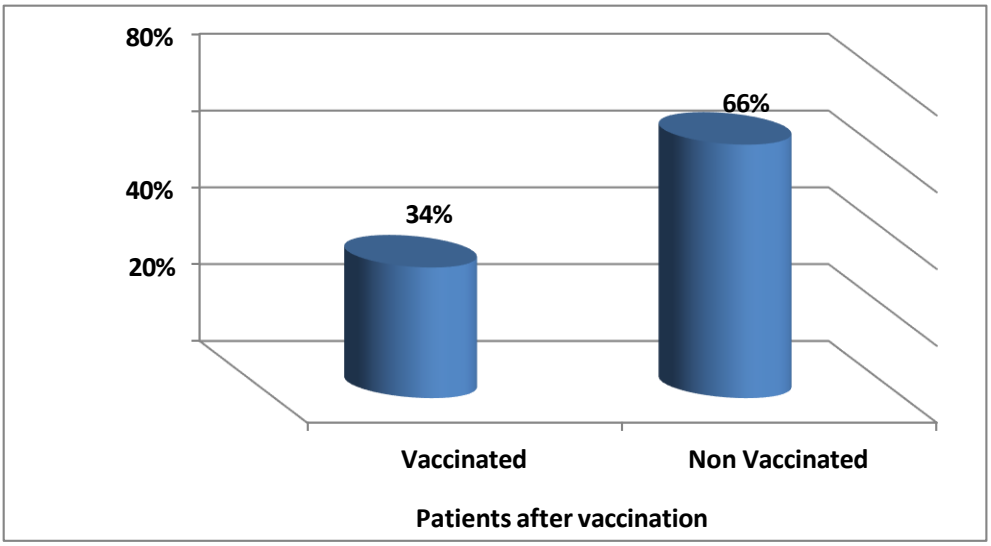


Figure 2. Vaccinated and non vaccinated patients.

When evaluating crown viral tainting, the assessment of all focused on boundaries with strong control found that, extended in IL-6 and IL-4. This finding could imply that the immune system serves as a protective mechanism, as well as a prognostic indicator for disease, development, and previous infection (inoculation). Table 1 displays the results (1). Serum levels of IL-6 and IL-4 were elevated in COVID-19 patients, and levels were generally higher in substantially and really affected COVID-19 patients compared to the control group. There were no significant differences between controls and those retrieved from the COVID-

19 social gathering, indicating that the severity of related RNA contaminations that cause illnesses in IL-6 and IL-4 was determined to be co-associated with the reality of COVID-19 [12].

Table 1. Comparison of studied parameters in patients and control.

Group Statistics	Variables	N	Mean ± SD	P. Value
IL-6	Patients	50	13.17 ±9.32	0.000
	Control	40	2.07 ±1.83	
IL-4	Patients	50	122.89 ±61.95	0.003
	Control	40	95.05± 10.40	

B. Age groups distribution of studied parameters

The repeat of patients age bundles were kept in the figure (3), the energetic patients at age range (20-29 years) are (36%) more rate than others, followed by grown-up age at (50-59 as well as > 60 years) at (18 % and 20 % The data for the research focus avowed COVID-19 cases from South Korea, Australia, New Zealand, Japan, and the Netherlands revealed fundamentally ambiguous profiles, with a bimodal assignment showing the more rate about attested SARS-CoV-2 pollutions among individuals in the 20-29 year age group (21 percent -27 percent of total), and a subsequent lower peak for the 50-59 or 60-69 year old groups (16-18 percent of t total) [13].

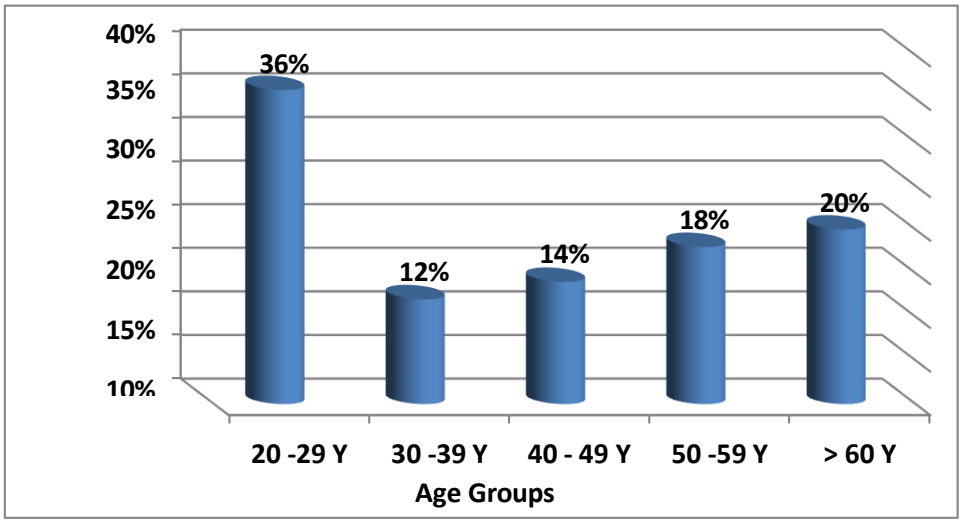


Figure 3. Age range frequency distribution.

C. Age range corresponding to Immunological markers

Certain cytokines (IL-6 and IL-4 level) were analyzed in SARS-CoV-2 patients, in which that the cytokines level are extended at all age bundles especially in young age (30-39 years) for IL-6 and 20-29 years of IL-4 ,since it have more huge level than other age range packs as shown in table (2). In numerous studies of COVID-19 patients, IL-4 levels have been found as a component of the cytokine storm connected to genuine respiratory adverse effects (18). Researchers showed that higher levels of IL-6 were linked to more severe in 452 patients can infected by SARS-CoV-2 [14].

Table 2. Age range and Immunological markers.

Age and immune markers		N	Mean ± SD	LSD Value
IL-6	20 -29 Y	18	7.06 ±2.56	5.44
	30 -39 Y	6	25.04 ± 4.38	
	40 - 49 Y	7	14.61 ±6.51	
	50 -59 Y	9	18.92 ± 4.17	
	> 60 Y	10	10.86 ±6.43	
	Control	40	2.07 ±1.83	
IL-4	20 -29 Y	18	146.98± 26.99	23.7
	30 -39 Y	6	105.26 ±16.11	
	40 - 49 Y	7	100.45 ±23.17	
	50 -59 Y	9	107.01 ±27.34	
	> 60 Y	10	120.10 ±53.30	
	Control	40	95.05 ±10.40	

D. Recurrence of Gender circulation

As indicated in Figure, both male and female patients were selected at a similar rate, with 27.8% for both in respect to direction matching control (1-4). In the current study, near-shortcoming to SARS-CoV-2 was seen in 1,019 patients (50.0 percent people) who persevered through the illness, gathered from a public educational collection and for a case series of 43 hospitalized patients. (51.2% folks) [15].

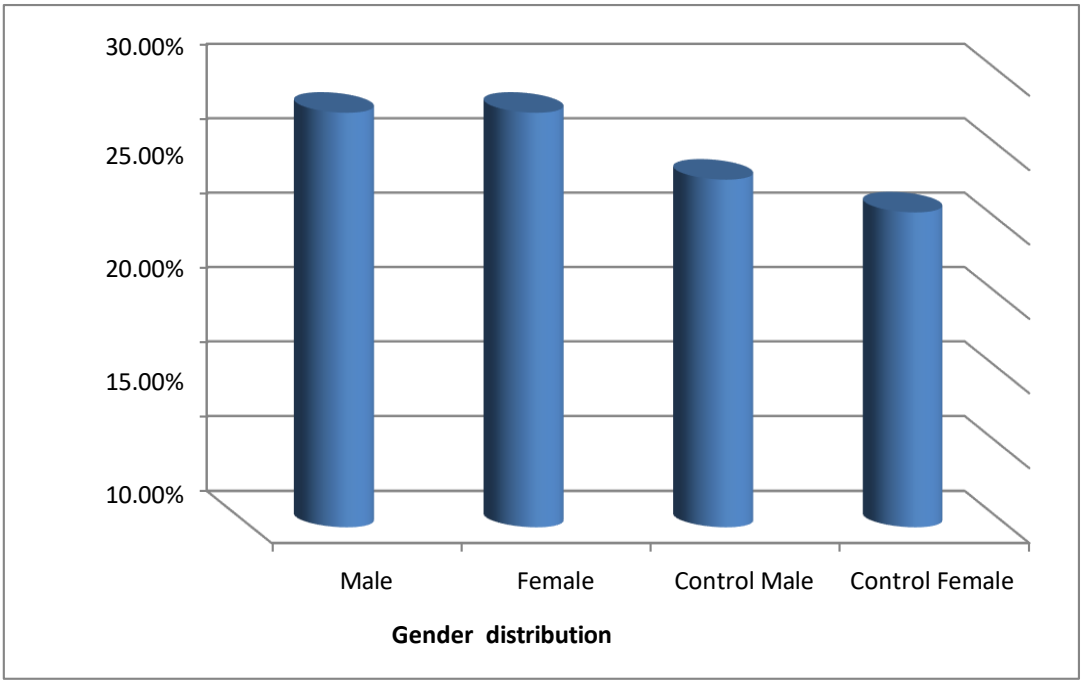


Figure 4. The frequency of Gender distribution.

E. Gender distribution in relation to immunological markers (IL-6, IL-4)

Male patients have higher levels of (IL-6, IL-4, PCT, and IgG) than female patients, but lower levels of IgM immunizer. This result may indicate that male patients have a

higher immunological development than female patients, as seen in table (3). Male COVID-19 patients had greater neutrophil, CRP, and combustible cytokine (IL-6) and lymphocyte levels, which were connected to the terrifying perception and bizarre clinical symptoms [16]. Higher neutralizer levels were discovered in recovered male patients by Klein SL et al., showing that recovery from COVID-19 may demand more antibodies [17].

Table 3. Gender distribution in relation to immunological markers (Il-6, IL-4, PCT, IgM and IgG).

Immunological Markers		N	Mean ± SD	P. Value
IL-6	Male	25	13.51 ± 7.84	1.23
	Female	25	12.83 ±2 .06	
	Con. Male	21	2.17 ±1.53	
	Con. Female	19	1.97 ±0.41	
IL-4	Male	25	142.40± 29.98	23.4
	Female	25	103.38 ±25.44	
	Con . Male	21	92.78 ±11.23	
	Con. Female	19	97.56 ±9.04	

F. Vaccinated patients in relation to Immunological markers (IL-6, IL-4)

The table (4) shows that non-immunized patients had higher IL-6 levels than inoculation and control patients, while vaccinated patients had higher IL-4, PCT, IgM, and IgG levels than non-vaccinated patients. This finding could mean that people who have been immunized are more resistant to SARS-CoV-2 infection than people who have not been immunized, and that cytokines and antibodies (IgM and IgG) play a role in disease protection. The CDC can discovered which people can there unvaccinated also another infection which likely by develop COVID-19 people completely vaccinated also had never been sick before in a separate MMWR study of more than 7,000 people hospitalized with COVID-like illness in nine states Inoculation can lead to a boost in overall immunity [18].

Table 4. Vaccinated patients in relation to immunological markers.

Immune Markers		N	Mean± SD	P, Value
IL-6	Vaccinated	17	7.73 ±1.70	.000
	Non Vaccinated	33	15.97 ± 3.05	
	Control	40	2.07 ±1.83	
IL-4	Vaccinated	17	148.14 ±18 .02	.000
	Non Vaccinated	33	109.88 ±23.80	
	Control	40	95.05 ±10.40	

As with PCT, the combination of IL-6 with IL-4 was shown to result in lower IL-4 levels as compared to IL-6 production. The IL-4 level could indicate a high level of sensitivity collaboration or a powerless state following a SARS-CoV-2 infection. Increased IL-6 in comparison to IL-4 at the underlying relatively short durations of infection could indicate that a safe response requires a more extreme stage reactive than

other or touchy reactants in the respiratory environment, as seen in Figure (5). A high level of interleukin-6 (IL-6) has been linked to a significant case setback from COVID-19 pollution in previous studies (Qingqing et al., 2021). IL-4 levels have been identified as a component of the cytokine storm linked to exaggerated respiratory symptoms in COVID-19 patients in various studies [14].

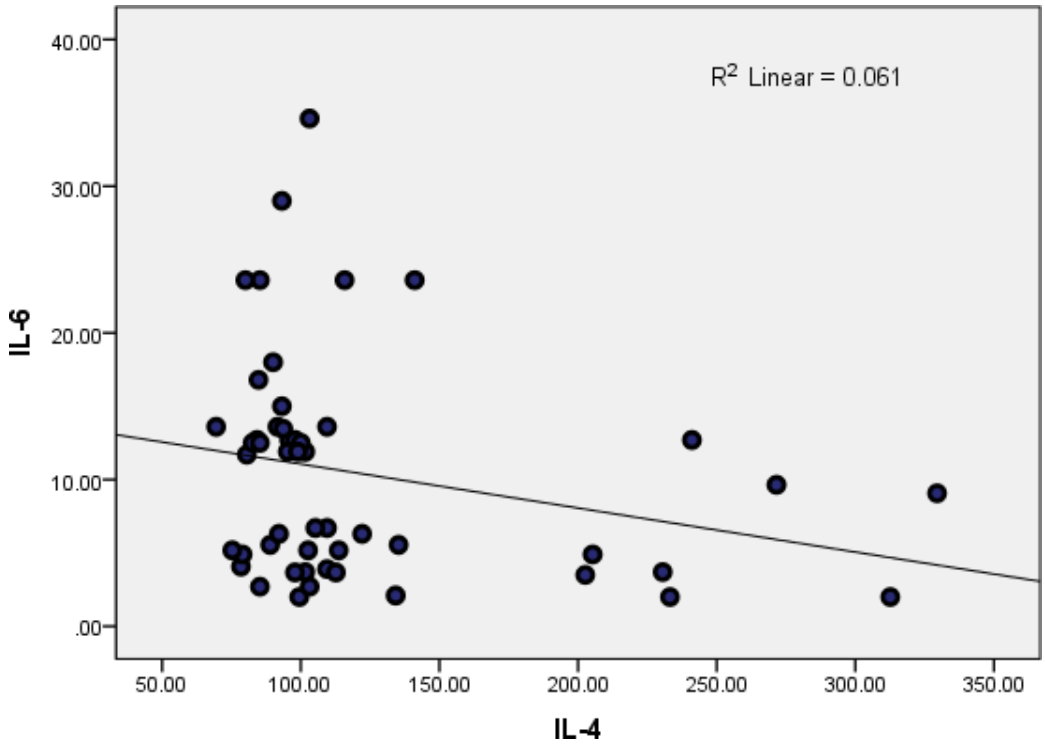


Figure 5. Correlation of IL-6 with IL- 4 in SARS-CoV-2 infected patients.

CONCLUSION

Fundamental Finding : Our findings suggest that at a reasonable cost, RT genotyping with a narrow panel of SNPs can add useful genotype information to PCR-positive data, indicating that SNPs in the S, E, and N genes may be linked to SARS-CoV-2 susceptibility and patient risk. **Implication :** Because of the quick turnaround time and ease with which this method may be automated, it has the potential to yield more information for epidemiological research and enhance genetic surveillance in pandemic management. **Limitation :** The current approach is limited by the focus on a narrow SNP panel and does not yet incorporate broader genomic variations or large-scale population diversity that might affect the generalizability of the findings. **Future Research :** We also advised considering IL6 markers as a major factor to understand therapeutic response against COVID-19 in infected human populations, emphasizing the need for population-based therapeutic development and personalized medicine discovery by combining immunological insights on IL6 and IL4 markers previously reported in lung and viral diseases.

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