**How to Cite**:
Tuamah, M. R. ., & Salih, I. A. A. (2022). SARS-CoV-2 detection using real-time RT-PCR and the relationship between immunological markers Interleukin -4, Interleukin -6 and SARS-CoV-2 patient groups. *International Journal of Health Sciences*, *6*(S1), 9508–9517.
https://doi.org/10.53730/ijhs.v6nS1.7192

# SARS-CoV-2 detection using real-time RT-PCR and the relationship between immunological markers Interleukin -4, Interleukin -6 and SARS-CoV-2 patient groups

**Mustafa Raheem Tuamaha**
Master student in Babylon University/ Collage Sciences for Woman
Corresponding author email: mustafabio224466@gmail.com

**Ishraq Abdul Ameer Salihb**
Department of Biology Science for Women, University of Babylon
Email: ishraqishraqsalih@gmail.com

***Abstract*---**Coronaviruses (CoVs) refer to one of the RNA virus infections can showed as one of diseases virus effect of humans. They're wrapped infections 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 focused sensory systems. Material and Method: viral 90 patients, a nasopharyngeal swab can be focused on a lot. COVID-19 and non-COVID-19 serum patients cytokines were identified by ELISA, while SARS-CoV-2 IL-4and, IL-6 were recognized by chemiluminescence method. Results: S quality is generally present in the early stages of contamination, with only a few extended stretches of contamination, whereas E quality is present in the late stages of disease, north of 1 - several weeks after the start of contamination, as well as N quality to a lesser extent. With 90% amino corrosive homology and less modifications over time, the N quality is more regulated and stable. The (S and/E quality) heterozygote has a higher rate than the others.

***Keywords*---** S gene, E gene, N gene, Interleukin-4, Interleukin-6, SARS-CoV-2.

## 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 (Guo *et al.*, 2020) (Peng *et al.*, 2020). 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. Trevor *et al.*, 2020).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. (WHO. 2020). (Yu *et al.*, 2020).

**Materials and Methods**

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.

**Result 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.

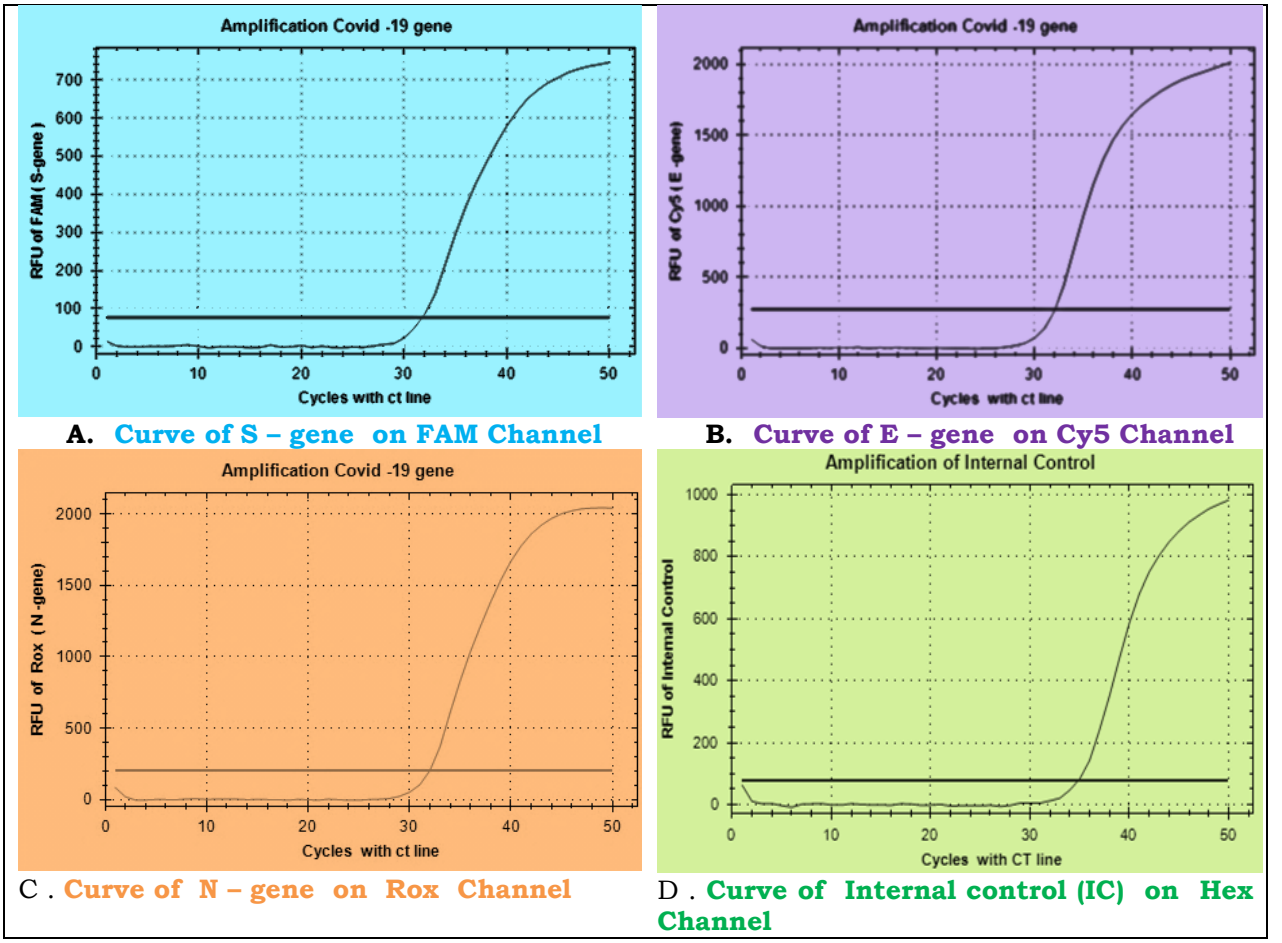


Fig. 1-1: Internal monitor used to monitor Amplification of Covid-19 genes using real-time PCR

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 (Grifoni *et al.*, 2020).

The N protein serves as a signaling molecule. (Rota *et al.*, 2003, Zhu *et al.*, 2005). Enhancement and cytotoxicity Anti-infection IgG antibodies were found in greater concentrations in the serum of SARS patients [ (Holmes *et al.*, 2003). 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 (Leung et al., 2004).

**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. (Günter, 2021).

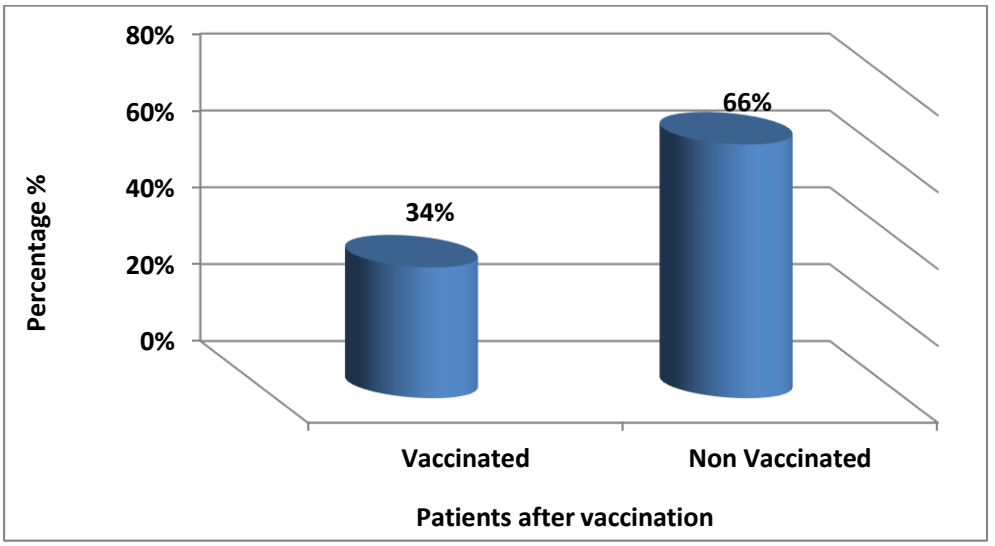


Figure (1-2): Vaccinated and Non vaccinated patients

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 (1-1). 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 (Mohammed *et al.*, 2021).

Table (1-1)
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 | |

**Age groups distribution of studied parameters**

The repeat of patients age bundles were kept in the figure (1-3), 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) (Dudley *et al.*, 2022).

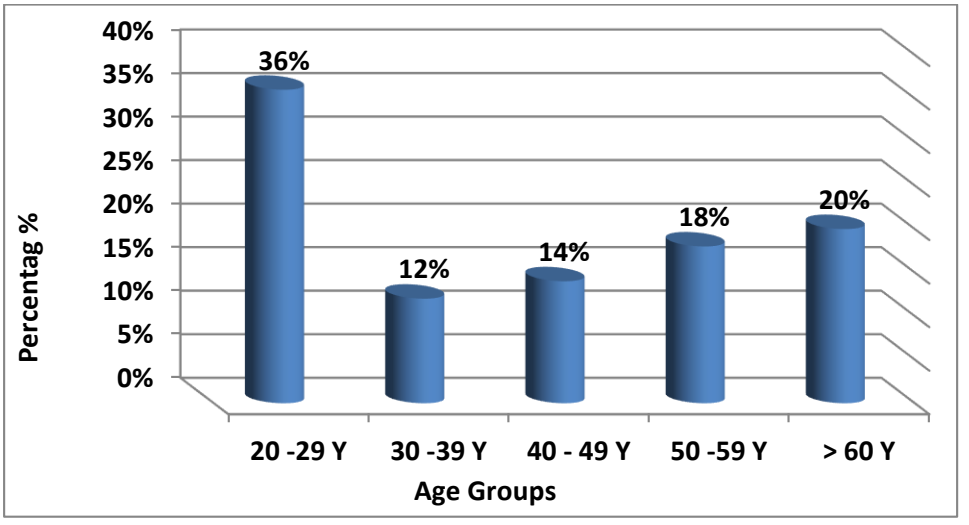


Figure (1-3): Age range frequency distribution

**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 (1-2). 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 (Victor *et al.*, 2020).

Table (1-2)
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 | |

**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) (Jian-Min *et al.*, 2020).

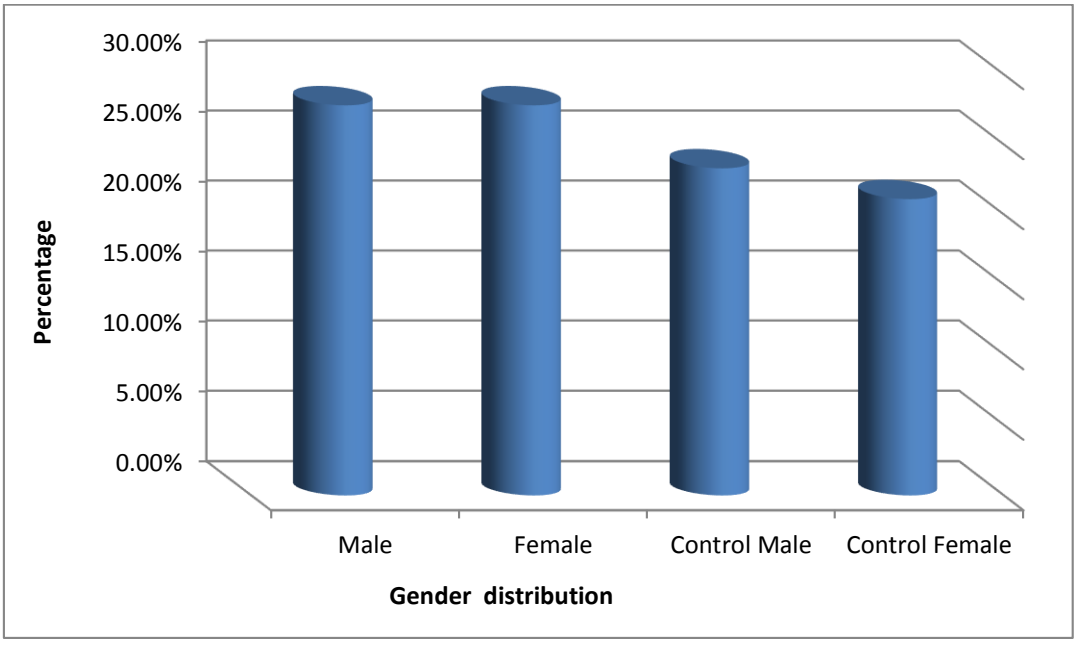


Figure (1-4): The frequency of Gender distribution

**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

(1-3). 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 (Ishraq ,2020).). Higher neutralizer levels were discovered in recovered male patients by Klein SL et al., showing that recovery from COVID-19 may demand more antibodies (Bin *et al.*, 2020).

Table (1-3)
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 | |

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

The table (1-4) 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. (New CDC Study, 2021).

Table (1-4)
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 1. (1-5). 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. (Víctor *et al.*, 2020).

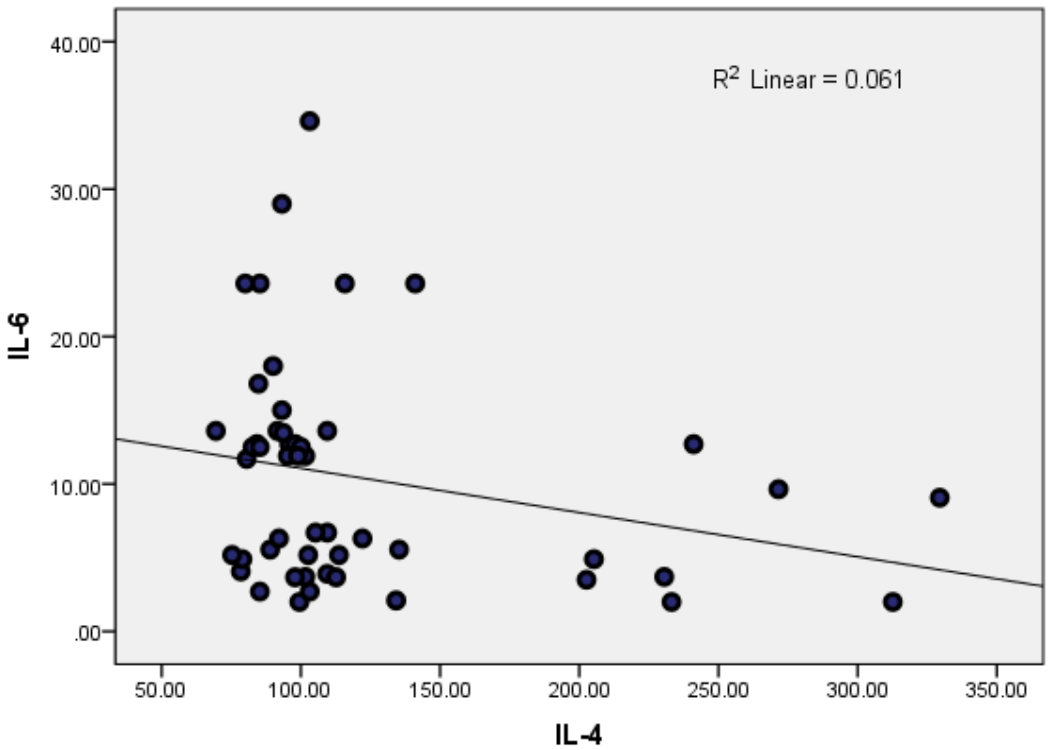


Figure (1-5): Correlation of IL-6 with IL- 4 in SARS-CoV-2 infected patients

**Conclusion**

Our findings suggest that at a reason able cost, RT genotyping with a narrow panel of SNPs can add useful genotype information to PCR-positive data. 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. Our findings imply that SNPs in the S gene, E gene, and N gene may be linked to SARS-CoV-2 susceptibility and patient risk.

We also advised considering IL6 markers as a major factor to understand therapeutic response against the COVID-19 in infected human populations to establish a population based therapeutic development as in personal medicine discovery by combining information about the immunological impact of IL6,il4 Immunological markers s as reported earlier in lung diseases and viral diseases.

**Acknowledgments**

At first of all, thanks to Allah the most gracious and the most merciful, who gave me the ability and desire to achieve this study. My sincere thanks to all staff of Merjan Hospital City for presenting all the facilities to finish this work. My special and sincere thanks to patients who i take the samples from and may Allah heal them and have mercy on those who died because of this epidemic. My sincere thanks are also to my family, friends and to anyone who helped and supported me by his pray for success in my work.

**Referances**

Guo, L., Ren, L., Yang, S., Xiao, M., Chang, D., Yang, F.,(2020). Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). *Clin. Infect. Dis.*71, 778–785.

Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. (2020). A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 395(10223):514–523 .

World Health Organization. (2020). Coronavirus disease (COVID-19) pandemic.

Yu Chen, Qianyun Liu, and Deyin Guo.(2020). Emerging coronaviruses: Genome structure, replication, and pathogenesis J Med Virol. Apr; 92(4): 418–423.

Peng Zhou, Xing-Lou Yang, Zheng-Li Shi. (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin Nature Research Journal , *Nature* volume. 579, pages270–273.

Trevor R. F. Smith, Ami Patel, Kate E. (2020). Broderick Immunogenicity of a DNA vaccine candidate for COVID-19 *Nature Communications* volume 11, Article number: 2601 .

Marra MA, Jones SJM, Astell CR, Holt RA, Brooks-Wilson A, Butterfield YSN, Khattra J, Asano JK, Barber SA, Chan SY, Cloutier A, Coughlin SM, Freeman D, Girn N, Griffith OL, Leach SR, Mayo M, McDonald H, Montgomery SB, Pandoh PK, Petrescu AS. (2003). The genome sequence of the SARS-associated coronavirus. Science .;300:1399–1404.

Drosten C, Gunther S, Preiser W, van der Werf S, Brodt HR, Becker S, Rabenau H, Panning M, Kolesnikova L, Fouchier RA, Berger A, Burguiere AM, Cinatl J, Eickmann M, Escriou N, Grywna K, Kramme S, Manuguerra JC, Muller S, Rickerts V, Sturmer M, Vieth S, Klenk HD, Osterhaus AD, Schmitz H, Doerr HW. (2003). Identification of a novel coronavirus in patients with severe acute respiratory syndrome. N Engl J Med.;348:1967–1976.

Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. (2020). A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. Cell Host Microbe. 8 April.

Rota PA, Oberste MS, Monroe SS, Nix WA, Campagnoli R, Icenogle JP, Penaranda S, Bankamp B, Maher K, Chen MH, Tong S, Tamin A, Lowe L, Frace M, DeRisi JL, Chen Q, Wang D, Erdman DD, Peret TC, Burns C, Ksiazek TG, Rollin PE, Sanchez A, Liffick S, Holloway B, Limor J, McCaustland K, Olsen-Rasmussen M, Fouchier R, Gunther S, Osterhaus AD, Drosten C, Pallansch MA, Anderson LJ, Bellini WJ. (2003). Characterization of a novel coronavirus associated with severe acute respiratory syndrome. Science.;300:1394–1399.

Zhu Y, Liu M, Zhao W, Zhang J, Zhang X, Wang K, Gu C, Wu K, Li Y, Zheng C, Xiao G, Yan H, Zhang J, Guo D, Tien P, Wu J. (2005). Isolation of virus from a SARS patient and genome-wide analysis of genetic mutations related to pathogenesis and epidemiology from 47 SARS-CoV isolates. Virus Genes.;30:93–102.

Holmes KV, Enjuanes L. (2003). Virology. The SARS coronavirus: a postgenomic era. Science.;300:1377–1378.

Ishraq Abdul Amir Saleh .(2020) , TUMOR NECROSIS FACTOR-± (TNF-±) 308 G/A PROMOTER POLYMORPHISMS IN GASTRIC CANCERS PATIENTS IN IRAQ *Plant Archives* Vol. 20 No. 2, pp. 8667-8670.

Cong Y, Ulasli M, Schepers H, Mauthe M, V'Kovski P, Kriegenburg F, Thiel V, de Haan CAM, Reggiori F. (2020). Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle. J Virol.;94:e01925-19.

Leung DT, Tam FC, Ma CH, Chan PK, Cheung JL, Niu H, Tam JS, Lim PL. (2004). Antibody response of patients with severe acute respiratory syndrome (SARS) targets the viral nucleocapsid. J Infect Dis. .;190:379–386.

Günter Kampf. (2021). COVID-19: stigmatising the unvaccinated is not justified.The Lancet..398,10314,1871.

Mohammed Yousif Merza, Rundk Ahmed Hwaiz, Badraldin Kareem Hamad, Karzan Abdulmuhsin Mohammad, Harmand Ali Hama, and Abdulkarim Yasin Karim. (2021). Analysis of cytokines in SARS-CoV-2 or COVID-19 patients in Erbil city, Kurdistan Region of Iraq. PLoS One.; 16(4): e0250330.

Dudley J, Leidos Inc, Alexandria VA. . (2022). COVID-19 Transmission Under the Public Health Radar: High Prevalence in Young Adults for COVID-19 Pandemic Wave 1Elsevier.

Víctor J. Costela-Ruiz, Rebeca Illescas-Montes, Jose M. Puerta-Puerta,[c] Concepción Ruiz, and Lucia Melguizo-Rodríguez. (2020). SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. Pudmed cental.

Jian-Min Jin, Peng Bai, Wei He, Fei Wu, Xiao-Fang Liu, De-Min Han, Shi Liu and Jin-Kui Yang. (2020). Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. Frontiers Public Health.

Bin Huang, Yun Cai, Ning Li, Kening Li, Zhihua Wang, Lu Li, Lingxiang Wu, Mengyan Zhu, Jie Li, Ziyu Wang, Min Wu, Wanlin Li, Wei Wu, Lishen Zhang, Xinyi Xia, Shukui Wang, Hongshan Chen & Qianghu Wang. (2021). Sex-based clinical and immunological differences in COVID-19. BMC Infectious Diseases.

New CDC Study.(2021). Vaccination Offers Higher Protection than Previous COVID-19 Infection. CDC Newsroom.

Víctor J. Costela-Ruiz, Rebeca Illescas-Montes, Jose M. Puerta-Puerta, Concepción Ruiz, and Lucia Melguizo-Rodrígueza. (2020). SARS-CoV-2 infection: The role of cytokines in COVID-19 disease. PMC.