Original Article

The Role of Ubiquitin-Like Interferon (IFN)-Stimulated 15 and IFN Gamma Genes Polymorphism with the Severity and Serum Biochemical Markers in COVID-19 Patients

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Abstract

Background: The ubiquitin-like interferon-stimulated 15 (*ISG15*) gene belongs to the group of "interferon-stimulated genes (ISGs)." These ISGs are powerful and rapid type 1 interferon (IFN)-stimulated response proteins that suppress viral replication. IFN-gamma (IFN-γ) is a kind of cytokine that plays a significant role in both the induction and regulation of a wide variety of immune responses. **Objectives:** This study examined the relationships between rs1921 and rs1861494 single nucleotide polymorphisms with the manifestation of coronavirus disease 2019 (COVID-19) symptoms in patients and the status of biochemical markers in previously diagnosed cases. **Materials and Methods:** A total of 100 blood samples were collected from COVID-19 patients in different cities in Iraq and tested for hematological parameters, including complete blood count, as well as biochemical markers, such as D-dimer, ferritin, and C-reactive protein (CRP). Additionally, the polymerase chain reaction-restriction fragment length polymorphism technique was used to analyze the genotyping of rs1921 and rs1861494 polymorphisms. **Results:** The results show that different rs1921 genotypes have significant associations with hemoglobin levels, white blood cell counts, basophil counts, and loss of smell. In contrast, there were no significant differences in red blood cell counts, monocyte counts, lymphocyte counts, platelet counts, CRP levels, D-dimer levels, ferritin levels, fever duration, or taste loss. Furthermore, rs1861494 genotypes revealed no significant differences in any of the patients' parameters. **Conclusion:** Our results indicate that rs1921 is more correlated with COVID-19 patients' parameters than rs1861494, suggesting the need for further investigation to enhance understanding.

Keywords: COVID-19, PCR-RFLP, polymorphism, rs1861494, rs1921

Introduction

Coronaviruses belong to a large family of viruses. Some of them, such as Middle East respiratory syndrome and severe acute respiratory syndrome (SARS), can cause severe illnesses. The coronavirus disease 2019 (COVID-19) pandemic has profoundly affected the dynamics and remains a significant threat to public health. Since the first diagnosis on November 30, 2021, more than 260 million cases and 5.2 million deaths have been reported due to this viral infection.[1,2] SARS coronavirus 2 (SARS-CoV-2), the causative agent of the COVID-19 pandemic, is a member of the Coronaviridae family. The transmission rate of SARS-CoV-2 is higher than that of SARS-CoV, possibly due to genetic recombination events in the spike protein within the receptor binding domains, which may enhance its ability to spread. Coronaviruses have been shown to suppress interferon (IFN)-mediated antiviral responses and reduced levels of IFN in patients infected with SARS-CoV-2 are associated with severe disease.[3,4] SARS-CoV-2 interacts with the amino-terminal domain of ubiquitin-like gene 15 (*ISG15*), an important regulator of host cell innate immunity and

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IFN.[5] The infection of human macrophages by SARS-COV-2 induces the release of intracellular *ISG15* through a viral papain-like mechanism, leading to increased secretion of proinflammatory cytokines and chemokines that contribute to the hyperinflammatory response observed in COVID-19.[6] While IFN induction typically establishes an antiviral state in humans, some viruses can evade this response in patients with chronic infections. The continuous increase of IFNs, a cytokine storm, and inflammation, impairing the function of multiple organs, which is common in severely ill COVID-19 patients.[7] IFN has been shown to enhance the activity of genes, with ISG15 being a member of the IFN-stimulated gene (ISG) family.[8] ISGs are potent and rapid type 1 IFN-stimulated response proteins that inhibit viral replication. The activity of ISGs these genes against viral invasions has been extensively studied.[9] ISG15 was the first protein demonstrated to have similarities to ubiquitin. It shares approximately 50% homology with ubiquitin and possesses two domains analogous to those found in ubiquitin.[10] Under physiological conditions, ISG15 precursor proteins can be cleaved into a mature form with a molecular weight of 15 kDa.[11] This study aims to investigate the associations between rs1921 and rs1861494 single nucleotide polymorphisms (SNPs) and the manifestation of COVID-19 symptoms in patients, as well as the relationship between these SNPs and the status of biochemical markers in previously diagnosed cases.

Materials and Methods

Sampling

A total of 100 samples were obtained from visitors to private labs in the cities of AL-Basrah, Kut, and Nasiriyah, Iraq. The sampling technique was approved by the research ethics committees of Ilam University and the University of Babylon (Babil, Iraq, project No: z220501, date: April 24, 2022). All samples were collected from individuals who self-reported as being of Arab ethnicity and residing in the cities of Al-Basrah, Kut, and Nasiriyah.

Inclusion and exclusion criteria

Inclusion criteria included the following:

- Age between 30 and 40 years
- Active infection with COVID-19, diagnosed by real-time polymerase chain reaction (PCR) technique
- First-time infection
- No chronic diseases
- Non-vaccinated
- Not pregnant (for female patients)
- Not taking immunosuppressant drugs
- No chronic diseases like diabetes mellitus

The exclusion criteria included any patient not meeting any of the inclusion criteria.

Laboratory tests

Blood samples were tested for hematological parameters, including complete blood count, and biochemical markers, such as D-dimer, C-reactive protein, and ferritin. The tests were performed using customized devices and reagents, following the manufacturers' instructions.

DNA extraction

DNA extraction was performed following the protocol outlined by Hashim and Al-Shuhaib.[12] The quality and quantity of the extracted DNA were assessed using a NanoDrop spectrophotometer (Scan Drop, Biometra, Göttingen, Germany). To evaluate the molecular weight and integrity of the extracted DNA, 1% agarose gel electrophoresis was conducted.[13]

Genetic study

rs1861494 and rs1921 polymorphisms were analyzed using a PCR-restriction fragment length polymorphism (RFLP) assay. The primers utilized[14] for detecting these polymorphisms were:

(1) rs1861494 (219bp):
5′-TCATCACAGTTCCTTGGTGGC-3′ (forward)
5′-TTGCGTAAAGACAGGTGAGTT-3′ (reverse)
(2) rs1921 (213 bp):
5′-CAGATCACCCAGAAGATCGGC-3′ (forward)
5′-CGTCAGCCGTACCTCGTAG-3′ (reverse).

The PCR conditions were as follows: an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, 60°C for 30 s, and extension at 72°C for 30 s, concluding with a final extension at 72°C for 5 min. The PCR products for rs1861494 and rs1921 genotyping were digested using restriction enzymes *BstDE* I and *BspAC* I, respectively.[15]

Statistical analysis

The phenotypic odds ratio was calculated using MedcCalc.net. software (https://www.medcalc.net/tests/oddsratio.php) according to the methodology described by Altman (1991).[16] Additionally, phenotypic means and standard deviation were compared using the Student *t* test, employing the IPM®SPSS® software (IBM Corp., Armonk, NY, USA).

Ethical approval

This study was conducted following the ethical principles derived from the Declaration of Helsinki. Verbal consent was obtained from all patients before sample collection. The study protocol, along with the subject information and consent form, was reviewed and approved by the University of Babylon, documented under number z220501 on April 24, 2022.

Results

The study utilized PCR-RFLP to genotype the SNPs rs1861494 and rs1921, with results depicted in Figures 1 and 2. For rs1861494, the restriction enzyme BspAC I effectively cleaved the C allele, yielding fragments of 159 and 60 bp, whereas the T allele remained uncut, resulting in a 219 bp fragment. In contrast, rs1921 was analyzed using BstDE I, which cut the G allele to produce 154 and 59 bp fragments, whereas the A allele produced the original 213 bp fragment.

In the present study, the results indicated significant differences in hemoglobin levels (g/dL) between the GG and AG genotypes of rs1921 ($P = 0.016$), individuals with the AG genotype exhibited the highest mean hemoglobin level (13.49 g/dL). Additionally, a significant difference was observed in white blood cell counts (cells 10 μL) between GG and AA genotypes ($P = 0.013$), with the AA genotype associated with the highest mean white blood cell count (9.37 cells × 10 μL). Moreover, there were significant differences in the number of basophiles (cell/μL) between the GG and AA genotypes ($P = 0.045$), where individuals with the AG genotype had the highest mean basophile count (1.84 cells/μL). A strong association was also found between the rs1921 genotype and the loss of smell ($P = 0.002$), with the GG genotype being more prevalent among individuals reporting smell loss, whereas the AA genotype was more common among those without this symptom. However, no significant differences in other patient parameters, including monocyte counts, lymphocyte counts, platelet levels, duration of fever, and taste loss [Tables 1 and 2].

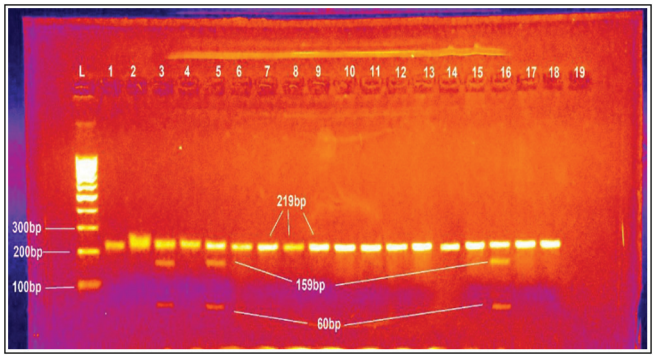

Figure 1: PCR-RFLP genotyping of rs1861494. Lane L 100 bp DNA ladder, lanes 3, 5, and 16 CT genotype; and other lanes TT genotype

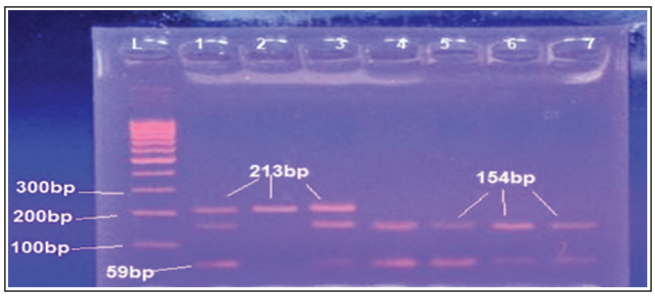

Figure 2: PCR-RFLP genotyping of rs1921. Lane L 100 bp DNA ladder, lanes 2 AA genotype; lanes 1 and 3 GA genotype; and lanes 4, 5, 6, and 7GG genotype

On the other hand, outcomes regarding rs1861494 showed there were no significant differences in the levels of hemoglobin ($P = 0.277$), white blood cells ($P = 0.945$), monocyte counts ($P = 0.277$), lymphocyte counts ($P = 0.913$), basophil counts ($P = 0.314$), platelets levels ($P = 0.260$), duration of fever ($P = 0.573$), taste loss ($P = 0.370$), and smell loss ($P = 0.553$) between TT genotype and TG genotype ($P \geq 0.05$) [Tables 3 and 4] and [Figures 3 and 4].

Discussion

Recent studies indicate that dysregulation of the *IFN-γ* gene may play a crucial role in determining the pathogenesis of COVID-19.[17] Evidence suggests that the response to class I IFNs in COVID-19 is impaired. In the blood of patients with severe COVID-19, levels of the *IFN-γ* gene are significantly lower compared with those in patients infected with highly pathogenic influenza viruses.[18] It has been demonstrated that *ISG15* can stimulate the production of *IFN-γ*. Additionally, *ISG15* enhances the proliferation of $CD56^+$ natural killer cells in the presence of $CD3^+$ T cells, resulting in increased target cell lysis.[19] This study aimed to investigate the associations between polymorphisms in the *IFN-γ* and *ISG15* genes and the severity of COVID-19, as well as serum biochemical markers in affected patients.

The findings revealed significant differences in hemoglobin levels (g/dL) between the GG genotype and AG genotype of rs1921 ($P = 0.016$). In contrast, our results regarding rs1861494 showed no significant differences in hemoglobin levels between TT and TG genotypes ($P = 0.277$). Vargas *et al.*[20] found that allele and genotype frequencies for rs8176740 T/A ($P = 0.002$) and rs512770 T/C ($P = 0.003$) differed between COVID-19 patients and healthy controls, while the rs495828 T/G and rs8176746 A/C SNPs were similar between the two groups.[20] To the best of our knowledge, this is the first study to explore the relationship between hemoglobin levels and polymorphisms in the *IFN-γ* and *ISG15* genes.

There were significant differences in white blood cell counts between the GG and AA genotypes of rs1921 ($P = 0.013$), whereas no significant differences were observed for TT and TG genotypes of rs1861494 ($P \geq 0.05$). Several recent studies[17,21] claim that IFN dysregulation is a defining feature of COVID-19 pathogenesis, whereas others state that the nonfunctional rs368234815-TT allele is exclusively found in humans.[22] In COVID-19 patients with severe illness who experience shortness of breath and may develop acute respiratory distress syndrome, higher neutrophil-to-lymphocyte ratios have been linked to poorer outcomes.[22]

Table 1: Association of hemoglobin concentration, white blood cell, and basophiles with rs1921 genotype

Genotype	(J)rs1291	Mean difference (I–J)	*P* value
Hemoglobin (g/dL mean ± SD)			
AA (12.9394 ± 1.49289)	AG	−0.55716	0.168
	GG	0.36113	0.318
AG (13.4966 ± 1.54469)	AA	0.55716	0.168
	GG	0.91829*	**0.016**
GG (12.5783 ± 1.65475)	AA	−0.36113	0.318
	AG	−0.91829*	**0.016**
White blood cells (cell/μL mean ± SD)			
AA (9.3788 ± 3.40778)	AG	1.00637	0.180
	GG	1.45922*	**0.031**
AG (8.3724 ± 2.70263)	AA	−1.00637	0.180
	GG	0.45285	0.516
GG (7.9196 ± 2.69061)	AA	−1.45922*	**0.031**
	AG	−0.45285	0.516
Basophiles (cell/μL mean ± SD)			
AA (.7609 ± 2.10174)	AG	−0.49875	0.404
	GG	−1.08170*	**0.045**
AG (1.2597 ± 2.28707)	AA	0.49875	0.404
	GG	−0.58295	0.296
GG (1.8426 ± 2.52686)	AA	1.08170*	**0.045**
	AG	0.58295	0.296

*reveals a significant relationship between biochemical markers and rs1921 (p-value < 0.05)

Table 2: Association of taste loss with rs1921 genotype

rs1921 genotype	Taste loss			*P* value
		No	Yes	
AA	Count	10	23	0.103
	Percentage ()	30.3%	69.7	
AG	Count	16	13	
	Percentage (%)	55.2	44.8	
GG	Count	23	23	
	Percentage (%)	50.0	50.0	

According to our current findings, there were no statistically significant differences in monocyte, lymphocyte, and red blood cell counts between rs1861494 and rs1921 genotypes. Our study showed significant differences in the number of basophils (cell/μL) between the GG and AA genotypes of rs1921, whereas rs1861494 showed no significant differences for TT and TG genotypes. El Bendary *et al.*[23] reported similar results indicating that SNPs in the IFN were associated with hepatitis C virus spontaneous viral clearance.[23] Meanwhile, Zhang and Zhang[24] found that ISG15 is crucial for several biological and cellular processes.[24] The present investigation found no statistically significant variations in platelet counts across any of the genotypes tested for rs1921 and rs1861494. Our results aligned with those from a cross-sectional study

Table 3: Association of hemoglobin concentration, white blood cell count, and basophils with rs1861494 genotype

Genotype	Mean g/dL	Std. deviation	Mean difference	*P*-value
Hemoglobin (g/dL mean ± SD)				
TT	12.8784	1.61737	0.558	0.277
TG	13.4364	1.48813		
White blood cells (cell/μL mean ± SD)				
TT	8.4804	3.05255	0.0651	0.945
TG	8.5455	2.20833		
Basophils (cell/μL mean ± SD)				
TT	2.0382	2.62893	0.7601	0.314
TG	1.3556	2.36404		

Table 4: Association of taste loss with rs1861494 genotype

rs1861494 genotype	Taste loss			*P* value
		No	Yes	
TT	Count	43	54	0.370
	Percentage (%)	44.3	55.7	
TG	Count	6	5	
	Percentage (%)	54.5	45.5	

conducted in Pakistan by Shamshad *et al.*[25] There were no statistically significant differences between the rs1921 and rs1861494 genotypes concerning fever duration. Our findings are consistent with those of Yang *et al.*[26] Our results suggested that there is no significant connection between the rs1921 and rs1861494 genotypes and the reduction in taste and smell. In the present study, there was no significant association between the rs1861494 genotype and smell loss. However, results concerning rs1921 indicated a highly significant association with smell loss ($P = 0.02$), with the GG genotype recorded as the most prevalent among individuals who experienced loss of smell. Research conducted by Landis *et al.*[27] predicts that the duration of symptoms associated with COVID-19 is typically just a few weeks.[27]

Conclusion

Gene polymorphisms may play an important role in many infections, including COVID-19. This study highlights the need for further investigation into gene polymorphisms to improve our understanding of their relationships with COVID-19 infections in Iraqi patients. Additionally, our findings suggest that the rs1921 SNP is more correlated with COVID-19 patient parameters than rs1861494 warranting further exploration. Future studies should also consider factors not included in this research, such as diabetes mellitus and the age of patients, which are important variables that must be correlated with SNPs associated with COVID-19. There is a need for studies involving a larger patient population to assess the incidence of COVID-19 in Iraq.

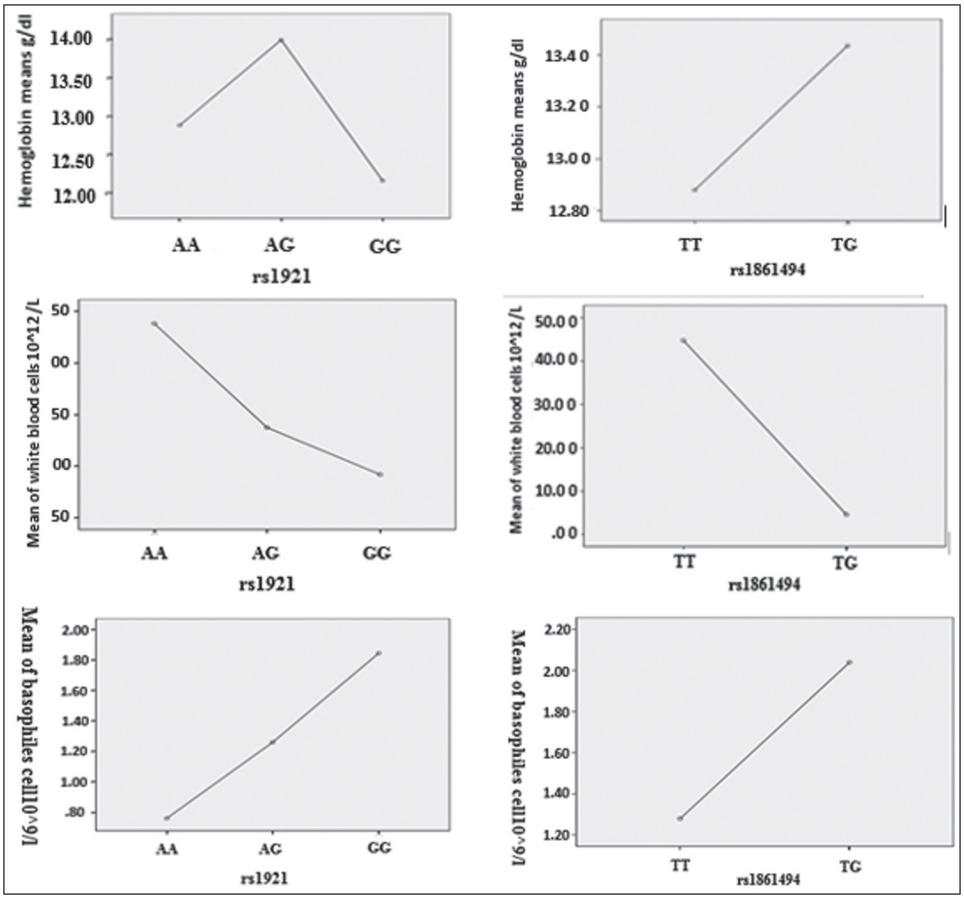

Figure 3: Association of hemoglobin concentration, white blood cell count, and basophil count with genotypes of *IFN-γ* and *ISG15* gene polymorphisms

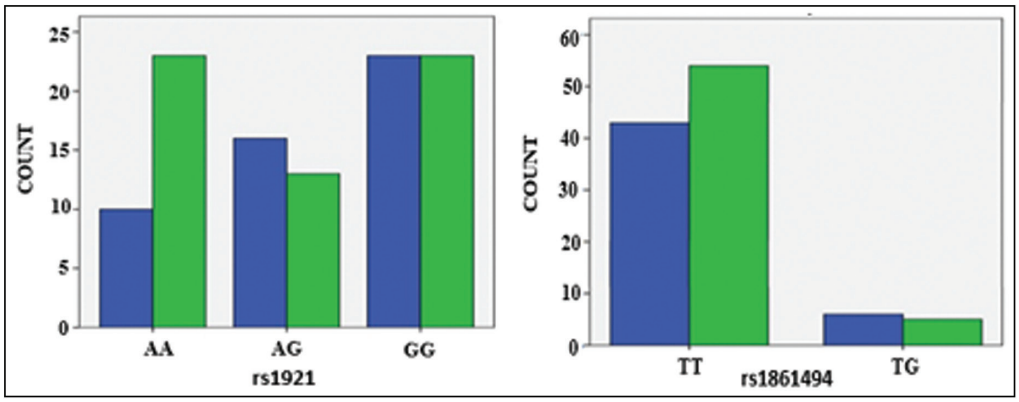

Figure 4: Association of taste loss with different genotypes of rs1921 and rs1861494

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Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Sahib ZH, Al-Zurfi AH, Al-Masoodi AT, Baay A, Al-Muhktar E. Remdesivir effects on COVID-19 infection in adult patients. Med J Babylon 2022;19:625-34.
2. Shrivastava SR, Shrivastava PS. Coronavirus disease-2019 infection among children. Med J Babylon 2022;19:102-4.
3. World Health Organization. Laboratory Testing for Coronavirus Disease 2019 (COVID-19) in Suspected Human Cases: Interim Guidance. Geneva: World Health Organization; 2020.
4. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, *et al*. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science 2020;369:718-24.
5. Shin D, Mukherjee R, Grewe D, Bojkova D, Baek K, Bhattacharya A, *et al*. Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity. Nature 2020;587:657-62.
6. Munnur D, Teo Q, Eggermont D, Lee HHY, Thery F, Ho J, *et al*. Altered ISGylation drives aberrant macrophage-dependent

immune responses during SARS-CoV-2 infection. Nat Immunol 2021;22:1416-27.
7. Murira A, Lamarre A. Type-I interferon responses: From friend to foe in the battle against chronic viral infection. Front Immunol 2016;7:609.
8. Takeuchi T, Koinuma S, Yokosawa H, Arata Y. An approach for the identification of proteins modified with ISG15. Methods Mol Biol 2019;1934:235-46.
9. Sooryanarain H, Rogers AJ, Cao D, Haac MER, Karpe YA, Meng XJ. ISG15 Modulates Type I interferon signaling and the antiviral response during hepatitis E virus replication. J Virol 2017;91:e00621-17.
10. Dos Santos PF, Mansur DS. Beyond ISGlylation: Functions of free intracellular and extracellular ISG15. J Interferon Cytokine Res 2017;37:246-53.
11. Zhao C, Sridharan H, Chen R, Baker DP, Wang S, Krug RM. Influenza B virus non-structural protein 1 counteracts ISG15 antiviral activity by sequestering ISGylated viral proteins. Nat Commun 2016;7:12754.
12. Hashim HO, Al-Shuhaib MBS. A novel DNA extraction protocol from frozen blood of normal individuals and patients who received systemic chemotherapy. J Appl Biotechnol Rep 2020;2020:90683.
13. Sambrook J, Russell D. SDS- gel electrophoresis of proteins, staining SDS-polyacrylamide gels and immunoblotting polyacrylamide. In: Cuddihy J, Janssen K, Zierler M, editors. Molecular Cloning: A Laboratory Manual. New York, NY: Cold Spring Harbor Laboratory Press; 2001. p. A840-A4854.
14. Hashim HO, Al-Saadi AH, Haider AH, Zaidan HK. Association of uromodulin rs13333226 and angiotensinogen rs699 genes variants with essential hypertension in Arab Iraqis of Babylon province. Res J Pharm Biol Chem Sci 2015;6:589.
15. Hashim HO, Al-Shuhaib MBS. Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: A review. J Appl Biotechnol Rep 2019;6:137-44.
16. Altman DG. Practical statistics for medical research. Chapman & Hall; 1991.
17. Andreakos E, Tsiodras S. COVID-19: Lambda interferon against viral load and hyperinflammation. EMBO Mol Med 2020;12:e12465.
18. Acharya D, Liu G, Gack MU. Dysregulation of type I interferon responses in COVID-19. Nat Rev Immunol 2020;20:397-8.
19. Qie S, Diehl JA. Cyclin D degradation by E3 ligases in cancer progression and treatment. Semin Cancer Biol 2020;67:159-70.
20. Vargas-Alarcón G, Ramírez-Bello J, Posadas-Sánchez R, Rojas-Velasco G, López-Reyes A, Martínez-Gómez L, *et al*. The rs8176740 *T*/*A* and rs512770 *T*/*C* genetic variants of the *ABO* gene increased the risk of COVID-19, as well as the plasma concentration platelets. Biomolecules 2022;12:486.
21. Entangled roots and otherwise possibilities. An anthropology of disasters COVID-19 research agenda. Hum Organ 2020;79:333-42.
22. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, *et al*. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study. Lancet (London, England) 2020;395:507-13.
23. El-Bendary M, Neamatallah M, Elalfy H, Besheer T, El-Setouhy M, Kasim N, *et al*. Association of interferon gamma gene polymorphism and susceptibility to hepatitis C virus infection in Egyptian patients: A multicenter, family-based study. JGH Open 2017;1:140-7.
24. Zhang D, Zhang D-E. Interferon-stimulated gene 15 and the protein ISGylation system. J Interferon Cytokine Res 2011;31:119-30.
25. Shamshad S, Khan S, Raja GK, Ahmad MS, Asad MJ, Zainab T. Correlation of C-reactive protein levels, gene polymorphism and platelets count in dengue infection. J Pak Med Assoc 2021;71:429-33.
26. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, *et al*. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: A single-centered, retrospective, observational study. Lancet Respir Med 2020;8:475-81.
27. Landis BN, Scheibe M, Weber C, Berger R, Brämerson A, Bende M, *et al*. Chemosensory interaction: Acquired olfactory impairment is associated with decreased taste function. J Neurol 2010;257:1303-8.