# The Prevalence and Implications of Factor V rs6687813 Mutation among COVID-19 Patients on D-dimer Levels

***Walid Aburayyan[1*], O'la Ahmad AL-Fawares[2], Mohammad Mansour Albalbaki[1], Nesrin Seder[3]***

[1]Department of Medical Laboratory Analysis, Faculty of Allied Medical Sciences, Al-Balqa Applied University, Al-Salt, Jordan.
[2]Department of Applied Biological Sciences, Faculty of Science, Al-Balqa Applied University, Al-Salt, Jordan.
[3]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmacy, Applied Science Private University, Amman, Jordan.

## ABSTRACT

COVID-19 has invaded human community worldwide threatening public health and the world economy. The confounder data about the pathogenesis of the disease progression has aroused prognostic theories concerning the implications of demographic data and the genetic variability of the disease prognosis. A total of 120 participants; 68 males and 52 females, aged from 15 to 82 years, among them 90 COVID-19-infected patients, and 30 COVID-19-free participants were enrolled in the current study. Blood samples were drawn from 120 participants and then analyzed for D-dimer levels using fluorescence immunoassay. The 120 DNA extracted samples were amplified by PCR using Factor V-specific primers. The PCR products were sequenced and examined for putative mutations in the Factor V gene. A significantly high mutation rate in Factor V **(**rs6687813) 82%, $p< 0.001$ was demonstrated in the study population. The mutations were significantly correlated with extremely high D-dimer levels of 2975 ng/ml versus 986 ng/ml in the wild type (r=0.228, $p<0.012$). Hospitalized COVID-19 patients expressed significantly higher levels of D-dimer compared to non-hospitalized patients 3353 ng/ml vs 513 ng/ml $p<0.001$, respectively. The age had an odds rate of 1.12 on the acquisition of COVID-19 infection as the ages above 35 were more vulnerable to acquiring the infection than younger ages (r=0.21, $p<0.022$). In conclusion, the study provides robust evidence about the direct association between the FVL mutation and elevated levels of D-dimer in COVID-19 patients and the implementation of the FVL mutation test as a prognostic marker is recommended for COVID-19 patients.

**Keywords:** Factor V, D-dimer, COVID-19, Jordan, Mutation, SNP.

## INTRODUCTION

The emergence of Corona Virus Disease (COVID-19) in late 2019, has prone millions of people to the threat of acquiring a series of respiratory infections such as severe acute respiratory syndrome and consequentially developing devastating pathological conditions [1]. Systematic multi-organ failure combined with thrombophilia disorders were concomitantly reported in COVID-19 hospitalized patients [1, 2]. The escalation in the coagulation byproducts as fibrin breakdown product D-dimer in COVID-19 patients is drastically correlated with increments in mortality and morbidity rates [3 4]. Moreover, escalated levels of D-dimer have been significantly associated with a high probability of hospitalization, admission to the intensive care unit (ICU), and consequently, higher prevalence in mortality rates by COVID-19 [5, 6]. COVID-19-infected patients are vulnerable to developing several disorders in the coagulopathy of the blood vessels or through an over-reactivity of the immune

[*]***Corresponding author: Walid Aburayyan***
Walid.aburayyan@bau.edu.jo
Received: 16/05/2024 Accepted: 12/12/2024.
DOI: https://doi.org/10.35516/jjps.v18i4.2652

system against the viral infection [7].

Additionally, COVID-19 patients imposed hyperimmunological responses including cytokines storm and disseminated intravascular coagulopathy [8]. Rationally, several scientists sought about the massive immune response encountered by COVID-19 patients [9]. The imputed values have highlighted the prognostic implication of genetic mutations in coagulation factors as Factor III (also known as tissue factor), fibrinogen alpha chain (FGA), fibrinogen gamma chain (FGG), and factor V on the pertinent implications. Nevertheless, the fundamental mechanisms underlying this association are not entirely understood [10].

During the COVID-19 pandemic, a drastic concern was appointed toward the D-dimer levels which were unprecedently elevated in severe infections [11]. This plausible feat was adopted as a clinical monitor for the evaluation of disease progression in hospitalized patients [12, 13]. Moreover, the clinical findings in D-dimer levels imposed the criteria for the discharge of COVID-19 patients or admission to the intensive care unit [14] . So far, D-dimer levels have been significantly higher in COVID-19 patients with severe illness compared to those with mild illness [15]. Moreover, in 2020, a retrospective study augmented the correlation between COVID-19 patients and the incidence of mortality in elevated D-dimer findings [16]. In the studies of the Genome-Wide Association (GWAS), a significant correlation has been shown between the single nucleotide mutations (SNPs) of the coagulation genes of fibrinogen, Alpha fibrinogen Gamma, MTHFR, KLKB1, KNG1, FII, FV, FVIII, FXI and the high D-dimer levels [17].

Factor V is indispensable in the hematological coagulation cascade [18]. It mediates the conversion of prothrombin to thrombin and stables fibrin clots. D-dimer is a byproduct formed as a consequence of the degradation of fibrin protein [19]. D-dimer monitoring is a useful tool for diagnosing Deep Vein Thrombosis (DVT) [20]. In infected patients with COVID-19, elevated D-dimer levels have been noticed in clinically severe patients encountering complications in the respiratory system or the cardiovascular system [21]. An estimated 10-fold probability of developing a threat of thrombus formation by activating Factor Va and VIIIa has been noticed in Factor V Leiden (FVL) mutant patients. The prevalence of FVL mutation in patients with an idiopathic case of stroke is estimated to be around 12 % [22]. A significant correlation between FVL mutation and unexplained venous thrombosis was demonstrated. The prevalence of FVL heterozygous patients showed a higher 10% risk of developing thrombosis. Meanwhile, the incidence of the FVL heterozygous mutation in the general population is 4.8% [23]. Consequently, COVID-19 patients with FVL heterozygous mutation are porn to the threat of coagulopathy during the infection.

In the current study, we will explore the D-dimer level in a population of COVID-19-positive patients and investigate the correlation of mutations in FVL with the levels of D-dimer in the patients and the effect of the demographic data on D-dimer levels.

## MATERIAL AND METHOD

### *Study Sample*

In the current study, 120 volunteers participated, 68 males and 52 females, aged from 15 to 82 years, 90 patients were previously diagnosed with COVID-19 infection and confirmed by Rt-PCR and 30 volunteers were COVID-19-free. The inclusion criteria for the study was a COVID-19-positive patient, hospitalized and admitted less than two days before blood collection. The patients on anticoagulant medications or cancer patients were excluded from the study. Blood samples were collected during three months from May to August 2022. A consent form was distributed to the volunteers and kindly requested to fill in their personal information. Blood samples of around 2.5 ml were phlebotomized in EDTA vacuum tubes from all participants from two distinct sites; The Hospital of Gardens and the

Laboratories of Consulting Medical group in the city of Amman, Jordan. A consent form was filled by each participant and the clinical data was extracted from the clinical records of the patient files. Blood samples were stored at -20 °C until the collection process was completed. The Institutional Review Board (IRB) of Al-Balqa Applied University has approved the current study under the approval number (26-3-1781). The approval is inserted in the Appendix A.

**DNA Extraction**

The blood samples were phlebotomized from the 120 patients and were processed for DNA extraction using (All Gene, South Korea) kit. The protocol of DNA extraction was implemented and performed according to the vendor's instructions in accordance with [24]. The extracted DNA was utilized for PCR amplification. DNA extracts were stored at -20 °C for 15 days until the whole DNA extraction process for all samples was finished.

**3.2.2 Quality control of DNA extraction**

The amount, purity, and integrity of the DNA extracts were estimated by measuring the concentration of DNA at 260 nm by using NanoDrop™ One (Thermo Fisher Scientific, USA). Additionally, to ensure DNA purity and roll out the presence of contaminants the ratio of absorbance at 230/260 and 260/280 was calculated according to [25].

Utilizing a 1% agarose gel electrophoresis (Invitrogen, USA) the quality and the integrity of the extracted DNA was examined. After loading the 5 µl of extracted DNA and 1µ of the loading buffer (Thermo, USA) in coombs the electrophoresis was run using a power pack at 100 volts for one hour (Bio-Rad, USA). Later on, the gel was stained in ethidium bromide for 15 minutes and then visualized under UV light [24].

**3.2.4 Primer Design**

The primer design for the target mutation sites of the Factor V gene has been performed using the official NCBI website for primer design (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). In particular, the primers for Factor V (Table 1) were used for the amplification of a target region of 113 nucleotides to seek an SNP rs6687813. The primers were ordered and purchased by Macrogen Company, (South Korea) and the purity was confirmed by HPLC certificate.

**Table 1: The sequence of primers utilized in DNA amplification**

| Primer | | Sequence (5'->3') | Primer Length | Tm | Product size (bp) |
|---|---|---|---|---|---|
| Factor V | Forward | GCAGTTGTTACACCTTGCAGA | 21 | 59.05 | 113 |
| | Reverse | TTCCTTCAGGCAAAGGCTCA | 20 | 59.52 | |

**3.2.5 Amplification Reaction**

Using a Rotor-Gene machine (QIAGEN, Model 6 Plex) (Hilden, Germany), a Master mix Solution of 2xPCR (iNtRON Biotechnology, South Korea) was used for performing a conventional PCR reaction. The procedure of a PCR program was implemented as follows: two minutes of initial denaturation at 94º C, thirty-two denaturation cycles at 94º C for twenty sec., annealing at 56º C for twenty sec., and elongation at 72º C for one min., with final elongation at 72º C for ten min. The reaction tube consisted of 1.25 µl of forward and reverse primers, 12.5 µM of the 2x buffer, 2 µl of DNA, and 9 µl of $H_2o$ to achieve a total volume of 25 µl.

**3.2.6 Purification of PCR Products**

The protocol of PCR product purification ensures a useful procedure to remove the byproduct contamination of PCR reaction ingredients such as dNTPs, primers, enzymes, and salts. The protocol of the company (Qiagen, Germany) was implemented in executing the PCR run. 50 µl of PB Buffer was added to 10 µl of the PCR sample to explicitly eliminate the residual mineral oil and kerosene. Later on, an amount of ten microliters of 3 M sodium

acetate were added to the PCR product and the mixture was inverted ten times until a yellow color was developed. The mixture was placed in a QIAquick spin column (Qiagen, Germany) in a provided 2 ml collection tube. The tubes were centrifuged at 13,000 rpm for 45 seconds and the flow-through was discarded. For DNA precipitation, 750 µl of PE Buffer (Hydrochloride and isopropanol) was pipetted to the QIAGEN quick column and then centrifuged at 13500 rounds per minute for 75 sec. and the flow was removed. Finally, DNA was eluted into a 1.5 ml Eppendorf tube by pipetting 100 µl of elution buffer and centrifuged at 130000 rpm for one minute. The samples were stored at -80C for the next step.

**3.2.7 DNA Sequencing**

The following protocol was conducted during the DNA sequencing process by the Macrogen Company (South Korea). In the Sequencing Kit of BigDye Terminator version 3.1 (Thermofisher, USA), 120 PCR products were examined for the mutations in the nucleotide sequence as SNPs. The BigDye Terminator v3.1 mix, which contains a reaction buffer, Taq DNA polymerase, and fluorescently labeled dideoxynucleosides, was mixed with purified PCR products. This produced a collection of DNA fragments with different fluorescent colors and lengths. The sequences of the genes were exported GC using software and saved as a Word document.

**3.3.1 D-dimer**

The vendor's instructions were followed in measuring the D-dimer level (Qdaisat, 2019). Ten microliters of human whole blood were transferred to a prefilled tube with a D-dimer detection buffer and the mixture was pipetted on a nitrocellulose matrix mixed for ten times. Finally, 75 µl of a sample was pipetted out onto the cartridge and measured after incubation for 10 minutes at 25℃. The cartridge was read and measured using iChromaTM II (Boditech Company, South Korea).

***3.4 Statistical Analysis***

The Statistical Package for the Social Sciences (SPSS) program Version 26 was used to study the statistical parameters of the generated data. The independent variables were tested for the normal distribution using the Kolmogorov-Smirnov test. Results were subjected to independent T-tests to reject the null hypothesis between the independent variables of gender, and the dependent variable of D-dimer level, whereas, the Weche t-test was performed to study the statistical difference among the independent variables of Covid-19 incidence, mutation of Factor V, and the dependent variableD-dimer. One-way ANOVA followed by a post hoc test was implemented to clarify the significant difference among the different age groups in relevance to D-dimer levels. A Bivariate analysis; of the two-tailed significance Pearson Correlation test was conducted to study possible correlation between the ordinal variables. The Chai square test was conducted to test the cross-tabbing of the independent variables. The charts and figures in this study were created using Excel as well.

***Results***

A significant difference $p< 0.001$ in D-dimer levels among the 120 participants was demonstrated as the mean level for D-dimer in the study population was 2643 ng/ml (Table 2); the D-dimer levels ranged between 145 ng/ml and 16949 ng/ml (Appendix B). D-dimer levels were significantly higher in COVID-19 patients than in the COVID-19-free participants as the mean levels were 3353 ng/ml versus 513 ng/ml $p>0.001$, respectively (Table 2). Nevertheless of gender, no significant distinction in D-dimer levels among females and males, the D-dimer levels were relatively similar in both genders $p=0.243$. Remarkably, there was a significantly high prevalence of Factor V mutation in comparison with the wild type among the study population (82.6% vs. 16.5%, $p<0.001$), respectively. A positive correlation was noticed between the mutations in Factor V and the high levels of D-dimer levels 2975 ng/ml whereas the wild-type patients reported mean D-dimer levels of 986 ng/ml, $p<0.001$ (Table 2). The mutation of Factor V has conferred high D-dimer levels $r=0.228$, $p=0.012$. Significantly, 86% of the high D-dimer

levels showed a mutant in Factor V $p<0.001$, whereas, around 80% of the normal D-dimer readings (less than 500 ng/ml) demonstrated a wild-type sequence of Factor V (Figure 1). Additionally, there was a significant escalation in mean D-dimer levels in concordance with the age ($r=0.21$, $p<0.022$) and the incidence of the COVID-19 infection ($r=0.378$, $p<0.001$) of the study population (Table 3). Moreover, there was no noteworthy association found between the mutation of Factor V and the prevalence of COVID-19 infection in the study population (Table 3).

**Table 2: The levels of D-dimer according to the demographic variables**

| | | **Frequency** | **Percent** | **D Dimer** | ***p*-Value** |
|---|---|---|---|---|---|
| **Factor V** | Wild type | 20 | 16.5 | 986 | **<0.001** |
| | Mutation | 100 | 82.6 | 2975 | |
| **Gender** | Male | 68 | 56.2 | 2655 | 0.243 |
| | Female | 52 | 43.0 | 2628 | |
| **Age** | <20 | 12 | 9.9 | 1363 | 0.716 |
| | <40 | 40 | 33.1 | 2799 | |
| | <60 | 29 | 24.0 | 2869 | |
| | <80 | 28 | 23.1 | 2670 | |
| | >80 | 11 | 9.1 | 2809 | |
| **Covid-19** | Negative | 30 | 24.8 | 513 | **<0.001** |
| | Positive | 90 | 74.4 | 3353 | |
| **D-dimer level** | <500 ng/ml | 31 | 25.1 | 126 | **<0.001** |
| | >500 ng/ml | 89 | 74.9 | 3464 | |
| | Total | 120 | 100 | 2643 | |

Bold*= Significant

***Sequencing analysis of Factor V***

The resulting gel showed clear and distinct DNA bands (Supplement Figure 1), with no evidence of degradation observed, indicating that the DNA extraction process was successful with high throughput. For the 230/260 and 260/280 ratios, all DNA samples met the quality approval requirements; the ratios were greater than 1.8 and less than 2.4 (The recorded measurements of DNA samples were summarized In Appendix C).

Table 1, Supplements 2 summarizes sequencing data for PCR products that were analyzed for SNPs in Factor V. Variations in the SNPs for every factor in the patient were found in the data. An A variant with A/C was found for the Factor V SNP (rs6687813) in place of the wild-type C allele. The GWAS Catalog was utilized to conduct additional analysis on the particular variants found in the sequencing data for every factor. (Figure Supplement (2))

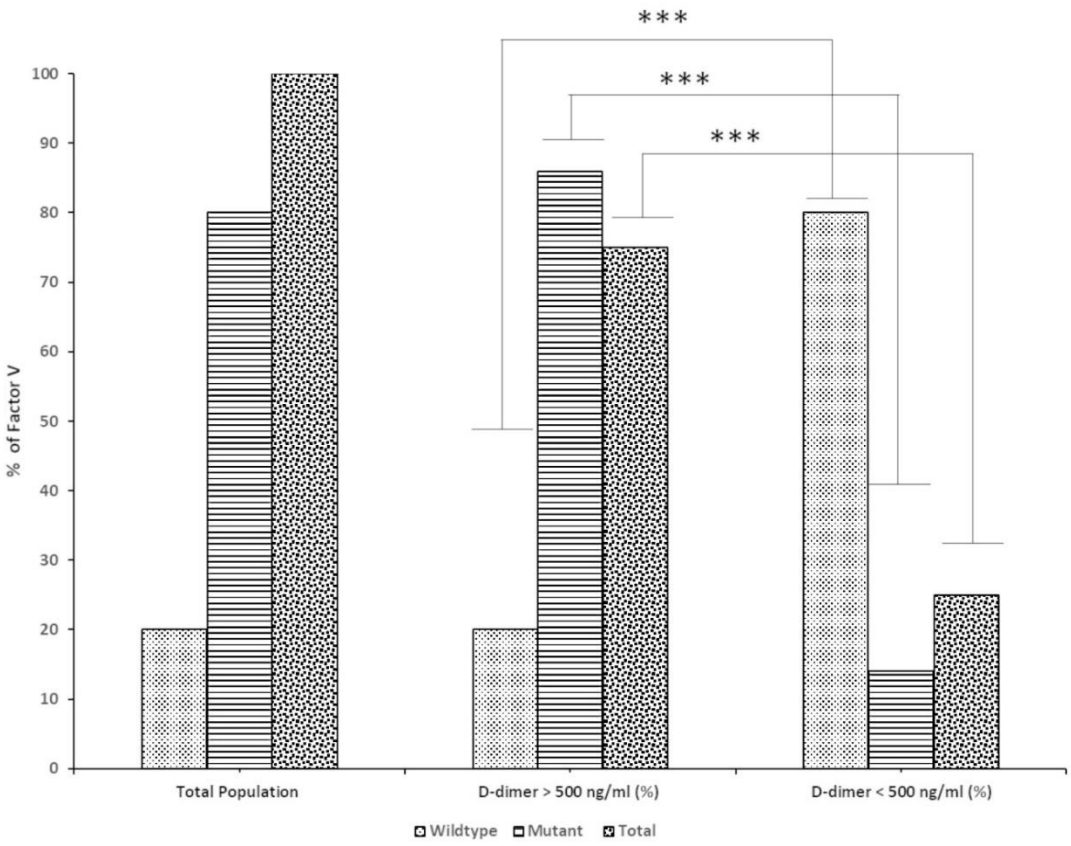


**Figure 1: The distribution of D-dimer levels in relevance to Factor V Mutation**

High D-dimer > 500 ng/ml, Normal D-dimer < 500 ng/ml

**Table 3: The correlation between the D-dimer levels and the demographic variables of the current study**

| | | Age | Gender | Covid-19 | D Dimer | Factor V |
|---|---|---|---|---|---|---|
| Age | Pearson Correlation | 1 | | | | |
| | Sig. (2-tailed) | | | | | |
| Gender | Pearson Correlation | -0.057 | 1 | | | |
| | Sig. (2-tailed) | 0.538 | | | | |
| Covid-19 | Pearson Correlation | 0.080 | -0.117 | 1 | | |
| | Sig. (2-tailed) | 0.383 | 0.205 | | | |
| D Dimer | Pearson Correlation | **.210*** | -0.004 | **.378**** | 1 | |
| | Sig. (2-tailed) | **0.022** | 0.965 | **0.000** | | |
| Factor V | Pearson Correlation | 0.019 | 0.120 | -0.052 | **.228*** | 1 |
| | Sig. (2-tailed) | 0.841 | 0.190 | 0.575 | **0.012** | |

Bold= statistically significant, *= $p<0.05$, ***=$p<0.001$

## DISCUSSION

The current research has highlighted the indispensable role of D-dimer as a reliable medical marker for monitoring the health status of COVID-19 patients. Specifically, among COVID-19-infected patients with elevated D-dimer. Statistical analysis revealed an Odds Ratio of 3.949, $p<0.001$ of having high D-dimer levels in COVID-19 patients indicating a higher risk of developing an intravascular coagulopathy. Substantially, COVID-19 infections have caused an increment in D-dimer levels up to six-fold higher than the non-infected samples during the study. Concomitantly, Lippi et al. measured the levels of D-dimer in a group of healthy populations and they reported levels less than 500 ng/ml [26]. The hallmark of the current study is the abundance of mutation in Factor V in the study population around 80% of the study samples have shown mutation in this Factor V gene and interestingly 100% of the mutations are associated with high D-dimer levels (r=0.228, $p<0.012$). The Odds Ratio for Factor V mutation and D-dimer levels was 4.37, $p<0.001$ which highlights the deleterious implication of genetic disorders in Factor V gene in increasing the potential risk of developing a thrombus formation in COVID-19 patients. Likewise, an exponential increase in levels of D-dimer in COVID-19 patients with mutant Factor V samples in comparison with COVID-19 patients without Factor V mutation [27]. Likewise, a study published BY Arsov et al., 2006 conducted on 390 participants has aroused the potential effect of Factor V mutation on escalating D-dimer levels [28]. FVL is a genetic disorder emanating from the superseding of arginine residue with a glutamine residue at position 506 in the factor V protein. This mutation leads to a hamper in the inactivation of the coagulation cascade by the activated protein C, which is a key regulator of the coagulation system. As a consequence, patients with the FVL mutation are exposed to a higher risk of thrombosis, or developing blood clots in their circulation [29]. The regulation process between factor V and protein C is dysregulated by FVL mutation and the tendency for thrombus formation is escalated 10 folds, and mutant factor V [30]. The pertinent correlation between COVID-19 infections and D-dimer levels is ultimately essential in severe cases, as high levels of D-dimer can be implemented as a prognostic indicator for early diagnosis of DVT and other clotting disorders [31].

Further studies evaluated the direct correlation between FVL mutations and elevations in D-dimer levels in a large population cohort study has concluded significantly higher levels of D-dimer in individuals with FVL mutation than individuals without the mutation [32]. This endows a robust correlation both increased risk of VTE incidence, elevated levels of D-dimer, and FVL mutation [32, 33].

Moreover, The age variable could significantly prominent the D-dimer levels, in concordance to our study, an increase of 1.579 for Odds Ratio was established for the risk of developing high D-dimer. The implications associated with the emergence of fibrin degradation products as the D-dimer protein in the events of coagulative disorders such as pulmonary embolism, DVT, and myocardial infarction [34]. A converge point of age-dependent factors may indirectly affect D-dimer levels such as kidney function failure due to geriatric, where the kidney is responsible for removing the D-dimer from the blood. This concludes that with increasing age, there may be a slight tendency for D-dimer levels to rise [35]. Regrettably, the implications of geriatric on D-dimer levels are deleterious and the conditions aroused by high D-dimer levels could be treated by several variables, including medical intervention and lifestyle modification [34].

In the current study, an internal comparison between the risk of developing high D-dimer levels in older people and young patients showed a significant escalation in D-dimer levels related to the increase in the age of the study group. There was an ascertainment of around 1.12 odds ratio in the levels of D-dimer above 500 ng/ml, especially in the ages between 35 and 60. The infected sample ages ranged from newborn to elderly people where 33.3% of

infected samples were under the age of 35 and two-thirds were above 35 years old; indicating a higher potential to get infected for mid-aged and above. A study by Statsenko *et al.* in 2022 found that older patients were more susceptible to severe cases of COVID-19 than their younger peers, with a greater likelihood of being admitted to the intensive care unit [36]. Another study that emphasizes the same result is conducted by Bruine de Bruin who summarized higher risks for mortality in older adults in relevance to a coagulopathy disorder [37]. According to the World Health Organization (WHO), aging is a substantial threat factor for the pathogenesis of COVID-19 patients, with older ages being vulnerable to severe illness consequences [38]. Numerous studies conducted during the pandemic demonstrated a direct correlation between age and the likelihood of contracting COVID-19 severity and mortality [39, 40].

## CONCLUSION

The results of this investigation confirm the link between the FVL mutation and high D-dimer levels in COVID-19 patients. Variable demographic factors as age incorporate a higher threat of developing deleterious coagulopathy events. Further concise research is crucial to investigate the underlying mechanisms of D-dimer elevation in Factor V mutant patients. In addition, the authors highlight the potent implementation of FVL mutation as a prognostic marker for D-dimer levels in COVID-19 patients.

**Author Contributions**

The research was designed by Walid AbuRayyan and O'la Ahmad AL-Fawares, with sample collection and experimentation handled by M. M. Albalbaki. The data interpretation and analysis was done by Nesrin Seder. The final version of the manuscript was approved by all authors who drafted it.

**Declaration of Conflicting Interests**

Regarding the research, writing, and/or publication of this article, the author(s) have disclosed no potential conflicts of interest.

**Ethical Approval**

The study has been approved by the Institutional Review Board (IRB) of Al-Balqa Applied University (26-3-1781).

**Funding**

The study was carried out at the expense of the researcher.

**Supplementary material:**

This manuscript included the supplementary file.

## REFERENCES

1. Yao Y., Cao J., Wang Q., et al. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case-control study. *Journal of Intensive Care* 2020;8:1–11.
2. Obeyat A.H.M. Association between ABO blood group system and the severity of COVID-19 in the West Bank: A case-control study. *Al-Quds University* 2021.
3. Soni M., Gopalakrishnan R., Vaishya R., et al. D-dimer level is a useful predictor for mortality in patients with COVID-19: Analysis of 483 cases. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 2020;14(6):2245–2249.
4. Taha M., Mansour I. Effect of COVID-19 on liver enzymes in hospitalized COVID-19 patients in the Gaza Strip: A retrospective study. *Jordan Journal of Pharmaceutical Sciences* 2023;258–267.
5. Iba T., Levy J.H., Levi M., et al. Coagulopathy in COVID-19. *Journal of Thrombosis and Haemostasis* 2020;18(9):2103–2109.
6. Al-Qerem W., Safar M., Safar I., et al. Identifying factors associated with increased rate of mortality of COVID-19 patients among Jordanian population: A multicenter case-controlled retrospective study. *Jordan Journal of Pharmaceutical Sciences* 2023;16(2):483–483.

7. The Lancet Haematology. COVID-19 coagulopathy: an evolving story. *The Lancet Haematology* 2020;7(6):e425.
8. Rayyan W.A., Hazzaa W.A., Seder N. The implications of COVID-19 infection on hematologic parameters and coagulation activity: A review. *Biomedical & Pharmacology Journal* 2022;15(4):1837–1851.
9. Sughayer M.A., Mansour A., Al Nuirat A., et al. Dramatic rise in seroprevalence rates of SARS-CoV-2 antibodies among healthy blood donors: The evolution of a pandemic. *International Journal of Infectious Diseases* 2021;107:116–120.
10. Uitte de Willige S., de Visser M.C., Houwing-Duistermaat J.J., et al. Genetic variation in the fibrinogen gamma gene increases the risk for deep venous thrombosis by reducing plasma fibrinogen γ′ levels. *Blood* 2005;106(13):4176–4183.
11. Johnson S.E., Pai E., Voroba A., et al. Examining D-dimer and empiric anti-coagulation in COVID-19–related thrombosis. *Cureus* 2022;14(7).
12. Farasani A. Biochemical role of serum ferritin and D-dimer parameters in COVID-19 diagnosis. *Saudi Journal of Biological Sciences* 2021;28(12):7486–7490.
13. Al-Ibadah M., Al-Qerem W. Investigating factors impacting hospitalization duration in COVID-19 patients: A retrospective case-control study in Jordan. *Jordan Journal of Pharmaceutical Sciences* 2025;18(1):1–9.
14. Tang N., Li D., Wang X., et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *Journal of Thrombosis and Haemostasis* 2020;18(4):844–847.
15. Godier A., Clausse D., Meslin S., et al. Major bleeding complications in critically ill patients with COVID-19 pneumonia. *Journal of Thrombosis and Thrombolysis* 2021;52:18–21.
16. Zhu J., Pang J., Ji P., et al. Elevated interleukin-6 is associated with severity of COVID-19: A meta-analysis. *Journal of Medical Virology* 2021;93(1):35.
17. Bosso M., Thanaraj T.A., Abu-Farha M., et al. The two faces of ACE2: the role of ACE2 receptor and its polymorphisms in hypertension and COVID-19. *Molecular Therapy – Methods & Clinical Development* 2020;18:321–327.
18. Rayyan W.A. Seroprevalence of SARS-CoV-2 antibodies among Jordanian citizens: A cross-sectional study of the demographic and clinical factors that ameliorate serum IgG concentration. *Journal of Applied Pharmaceutical Science* 2022;12(11):151–156.
19. Deme D., Kovacs S., Telekes A. Overall survival prediction of advanced cancer patients by selection of the most significant baseline serum biomarker combination. *Pathology & Oncology Research* 2022;28:1610004.
20. Konstantinides S.V., Meyer G., Becattini C., et al. 2019 ESC Guidelines for the diagnosis and management of acute pulmonary embolism. *European Heart Journal* 2020;41(4):543–603.
21. Seder N., Rayyan W.A., O'la Al-Fawares M.H., et al. Pseudomonas aeruginosa virulence factors and antivirulence mechanisms to combat drug resistance: A systematic review. *Sapporo Medical Journal* 2023;10:11.
22. Albalbaki M.M., Al-Fawares O., Aburayyan W., et al. The correlation of gene mutation of coagulopathy cascade with elevated D-dimer levels in COVID-19 patients. *Journal of Applied Pharmaceutical Science* 2023;0(0):0. http://doi.org/10.7324/JAPS.2023.145308
23. Yusuf M., Gupta A., Kumar A., et al. Mechanism and pathophysiology of activated protein C-related Factor V Leiden in venous thrombosis. *Asian Journal of Transfusion Science* 2012;6(1):47.
24. Aburayyan W.S. High-throughput RNA extraction method for *P. aeruginosa* and *S. pyogenes* biofilms. *Journal of Applied Pharmaceutical Science* 2023;13(3):097–105.
25. Seder N., Abu Bakar M.H., Abu Rayyan W.S. Transcriptome analysis of *Pseudomonas aeruginosa* biofilm following exposure to Malaysian stingless bee honey. *Advances in Applied Bioinformatics and Chemistry* 2021;14:1–11. 10.2147/AABC.S292143
26. Lippi G., Plebani M., Henry B.M. Thrombocytopenia is associated with severe COVID-19 infections: a meta-analysis. *Clinica Chimica Acta* 2020;506:145–148.
27. Eppenberger D., Nilius H., Anagnostelis B., et al. Factor V Leiden mutation as a risk factor for recurrent venous thromboembolism: A systematic review and meta-analysis. *Frontiers in Cardiovascular Medicine* 2022;9:883986.

28. Arsov T., Miladinova D., Spiroski M. Factor V Leiden is associated with higher risk of deep venous thrombosis of large blood vessels. *Croatian Medical Journal* 2006;47(3):433–439.
29. Lee S., Lee C.H., Seo M.S., et al. Integrative analyses of genes about venous thromboembolism: An umbrella review of systematic reviews and meta-analyses. *Medicine* 2022;101(43).
30. Hamedi B., Feulefack J., Khan A., et al. Association between Factor V Leiden mutation and recurrent pregnancy loss in Middle Eastern countries: A Newcastle–Ottawa meta-analysis. *Archives of Gynecology and Obstetrics* 2020;302:345–354.
31. Nasif W.A., El-Moursy Ali A.S., Hasan Mukhtar M., et al. Elucidating the correlation of D-dimer levels with COVID-19 severity: A scoping review. *Anemia* 2022;2022.
32. Wang H., Rosendaal F.R., Cushman M., et al. D-dimer, thrombin generation, and risk of a first venous thrombosis in the elderly. *Research and Practice in Thrombosis and Haemostasis* 2021;5(5):e12536.
33. Bikdeli B., Madhavan M.V., Jimenez D., et al. COVID-19 and thrombotic or thromboembolic disease: Implications for prevention, antithrombotic therapy, and follow-up. *Journal of the American College of Cardiology* 2020;75(23):2950–2973.
34. Kumar K., Afonso L. Cardiovascular risk prediction in the elderly. *Journal of Geriatric Cardiology (JGC)* 2019;16(5):414.
35. Fan B.E., Umapathi T., Chua K., et al. Delayed catastrophic thrombotic events in young and asymptomatic post-COVID-19 patients. *Journal of Thrombosis and Thrombolysis* 2021;51:971–977.
36. Statsenko Y., Al Zahmi F., Habuza T., et al. Impact of age and sex on COVID-19 severity assessed from radiologic and clinical findings. *Frontiers in Cellular and Infection Microbiology* 2022;11:1395.
37. Bruine de Bruin W. Age differences in COVID-19 risk perceptions and mental health: Evidence from a national US survey conducted in March 2020. *The Journals of Gerontology: Series B* 2021;76(2):e24–e29.
38. WHO. WHO Coronavirus (COVID-19) Dashboard 2019; Available from: https://covid19.who.int/
39. Poudel A., Poudel Y., Adhikari A., et al. D-dimer as a biomarker for assessment of COVID-19 prognosis: D-dimer levels on admission and its role in predicting disease outcome. *PLOS One* 2021;16(8):e0256744.
40. Wolfe K., Sirota M., Clarke A.D. Age differences in COVID-19 risk-taking and the relationship with risk attitude and numerical ability. *Royal Society Open Science* 2021;8(9):201445.

# انتشار وآثار طفرة العامل الخامس rs6687813 على مستويات دي–ديمر بين مرضى كوفيد–19

***وليد أبو ريان[1]* ، علا أحمد الفوارس [2]، محمد منصور البعلبكي[1]، نسرين سدر[3]***

[1]قسم التحاليل الطبية المخبرية، كلية العلوم الطبية المساندة، جامعة البلقاء التطبيقية، السلط، الأردن.

[2]قسم العلوم الحياتية التطبيقية، كلية العلوم ، جامعة البلقاء التطبيقية، السلط، الأردن.

[3]قسم الكيمياء الصيدلية وعلم العقاقير، كلية الصيدلة، جامعة العلوم التطبيقية الخاصة، عمان، الأردن.

## ملخص

اجتاح فيروس كوفيد–19 المجتمع البشري في جميع أنحاء العالم، مهددًا الصحة العامة والاقتصاد العالمي. وقد أثارت البيانات المُربكة حول آلية تطور المرض نظريات تنبؤية تتعلق بآثار البيانات الديموغرافية والتنوع الجيني لتشخيص المرض. شارك في الدراسة الحالية 120 مشاركًا؛ 68 ذكرًا و52 أنثى، تتراوح أعمارهم بين 15 و82 عامًا، من بينهم 90 مريضًا مصابًا بكوفيد–19، و30 مشاركًا سليمًا. تم سحب عينات دم من 120 مشاركًا، ثم تم تحليلها لمستويات دي–دايمرباستخدام مقايسة المناعة الفلورية. تم تضخيم عينات الحمض النووي الـ 120 المستخرجة بواسطة تفاعل البوليميراز المتسلسل (PCR) باستخدام بادئات خاصة بالعامل الخامس. تم تسلسل نواتج تفاعل البوليميراز المتسلسل (PCR) وفحصها للكشف عن الطفرات المحتملة في جين العامل الخامس. وُجد معدل طفرة مرتفع بشكل ملحوظ في العامل الخامس (rs6687813) بنسبة 82%، قيمة P<0.001 في عينة الدراسة. وارتبطت الطفرات بشكل ملحوظ بمستويات عالية للغاية من دي–دايمر، بلغت 2975 نانوغرام/مل، مقابل 986 نانوغرام/مل في النوع البري (r=0.228، قيمة P<0.012). أظهر مرضى كوفيد–19 المنومون في المستشفيات مستويات أعلى بكثير من دي–دايمر، مقارنةً بالمرضى غير المنومين، حيث بلغت 3353 نانوغرام/مل مقابل 513 نانوغرام/مل، قيمة P<0.001، على التوالي. وكان معدل احتمال الإصابة بعدوى كوفيد–19 للعمر 1.12، حيث كان من تزيد أعمارهم عن 35 عامًا أكثر عرضة للإصابة بالعدوى من الفئات العمرية الأصغر (r=0.21، قيمة P<0.022). في الختام، تُقدم الدراسة أدلة قوية على الارتباط المباشر بين طفرة العامل الخامس وارتفاع مستويات دي–دايمر لدى مرضى كوفيد–19، ويُوصى بتطبيق اختبار طفرة العامل الخامس كعلامة تشخيصية لمرضى كوفيد–19.

**الكلمات الدالة:** العامل الخامس، دي–دايمر ، كوفيد–19، الأردن، الطفرة، تعدد أشكال النوكليوتيدات المفردة.

---

* ***المؤلف المراسل: وليد أبو ريان***

Walid.aburayyan@bau.edu.jo

تاريخ استلام البحث 2024/05/16 وتاريخ قبوله للنشر 12/12/2024.