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TIME-DEPENDENT VARIABILITY OF D-DIMER LEVEL IN BLOOD SAMPLE OF COVID POSITIVE PATIENT

Original Research

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ABSTRACT

Background: Coronavirus disease 2019 (COVID-19) has emerged as a global health crisis, causing significant morbidity and mortality. Beyond its respiratory manifestations, it has profound systemic effects, particularly on the cardiovascular system, where it predisposes patients to hypercoagulability and disseminated intravascular coagulation (DIC). D-dimer, a fibrin degradation product, is widely used as a biomarker to assess thrombotic activity and disease progression. Establishing whether storage time influences D-dimer values is essential for ensuring laboratory accuracy and reliability, especially under strained healthcare conditions.

Objective: This study aimed to evaluate the effect of short-term storage time on D-dimer levels in samples from COVID-19–positive patients compared with healthy controls.

Methods: A case–control study was conducted at Hayatabad Medical Complex, Peshawar, between June and August 2021. A total of 100 participants were enrolled, including 50 confirmed COVID-19–positive patients and 50 healthy controls. Venous blood samples (2 mL) were collected in sodium citrate tubes from the antecubital vein of each participant and analyzed using a Cobas c501 analyzer. Samples were tested immediately (0 hours), after 4 hours, and after 8 hours at room temperature. Patients with comorbidities were excluded. Paired sample t-tests were applied to assess time-related differences in D-dimer values, and Pearson correlation was used to explore associations between time and D-dimer levels.

Results: In COVID-19–positive patients, the mean baseline D-dimer level was 3.77 ± 3.71 µg/mL FEU, increasing slightly to 3.81 ± 3.73 at 4 hours and 4.00 ± 3.80 at 8 hours. The corresponding two-tailed p-values were 0.366 and 0.174, both above the 0.05 threshold, indicating no statistically significant variation over time. Controls maintained consistently low D-dimer levels (0.69 ± 0.40 at baseline, 0.68 ± 0.39 at 4 hours, and 0.70 ± 0.39 at 8 hours) with p-values of 0.159. Pearson correlation revealed a weak, clinically negligible association between time and D-dimer levels.

Conclusion: D-dimer values in COVID-19–positive samples remained stable over 8 hours of storage at room temperature, confirming that short-term delays in analysis do not compromise test accuracy. This stability enhances the practical utility of D-dimer testing in clinical laboratories, particularly during peak workloads in pandemics.

Keywords: COVID-19, D-Dimer, Fibrinolysis, Prognosis, SARS-CoV-2, Thrombosis, Time Factors.

INTRODUCTION

The coronavirus is an enveloped, positive-stranded RNA virus belonging to the viridae family, known for causing a spectrum of illnesses ranging from the common cold to severe respiratory syndromes (1,2). Earlier outbreaks of coronaviruses, including Severe Acute Respiratory Syndrome (SARS) in 2002–2003 and Middle East Respiratory Syndrome (MERS) in 2012, highlighted their pandemic potential, with SARS alone affecting 29 countries and causing over 8,000 cases and 770 deaths (2-4). At the end of 2019, a cluster of pneumonia cases of unknown origin was reported in Wuhan, China, later identified as Coronavirus Disease 2019 (COVID-19), which spread rapidly across the globe, including the first confirmed case in North America in January 2020 and in Pakistan by February 2020 (5,6). Since then, the disease has become a global health emergency, with more than 33 million reported cases worldwide by mid-2021 (7). Structurally, coronaviruses are characterized by spike-like glycoproteins essential for binding and entry into host cells (8,9). SARS-CoV-2, the causative agent of COVID-19, possesses one of the largest viral RNA genomes, ranging between 30–32 kb, with unique end caps and poly-A tails that contribute to its replication and pathogenicity (10,11). The infection manifests with diverse clinical presentations, from asymptomatic or mildly symptomatic individuals to those with severe respiratory failure and multi-organ involvement. Pneumonia with bilateral infiltrates remains the most critical clinical feature, though gastrointestinal manifestations such as diarrhea and nausea are also observed (12–14). The asymptomatic ratio is difficult to quantify, as undiagnosed carriers contribute significantly to community transmission through droplets, aerosols, and fomites (13,14).

The incubation period typically spans 7–10 days, though re-exposure may extend this period, leading to the current recommendation of 14 days of observation for exposed individuals (14,15). Beyond respiratory complications, COVID-19 has been linked to systemic effects, particularly hypercoagulability. The disease induces a cytokine storm that triggers coagulation pathways, elevating D-dimer levels and predisposing patients to thromboembolic events such as deep vein thrombosis and disseminated intravascular coagulation (14,16). D-dimer testing therefore plays a vital role in prognosis, especially among critically ill patients, as elevated levels often indicate poor clinical outcomes (16). Despite significant advances in understanding the epidemiology, virology, and clinical manifestations of COVID-19, substantial gaps remain in predicting disease severity, guiding timely interventions, and reducing mortality. This study aims to investigate the prognostic significance of D-dimer levels in patients with COVID-19, with the objective of establishing their role as a reliable biomarker for early risk stratification and improved clinical management.

METHODS

The present study was designed as a case–control study and was conducted at the Hayatabad Medical Complex, Peshawar, between June 2021 and August 2021. A total of 100 participants were enrolled, comprising 50 patients admitted to the intensive care unit (ICU) with confirmed SARS-CoV-2 infection and 50 healthy individuals who served as controls. The diagnosis of COVID-19 in the patient group was established using real-time qualitative polymerase chain reaction (RT-qPCR). To reduce confounding factors, strict inclusion and exclusion criteria were applied. Only ICU patients without any known comorbidities were included, while those with comorbid conditions were excluded. Similarly, the control group consisted exclusively of healthy, COVID-19–negative individuals without comorbidities. Individuals with SARS-CoV-2 infection and comorbidities, as well as those with comorbidities in the absence of infection, were excluded from participation. For sample collection, two milliliters of venous blood were drawn aseptically from the antecubital vein of each participant into sodium citrate tubes. The samples were transported to the laboratory using an appropriate transport medium and maintained at room temperature. Following centrifugation, plasma was separated for the assessment of D-dimer levels. The testing protocol was standardized and included three runs: the first analysis was performed immediately after sample collection (0 hours), the second after storage for 4 hours, and the third following an additional 4 hours of storage (8 hours in total). Identical handling and testing procedures were applied to both case and control groups to ensure consistency and minimize bias. D-dimer levels were quantified using routine laboratory methods. Ethical approval was obtained from the institutional review board of Hayatabad Medical Complex. Written informed consent was obtained from all participants or their legal guardians prior to enrollment, ensuring adherence to ethical standards for human research in accordance with the Declaration of Helsinki.

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RESULTS

The study enrolled 100 participants, equally divided between COVID-19–positive patients and healthy controls. Within the control group, 9 participants (18.0%) were male and 41 (82.0%) were female, whereas in the case group, 26 (52.0%) were male and 24 (48.0%) were female. Age distribution analysis showed that 8 participants (8.0%) were between 18–30 years, 57 participants (57.0%) were between 31–60 years, and 35 participants (35.0%) were above 60 years. The D-dimer values in the control group remained stable across all time intervals. At baseline, the mean D-dimer level was 0.6884 ± 0.40429 µg/mL FEU. After 4 hours, the mean was 0.6827 ± 0.39488, and after 8 hours, it was 0.6960 ± 0.39525. Paired-sample analysis revealed no statistically significant difference in values at baseline compared with 4 hours ($p = 0.159$) or 8 hours ($p = 0.159$). Among COVID-19–positive patients, the baseline D-dimer level was 3.7718 ± 3.71755 µg/mL FEU. After 4 hours, the mean level was 3.8061 ± 3.73460, and after 8 hours it increased slightly to 4.0030 ± 3.80267. However, the paired-sample t-test again demonstrated no statistically significant difference in D-dimer levels between baseline and 4 hours ($p = 0.366$) or baseline and 8 hours ($p = 0.174$) Correlation analysis between D-dimer levels and time indicated a weak, non-significant association in COVID-19–positive patients ($r = 0.013$, $p = 0.874$). A slight but statistically significant correlation was observed between patient and control D-dimer values ($r = 0.189$, $p = 0.021$). Overall, these findings confirm that time did not significantly influence D-dimer levels in either group, and the null hypothesis was retained.

When absolute D-dimer levels were compared between COVID-19–positive patients and healthy controls, a marked difference was observed. At baseline, the mean D-dimer level in patients was 3.77 ± 3.71 µg/mL FEU, whereas the control group demonstrated a mean of only 0.69 ± 0.40. This significant disparity persisted across subsequent time intervals, with patients showing means of 3.81 ± 3.73 after 4 hours and 4.00 ± 3.80 after 8 hours, compared to 0.68 ± 0.39 and 0.70 ± 0.39 in controls. These findings indicate that COVID-19 infection was consistently associated with substantially elevated D-dimer concentrations, independent of sample stability over time. While stability testing revealed no significant time-dependent variability within each group, the absolute intergroup difference strongly supports the potential prognostic value of D-dimer in distinguishing infected patients from healthy individuals. However, results linking D-dimer levels to ICU outcomes, severity grading, or thromboembolic complications were not analyzed, leaving the prognostic interpretation incomplete.

Table 1: Gender-based distribution of control group and COVID-19–positive cases

Gender	Control group	Percentage (%)	Cases	Percentage (%)
Male	9	18.0	26	52.0
Female	41	82.0	24	48.0
Total	50	100	50	100

Table 2: Age-wise distribution of COVID-positive control group

Age	Case-control patients	Percentage (%)
18-30	8	8.0
31-60	57	57.0
above 60	35	35.0
Total	100	100.0

Table 3: One sample statistic of the control group

	N	Mean	Std. Deviation	Std. Error Mean
Control result Time After 8hours	50	.6960	.39525	.05590
Control result Time After 4 hours	50	.6827	.39488	.05584
Control result Time-0	50	.6884	.40429	.05717

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Table 4: One sample statistic of COVID positive patient

	N	Mean	Std. Deviation	Std. Error Mean
Patient results in Time-0	50	3.7718	3.71755	.52574
Patient result Time After 4 hours	50	3.8061	3.73460	.52815
Patient result Time After 8 hours	50	4.0030	3.80267	.53778

Table 5: Paired sample test of the control group

Paired Samples Test									
		Paired Differences					T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	1st result - 2nd result	-.040	.198	.028	-.096	.016	-1.429	49	.159
Pair 2	1st result - 3rd result	-.040	.198	.028	-.096	.016	-1.429	49	.159

Table 6: Paired sample test of COVID-19-positive patient

Paired Samples Test									
		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Immediate result vs. 2ND RESULT	-.0343400	.2659763	.0376147	-.1099296	.0412496	-.913	49	.366
Pair 2	Immediate result - 3RD RESULT	-.2312000	1.1860716	.1677359	-.5682778	.1058778	-1.378	49	.174

Table 7: Two-tailed Pearson correlation between the time and patients

Correlations				
		Patients D Dimer	Time	Control D Dimer
Patients D Dimer	Pearson Correlation	1	.013	.189*
	Sig. (2-tailed)		.874	.021
	N	150	150	150
Time	Pearson Correlation	.013	1	.029
	Sig. (2-tailed)	.874		.721
	N	150	150	150
Control D Dimer	Pearson Correlation	.189*	.029	1
	Sig. (2-tailed)	.021	.721	
	N	150	150	150

*. Correlation is significant at the 0.05 level (2-tailed).

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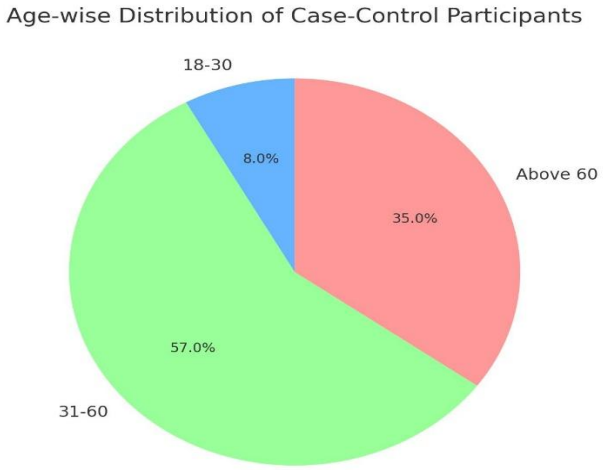


Figure 1 Age-wise Distribution of Case-Control Participants

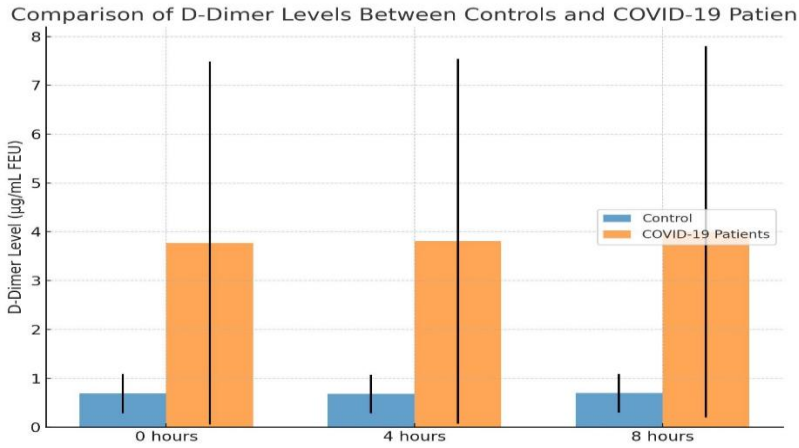


Figure 2 Comparison of D-Dimer Levels Between Control and COVID-19 Patients

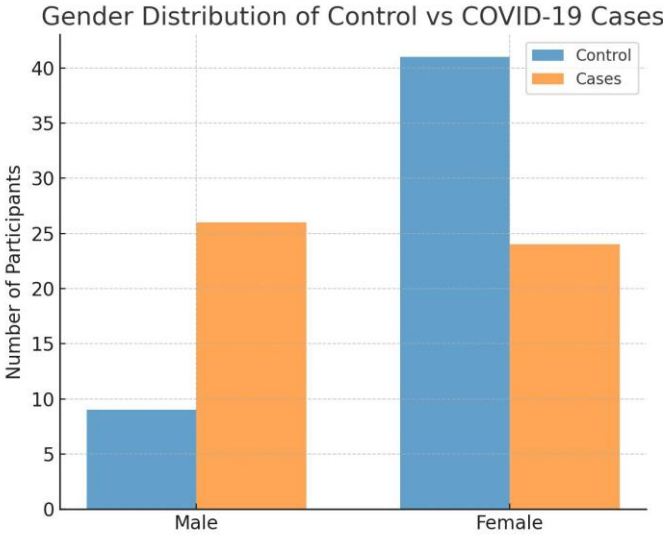


Figure 3 Gender Distribution of Control vs COVID-19 Cases

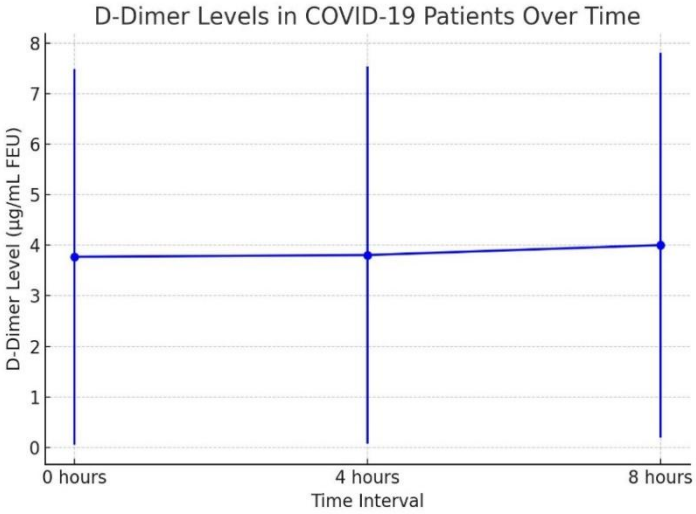
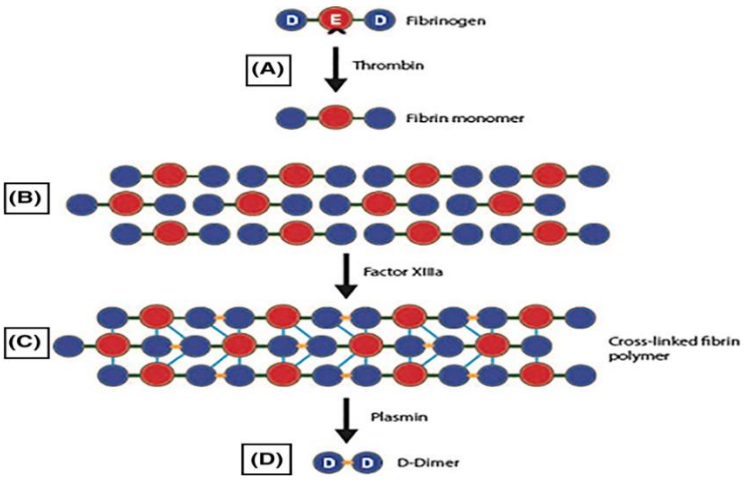


Figure 4 D-Dimer Levels in COVID-19 Patients Over Time



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DISCUSSION

D-dimer is a well-established marker of fibrinolytic activity, widely used in the diagnosis and monitoring of venous thromboembolism prior to the emergence of COVID-19. The present study focused on evaluating the effect of time on D-dimer stability in samples collected from COVID-19–positive patients. The findings demonstrated that D-dimer levels did not vary significantly over short-term storage, with mean values remaining consistent at 0, 4, and 8 hours. Statistical testing confirmed no significant changes, with p-values of 0.366 and 0.177 at 4 and 8 hours respectively, both well above the conventional threshold of 0.05. This suggests that D-dimer testing in COVID-19 samples can be reliably performed within this time frame without concern for degradation or loss of accuracy. These findings are consistent with previous reports that examined D-dimer stability under different storage conditions. Studies investigating plasma storage at room temperature have shown minimal change within the first 24 hours, with significant variability only emerging after extended durations such as 120 hours (15,16). Similar evidence indicated that percentage changes in D-dimer values remained below 10% up to 24 hours of storage, even at room temperature, supporting the conclusion that short-term storage does not compromise sample integrity (17,18). Comparable results have been observed when samples were kept at slightly elevated temperatures, where variations after 24 hours still remained within acceptable ranges (19). The study also found a weak positive correlation between D-dimer values and time in both patient and control groups. However, this association was clinically negligible, reinforcing the conclusion that storage time within the studied interval does not influence D-dimer results. Importantly, this suggests that in clinical practice, COVID-19 samples can be safely stored for up to 8 hours before testing, which provides practical flexibility in laboratory workflows, particularly under resource-constrained settings (20,21).

One of the strengths of this study was the controlled design, in which COVID-19–positive patients were compared against healthy controls under identical laboratory conditions. The repeated testing protocol allowed for careful observation of D-dimer trends over time. However, several limitations must be acknowledged. The analysis was restricted to an 8-hour observation period due to limited resources, which does not allow for conclusions regarding longer storage intervals. Furthermore, while the study established stability, it did not extend to evaluating the prognostic implications of absolute D-dimer levels in relation to disease severity, thromboembolic complications, or patient outcomes. The exclusion of patients with comorbidities, though methodologically aimed at reducing confounding, also limits the generalizability of results, as most critically ill COVID-19 patients often present with multiple comorbidities. Overall, the findings confirm that D-dimer values in COVID-19–positive samples remain stable for at least 8 hours at room temperature. This supports the reliability of delayed testing within this period, which is of practical significance in clinical laboratories. Future studies should extend the observation window to 16 and 24 hours and incorporate larger sample sizes to assess long-term stability (22). In addition, research linking D-dimer concentrations with clinical outcomes such as ICU stay, severity indices, and thromboembolic events would be essential to establish the true prognostic role of this biomarker in COVID-19.

CONCLUSION

This study concluded that the stability of D-dimer values in COVID-19–positive samples was not influenced by short-term storage, indicating that time had no marked effect on the accuracy of results within the tested duration. These findings highlight the reliability of D-dimer testing in routine clinical practice, even when immediate analysis is not possible, and underscore its practical utility in managing COVID-19 patients. By confirming that sample integrity is maintained over several hours, this research provides valuable assurance for laboratories and clinicians working under time and resource constraints, supporting the continued use of D-dimer as a dependable biomarker in the assessment of COVID-19.

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AUTHOR CONTRIBUTION

Author	Contribution
Said Wali	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Emad Ud Din	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Umar Gul	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Rizwan Ullah	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Abdur Rehman*	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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