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RESEARCH ARTICLE

Evaluation of Immune-Inflammatory and Other Biomarkers in Severe COVID-19 Patients with Diabetes Mellitus: A Cross-Sectional Study

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ABSTRACT:

Diabetes mellitus (DM) is a risk factor for severe coronavirus disease 2019 (COVID-19) and even death. This study assessed the blood levels of immune-inflammatory and other biomarkers in severe COVID-19 diabetic patients and identified possible predictive biomarkers for disease severity. The cross-sectional study was conducted in a tertiary hospital in Gaza from 15 February 2022 to 15 July 2022. The study patients were in three Groups (each group 14 patients): ICU severe COVID-19 diabetic patients (Group 1), non-hospitalized non-diabetic patients with mild/moderate COVID-19 (Group 2) and non-COVID-19 diabetic patients (Group 3). The evaluated blood biomarkers and statistically analyzed were CRP, LDH, PCT, Ang-II, IL-6, ferritin, ESR (immune-inflammatory biomarkers), D-dimer (coagulative biomarker), CK-MB and cTn I (cardiac biomarkers), AST, ALT (liver biomarkers), HbA1c, RBC, HGB, WBC and LYM (hematologic biomarkers). ICU diabetic patients (Group 1) had the highest average age. Group 1 patients had significantly more elevated CRP, LDH, PCT, AngII, Ferritin, IL-6 and ESR, D-dimer and CK-MB levels than the other two groups at $P< 0.05$. Group 2 patients showed higher levels than Group 3 patients. Haematological biomarkers, except WBC, were higher among patients in Group 3 than in the other two groups. There was no significant difference between the three patient groups regarding AST and ALT levels. The study results showed a high association between LDH, PCT, Ang II, ferritin, IL-6, CK-MB, cTn I, D-dimer and LYM levels and the severity of COVID-19 in diabetic patients. These findings support the notion that DM can lead to the rapid progression of COVID-19 and make it more prone to an inflammatory cytokine storm and cardiac and coagulation problems, which could lead to various organ failures and a bad prognosis of COVID-19.

KEYWORDS: COVID-19, diabetes, biomarkers, IL-6, immune-inflammatory, D-dimer, ICU.

INTRODUCTION:

COVID-19 patients most frequently suffer from fever, cough, expectoration, fatigue, dyspnea, conjunctivitis, malaise, myalgia and pneumonia. However, patients may present symptoms like headache, hemoptysis and diarrhoea. Most patients (up to 80%) develop mild symptomatic disease or remain asymptomatic, while up to 10 – 20% develop severe pneumonia. Almost 5% of the cases develop acute respiratory distress syndrome (ARDS), septic shock and multiple organ failure[1,2,3]. Biomarkers are protein molecules that occur naturally in humans through which

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pathological or disease conditions can be diagnosed and monitored[4]. Biomarkers could recognize COVID-19 patients suffering rapid progression or severe complications that could lead to death. However, understanding the virus pathogenesis and cellular or organ damage mechanisms is essential to identify valuable biomarkers[5]. The immune/inflammatory responses play a critical role in the progression of COVID-19. The inflammatory reactions triggered by rapid viral replication and cellular destruction can stimulate monocytes and macrophages and excessively release cytokines, causing a cytokine storm that further activates other immune responses, causing exacerbations[6,7]. The immune system overactivation in severe COVID-19 results in the release of large amounts of cytokines into the blood, particularly interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), triggering diverse local and systemic inflammatory responses. Therefore, several immune-inflammatory biomarkers, such as IL-6, are valued for indicating disease COVID-19 severity and fatality[8,9]. In addition, severe COVID-19 has been significantly associated with high levels of biomarkers like procalcitonin (PCT), serum ferritin, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)[10,11]. Thus, efficient biomarkers would be helpful for early detection and rapid management, avoiding severe complications and reducing mortality[6,7]. However, this concept might be controversial as no significant abnormalities existed in some studies[12,13].

Diabetes mellitus (DM) is a metabolic disease resulting from abnormal insulin production, impaired insulin utilization, or both[14,15]. DM and cardiovascular chronic diseases are co-morbidities in COVID-19 patients. Early studies found increased COVID-19 severity in diabetic patients. The immune and inflammatory responses are modulated by hyperglycemia; thus, COVID-19 in diabetic patients develops into severe disease and may lead to death[16,17]. In addition, hyperglycemia directly supports SARS-CoV-2 replication in human monocytes, and glycolysis upholds SARS-CoV-2 reproduction by stimulating hypoxia-inducible factor 1, and thus, reactive oxygen species are produced in the mitochondria[18].

The spike receptor-binding domain (RBD) is an essential peptide in SARS-CoV-2 pathophysiology. The spike RBD enables virus binding to the angiotensin-converting enzyme 2 (ACE2), the primary receptor or entry site for SARS-CoV-2, in the lungs and other tissues[19]. Therefore, SARS-CoV-2 mainly infects the respiratory tract and other vital organs, including the cardiovascular, gastrointestinal tract (GI), hepatobiliary, renal and central neurological systems. However, SARS-CoV-2–induced organ dysfunction is possibly owing to different passways, including direct viral toxicity, ischemic injury (due to vasculitis, thrombosis, or thrombo-inflammation), immunological irregularities, or renin-angiotensin-aldosterone system (RAAS) dysregulation[20]. The complication of SARS-CoV-2 is centred on the unpredictable rapid progress of the disease to severe conditions such as respiratory failure and multi-organ injuries that mainly happen in patients with high-risk factors, such as DM[21]. Although COVID-19 infections increased worldwide, there is limited information on predictive biomarkers of disease severity in diabetic patients. Hence, identifying efficient predictive laboratory biomarkers, particularly in high-risk patients, is necessary for prompt treatment and reducing mortality. Accordingly, this study aimed to assess the performance of immune-inflammatory, cardiac, coagulation, hepatic and haematological biomarkers in severe COVID-19 ICU patients with DM to find potential predictive biomarkers for COVID-19 severity in diabetic patients.

MATERIALS AND METHODS:

Study design and subjects:

It is a cross-sectional study to determine blood levels of essential biomarkers among critically ill COVID-19 ICU patients with DM. The study was conducted at Al-Shifa tertiary care hospital in the Gaza Strip from 15 February 2022 to 15 July 2022 according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Pharmacy, Al-Azhar University-Gaza, Gaza (Ethics code: PHRC/HC/1057/22). The study included 42 adult patients aged ≥ 18 years and a body mass index (BMI) of 25-30. The 42 patients were grouped into three comparable groups regarding gender and age (Table 1). The study groups were Group 1: severe COVID-19 diabetic ICU patients, Group 2: non-hospitalized mild/moderate COVID-19 non-diabetic patients, and Group 3: non-COVID-19 diabetic patients. Groups 1 and 2 patients were confirmed positive for SARS-CoV-2 by RT-PCR using nasopharyngeal swabs (Cobas SARS-CoV-2 kit, Roche; Cobas 6800 Roche). Group 3 patients had not had coronavirus infection for the six months before taking the blood samples nor being vaccinated against COVID-19. The exclusion criteria included patients < 18 years old, vaccinated against COVID-19, with chronic diseases other than DM, or patients taking immunomodulators. Demographic data, co-morbidities and clinical characteristics of patients were collected.

Table 1. Demographic characteristics of study groups of patients.

Groups	Patients groups	No.	Age range	Mean age ±SD	Gender	
					F	M
Group 1	Severe COVID-19 ICU diabetic patients	14	37 – 84	65.9 ± 12.2	7	7
Group 2	Mild/moderate non-hospitalized COVID-19 patients, non-diabetic	14	20 – 85	46.3 ± 17.1	8	6
Group 3	Non-COVID-19 diabetic patients	14	55 – 71	61.1 ± 4.8	9	5
Total No. of patients		42	20 – 85	57.7 ± 14.8	24	18

DM: Diabetes mellitus, SD: Standard deviation, F: Female, M: Male

Study biomarkers and blood sample processing:

The blood biomarkers evaluated in this study included immune-inflammatory biomarkers (CRP, LDH, PCT, Ang-II, IL-6, ferritin, ESR), coagulation biomarkers (D-dimer), cardiac biomarkers (CK-MB, cTn I), liver biomarkers (AST, ALT) and hematologic biomarkers (HbA1c, RBC, HGB, WBC and LYM). The blood sample was collected when the patient was in the symptomatic phase. The categorization of COVID-19 patients was based on WHO guidelines. The criteria for moderate COVID-19 were clinical signs of fever, cough, dyspnea, and fast breathing but no signs of severe pneumonia, including an oxygen saturation ≥ 90%. The criteria for severe COVID-19 were clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following: respiratory rate > 30 breaths/minute, severe respiratory distress, or oxygen saturation < 90%.[1]

Biomarkers laboratory analysis:

The blood sample drawn from each patient was divided into three tubes. One tube is the EDTA tube, which is directly transferred to CBC Orphee® for the CBC test. ESR test was performed manually by adding 0.2ml of ESR solution to 1.8 ml of blood in EDTA and filling in a wintrobe ESR tube at room temperature. The Plane Tube and centrifugation for 4 min at 4000 rpm and then to the automated Diasys® Diagnostic system (respons 920) for the quantitative determination of CK-MB, CRP, LDH, ALT and AST in serum, and to MAGUMI®4000 equipment to measure IL-6, PCT, Cardiac troponin I (cTn I), Ferritin and Angiotensin II. The third tube is a sodium citrate tube, and the blood sample was centrifuged for 10 min at 4000rpm, then to MAGUMI®4000 equipment to perform the D-dimer test. The biochemical parameters studied are summarized in Table 2.

Table 2. Biochemical parameters measured in this study

Biomarker	Test	Measurement method and reference range
Immune-inflammatory biomarkers	C-reactive protein (CRP)	Diasys® Diagnostic photometric system (respons 920). RR: <5 mg/L
	Lactate dehydrogenase (LDH)	Diasys® Diagnostic photometric system (respons 920). RR: < 248 U/L (male); < 247 U/L (female)
	Procalcitonin (PCT)	Quantitative CLIA using MAGUMI®4000 plus from Snibe. RR: < 0.05 ng/ml
	Angiotensin II (Ang II)	Quantitative CLIA using MAGUMI®4000 plus from Snibe. RR: 25-60 pg/ml (lying position); 50-120 pg/ml (standing position)
	Ferritin	Quantitative CLIA using MAGUMI®4000 plus from Snibe. RR: 25-350 ng/ml (male); 13-232 ng/ml (female)
	Interleukins (IL-6)	Sandwich chemiluminescence immunoassay (CLIA) using MAGUMI®4000 equipment from Snibe. Positive if it is above 7.00 pg/ml
	ESR	Measure the rate of fall of RBCs manually in mm after one hour. RR: < 15 mm/h
Coagulation biomarker	D-dimer levels	Quantitative CLIA using MAGUMI®4000 plus from Snibe. RR: < 500 ng FEU/ml
Cardiac biomarkers	Creatine Kinase MB (CK-MB)	Diasys® Diagnostic photometric system (respons 920). RR: > 24 U/L
	Cardiac troponin I (cTn I)	Quantitative CLIA using MAGUMI®4000 plus from Snibe. RR: < 0.1 ng/ml
Liver biomarkers	Alanine aminotransferase (ALT)	Diasys® Diagnostic photometric system (respons 920). RR: < 45 U/L (male); < 34 U/L (female)
	Aspartate aminotransferase (AST)	Diasys® Diagnostic photometric system (respons 920). RR: < 35 U/L (male); < 31 U/L (female)
Haematological markers	Hemoglobin A1c (HbA1c%)	The percentage was measured using HemoCue® HbA1c 501. RR: 4.6 – 6.4 %
	Complete blood count (CBC)	3-DIFF haematological analyzerOrphee® mythic 22. Red blood cells (RBC): (4–6.2)*10^6 µl - Haemoglobin (HGB): (11–17) g/dL White blood count (WBC):(4–12) *10^3 µl - Lymphocytes (LYMs):(1–5) *10^3 µl

RR: reference range, CLIA: chemiluminescence immunoassay, ESR: Erythrocyte sedimentation rate

Data statistical analyses:

Statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS), version 27(IBM Corp., Armonk, NY, USA). For descriptive analysis and tabular presentation, data were presented as numbers and percentages (for qualitative variables) or mean and standard deviations (SD) (for quantitative variables) where appropriate. The *chi*-square or Fisher's exact test was used to compare qualitative categorical variables. The Kruskal-Wallis test was used to examine the difference between categorical variables means of different groups. The student's *t*-test or the Mann–Whitney U test was used to compare quantitative categorical variables between two groups. The significant difference was considered when the *P* value < 0.05.

RESULTS:

Descriptive statistics of biomarker levels among the study groups:

The blood levels of immune-inflammatory biomarkers (CRP, LDH, PCT, AngII, Ferritin, IL-6 and ESR), D-dimer, CK-MB and Tn 1 were significantly higher in Group1 patients compared to the other two groups ($P < 0.05$). The haematological biomarkers (except WBCs) were more elevated in Group 3 than in Groups 1 and 2, with statistically significant differences between the study groups ($P < 0.05$), except for WBCs ($P = 0.178$). There was no significant difference between the three groups ($P > 0.05$) regarding liver biomarkers AST and ALT levels. However, AST and ALT levels were higher in Groups 1 and 3 patients, respectively (Table 3).

Based on the reference range, most patients in the three study groups had abnormally high CRP, LDH, IL-6, ESR and CK-MB levels and an average level of each AST, ALT and Tn 1. However, most patients in Group 1 exhibited more abnormal levels in Ang-II, ferritin, RBC and LYM than in Groups 2 and 3. Regarding D-dimer, most Groups 1 and 2 patients had high levels, but all Group 3 patients showed a normal range. Whereas most Groups 1 and 3 patients had high PCT and HbA1c levels. Most Group 1 and Group 3 patients showed abnormally low levels under the average references of LYM and Ang-II, respectively. The higher percentage of patients in each group showed the normal range of WBCs (Table 4).

Table 3. The descriptive data of each biomarker level in the study groups

Test	Group 1			Group 2			Group 3			[a]*P* value
	Min.	Max.	Mean± SD	Min.	Max.	Mean ± SD	Min	Max.	Mean ± SD	
Immune-inflammatory biomarkers										
CRP	16	120	66.6 ± 27.7	4.6	95	42.7 ± 33.2	5	14	9.14 ± 2.56	0.000*
LDH	340	600	451.4 ± 90.6	276	480	336 ± 61	240	360	306.6 ± 28.5	0.000*
PCT	0.1	2.2	0.69 ± 0.73	0.01	0.15	0.47 ± 0.054	0.01	0.9	0.27 ± 0.29	0.002*
Ang II	77	404	167.8 ± 111.6	15.8	115.3	52.2 ± 29.4	12	70	36.1 ± 17.8	0.000*
Ferritin	350	1312	972 ± 290	24	350	126.9 ± 90.1	15.7	180.0	95.2 ± 59.9	0.000*
IL-6	109.5	1209	736.1 ± 347.6	0.5	64.3	22.5 ± 19.9	2.8	22	10.7 ± 5.6	0.000*
ESR	19	95	55.4 ± 26.3	13	88	37.9 ± 24.1	16	36	22.2 ± 5.4	0.001*
Coagulation biomarker										
D-dimer	323	1555	967.4 ± 418.2	188	1140	636.2 ± 270.8	197	419	309.1 ± 73.9	0.000*
Cardiac biomarkers										
CK-MB	12.1	23	17.6 ± 3.1	11.2	19.4	14.7 ± 2.4	12.9	20.0	15.8 ± 2.1	0.023*
cTn I	0.02	2.1	0.81 ± 0.89	0.01	0.03	0.016 ± 0.008	0.01	0.14	0.054 ± 0.05	0.000*
Liver biomarkers										
ALT	14	40	22.5 ± 9	15	36	23.7 ± 6.7	15	40	25.2 ± 7	0.315
AST	16	55	27.2 ± 12.2	17	34	22.9 ± 5.2	14	35	23.1 ± 5.8	0.980
Haematological markers										
HbA1c (%)	5.6	8.7	7.02 ± 0.78	5.1	6.4	5.6 ± 0.4	6.2	9.8	7.7 ± 0.95	0.000*
RBC (*10^6 µl)	2.4	5.8	3.7 ± 1.0	3.3	5.2	4.2 ± 0.6	3.8	5.9	4.7 ± 0.6	0.007*
HGB (g/dl)	5.8	14.1	10.4 ± 2.2	8.9	13.3	11.5 ± 1.24	10.4	16.9	13.7 ± 1.8	0.000*
WBC (*10^3µl)	4.1	17.1	9.49 ± 3.8	2.9	14.2	7.3 ± 3.04	4.2	9.6	7.01 ± 1.41	0.178
LYMs (*10^3µl)	0.7	0.96	0.84 ± 0.07	0.5	4.5	1.76 ± 1.02	1.3	3.4	2.3 ± 0.6	0.000*

N: Number of participants; SD: Standard deviation, [a]*P* Values were calculated using the Kruskal-Wallis test; *: *P* Value < 0.05 Was considered statistically significant.

Table 4. Distribution of normal and abnormal levels of biomarkers in patient groups compared to reference ranges

Biomarker Test	Result	Group 1		Group 2		Group 3	
		N	%	N	%	N	%
CRP (mg/L)	Normal	-	-	1	7.1	-	-
	Abnormal	14	100	13	92.9	14	100.0
LDH (U/L)	Normal	-	-	-	-	1	7.1
	Abnormal	14	100	14	100	13	92.9
PCT (ng/mL)	Normal	1	7.1	9	64.3	5	35.7
	Abnormal	13	92.9	5	35.7	9	64.3
Ang II (pg/mL)	Normal	4	28.6	2	14.3	3	21.4
	Abnormal	10	71.4	7	50	0	-
	Abnormal*	-	-	5	35.7	11	78.6
Ferritin (ng/mL)	Normal	1	7.1	13	92.9	14	100
	Abnormal	13	92.9	1	7.1	-	-
IL-6 (pg/mL)	Normal	-	-	3	21.4	3	21.4
	Abnormal	14	100	11	78.6	11	78.6

ESR (mm/h)	Normal	-	-	1	7.1	-	-
	Abnormal	14	100	13	92.9	14	100
D-dimer (ng FEU/mL)	Normal	4	28.6	3	21.4	14	100
	Abnormal	10	71.4	11	78.6	-	-
CK-MB (U/L)	Normal	-	-	1	7.1	-	-
	Abnormal	14	100	13	92.9	14	100
cTn I (ng/mL)	Normal	8	57.1	14	100	9	64.3
	Abnormal	6	42.9	-	-	5	35.7
ALT (U/L)	Normal	12	85.7	13	92.9	12	85.7
	Abnormal	2	14.3	1	7.1	2	14.3
AST (U/L)	Normal	9	64.3	12	85.7	13	92.9
	Abnormal	5	35.7	2	14.3	1	7.1
HbA1c (%)	Normal	2	14.3	14	100	1	7.1
	Abnormal	12	85.7	-	-	13	92.9
RBC (*10^6 µl)	Normal	5	35.7	7	50.0	13	92.9
	Abnormal*	9	64.3	7	50.0	1	7.1
HGB (g/dl)	Normal	7	50.0	9	64.3	13	92.9
	Abnormal	7	50.0	5	35.7	1	7.1
WBC (*10^3 µl)	Normal	10	71.4	11	78.6	12	85.7
	Abnormal	4	28.6	3	21.4	2	14.3
LYM (*10^3 µl)	Normal	1	7.1	11	78.6	14	100
	Abnormal*	13	92.9	3	21.4	-	-

N: Number of cases. %: Percentage calculated by dividing the number of cases by the sample size multiplied by 100%. Abnormal *: Outside the normal reference range.

Comparative analyses of biomarkers between diabetic patients with severe COVID-19 (Group 1) and non-COVID-19 diabetic subjects (Group 3):

Comparing Group 1 patients to Group 3 patients, the blood levels of the biomarkers CRP (66.6vs 9.14mg/L), LDH (451.4 vs 306.6 U/L), Ang II (167.8pg/ml vs 36.1 pg/ml), ferritin (972 vs 95.2ng/ml), IL-6 (736.1 vs 10.7 pg/ml), ESR (55.4 vs 22.2 mm/h), D-dimer (967.4 vs 309.1ng FEU/ml), cTn I (0.81 vs 0.054 ng/ml), RBC (3.7 vs 4.7 *10^6µl), HGB (10.4 vs 13.7 g/dl) were higher in Group 1 compared to Group 3 patients with a statistically significant difference at $P<0.05$. Lymphopenia was observed in Group 1 patients as the lymphocyte count was higher in Group 3 (LYM, 0.84 vs 2.3 *10^3µl) with a statistically significant difference at $P = 0.0001$ (Table 5).

Comparative analyses of biomarkers between diabetic patients with severe COVID-19 (Group 1) and non-diabetic patients with mild COVID-19 (Group 2):

Comparing Group 1 patients to Group 2 patients, the blood levels of the biomarkers LDH (451.4 vs 336U/L), PCT (0.69 vs 0.47), Ang II (167.8pg/ml vs 52.2 pg/ml), ferritin (972 vs 126.9ng/ml), IL-6 (736.1 vs 22.5 pg/ml), D-dimer (967.4 vs 636.2ng FEU/ml), CK-MB (17.6 vs 14.7U/L), Tn 1 (0.81 vs 0.016ng/ml), HbA1c (7.02 vs 5.6) were higher in Group 1 compared to Group 2 patients with a statistically significant difference at $P < 0.05$. The lymphocyte count was higher in Group 2 compared to Group 1 patients who showed lymphopenia (LYM, 0.84 vs 1.76 *10^3µl) with a statistically significant difference at $P = 0.004$ (Table 6).

Table 5. Comparison between the levels of tested biomarkers in Group 1 of diabetic severe COVID-19 patients and Group 3 of non-COVID diabetic patients

Test	Group 1 (N = 14) Mean ± SD	Group 3 (N = 14) Mean ± SD	[a]*P* value
CRP (mg/L)	66.6 ± 27.7	9.14 ± 2.56	0.0001*
LDH (U/L)	451.4 ± 90.6	306.6 ± 28.5	0.0001*
PCT (ng/ml)	0.69 ± 0.73	0.27 ± 0.29	0.166
Ang II (pg/mL)	167.8 ± 111.6	36.1 ± 17.8	0.0001*
Ferritin (ng/mL)	972 ± 290	95.2 ± 59.9	0.0001*
IL-6 (pg/mL)	736.1 ± 347.6	10.7 ± 5.6	0.0001*
ESR (mm/h)	55.4 ± 26.3	22.2 ± 5.4	0.0001*
D-dimer (ng FEU/mL)	967.4 ± 418.2	309.1 ± 73.9	0.0001*
CK-MB (U/L)	17.6 ± 3.1	15.8 ± 2.1	0.066
cTn I (ng/mL)	0.81 ±0.89	0.054 ± 0.05	0.006*
ALT (U/L)	22.5 ± 9	25.2 ± 7	0.146
AST (U/L)	27.2 ± 12.2	23.1 ± 5.8	0.729
HbA1c (%)	7.02 ± 0.78	7.7 ± 0.95	0.056
RBC (*10^6µl)	3.7 ± 1	4.7 ± 0.6	0.004*
HGB (g/dl)	10.4 ± 2.2	13.7 ± 1.8	0.0001*
WBC (*10^3µl)	9.49 ± 3.8	7.06 ± 1.41	0.089
LYMs (*10^3µl)	0.84 ± 0.07	2.3 ± 0.6	0.0001*

N: Number of participants; SD: Standard deviation; [a]*P* Value was calculated using the Mann-Whitney-U test; *: *P* Value < 0.05 Was considered statistically significant.

Table 6. Comparison between the levels of tested biomarkers in Group 1 patients with severe COVID-19 and Group 2 patients with mild COVID-19

Biomarker test	Group 1 (N = 14) Mean ± SD	Group 2 (N = 14) Mean ± SD	[a]*P* value
CRP (mg/L)	66.6 ± 27.7	42.7 ± 33.2	0.066
LDH (U/L)	451.4 ± 90.6	336 ± 61	0.001*
PCT (ng/mL)	0.69 ± 0.73	0.47 ± 0.054	0.0001*
Ang II (pg/mL)	167.8 ± 111.6	52.2 ± 29.4	0.0001*
Ferritin (ng/mL)	972 ± 290	126.9 ± 90.1	0.0001*
IL-6 (pg/mL)	736.1 ± 347.6	22.5 ± 19.9	0.0001*
ESR (mm/h)	55.4 ± 26.3	37.9 ± 24.1	0.066
D-dimer	967.4 ± 418.2	636.2 ± 270.8	0.027*

(ng FEU/mL)			
CK-MB (U/L)	17.6 ± 3.1	14.7 ± 2.4	0.014*
cTn I (ng/mL)	0.81 ±0.89	0.016 ± 0.008	0.0001*
ALT (U/L)	22.5± 9	23.7 ± 6.7	0.355
AST (U/L)	27.2 ± 12.2	22.9 ± 5.2	1.00
HbA1c (%)	7.02± 0.78	5.6 ± 0.4	0.0001*
RBC (*10^6µl)	3.7 ± 1	4.2 ± 0.6	0.103
HGB (g/dl)	10.4 ± 2.2	11.5 ± 1.24	0.174
WBC (*10^3µl)	9.49 ± 3.8	7.3 ± 3.04	0.135
LYMs (*10^3µl)	0.84 ± 0.07	1.76 ± 1.02	0.004*

N: Number of participants; SD: Standard deviation; [a]*P* Value was calculated using the Mann-Whitney-U test; *: *P* Value < 0.05 Was considered statistically significant

DISCUSSION:

DM is commonly associated with high death rates when comorbid with infectious diseases. Recent studies revealed that about one-third of COVID-19 patients who died had DM. Compared to non-diabetics, diabetic COVID-19 patients had a twofold increase in fatal outcomes[22,23,24]. The severity of COVID-19 in diabetics could be explained by the increased vulnerability to infections, defects in innate or cell-mediated immunity, old age and the inflammatory state owing to DM[23,24]. Consistently, in the current study, the severe COVID-19 ICU patients of Group 1 were older diabetic patients with a mean age of 65.9 years, higher than the mean age of 46.3 years of non-diabetic mild COVID-19 patients of Group 2. Biomarkers are proteins measured in the blood whose concentration indicates the presence or severity of a disease[25]. The current study assessed the performance of diverse biomarkers to find predictive biomarkers for COVID-19 severity in diabetic patients.

Immune-inflammatory biomarkers and severity of COVID-19:

The blood inflammatory biomarkers are valuable in identifying and categorizing the COVID-19 severity[26]. Notably, high levels of IL-6 were verified in severe COVID-19 patients with clinical outcomes like pulmonary inflammation, lung destruction and multiple organ injury. IL-6, a pro-inflammatory cytokine, is produced by different cells in the innate immune response when SARS-CoV-2 is attached to cell surfaces[27]. In this study, all Group 1 and most Group 2 patients showed high levels of IL-6; however, its elevation in Group 1 was massive. The combination of DM and COVID-19 might cause this noticeable elevation with a statistically significant difference between the three study groups (*P* = 0.0001). Therefore, high IL-6 levels can be correlated to the severity of COVID-19 in diabetic patients. Similarly, a previous study reported high levels of IL-6 in severe COVID-19 patients, which may predict severe disease or even death[28]. LDH is an intracellular enzyme correlated with COVID-19 because it is a lung and tissue damage marker, and COVID-19 infects mainly the lower respiratory tract. Therefore, LDH might suggest the degree of lung destruction and predict worse consequences of COVID-19[29]. In the current study, LDH levels were elevated in all groups 1 and 2 patients, with significantly higher levels in Group 1 patients (*P* = 0.001). In addition, LDH levels elevated in 92.9% of Group 3 patients, but with significantly higher levels in Group 1 patients compared to Group 3 (*P* = 0.0001). Thus, the combination of DM and severe COVID-19 causes an apparent increase in LDH levels. Therefore, LDH level can be considered a predictor of the severity of COVID-19 in diabetic patients. This finding matches the results of the Wang *et al*. study, which included diabetic patients; LDH level was remarkably increased in ICU patients compared to non-ICU patients[9]. In addition, a prospective cohort study indicated that LDH could be a prognostic biomarker of COVID-19 severity in metabolic disease patients[30].

PCT is the pro-hormone of calcitonin produced in inflamed tissues. IL-6 and TNF-α upregulate PCT synthesis. High levels of IL-6 and TNF-α cytokines have been detected in severe COVID-19; thus, the PCT level may increase owing to the hyperinflammatory state[27,31]. Consequently, PCT may be an excellent early indicator of identifying patients with hyperinflammation at high risk of developing ARDS, the most critical complication of COVID-19[32]. Higher PCT serum level is also a marker of myocardial damage and a predictor of cardiogenic shock[33]. The current study found PCT high levels in 92.9% of Group 1 and 35.7% of Group 2 patients, with a significant difference (*P* = 0.0001). However, compared to Group 1, most Group 3 patients had an elevation in PCT level, and there was no significant difference between the two groups (*P* = 0.166). This finding indicates that combining DM and COVID-19 leads to a highly elevated level of PCT, and thus, PCT can be considered a biomarker of severe COVID-19 in diabetic patients. This finding is consistent with data reported by Tong-Minh *et al*., which revealed that high PCT level is associated with admission to ICU and mortality[34]. Kaal *et al*. cohort study showed that patients with PCT levels above 0.1 ng/mL have an increased risk of severe COVID-19[35].

ACE2 is the cellular receptor for SARS-CoV-2 in the lungs, heart, kidney, brain, blood vessels, gut, adipose tissue and testis. Following cell entry, SARS-CoV-2 downregulates ACE-2 expression, thus enhancing the ACE/AngII/AT1R axis, raising Ang II levels and acute lung injury[36]. In the current study, there were significantly higher Ang II levels (167.8 pg/ml) in 71.4% of Group 1 patients compared to other groups (*P* = 0.0001). Notably, most Group 3 patients (78.6%) had Ang II levels below the normal range, which can be explained by the significantly decreased plasma Ang II in non-complicated diabetics compared to healthy

subjects[37]. The current study finding agrees with the Guzzi *et al*. study, which indicated that a decline in ACE2 expression during COVID-19 results in excessive AT1R activation by Ang II. Increased AT1R activation leads to pro-inflammatory, prothrombotic and pro-apoptotic effects, perhaps the cause of severe illness and death in COVID-19 patients[38].

Serum ferritin is a biomarker whose level increases with viral or bacterial infections due to immune and inflammatory responses. It also has a significant role in immune dysregulation, particularly extreme hyperferritinemia, which has pro-inflammatory effects leading to the cytokine storm. As the severity of COVID-19 depends on cytokine storm, the elevated serum ferritin levels in COVID-19 patients indicate that ferritin might be a potential biomarker for the severity of COVID-19[39]. In this study, the ferritin level increased significantly in 92.9% of Group 1 compared with 7.1% of Group 2 patients (P = 0.0001). In addition, there was a significant difference in ferritin levels between Group 1 and Group 3 patients (P = 0.0001). Consequently, ferritin can be considered a marker of disease severity. This result is consistent with other studies, which reported that elevated ferritin levels might be associated with a poor disease outcome[40,41]. Furthermore, Malik *et al*. study indicated that serum ferritin is significantly higher in diabetic COVID-19 patients compared to non-diabetic COVID-19 patients and hypothesized serum ferritin could be a routine biomarker for the severity of the disease[41].

CRP is a biomarker for inflammation, infection, or cardiovascular disease[42]. In this study, the level of CRP was elevated among all patients, with a significant difference between Group 1 and Group 3 (P = 0.0001) and no significant difference between Group 1 and Group 2 (P = 0.066). However, the highest level was among Group 1 patients compared to other groups (P = 0.0001), indicating that CRP is a decisive biomarker in diabetic COVID-19 patients. These results align with a previous study that revealed a higher trend in COVID-19-positive patients with or without diabetes[41]. Additionally, Mazaheri *et al*. found no significant differences in CRP in COVID-19 ICU versus non-ICU patients[27]. IL-6 could explain this finding by stimulating CRP production and release from the liver[10]. However, despite the significant increase in IL-6 in Group 1 patients versus Group 2 patients, there was no significant difference in CRP levels between the two groups. On the other hand, Huang *et al*. reported that the elevated CRP level is linked to the severity of COVID-19[40]. The ESR level commonly indicates body inflammation and suggests the patient's progression to a critical condition[43]. In this study, the level of ESR was abnormally elevated among most patients in the three study groups, with a significant difference between Group 1 and Group 3 (P = 0.0001) and no significant difference between Group 1 and Group 2 patients (P = 0.066). However, the highest level was among Group 1 patients, which may indicate that ESR level is a biomarker in diabetic COVID-19 patients.

Assessment of cardiac biomarkers

It was estimated that around 60% of severe COVID-19 patients have an acute cardiac injury with increased cardiac biomarkers and electrocardiographic abnormalities, whether or not the patient has a history of heart illness. CK-MB and cardiac cTn I are two biomarkers of myocardial injury and prognosis biomarkers in cardiac diseases that have been far studied in COVID-19 patients to support clinical assumptions[44,45]. The current study recorded a significant elevation in CK-MB in 100% of Group 1 and Group 3 patients and 92.9% of Group 2 patients, with a statistically significant difference between the three groups (P = 0.023). However, the higher levels were among Group 1 patients, with significant differences between Group 1 and Group 2(P = 0.014) and no significant difference between Group 1 and Group 3 (P = 0.066). Accordingly, high CK-MB levels can be associated with DM and severe COVID-19 and could indicate the severity of COVID-19 in diabetic patients. This finding agrees with the meta-analysis with meta-regression study, verifying that blood CK-MB values in COVID-19 patients are significant and substantially correlated to worse clinical status and death[44]. The higher cTn I level would be linked with severe COVID-19 and may help predict a poor prognosis[46]. The current study recorded elevated cTn I levels in 6(42.9%) patients of Group 1 and 5(35.7%) of Group 3 patients who had increased cTn I levels with a statistically significant difference (P = 0.0001); however, all Group 2 patients showed average level. This may indicate that DM also affected cardiac markers in COVID-19 patients. Therefore, cTn I should be observed in diabetic COVID-19 patients to predict the severity. These findings are consistent with a previous meta-analysis and study, which also provided evidence of high levels of cardiac markers cTn I and CK-MB associated with severe COVID-19 and death of patients[11,47]. The elevation of cTn I and CK-MB cardiac markers in severe COVID-19 is explained by several mechanisms, including viral myocarditis, cytokine storm-driven myocardial injury, microangiopathy, and direct viral myocardial damage due to the viral entrance mechanism through the ACE2 receptor[48].

Assessment of coagulation biomarker (D-dimer):

D-dimer is an indirect biomarker of the activation of the hemostatic system. It is a sensitive but not specific biomarker of venous thromboembolism. Elevated D-

dimer levels in COVID-19 patients have been related to disease severity, pulmonary complications and increasing risk of venous thromboembolism or COVID-19-associated coagulopathy[49]. This study detected elevated D-dimer levels in 78.6% of Group 1 patients and 71.4% of Group 2 patients, without any abnormal increase in Group 3 patients. However, the increase in Group 1 patients was huge compared with Group 2 (P = 0.027). Thus, the D-dimer high value can indicate severe COVID-19 and is associated with poor prognosis[50]. The D-dimer testing outcomes of the current study agree with those of Mishra et al., who revealed that diabetic COVID-19 patients exhibited statistically significant elevated D-dimer levels[51].

Liver biomarkers (AST and ALT) in COVID-19 patients:

The AST and ALT biomarkers measure liver functionality and injury[52]. Most COVID-19 patients have liver biochemistry abnormalities and increased AST and ALT levels. According to a multicenter retrospective study, acute liver damage develops later in the COVID-19 course. In a Chinese cohort study, the frequency of ALT elevations among COVID-19 patients ranged from 4% to 33% (average: 19%), reaching as high as 39% in a large cohort from New York City[53]. However, diminishing liver function is not a prominent characteristic of COVID-19 and may not have significant clinical outcomes[54]. The current study found that on hospital admission, increased ALT and AST levels in Group 1 were observed in 2 (14.3%) and 5 (35.7%) patients, respectively. There were no significant differences in ALT (P = 0.355) or AST levels (P = 1.00) between Group 1 and Group 2. Likewise, there were no significant differences in ALT (P = 0.146) and AST (P = 0.729) in Group 1 and Group 3 patients. These findings demonstrate that the liver is not the primary target tissue of SARS-CoV-2. In addition, previous studies reported low ACE2 expression in human hepatocytes, which may explain why SARS-CoV-2 infection had little impact on liver function. However, elevated liver enzymes are a sign of damage to the liver, which may be caused by immune-related injury. In addition, several antimicrobial drugs used during COVID-19 can also cause elevated liver enzyme levels[55]. Thus, there is a discrepancy in the clinical significance of liver function test abnormalities for COVID-19[54].

Assessment of haematological parameters:

Haematological alterations increase in COVID-19 patients, showing a progressive deterioration as COVID-19 severity increases. Therefore, monitoring haematological changes in COVID-19 patients may help manage the disease and lower the likelihood of a severe disease course[56]. Lymphopenia seen in severe COVID-19 patients is due to a downregulation in genes involved in T-cell activation, function and mortality. In addition, a study assumed that SARS-CoV-2 directly infects T-cells[56]. In the current study, 92.9% of the Group 1 patients had severe drops in their LYM counts, 21.4% of Group 2 and 0% of Group 3 patients, with a statistically significant difference between the three Groups (P = 0.000). Consequently, in this study, lymphopenia was markedly associated with the severe phase of COVID-19, consistent with previous studies[57,58]. RBCs function or structure alterations may provide decisive data about the severity and progress of a disease. Viral infections may cause noteworthy RBCs changes, including size, rigidity and distribution width. In addition, some diseases, like DM and sickle cell anaemia, have been associated with pathological RBCs changes affecting cellular function and/or deformability. SARS-CoV-2 infects RBCs by binding the virus S1 spike protein to RBC band 3 surface proteins. This interaction can affect the RBC functionality, many RBC properties, and the release of oxygen. Thus, erythrocytes play a role in coronavirus infection[59]. In the current study, 64.3% of Group 1 patients had a drop in RBCs count, 50% of Group 2 and 7% of Group 3, with no significant differences (P = 0.103) between Groups 1 and 2. However, there is a significant difference (P = 0.004) between Groups 1 and 3. Thus, in a COVID-19 patient evaluation, a drop in RBCs count should be considered.

A few studies examined the relationship between anaemia and COVID-19. Tao *et al*. study revealed a significant percentage of severe COVID-19 among older patients with anaemia identified within 24 hours of admission; haemoglobin levels of less than 12 g/dl in women and less than 13 g/dl in men[60]. On the contrary, a prospective analysis revealed that anaemia was not associated with severe COVID-19[61]. In the current study, 50% of Group 1 patients had low haemoglobin levels and 35% of Group 2, with no significant difference (P = 0.174). These results propose that the anaemia among patients noted at admission is due to chronic disorders and can be complicated by COVID-19 and the inflammatory response in each person. WBCs count indicates the presence of infection if there is an abnormally high number of these cells. WBCs count accuracy as a COVID-19 biomarker has not yet been recognized. According to a retrospective study, leucocytes increased in both severe and non-severe COVID-19 conditions, although the severe COVID-19 patients' upsurge was more significant[62]. The present study found some Group 1 and Group 2 patients had elevated WBCs. However, the peak was higher in Group 1, with no significant differences between the two groups (P = 0.135) and even no considerable difference between the three groups (P = 0.178).

The relationship between poor glycemic control, as determined by higher HbA1c numbers, and the emergence of severe COVID-19 or probable death is conflicting. Increased mortality was linked to worse long-term diabetes control, according to population-based studies in the United Kingdom[63]. The current study found that the HbA1c value was significantly higher in Group 1 than in Group 2 patients ($P = 0.0001$). It is a logical result because Group 2 includes non-diabetic patients. However, no significant difference was observed in HbA1c levels in DM patients, either COVID-19 positive (Group 1) or COVID-19 negative (Group 3) ($P = 0.056$). Therefore, these findings support the idea that multiple pathophysiological events brought on by DM may be due to the progression to severe COVID-19 rather than that of diabetes itself. This may explain why diabetic COVID-19 patients were more likely to develop severe complications. Additionally, a retrospective study in the UK found that HbA1c in diabetic COVID-19 patients did not indicate severe disease or mortality, which agrees with the current study[64]. Consistent with current research, the study by Agarwal *et al*. showed no association between long-term glycemic management and COVID-19 severity or mortality[65]. The causes of these discrepancies are unclear, given the mechanistic relationship between hyperglycemia and a dysregulated immune response[6].

CONCLUSION:

The blood biomarkers analysis revealed statistically significant changes in the levels of tested biomarkers among ICU diabetic COVID-19 patients compared to non-COVID-19 patients. The study findings showed a high association between LDH, PCT, Ang II, ferritin, IL-6, CK-MB, cTn I, D-dimer and LYM levels and the severity of COVID-19 in diabetic patients. These findings endorse diabetic COVID-19 patients are more predisposed to inflammatory storms, cardiac and coagulation problems, organ failure and worse disease outcomes. Thus, the medical history of SARS-CoV-2 infected patients should be investigated for pre-existing, long-term, or chronic abnormalities of these biomarkers. There is a need for further large-scale studies on the clinical effectiveness of the proposed biomarkers in other geographic regions. This study remarks that blood analysis can be utilized in developing countries as a low-cost and efficient alternative for RT-PCR.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

ETHICAL APPROVAL:

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Pharmacy, Al-Azhar University-Gaza (Ethics code: PHRC/HC/1057/22). The patient or a trusted person signed the informed consent form with precise and faithful information before being included in the study.

DATA AVAILABILITY:

The authors confirm that the data supporting the findings of this study are available within the article.

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AUTHOR CONTRIBUTIONS:

MHT and IMA contributed to the conception and design of the study; EKS conducted the experimental work; EKS, MHT and IMA collected and organized the data; EKS, MHT, IMA and MMT contributed to data analysis and interpretation; EKS, MHT, IMA, MH and MMT wrote the manuscript draft; MMT revised and edited the manuscript; All authors read and approved the final manuscript.

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