



## ASSOCIATION OF SERUM C-REACTIVE PROTEIN AND D-DIMER CONCENTRATION WITH CLINICAL SEVERITY CAUSED BY CORONAVIRUS IN HOSPITALIZED PATIENTS WITH DIABETES

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**ABSTRACT** **BACKGROUND:** The aim of this research was to compare the levels of inflammatory markers (D-dimers and C-reactive protein) in diabetic and non-diabetic patients depending on severity of clinical manifestation, therefore based on such, define them as potential predictors of lethal outcome and clinical severity. **METHODS:** A total of 422 subjects were included in this research by retrospective review of the documentation at the Clinic for Infectious Diseases of University Clinical Hospital Mostar. While reviewing the medical records, the following data were filled in: demographic (age, sex, diabetes mellitus), oxygen saturation and  $paO_2/fiO_2$  ratio, inflammatory markers (C-reactive protein and D-dimers), used therapy (non-invasive ventilation, oxygen therapy, invasive mechanical ventilation) and final outcome of disease. Based on the presence of diabetes mellitus the patients were divided into two groups: diabetic and non-diabetic patients. Data entry into tables was followed by statistical data processing using IBM SPSS Statistics v.26 and Microsoft Excel 2016. **RESULTS:** Out of total number of subjects, 161 had diabetes and 261 did not. Surprisingly, women statistically had a higher average value of D-dimers in comparison to men and they had a higher percentage of lethal outcome compared to men. Deceased patients had a higher average value of CRP (126,25) and D-dimers (2,20) in comparison to survivors. Also, the deceased had a lower average of oxygen saturation (83,07), lowered  $paO_2/fiO_2$  ratio (131,5) and more liters of oxygen used in oxygen therapy (11,59). Statistically significant higher lethality was noted in patients with diabetes compared to non-diabetic patients. **CONCLUSION:** Levels of C-reactive protein and D-dimers were higher in patients with a more severe clinical manifestation. Since lethal outcome was more statistically prevalent in those subjects, CRP and D-dimers are stated as prognostic factors of clinical severity and lethal outcome in patients with COVID-19. Lastly, there was no notably increased difference in CRP and D-dimer levels between diabetic and non-diabetic patients.

**KEYWORDS :** D-dimers, C-reactive protein, COVID-19, diabetes mellitus

### INTRODUCTION:

SARS-CoV-2 first occurred at the end of 2019. in Wuhan, at the south of China. Few Health Institutions have reported new clusters of patients with pneumonia of unknown etiology. Similarly, like patients with SARS and MERS (Middle East respiratory syndrom), these patients showed signs of viral pneumonia, which included fever, cough and chest discomfort (1). Amongst the first documented hospitalized patients same epidemiological link was traced to a small sea food market of Huanan (2). With further tests the culprit was identified and was named SARS-CoV-2. Rapid spread proved the possibility of interpersonal transmission and the incubation period is between 2 and 14 days (3). Main pathway of spread is through the respiratory tract by respiratory secretions (infected droplets) or direct contact. Viral particles were isolated from fecal matter and blood, implying other means of possible transmission (4). Filogenetic analysis showed that SARS-CoV-2 shared much of its genom with coronaviruses isolated from bats, whom are known to be their natural reservoirs (3). Compared to the flu virus, SARS-CoV-2 has a higher reproduction number of 2.2. Which is an indicator for contagiousness, the higher the number, the more contagious the disease. Unlike the flu virus, SARS-CoV-2 has a higher percentage of lethal outcome (5).

Manifestation of COVID-19 is linked to a broad spectrum of symptoms, that can be divided to common and rare ones. Most common symptom include fever (87.9%), dry cough (67.7%), fatigue (38.1%), production of sputum (33.4%), dyspnea (18.6%), sore throat (13.1%), chills (11.4%), nasal congestion (4.8%) and haemoptysis (0.9%) (6). Some patients would rapidly progress to acute lung failure and ARDS (Acute Respiratory Distress Syndrome) with septic shock (7). Most unusual symptoms include deterioration of sensations of taste (dysgeusia) and smell (anosmia) and can be present at earlier stages of disease (8). Gastrointestinal symptoms are diarrhoea, vomiting and nausea. Interestingly, one study of 81 COVID-19 positive men compared hormone levels with 100 healthy age compatible men. The study showed lower levels of testosterone and increased levels of FSH and prolactin, which could be indicators of detrimental effect on Leydig cells. All of this is linked to temporary decrease of fertility in the time period of up to 3 months post infection (9).

Many fatal forms of COVID-19 have been linked to different comorbidities, one of which and being most significant is diabetes mellitus. Obesity is the most important factor that leads to development of insulin resistency. Due to overburdening of fat tissue, adipocytes are in constant state of low intensity, chronic inflammation, where they release cytokines which make the person more susceptible

to inflammatory reactions (10). State of hyperglycemia encourages the body to get rid of excess glucose with non enzymatic glycation. Binding of glucose to various proteins (immunoglobulins, complements) creates new compounds that lose their original function and they are called advanced glycation end products (AGE). In conclusion, state of hyperglycemia disturbs normal cytokine production, inhibits antibody and complement systems and causes dysfunction in phagocytosis (11).

Inflammatory parameters are greatly increased in critically ill patients and their early detection can give us an insight in patient's current state and possible prevention of lethal outcome. These parameters of great importance include: C-reactive protein (CRP) and D-dimers. Increased CRP values are linked to secondary bacterial superinfection and with higher chances of developing ARDS (12). By comparing levels of CRP between the survivors and deceased patients, the mean value of CRP in survivors was calculated to be around 40 mg/L, while the deceased had a mean value of 125 mg/L. The deceased also had increased value of D-dimers, which supports the possibility of developing coagulopathies and disseminated intravascular coagulation (13).

### MATERIALS AND METHODS

This was a one-center retrospective study which included 422 patients treated at the COVID department as a part of the Department of Infectious Diseases in the University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina. This study was approved by the Institutional Ethics Board of University Clinical Hospital Mostar and is in full compliance with the Helsinki Declaration.

### Participants

The study included 422 patients treated for COVID-19 infection at the Clinic for Infectious Diseases in University Clinical Hospital Mostar during the period between 1st January and 1st May 2021. The main criterion for inclusion of subjects is a positive PCR (polymerase chain reaction) finding from nasopharyngeal or oropharyngeal swab samples. The second inclusion criterion is a positive diagnosis of type I. or type II. diabetes mellitus. Only those patients who had complete medical documentation were included in the research.

### Methods

Data was collected from the medical documentation of the respondents at the Clinic for Infectious Diseases of the University Clinical Hospital Mostar. While reviewing medical records, the following data were filled in: demographic (age, sex, diabetes mellitus), clinical symptoms (fever, dyspnea with low blood oxygen saturation), complications

(Acute Respiratory Distress Syndrome – ARDS), methods of treatment (invasive or non-invasive ventilation) and laboratory markers (C-reactive protein, D-dimers). The BCS XP Siemens analyzer was used to detect the levels of D-dimers in serum samples. The device works on the principle of immunoturbidimetry for quantitative measuring of fibrin degradation products (D-dimers). Other analyzer used for measuring CRP in plasma samples was Beckman Coulter DXC 700AU.

**Statistical Analysis**

In preparation for the statistical analysis, all the above data were filled into previously created table in MS Office Excel. Using the formulas of the Excel program the mean value of CRP and D-dimers was calculated. The patients were divided into two groups : the ones with diabetes and the non diabetic group. IBM SPSS Statistics v.26 was used. The results are presented in tables graphically and are interpreted at the significance level P < 0.05. The significant differences between the groups for continuous variables were compared using one-way analysis of variance when the data was normally distributed, otherwise, the Kruskal-Wallis H-test was used. Categorical variables were described as frequency and percentages. The X2 (chi square) test for independent samples and the Fisher exact test were used to process the data.

**RESULTS**

**Respondent characteristics**

The study included 422 respondents in the time period between 1st January and 1st May 2021. that were hospitalised at the COVID department in Clinic for Infectious Diseases of the University Clinical Hospital Mostar. Out of total number of participants 160 (37.9%) were female and 262 (62.1%) were male. Higher values of D-dimers in women, compared to men, was proven to be statistically significant. Mean value of D-dimers amongst female patients was 1.96 ng/mL, while men had a mean value of 1.67 ng/mL. Another result showed that men were more likely to be hospitalised at a younger age than women, as shown in Table 1.

	Sex				t <sup>a</sup>	p
	Female		Male			
	X□	SD <sup>b</sup>	X□	SD		
Age	73,36	11,99	69,02	13,27	3,379	0,001
sO2% <sup>c</sup>	88,86	9,92	88,89	11,47	0,024	0,981
Oxygen therapy	8,58	5,07	9,26	5,37	1,029	0,304
D-dimers	1,96	1,44	1,67	1,41	2,018	0,044
CRP	101,19	71,06	105,75	74,11	0,622	0,534
paO2/fiO2 <sup>d</sup>	164,30	75,38	189,02	94,66	1,962	0,052

- a Chi square test
- b Standard deviation
- c Blood oxygen saturation
- d ARDS severity ratio

**Comparison between diabetic and non-diabetic patients**

The total number of patients with diabetes mellitus was 161 and the rest 261 were without diabetes. No major statistical relevance was reported in the comparison between these two groups. Surprisingly, even the difference in levels of CRP and D-dimers was insignificant, which is shown in Table 2.

	NDM <sup>a</sup>		DM <sup>b</sup>		t <sup>c</sup>	p
	X□	SD <sup>d</sup>	X□	SD		
Age	69,62	14,02	72,35	10,86	2,245	0,025
sO2% <sup>e</sup>	89,40	10,03	88,04	12,14	1,194	0,233
Oxygen therapy	8,82	5,32	9,27	5,17	0,691	0,490
D-dimers	1,71	1,39	1,90	1,48	1,323	0,187
CRP	99,78	74,01	110,91	70,77	1,522	0,129
paO2/fiO2 <sup>f</sup>	178,90	89,12	184,13	90,08	0,389	0,698

- a No diabetes mellitus
- b Diabetes mellitus
- c Chi square test
- d Standard deviation
- e Blood oxygen saturation
- f ARDS severity ratio

The number of survivors was higher (260) compared to deceased patients (162). Comparing the levels of D-dimers, CRP, oxygen saturation (sO2%), paO2/fiO2 and respondent age there were many statistical significants with the outcome of treatment. Respondents with a higher average of age (75 years) had a lethal outcome compared to younger survivors with an average age of 67 years. Lowered average levels of oxygen saturation were present in patients with poor outcome (83.07%) and therefore they required more litres of oxygen (11.5L). Higher mean value of D-dimers was connected with poor outcome (2,20), as well as higher mean value of CRP (126.25). Lastly, based upon the values of paO2/fiO2 we can conclude that most patients with lethal outcome had a mild form of ARDS.

	Outcome				t <sup>a</sup>	p
	Deaths		Survivors			
	X□	SD <sup>b</sup>	X□	SD		
Age	75,26	10,74	67,80	13,41	6,298	<0,001
sO2% <sup>c</sup>	83,07	14,10	92,47	5,98	8,026	<0,001
Oxygen therapy	11,59	5,07	6,85	4,38	8,180	<0,001
D-dimers	2,20	1,47	1,52	1,34	4,759	<0,001
CRP	126,25	77,97	90,20	66,06	5,070	<0,001
paO2/fiO2 <sup>d</sup>	131,50	81,11	203,18	83,89	5,544	<0,001

- a Chi square test
- b Standard deviation
- c Blood oxygen saturation
- d ARDS severity ratio

**DISCUSSION**

This study included 422 participants, of the total number the majority were male, non diabetic patients and survivors of COVID-19 infection. Patients with diabetes were more likely to be hospitalised in older age in comparison to patients without diabetes. Based on the results the initial assumption was confirmed that diabetic patients had more severe coronavirus infections and this was in close correlation with higher values of CRP and D-dimers.

Ruan and colleagues in their study of 150 patients in Wuhan had concluded that the mean value of CRP in surviving patients was 40 mg/L, while in deceased patients it was 125 mg/L (14). These values are in close correlation with our results. Our patients with lethal outcome had mean values of CRP around 126 mg/L.

Furthermore, mean values of D-dimers were around 2,20 µg/mL in deceased and around 1,52 µg/mL in surviving patients. Litao and colleagues have conducted a study with 343 people, that they divided into two groups. In the first group were those with increased D-dimers and they had 12 lethal outcomes, the second group was more numerous with low D-dimers and they only had one lethal outcome (15). Same results were found by Guan and colleagues in their study of 1099 people, with mean value of D-dimers around 2,12 µg/mL in deceased people (12). Similarly, Ning and colleagues found abnormal coagulation parameters and they accentuate the increased values of D-dimers in COVID-19 patients (16). From all of this we can easily deduce that values of D-dimers above 2 µg/mL carry a predictive value for lethal result. Interestingly, in our research we found that the female population was more prone to higher D-dimers and therefore had higher mortality rates compared to men.

In patients with diabetes mellitus, due to hyperglycemic burdening, endothelial dysfunction happens and therefore easier formations of thrombi, because of this we can always expect a certain increase in D-dimers. Yogendra and colleagues researched the correlation of D-dimer values in patients with diabetes and COVID-19 patients and they noticed high increase of D-dimers in diabetic patients compared to non-diabetic (17). Surprisingly, in our research we could not find statistical relevance in D-dimer levels between these two groups. Diabetes mellitus can worsen COVID-19 outcome, but COVID-19 can also worsen or even cause newly developed diabetes mellitus in genetically predisposed patients. It is proven that the virus can precipitate acute metabolic complications by negatively and directly affecting pancreatic beta cells (18).

**CONCLUSION**

C-reactive protein and D-dimers show a trend of increase in mild to severe clinical presentation, especially in patients with poor outcome. Correlation of CRP and D-dimer concentrations with severe clinical

presentation has a proven predictive value for lethal outcome. Patients with diabetes mellitus treated for coronavirus infection have severe clinical manifestations and frequent lethal results in regards to patients without diabetes treated for coronavirus infections.

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