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VASCULAR CELLULAR ADHESION MOLECULES ARE ELEVATED UP TO 23 MONTHS AFTER ACUTE COVID-19 INFECTION IN PATIENTS WITH TYPE 2 DIABETES: A CROSS-SECTIONAL STUDY

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Abstract: The aim of our study was to evaluate the level of vascular cell adhesion molecules VCAM-1 in patients with type 2 diabetes mellitus in one year after COVID-19 infection.

Keywords: diabetes, COVID-19, vascular adhesion molecules, endothelial dysfunction

Methods. We studied 136 type 2 diabetes patients who had COVID-19 in 2020 in 1-23 months after the acute infection. VCAM-1 was tested using the Human ELISA Kit assay (Elabscience) in the laboratory of the Republican Centre of Endocrinology. Statistical analysis was performed using STATA v.16.0.

Results. The level of VCAM-1 remained elevated up to 23 months after the COVID-19 onset. It was significantly lower in patients regularly taking rivaroxaban, beta-blockers, ACE inhibitors, statins, and fibrates and had no difference with any glucose-lowering therapy, aspirin or clopidogrel, or coagulogram parameters. VCAM-1 level was significantly lower in those patients who received dexamethasone and remdesivir but not favipiravir during the acute COVID-19 infection.

Conclusion. Endothelial dysfunction may be present up to 23 months after COVID-19, and patients with type 2 diabetes should be monitored closely for post-COVID vascular complications. Dexamethasone use during COVID-19 (when indicated) may have a beneficial long-term effect on endothelial cells. Oral anticoagulants, beta-blockers, ACE inhibitors, statins, and fibrates may have a potential protective effect on the endothelium in the post-COVID-19 period, but further studies are needed.

Background & Objectives

Despite the primary consideration that the lungs are the primary target organs in COVID-19, great attention is paid nowadays to endothelial dysfunction. Virus plays the role of a trigger to angiontensin converting enzyme two receptors activation on endothelial cell (EC) surface, resulting in the increase of pro-inflammatory cytokines, molecules of adhesion, chemokines, von Willebrand factor (vWF) antigen, vWF activity, factor VIII, acute phase reactants, and endothelial dysfunction (ED). Consequently, ED contributes to COVID-19-associated endotheliitis in the lungs, heart, and kidney, as well as COVID-19-associated coagulopathy [1].

Molecules of adhesion are a group of glycoproteins expressed on the cell surface and play a pivotal role in inflammatory and oncological processes. Some studies showed the increase of these molecules within 6-12 hours after SARS-CoV-2 contamination. Vascular

cell adhesion molecules (VCAM-1) reach their peak level in 24 hours and remain elevated. The level of molecules of adhesion correlates with disease severity and mortality rate [1]. Enhancing of VCAM-1 synthesis is attributed to irreversible EC injury through participating in leukocyte recruitment in the microvasculature [2].

The questions are: how long is the adhesion molecules level elevated after reconvalescence after acute COVID-19, and what are possible mechanisms to cope with endotheliitis and endothelial dysfunction in patients with diabetes after COVID-19? Some clinical studies indicate the role of heparin in preventing VCAM-1 elevation [3]. Another agent to decrease VCAM-1 is simvastatin [4]. A clear understanding of pathophysiological mechanisms may lead practicing physicians to choose the right methods of treatment.

The objectives of our study were to evaluate the level of VCAM-1 in patients with type 2 diabetes mellitus after COVID-19 infection and assess the influence of the regularly taken medications on the level of VCAM-1 in patients with diabetes.

Materials and methods

In our cross-sectional study, we examined type 2 diabetes patients who had COVID-19 in 2020, were referred to an endocrinologist in the Republican Centre of Endocrinology in 2021 (from January to December) and gave their written informed consent to use their data in the study and analysis. The fact of COVID-19 was confirmed by medical records of patients (by PCR and/or chest CT scan during the onset of COVID-19) and the presence of neutralizing antibodies to COVID-19. VCAM-1 was measured in ng/mL using a Human ELISA Kit assay (Elabscience) in the laboratory of the Republican Centre of Endocrinology. In brief, blood samples were collected in the fasting state and centrifuged immediately at 1000 rpm for 20 minutes at 2-8ºC. Samples and standards were added in a volume of 100 µL onto the bottom of the plate, avoiding touching the wall and foaming, and incubated at 37 ºC for 90 min. After decanting the liquid, 100 µL of Biotinylated Detection Ab working solution was added and incubated, covered with a sealer, at 37 ºC for 1 hour. After decanting the solution, the wells were washed thrice with 350 µL of wash buffer. Then 100 µL of HRP Conjugate working solution was added and incubated at 37 ºC for 30 min, followed by washing five times with buffer. After that, 90 µL of Substrate reagent was added and incubated at 37 ºC for 15 min, protected from the light. Then, 50 µL of Stop solution was added, and the optical density was measured at once with a micro-plate reader set to 450 nm. The ELISA Kit was used, according to instruction, for research only, not for diagnostic procedures.

Statistical analysis was performed using STATA v.16.0. Parametric parameters were analyzed using a t-test in case of the normal distribution; the data are shown in mean and standard deviation and were considered statistically significant in $p<0.05$. Non-parametric criteria were analyzed using χ^2 or Fisher exact test and are shown in numbers and/or percent.

COVID-19 was classified as mild, moderate, or severe based on the local recommendations on the management of patients with COVID-19.

Ethical approval was taken at the ethical committee of the Republican Centre of Endocrinology #1/A-CC-2021-139.

Results

In total, we studied 136 type 2 diabetes patients. The time after the acute infection was 12.46±4.61 months (from 1 to 23 months). The mean age was 59.81±7.96 years (from 43 to 80 years), and 41.18% were male. The mean diabetes duration was 10.38±6.74 years (min 0, max 35 years). The mean BMI was 29.61±5.27 kg/m^2.

8.08 % of patients had severe COVID-19, 46.46 % - moderate, and 60.61 % - mild COVID-19. 22 (16.18%) patients did not know about the viral disease, had not been vaccinated by the time of the study, and had a high level of neutralizing IgG antibodies to COVID-19.

The data on concomitant diseases, complications, and regularly prescribed medicines are provided in table 1.

The main laboratory tests are provided in table 2.

The level of VCAM-1 remained elevated up to 23 months after the COVID-19 onset (Fig.1). It was significantly lower in patients regularly taking rivaroxaban, beta-blockers, ACE inhibitors, statins, and fibrates, and had no difference with any glucose-lowering therapy, aspirin or clopidogrel, or coagulogram parameters.

Interestingly, VCAM-1 level was significantly lower in those patients who received dexamethasone and remdesivir, but not favipiravir during the acute COVID-19 infection. Patients who had arterial hypertension and did not take regular antihypertensive therapy had significantly higher levels of VCAM-1 (851.09, 95% CI 597.77-1104.42 vs 527.93, 95% CI 461.99-593.86).

Discussion

One of the key pathophysiological pathways in COVID-19 is endothelial dysfunction, which has complex pathogenesis and multiple molecules involved [1]. The increase in the level of VCAM-1 - one of the mediators of vascular inflammation - is a result of reversible activation of vascular endothelial cells within hours or days after the onset of COVID-19 in type II endothelial cells activation. The latter results in procoagulant molecules formation and an increase in the risk of thrombosis. The key message is that this stage (type II activation of endothelial cells) is still reversible compared to further apoptosis and necrosis.

In our study, the VCAM-1 level was increased up to 21 months after COVID-19 onset and was not associated with von Willebrand's factor or D-dimer increase or other disorders in the coagulogram. This means that routine check of coagulogram, D-dimer, and vWF may miss pro-thrombotic changes in microvasculature.

Tong M. and coauthors showed a significant increase of endothelial cell adhesion molecules depending on the severity of COVID-19 with a further decrease of its level with convalescence [2].

Vinayagam S., in the review article, clearly showed the benefits of anticoagulant treatment for patients with COVID-19 [5]. The question is how long one should use and which anticoagulant. And another issue is hypocoagulation cases, which, being rare, are of great importance and difficult to manage. Earlier, we have described the case of severe Fisher-Evans' syndrome in a patient after COVID-19 associated with subcutaneous and submucous hemorrhages and lethal outcomes [6].

Salas A. and coauthors, in their study on rats, showed that pre-treatment of heparin did not affect the expression of VCAM-1 in response to induction of inflammation with tumor necrosis factor alpha. Interestingly enough, the authors showed in their work that the level of VCAM-1 was different in different tissues, but its increase was in all tissues (lung, heart, pancreas, stomach, small bowel, caecum, colon, kidney, and muscle), and the level was the highest in lung and kidney followed by heart tissue and pancreas [7]. The latter may be one of the reasons for arrhythmia and diabetes onset after COVID-19.

Hippensteel JA and coauthors, in their mini-review, showed the potential role of heparin in the prevention of severe COVID-19: together with anti-coagulant action, heparin is proposed to have an anti-viral effect due to inhibition of the interaction of viral Spike protein with endothelial or epithelial cell surface; and inhibition of inflammatory cells infiltration and dampening of pro-inflammatory signaling. Still, the question of the timeline of heparin use after COVID-19 is open [3].

Regarding statins, Qian Y and coauthors showed that simvastatin - compared to other statins - inhibited in vitro activation of endothelial cells triggered by SARS-CoV-2 nucleocapsid protein. The authors also showed that the activation of endothelial cells was only with SARS-CoV-2 but not with the other six types of coronaviruses infecting

humans [4].

Some authors showed a decrease in VCAM-1 level in patients taking metformin [8,9], DPP4 inhibitors [10,11], and GLP-1ra [12,13]. Regarding SGLT2 inhibitors, there are data showing that this class of glucose-lowering medications does not affect the level of VCAM-1 [14-16]. In our study, we did not see any statistically significant difference in VCAM-1 level depending on glucose-lowering therapy.

Limitations of the study: patients in our study were not randomized to any group, and the level of VCAM-1 may have been influenced by other factors, so further, more precise studies are needed to prove our findings and also to find the exact mechanisms of potential benefits of the described medicines. Therefore, the external validity of the results should be confirmed in further studies.

Also, as we have no data before the infection, the elevated level of VCAM-1 in patients with arterial hypertension who did not take regular antihypertensive therapy may also be related to reasons other than COVID-19.

Twenty-two patients who did not know that they had COVID-19 and had not been vaccinated by the time of the study had a high level of neutralizing antibodies (IgG) to COVID-19; thus, the presence of antibodies is an indirect sign of possible COVID-19 before enrolment to the study.

Conclusion

Endothelial dysfunction may be present up to 23 months after COVID-19, and patients with type 2 diabetes should be monitored closely for post-COVID vascular complications. Dexamethasone use during COVID-19 (when indicated) may have a beneficial long-term effect on endothelial cells. Oral anticoagulants, beta-blockers, ACE inhibitors, statins, and fibrates may have a potential protective effect on the endothelium in the post-COVID-19 period, but further studies are needed.

Figures and Tables

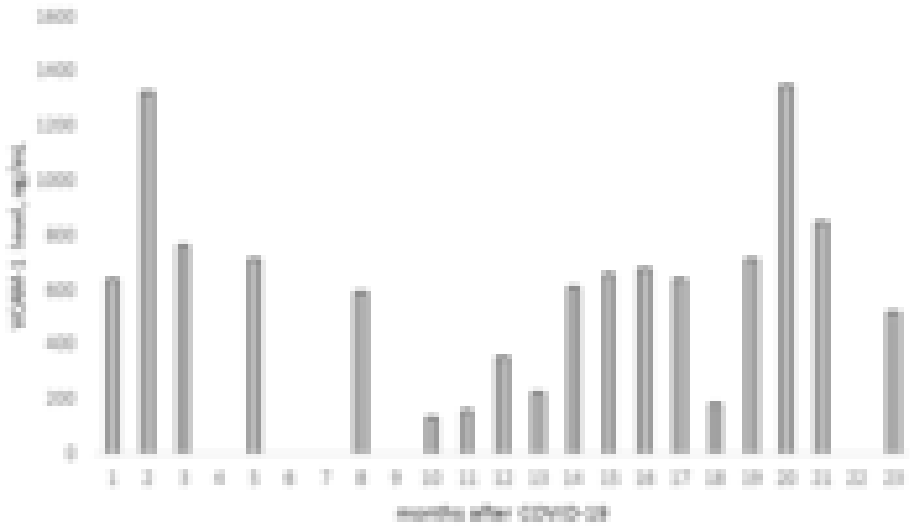


Fig.1. The mean level of VCAM-1 in 1-23 months after COVID-19 (ng/mL).

Table 1.

Characteristics of patients with type 2 diabetes: concomitant diseases, complications, and regularly prescribed medicines.

Parameter	**n (%)**
Overweight	55 (40.4)
Obesity I	30 (22.1)
Obesity II	18 (13.2)
Obesity III	4 (2.9)
Arterial hypertension	76 (55.9)
Systolic	37 (27.2)
Diastolic	48 (35.3)
Had arterial hypertension but did not take any antihypertensive therapy	11 (8.1)
Anamnesis	
Family history of DM	79 (58.1)
Family history of obesity	66 (48.5)
Family history of arterial hypertension	78 (57.4)
Treatment for diabetes:	
Insulin analogue	50 (36.8)
Human insulin	47 (34.6)
Insulin dose, U	27.18±18.73
Metformin	80 (58.8)
DPP4i	22 (16.2)
GPP1ra	2 (1.5)
SU	36 (26.5)
SGLT2	15 (11.0)
Treatment for concomitant diseases:	
Beta-blockers	70 (51.5)
ACEi	54 (39.7)
BRA	10 (7.4)
Ca channel blockers	23 (16.9)
Aspirin	50 (36.8)
Rivaroxaban	2 (1.5)
Clopidogrel	10 (7.4)
Verspirone	7 (5.1)
Diuretics (thiazides and furosemide)	18 (13.2)
Statins	56 (41.2)
Fibrate	9 (6.6)
Alfa-lipoic acid	4 (2.9)
Iodine	1 (0.7)
Vit D pills	4 (2.9)
PostCOVID and diabetes complications	
PostCOVID depression	14 (10.3)
Muscle weak	56 (41.2)
Smell loss	3 (2.2)
Cognitive impairment	7 (5.1)
Acute kidney failure	3 (2.2)
Purulent complications	10 (7.4)
Thrombosis	15 (11.0)
Vasculitis	42 (30.9)
Neuropathy 1 2	87 (64.0) 5 (3.7)
Pulse loss	23 (16.9)

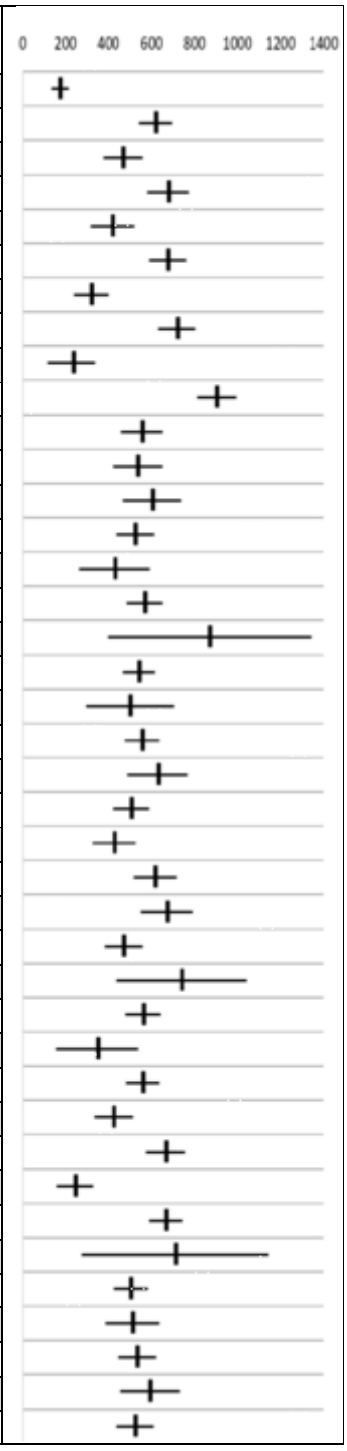
Feet ulcers	17 (12.5)
MI	9 (6.6)
Stroke	28 (20.6)
DR 0 1 2 3	77 (56.6) 42 (30.9) 15 (11.0) 1 (0.7)
Nephropathy 0 1 2 3 4 5	68 (50.0) 54 (39.7) 6 (4.4) 3 (2.2) - 2 (1.5)
Autonomous neuropathy	45 (33.1)
IHD	103 (75.7)
Disability	11 (8.1)
Vaccinated	11 (8.1)
Laboratory results	
LII high	14 (10.3)
Willebrand low	1 (0.7)
Willebrand high	44 (32.4)
D-dimer high	48 (35.3)
IL6 high	99 (72.8)
Renin high	49 (36.0)
Aldosterone high	101 (74.3)
Renin/aldosterone high	22 (16.2)

Table 2. Laboratory test results of patients with type 2 diabetes after COVID-19

Parameter	U	Mean	SD
Fasting glycemia	mmol/l	10.617	4.099
Postprandial glycemia	mmol/l	13.146	4.260
HbA1c	%	9.064	2.056
Hemoglobin	g/L	119.39	19.491
WBC	*10^9/L	8.119	3.970
Erythrocytes sedimentation rate	mm/h	26.01	18.444
Total cholesterol	mmol/l	5.078	1.422
Triglycerides	mmol/l	2.585	1.941
HDL	mmol/l	1.278	0.714
LDL	mmol/l	2.692	1.138
VLDL	mmol/l	1.112	0.940
Urea	mmol/l	6.538	3.402
Creatinine	µmol/l	93.83	73.677
ALT	U/L	31.39	31.868
AST	U/L	27.59	37.511
GGT	U/L	69.07	95.936
MNO		1.018	.270
Fibrinogen	g/L	3.732	1.342
Willebrand factor (50-150%)	%	145.067	56.857
D-dimer (0-0.5)	mg/L	0.680	0.877
TSH (0.27-4.0)	mIU/mL	2.446	2.383
BitB12 (197-771)	pg/mL	835.242	868.381
Ferritin (f 13.0-150.0 ng/mL, m 30.0-400.0 ng/mL)	ng/mL	336.828	404.647
Insulin (2.6-24.9)	ng/mL	15.950	22.076
Procalcitonin (<0.05)	ng/mL	.118	.199
Vit D3 (30-100)	ng/mL	18.470	14.152
C-peptide (0.01-40.0)	ng/mL	4.165	3.399
IL6 (1.5-7.0)	pg/mL	32.917	51.442
IgG Ab for SARS-CoV-2 (<1.0)	U	1082.169	951.857
VCAM1 (ng/mL)		546.186	417.881

Table 3. VCAM-1 level in patients with type 2 diabetes depending on taking other medicines.

Factor	VCAM-1 level	95% CI
Taking rivaroxaban (n=2)	174.65	134.2-215.1
Not taking rivaroxaban (n=134)	618.12	542.04-694.19
Taking beta-blockers (n=70)	466.39	373.91-558.87
Not taking beta-blockers (n=66)	676.35	578.15-774.56
Taking ACE inhibitors (n=54)	417.76	314.72-520.81
Not taking ACE inhibitors (n=82)	674.82	586.86-762.77
Taking statins (n=56)	318.65	238.54-398.75
Not taking statins (n=80)	717.02	630.12-803.91
Taking fibrates (n=9)	235.35	133.96-336.75
Not taking fibrates (n=127)	902.53	812.43-992.63
Taking metformin (n=80)	555.22	458.99-651.46
Not taking metformin (n=56)	534.84	418.44-651.25
Taking SU (n=36)	602.48	465.97-738.99
Not taking SU (n=100)	524.09	436.06-612.13
Taking DPP4i (n=22)	427.07	263.55-590.60
Not taking DPP4i (n=114)	567.69	485.95-649.43
Taking GLP-1ra (n=2)	868.5	394.49-1342.50
Not taking GLP-1ra (n=134)	541.29	466.69-615.91
Taking SGLT2i (n=15)	500.03	295.02-705.04
Not taking SGLT2i (n=121)	555.97	476.17-635.77
Taking human insulin (n=47)	628.48	487.73-769.23
Not taking human insulin (n=89)	504.01	419.44-588.58
Taking analogue insulin (n=50)	425.32	325.65-524.98
Not taking analogue insulin (n=86)	616.16	517.56-714.77
Taking aspirin (n=50)	671.65	550.32-792.97
Not taking aspirin (n=86)	469.69	381.51-558.40
Taking clopidogrel (n=10)	738.18	436.55-1039.81
Not taking clopidogrel (n=126)	560.71	479.10-642.32
Taking spironolactone (n=7)	348.03	157.28-538.77
Not taking spironolactone (n=129)	557.75	481.28-634.21
Received dexamethasone* (n=49)	423.76	332.37-515.14
Didn't receive dexamethasone* (n=87)	664.26	572.79-755.72
Received remdesivir* (n=20)	244.29	160.25-328.32
Didn't receive remdesivir* (n=116)	666.07	586.89-745.25
Received favipiravir* (n=6)	709.5	273.82-1145.18
Didn't receive favipiravir* (n=130)	502.45	422.61-582.30
von Willebrand's factor high (n=44)	510.86	385.54-636.17
von Willebrand's factor normal (n=92)	532.61	445.85-619.37
D-dimer high (over 0.5 mg/L, n=48)	591.99	454.55-729.44
D-dimer normal (0-0.5 mg/L, n=88)	522.74	436.78-608.69



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