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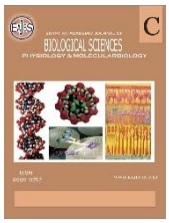

Altered Insulin, IRS/PI3K/AKT/mTOR Signaling, ACE2 and Angiotensin II, Correlating with Vitamin K Status and Severity in Metabolically Disordered COVID-19 Egyptian patients

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ABSTRACT

Aims: Metabolic disorders are essential risks of adverse outcomes in coronavirus disease 2019 (COVID-19). Vitamin K status might play an important role in metabolic regulation and influence the severity of COVID-19. This research shed light on vitamin K status in association with some metabolic pathways; pancreatic beta-cell function, insulin resistance, PI3K/AKT/mTOR signaling, and the angiotensin-converting enzyme 2 (ACE2)/angiotensin II in the context of COVID-19 and its metabolic effects in metabolically disordered patients. *Main methods:* This study included hospital-confirmed COVID-19 patients with or without obesity and/or diabetes mellitus (DM) and control subjects. The effect of viral infection on PERK double-stranded RNA-dependent protein kinase (PKR) and ACE2 was regressed against insulin resistance and blood pressure, respectively. Demographic and clinical characteristics, blood, and, clinical indices, were collected. **Key findings:** A total of 90 hospital-confirmed COVID-19-positive patients were eligible for our study. COVID-19 patients with obesity and DM showed poor vitamin K status which is correlated with severely compromised insulin signaling and inflammatory and immune states. It is also adversely associated with the existence of hypertension comorbidities. PKR levels significantly ($P<0.05$) and negatively predicted IRS ($R^2= 0.598$) explaining 59.8% of the variance in IRS. Also, ACE2 is significantly ($P<0.05$) and negatively predicted systolic blood pressure and explained 45.6% of the variance in SBP ($R^2=0.456$). *Significance:* Our study indicated that poor vitamin K status might be an important risk factor for severe adverse outcomes in COVID-19 patients with pre-existing obesity and type 2 diabetes. Also, metabolically disordered patients are highly susceptible to COVID-19 infection and its related adverse metabolic effects.

INTRODUCTION

In 2019, a novel coronavirus emerged and was named SARS-CoV-2 due to its genetic similarity to the previous coronavirus responsible for the severe acute respiratory syndrome (SARS) that evolved in 2003. The disease caused by SARS-CoV-2 was named Coronavirus Disease 2019 (COVID-19) (Andersen *et al.,* 2020). SARS-CoV-2 is one of the deadliest viruses in human history, having caused more than 6.8 million fatalities since its discovery in December 2019 (Li *et al.,* 2023). Coronaviruses are responsible for a range of illnesses, from mild colds to severe respiratory diseases (Andersen *et al.,* 2020). Coronaviruses

are enveloped viruses with a positive-sense, single-stranded RNA genome (F. Wu *et al.,* 2020).

The severity of the disease can vary significantly, ranging from asymptomatic or mild symptoms to severe respiratory distress and even death (S. Tamblyn, 2020). Certain pre-existing health conditions, such as metabolic disorders, play a crucial role in shaping the outcomes of COVID-19 (Pal & Banerjee, 2020). Particularly in vulnerable populations such as the elderly and those with pre-existing health conditions (S. Tamblyn, 2020). Along with the severe lung condition, COVID-19 can cause damage to various organs, including the heart, kidneys, and liver, leading to organ failure (Nalbandian *et al.,* 2021).

Some of the most prevalent metabolic disorders, including type 2 diabetes, obesity, dyslipidemia, and metabolic syndrome, contribute significantly to an individual's susceptibility to severe COVID-19 (Z. Hong Wu *et al.,* 2021). The interplay between metabolic disorders and COVID-19 is a substantial topic of research as individuals with pre-existing metabolic conditions are at an increased risk of developing severe forms of COVID-19 with worsened prognostics and mortality outcomes (Steenblock et al., 2021). Chronic low-grade inflammation and compromised immune function, found in metabolic disorders, may contribute to the excessive cytokine response observed in severe COVID-19 cases making individuals more susceptible to viral infections (Z. Hong Wu *et al.,* 2021). Additionally, the expression of angiotensin-converting enzyme 2 (ACE2) receptors, which SARS-CoV-2 uses to enter host cells, is influenced by metabolic dysregulation potentially exacerbating COVID-19 outcomes (Pal & Banerjee, 2020).

Studies have shown that individuals with impaired insulin sensitivity may be at an increased risk of severe COVID-19 outcomes (Drucker, 2021). The phosphatidylinositol-3-kinase/ AKT/ mammalian target of rapamycin (PI3K/AKT/mTOR) signaling pathway is intricately linked to insulin signaling and is involved in cell growth, proliferation, and survival. Dysregulation of this pathway has been implicated in various diseases, including cancer, metabolic disorders, and viral infections (Solerte et al., 2020). Obesity is often accompanied by other comorbidities, such as type 2 diabetes, cardiovascular diseases, and hypertension, which are also risk factors for severe COVID-19 outcomes (Stefan *et al.,* 2020). Thus understanding the intricate relationship between metabolic disorders and COVID-19 is crucial for optimizing patient care and implementing targeted preventive measures (Steenblock *et al.,* 2021).

Vitamin K is a fat-soluble vitamin found as phylloquinone in green leafy vegetables and menaquinones in animal products (Dam *et al.,* 2015). Vitamin K is stored in all body tissues, especially in the liver, heart, brain, kidneys and pancreas (Ho *et al.,* 2020) Vitamin K intake was associated with several components of metabolic disorders. High intakes have been associated with improved insulin sensitivity, glycemia, lipoprotein profile, lower body mass index (BMI) (Dam *et al.,* 2015) and lower severity of COVID-19 onset (Desai *et al.,* 2021). An important function of vitamin K is acting as a cofactor for gamma-glutamate carboxylase during the conversion of glutamate (protein-bound residues) into gamma carboxyglutamate (Gla). Poor vitamin K status can result in the appearance of inactive uncarboxylated species of these Gla proteins And its poor status, can be detected from high blood levels of uncarboxylated matrix Gla proteins (Dam *et al.,* 2015).

This research studies the correlation between vitamin K status, pancreatic beta-cell function, insulin resistance, PI3K/AKT/ mTOR signaling, and ACE2/angiotensin II in the context of COVID-19 along with pre-existing metabolic disorders, shedding light on potential metabolic effects of COVID-19 infection contributing to the severity in these conditions.

MATERIALS AND METHODS

1-Study Subjects:

The investigation is hospital-based, and conducted at the hospitals of Cairo, Egypt, from October 2021 to January 2022. It was done to study some metabolic pathways in the context of COVID-19 infection and its metabolic effects among metabolically disordered confirmed COVID-19-positive hospitalized cases and negative subjects. A total of 88 COVID-19-positive patients with or without pre-existing obesity and/or type 2 diabetes (male: female = 50:38) and 28 controls (male: female = 18:10) were enrolled. Patients with COVID-19 were confirmed to be SARS-CoV-2 positive using a polymerase chain reaction (PCR) test. COVID-19-positive patients were divided as normal (n=24), obese (n=23), diabetes (n=19), and diabesity (n=22) (suffering from obesity and type 2 diabetes mellitus (DM)). The age range for all participants was restricted to be ≥35 and ≤70 years old.

Demographic data of patients including age, sex, and presence of hypertension and disease histories were collected. Measurements of capillary oxygen saturation were done using pulse oximetry (model: JZK-303). Blood pressure was also measured using an automatic wrist blood pressure monitor (USA, eB-G0I0F2Z10255). Blood samples were collected from all included subjects under aseptic conditions. Erythrocyte sedimentation rate (ESR) measurement (mm/hr) was made using a fully automated ESR analyzer (the reference value for ESR was 0-20 mm/h for males and 0-30 mm/h for females).

2-Inclusion Criteria:

Patients with confirmed COVID-19 had positive real-time reverse transcription-polymerase chain reaction (RT-PCR) results for coronavirus RNA in suspected cases (cases with epidemiological history of contact with confirmed cases in the previous 14 days), or clinical presentations (having fever and/or respiratory tract symptoms, or loss of taste or smell). COVID-19 patients were confirmed to be obese and have T2DM according to the diagnostic criteria of the World Health Organization (Alberti & Zimmet, 1998). Obesity could be defined by body mass index (BMI) ≥30 kg/m^2 and waist circumference (≥88 cm in females and ≥104 cm in males). T2DM is defined by fasting blood glucose ≥126 mg/dL; or random blood glucose ≥200 mg/dL. Controls were recruited within the same geographical area of COVID-19 patients. Only those subjects qualified as controls had normal blood sugar based on the updated American Diabetes Association standards (Niroomand et al., 2019) and reported no history of developing obesity, type 2 diabetes, and hypertension were included in the study.

3-Subjects' Laboratory Evaluation:

Laboratory measurements included blood glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR), glycated hemoglobin (HbA1c), pancreatic beta cell function, blood routine (hemoglobin, red blood cells (RBCs), platelet, white blood cells (RBCs), lymphocytes, monocytes, and neutrophils counts) were measured by routine laboratory testing. Also, other biomarkers including; PKR (PERK double-stranded RNA-dependent protein kinase), insulin receptor substrate 1 (IRS1) insulin resistance signaling (PI3K/AKT/mTOR), inflammation factors (monocyte chemoattractant protein 1 (MCP1), C-reactive protein (CRP), ferritin) and blood pressure indicators (ACE2 and angiotensin II) were measured by the corresponding human ELISA kits.

3.1.Dephosporylated Uncarboxylated Matrix Gla Protein (dp-ucMGP):

This protein is used as an indicator of vitamin K status in the body because of the inability to directly quantify blood vitamin K levels. Serum dp-ucMGP level was used to indirectly measure extrahepatic vitamin K status. Low vitamin K status is indicated by High dp-ucMGP levels. Vitamin K (dp-ucMGP) status was categorized as; low (dp-ucMGP > 897 ng/mL), mid (dp-ucMGP = 520-897 ng/mL), and high (dp-ucMGP <520 ng/mL) (Desai et al., 2021).

3.2.Ethical Approval:

Written and informed consent from participants enrolled in the study was obtained and the study was approved by the

ethical committee of the Faculty of Women for Arts, Science and Education, Ain Shams University (Approval No: sci1432307002).

3.3.Statistical Analysis.

The data between groups were compared using the student's t-test or Mann-Whitney U test. The data were entered into the software of Statistical Package for the Social Sciences (SPSS) version-20; IBM Corp., Armonk, NY, USA. The data was then revised, and coded. Categorical data is presented as numbers (%). Continuous data are presented as mean ±standard deviation (±SD) or median (interquartile range IQR) with the data distributed normally according to the significance of the test of normality. Data are shown as n (%) for categorical variables. Linear regression analysis was conducted to determine the associations between some variables that demonstrated significant associations with COVID-19 and metabolic comorbidities. Pearson's correlation analysis was done to represent the correlation between metabolic disorders and COVID-associated variables. Median values and interquartile range were also determined by SPSS software. A *p-value* $\leq$ 0.05 was selected as the threshold of significance.

RESULTS

1-Patients' Characteristics and Comorbidities:

A total of 116 subjects were enrolled in the study (Table 1). The median age of all cases selected was between ≥35 years and ≤70 years. Of them, 28 subjects were control (median age 49 yrs, IQR= 41.5-60 yrs) without pre-existing or previous history of obesity (their mean BMI= 22.94±0.92 kg/m^2, and waist circumference= 88.29±5.13 cm) type 2 diabetes or hypertension and were negative at the COVID-19 testing. All the remaining 88 patients were selected after being positively checked for COVID-19 infection and 24 cases (median age= 48 yrs, IQR= 41.5-56 yrs) were included to be without pre-existing or previous history of obesity (their mean BMI= 23.09±1.08 kg/m^2, and waist circumference= 87.04±5.18cm), type 2 diabetes or hypertension [13 (54.2%) male cases and 11 (45.8%) female]. 23 obese patients (median age= 46 yrs, IQR= 39-62 yrs), with 14 (60.9%) male and 9 (39.1%) female cases were enrolled, and their mean BMI recorded 35.87±2.99 kg/m^2 and waist circumference= 103.9±5.43 cm. Also, 19 patients (median age= 48 yrs, IQR= 41-62 yrs) were selected with pre-existing type 2 diabetes mellitus without obesity (their mean BMI= 27.52±1.45 kg/m^2, and waist circumference= 93.74±5.54 cm) of them 11 (57.9%) male and 8 (42.1) female cases. The remaining 22 patients (median age=50 yrs, IQR= 44-58 yrs) were suffering from pre-existing obesity and type 2 DM (their mean BMI= 38.06±4.72 kg/m^2, and waist circumference= 104.6±3.53 cm), 12 (54.5%) male and 10 (45.5%) female cases.

Considering the blood pressure levels, 62.5% of normal COVID-19 patients were hypertensive (median systolic pressure=123 mmHg (IQR=120-125) vs. 117.5 mmHg (IQR=109-120) in control subjects, and diastolic pressure =78 mmHg (IQR=76-79.5) vs. 83.5 mmHg (IQR= 78.5-87.5) in control subjects). Among the patients suffering from pre-existing obesity and /or DM, about 81.8 % of patients with diabesity were hypertensive with a median systolic pressure =129.5 mmHg (IQR= 127-134) and diastolic pressure =80 mmHg (IQR= 79-82) as compared with 65.2% of obese cases and 42.1 % of the diabetic cases as indicated in Table (1). The hypertensive case was significantly and positively correlated with the severity of insulin resistance (table 3). Collectively the co-existence of obesity and diabetes mellitus with COVID-19 infection severely impacts insulin resistance, inflammation, and hypertension in those patients.

Table 1: Characteristics of COVID-19 +ve patients with or without obesity and/or T2DM.

	Control subjects (n= 28)	Normal with covid-19 (n=24)	Obese with covid-19 (n=23)	Diabetic with covid-19 (n=19)	Diabesity with covid-19 (n=22)	P value
Characteristics						
Age (yrs), median (IQR)	49 (41.5-60)	48(41.5-56)	46(39-62)	48(41-62)	50(44-58)	0.892
Male, n (%)	18 (64.3)	13 (54. 2)	14 (60.9)	11 (57.9)	12 (54.5)	0.741
Female, n (%)	10 (35.7)	11 (45.8)	9 (39.1)	8 (42.1)	10 (45.5)	0.741
BMI (kg/m^2)	22.94±0.92	23.09±1.08	35.87±2.99	27.52±1.45	38.06±4.72	<0.001
Waist circumference (cm)	88.29±5.13	87.04±5.18	103.9±5.43	93.74±5.54	104.6±3.53	<0.001
Symptoms of COVID infection, n (%)						
Fever	-	13 (54.2)	13 (56.5)	10 (52.6)	13 (59.1)	<0.001
Sore throat	-	12 (50.0)	12 (52.2)	11 (57.9)	14 (63.6)	<0.001
Cough	-	10 (41.7)	9 (39.1)	9 (47.4)	7 (31.8)	0.001
Shortness of breath	-	7 (29.2)	7 (30.4)	6 (31.6)	7 (31.8)	0.022
Headache	-	8 (33.3)	15 (65.2)	10 (52.6)	16 (72.7)	<0.001
Fatigue	-	13 (54.2)	15 (65.2)	13 (68.4)	17 (77.3)	<0.001
Abdominal pain	-	12 (50.0)	6 (26.1)	8 (42.1)	10 (45.5)	0.005
diarrhea	-	12 (50.0)	7 (30.4)	8 (42.1)	10 (45.5)	<0.001
Comorbidities						
Hypertensive subjects, n(%)	-	15 (62.5)	15 (65.2)	8 (42.1)	18 (81.8)	<0.001
Systolic blood pressure (mmHg), Median (IQR)	117.5 (109-120)	123 (120-125)	128 (124-129)	120 (119-126)	129.5 (127-134)	<0.001
Diastolic blood pressure (mmHg), Median (range)	83.5 (78.5-87.5)	78(76-79.5)	79(75-80)	78(70-80)	80(79-82)	<0.001

BMI: body mass index, IQR: interquartile range, yrs: years. Categorical data is presented as number (%), while continuous data is presented as mean ±standard deviation (±SD) or median (interquartile range IQR). Significance is determined at P<0.05.

2-Laboratory Measures:

Considering the laboratory data in Table (2) including the patient's blood sugar, insulin, and insulin resistance measures along with blood cell count. Patients with positive COVID-19 tests had higher and comparable blood glucose and insulin levels to the control subjects. In Normal cases with COVID-19, the levels were 117.7±7.55 mg/dl and 13.57±1.54 µU/ml respectively. It was reflected in significantly higher insulin resistance (HOMA-IR values 3.95±0.53 vs. 2.23±0.63 in COVID-ve subjects) than control counterparts. Although glycated hemoglobin levels were non significantly comparable between those cases. The blood glucose, insulin, and HOMA-IR levels in obese patients were significantly higher. The severity of insulin resistance was found to be greatly increased in COVID-19 patients suffering from diabesity, HOMA-IR was about 5-fold the values in patients without obesity and DM. They also have higher levels of glycated hemoglobin. Importantly testing pancreatic beta-cell function indicated significantly compromised beta function in COVID-19-positive patients (38.2±4.99 vs. 51.1±18.8 in control subjects). Moreover, the beta-cell function is severely compromised with the co-existence of obesity and DM in COVID patients, adding more burden on the severity and disease progression in COVID patients.

The values of blood oxygen saturation were significantly reduced by COVID-19 infection as compared with control cases. The severity of hypoxia was worsened by the existence of obesity and or DM, especially in patients with pre-existing diabesity (84.73±2.69 % vs 89.29±2.03 % in COVID patients without obesity or DM) indicating the worsened conditions related to metabolic disorders.

One of the proposed molecular mechanisms that might be included in the development and worsening of insulin

resistance in COVID-19 patients is included in this study. It was found that the blood level of double-stranded RNA-dependent protein kinase (PKR) was significantly upregulated in COVID-positive patients either with or without obesity and/or T2DM. IRS1 values were significantly compromised by COVID-19 infection in patients suffering from pre-existing obesity and/or T2DM. The values were reduced in COVID patients significantly as compared with control subjects. More worsened and severe insulin resistance is found correlated with the presence of obesity and T2DM along with COVID-19 infection. In the same manner, this was reflected in the levels of insulin signaling and inflammatory pathway markers PI3K/AKT/mTOR/MCP1. Insulin signaling was significantly suppressed in COVID-positive patients and the case is worsened by the co-existence of obesity and/or T2DM. While the inflammatory marker MCP1 levels were significantly higher in COVID patients along with severely compromised and worsened inflammatory cases in patients suffering from diabesity. In the same manner, the levels of CRP, ferritin, and ESR were also significantly increased in COVID patients, and the inflammatory condition is severely increased by the co-existence of obesity and T2DM (Figs. 1- 3).

Correlating the levels of PKR and other metabolic variables, a significant correlation was found between PKR and insulin resistance signaling, blood glucose level, MCP1, ACE2, and blood pressure. Also, the insulin resistance indicator is found to be significantly correlated with PKR and other metabolic variables as well as hypertension markers (Table 3).

Focusing on the status of vitamin K (serum dp-ucMGP), our study found a significantly mild vitamin K status in all COVID-19-positive patients as compared with control persons. Although patients with obesity and DM have lower vitamin K status which is significantly comparable to patients without metabolic comorbidities. In our study, serum dp-ucMGP is significantly correlated with BMI, blood insulin and CRP values (Table 4). This might indicate to some extent its role in insulin resistance, inflammation and thrombotic changes correlated to these comorbidities especially worsened when combined with viral infection.

Blood hemoglobin and RBCs count in Table (2) were significantly reduced by COVID-19 infection and worsened condition was found in patients suffering from pre-existing obesity and/or DM. hemoglobin values recorded 8.76±0.98 g/dl vs. 12.05±1.48 g/dl and RBCs =2.74±0.08 10^{12}/L vs. 3.66±0.25 10^{12}/L in patients suffering from diabesity vs. those without obesity or DM. platelets and white blood cell count were non-significantly comparable between groups. While neutrophils count was significantly increased and lymphocytes and monocytes count significantly reduced by COVID-19 infection (6.31±0.20 10^{9}/L vs 2.27±0.14 10^{9}/L, 0.97±0.07 10^{9}/L vs. 1.55±0.10 10^{9}/L, and 0.22±0.02 10^{9}/L vs. 0.26±0.03 10^{9}/L in normal COVID-19 patients vs. control subjects, respectively) the reduced values of lymphocyte/monocyte ratio indicating compromised inflammatory condition and immunity.

On the other hand, the hypertensive cases found in COVID patients might be explained by highlighting ACE2/angiotensin II pathway (figure 4). It was found that blood levels of ACE2 were significantly downregulated and angiotensin II upregulation by COVID infection in patients with or without obesity and/or T2DM as compared with control subjects. The most severe vasoconstrictive and hypertensive cases could be found by the co-existence of diabesity with COVID-19 infection.

Table 2: Some laboratory indices of COVID-19 +ve patients with or without obesity and/or T2DM.

	Normal range	Control subjects (n= 28)	Normal with covid-19 (n=24)	Obese with covid-19 (n=23)	Diabetic with covid-19 (n=19)	Diabesity with covid-19 (n=22)	P value
Blood glucose (mg/dl)	70 - 110	78.50±9.89	117.7±7.55**	122.4±7.97*	301.5±9.81*	344.4±17.76*	<0.001
Blood insulin (µU/ml)	<17	11.63±3.39	13.57±1.54**	14.20±2.11	18.26±1.57*	20.44±3.91*	<0.001
β-cell function		51.1±18.8	38.2±4.99**	38.4±6.20	18.3±1.84*	17.9±1.54*	<0.001
HbA1c (%)	<5	3.00±0.55	3.25±0.82	4.57±0.88*	6.86±0.96*	7.15±1.14*	<0.001
HOMA-IR		2.23±0.63	3.95±0.53**	4.30±0.75*	13.59±1.32*	17.38±1.31*	<0.001
Blood oxygen saturation (%)	95-100	96.96±1.69	89.29±2.03**	88.69±1.06	87.42±1.35*	84.73±2.69*	<0.001
Vitamin K status:							
Dp-ucMGP (ng/ml)		575±157	681±49.9**	756±78.2*	722±88.5*	803±62.7*	<0.001
Routine blood analysis:							
Hemoglobin (g/dl)	12-16	15.13±0.13	12.05±1.48**	11.60±1.11	11.28±1.18*	8.76±0.98*	<0.001
Red blood cells (10^{12}/L)	3.92 – 5.65	4.85±0.19	3.66±0.25**	3.50±0.22*	3.28±0.07*	2.74±0.08*	<0.001
Platelets (10^9/L)	150- 450	165.4±8.54	168.2±13.5	174.4±12.3	173.1±11.3	169.4±14.18	0.066
White blood cells (10^9/L)	3.50–9.50	8.32±0.56	8.48±0.55	8.37±0.45	8.35±0.56	8.29±0.48	0.775
Neutrophils (10^9/L)	1.80–6.30	2.27±0.14	6.31±0.20**	6.74±0.25*	6.26±0.07	6.11±0.69*	<0.001
Lymphocytes (10^9/L)	1.10–3.20	1.55±0.10	0.97±0.07**	0.96±0.04	0.96±0.6	0.98±0.06	<0.001
Monocytes (10^9/L)	0.12–1.20	0.26±0.03	0.22±0.02**	0.23±0.01	0.24±0.06	0.22±0.06	0.022
Lymphocyte/ Monocyte ratio		6.09±0.66	4.28±0.39**	4.18±0.26	4.34±0.98	4.68±1.56*	<0.001

Values are presented as mean ±SD. ND; not determined *means are significant at (P<0.05) as compared with normal-COVID subjects. **means are significant at (p<0.05) as compared with the control subjects.

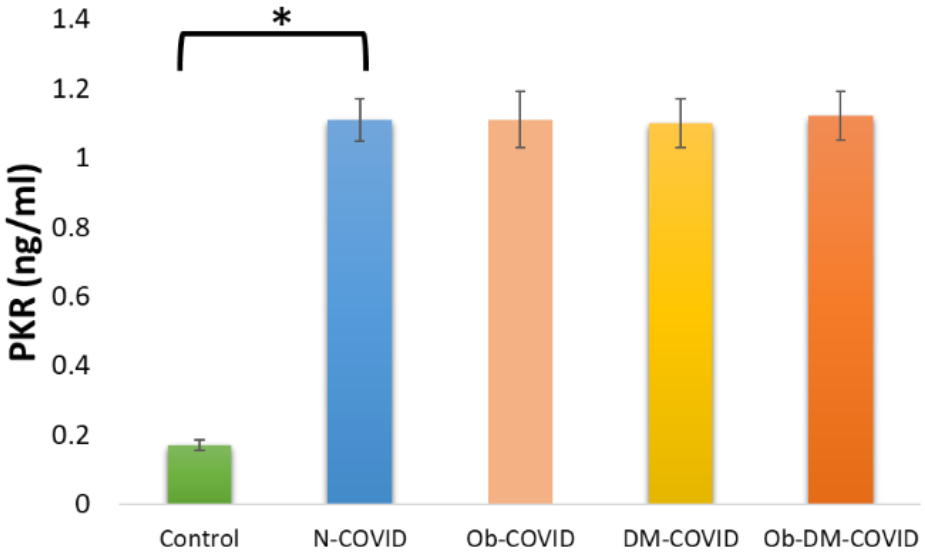

Fig.1: the level of PKR in COVID-19 +ve patients with or without obesity and/or T2DM. *values are statistically significant at P<0.05.

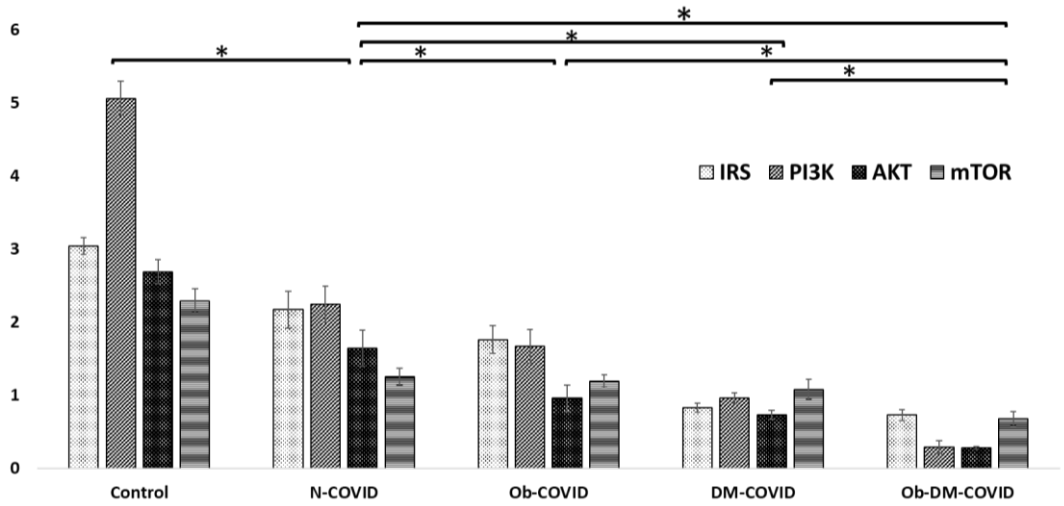

Fig.2: the level of insulin resistance signaling (IRS1/PI3K/AKT/mTOR) in COVID-19 +ve patients with or without obesity and/or T2DM.*values are statistically significant at P<0.05 between groups.

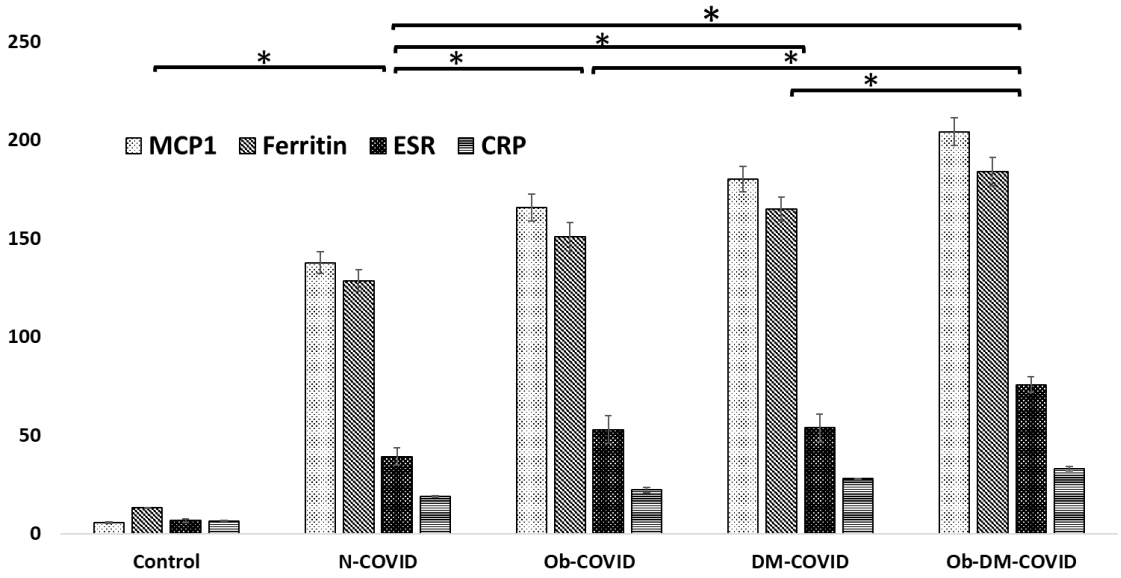

Fig. 3: the level of MCP1, ferritin, ESR, and CRP in COVID-19 +ve patients with or without obesity and/or T2DM.*values are statistically significant at P<0.05 between groups.

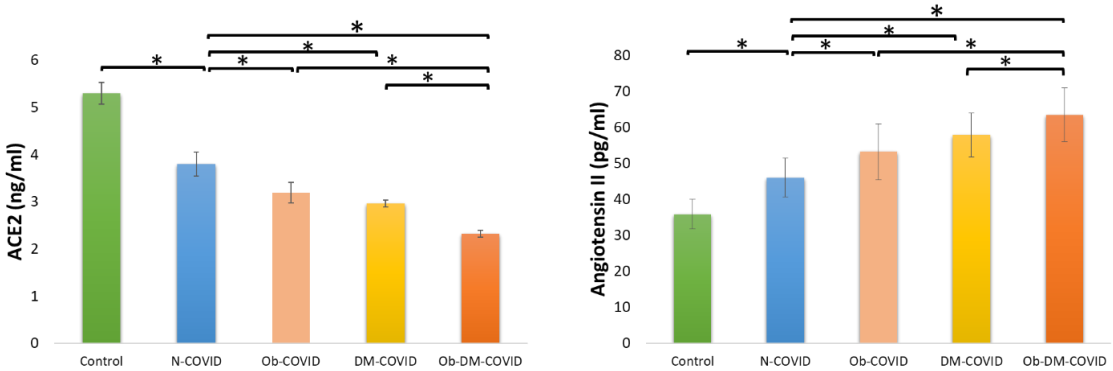

Fig.4: the blood level of ACE2 and angiotensin II in COVID-19 +ve patients with or without obesity and/or T2DM.*values are statistically significant at P<0.05.

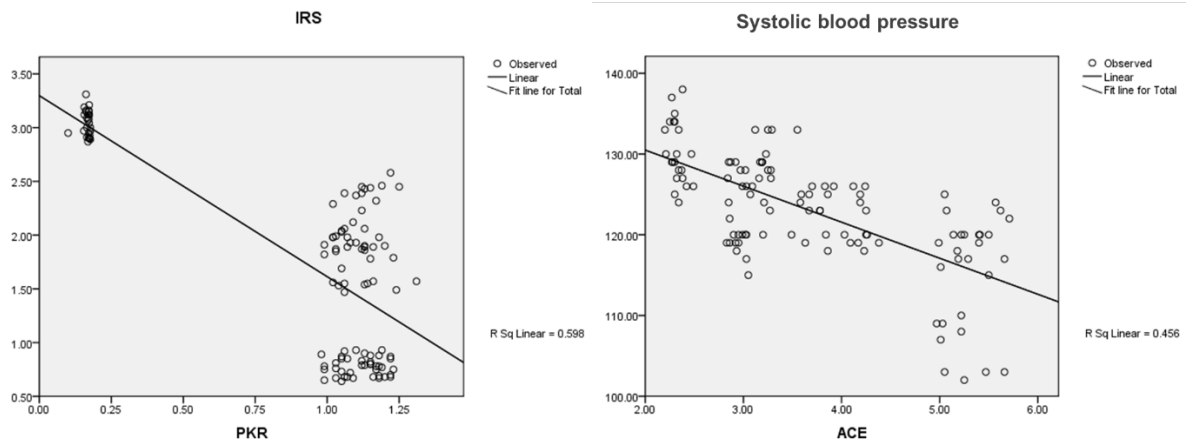

Fig. 5: linear regression between the dependent variables IRS and systolic blood pressure and the independent variables PKR and ACE2, respectively. The results indicated that PKR levels significantly (P<0.05) and negatively predicted IRS. Moreover, the R^2= 0.598 depicts that the model explains 59.8% of the variance in IRS. Also, ACE2 is significantly (P<0.05) and negatively predicted systolic blood pressure and explained 45.6% of the variance in SBP (R^2=0.456).

Table 3: Pearson's correlation between insulin resistance, inflammation, and blood pressure variables in metabolically disordered COVID patients.

Pearson's correlation											
	Glucose	**Insulin**	**HbA1c**	**HOMAIR**	**β-Cell Function**	**IRS**	**PKR**	**ACE2**	**MCP1**	**Systolic BP**	**P values**
Glucose	1										<0.001
Insulin	.861	1									<0.001
HbA1c	.833	.752	1								<0.001
HOMAIR	.987	.914	.822	1							<0.001
β-Cell Function	-.860	-.549	-.715	-.802	1						<0.001
IRS	-.918	-.831	-.840	-.898	.833	1					<0.001
PKR	.428	.444	.411	.404	-.507	-.649	1				<0.001
ACE2	-.812	-.775	-.750	-.809	.743	.932	-.741	1			<0.001
MCP1	.725	.699	.682	.712	-.719	-.878	.877	-.944	1		<0.001
Systolic BP	.432	.485	.338	.472	-.361	-.478	.474	-.631	.604	1	<0.001

Variables are significantly correlated at P<0.05. A severe correlation is present between IRS, ACE2, MCP1, β-cell function & PKR. Also, blood glucose levels, insulin levels, IRS & ACE2 are severely correlated with each other. A moderate correlation is found between PKR, HOMA-IR, blood glucose & insulin. Blood pressure is moderately correlated with PKR, HOMA-IR, IRS, and ACE2 values.

Table 4: Pearson's correlation between vitamin K status and some metabolic variables.

Pearson's correlation		
	Vitamin K status (dp-ucMGP)	**P value**
BMI	.532	<0.001
Blood glucose	.418	<0.001
Blood Insulin	.560	<0.001
CRP	.569	<0.001

Variables are significantly correlated at P<0.05. A moderate and significant correlation is present between blood levels of dp-ucMGP, insulin, CRP as well and BMI.

DISCUSSION

This study aims to shed light on some metabolic effects of COVID-19 infection among the pancreatic beta cell function and the signaling mechanisms correlating insulin resistance, inflammation, and blood pressure markers in Egyptian COVID-19-positive patients with/without pre-existing obesity and/or diabetes mellitus. Highlighting the correlation of vitamin K status with the severity and adverse outcomes of COVID-19 and metabolic disorders.

Previous research suggested that those with diabetes are more likely to get COVID-19 and experience more severe illness. This is in part because of the metabolic syndrome's related systemic inflammatory state and pro-thrombotic environment. Obesity and type 2 diabetes are metabolic syndromes that include insulin resistance as their underlying disease. Insulin resistance impacts metabolic and cardiovascular balance by impairing insulin signaling pathways (Gangadharan *et al.,* 2021). It is now widely accepted that people with COVID-19 have higher hospital admission rates, morbidity, and death when they are older, have diabetes mellitus, have hypertension, and are obese (Jain & Yuan, 2020). In our study, COVID infection is markedly associated with severe and more adverse metabolic effects, especially in patients with pre-existing obesity and diabetes mellitus.

It has been shown that a variety of physiological and pathological stresses, including viral infection and hypoxia, can trigger the cellular integrated stress response (ISR). Different stresses trigger the family of four serine/threonine kinases—PKR-like ER kinase (PERK double-stranded RNA-dependent protein kinase (PKR), heme-regulated eIF2a kinase (HRI), and general control non-derepressible 2 (GCN2)—to activate at least one of their members (Leiria *et al.,* 2015)-(Santos *et al.,* 2021). Specifically in our findings, PKR is significantly increased in all COVID-positive patients included in the study. Moreover, the increase in PKR is found to be an independent factor for IRS-1 and depicted about 59.8% in IRS-1 reduction. For COVID-19 specifically, viral RNA fragments can activate PKR, causing IRS-1 serine phosphorylation and subsequently insulin resistance (Santos *et al.,* 2021). We, therefore, suggest that insulin resistance related to COVID infection accompanies ISR. Also, a significant correlation is found between PKR, blood glucose, insulin, HOMA-IR, and pancreatic β-cell function. Indicating progressive correlation between the severity of insulin resistance, pancreatic beta cell function, and COVID-19 infection in patients with pre-existing obesity and/or DM.

Numerous studies have shown that insulin resistance or hyperglycemia are independent risk factors for the development and death in individuals with a variety of infectious illnesses, including SARS and COVID-19 (Singh & Khunti, 2020) (S. Wang *et al.,* 2020), (Zhu et al., 2020). hyperglycemia is always associated with elevated levels of glycated hemoglobin thus harming its ability to carry oxygen. In our findings oxygen saturation is significantly lowered with COVID-19 infection and the severity was greater with the co-existence of obesity and T2DM. It has shown that SARS-CoV-2 non-structural proteins can target the b1- chain of hemoglobin leading to the dissociation of iron from porphyrin, therefore impairing the ability of hemoglobin to carry oxygen and it might have a higher affinity to bind to glycated hemoglobin than non-glycated hemoglobin which is just a hypothesis (Pal & Bhadada, 2020).

One of the proposed mechanisms by which the SARS-CoV-2 virus could enhance insulin resistance in pre-existing metabolic comorbidities might be through the upregulation of PKR in infected cells. PKR can induce IRS-1 suppression through serine phosphorylation which subsequently downregulates cellular insulin signaling and leads to insulin resistance. Although the underlying molecular processes for this association are still being elucidated, it is significant to note that the insulin signaling

pathway may, in theory, be regulated by either PI3K/Akt or ERK signaling (Santos *et al.,* 2021), and our results showed that the PI3K/AKT/mTOR pathway is responsible for this insulin action in glucose metabolism. The activation of PKR by viral RNA resulted in worsened insulin resistance and hyperglycemia in patients with pre-existing obesity and T2DM. Furthermore, One important gene found to be suppressed by mTORC2 is monocyte chemoattractant protein 1 (MCP1) (Santos *et al.,* 2021). Derepressed MCP1 will attract monocytes to adipose tissue, which will be converted into M1 macrophage with subsequent production of inflammatory cytokines and aggravation of the inflammatory state (Shimobayashi *et al.,* 2018). We found a significant correlation between the MCP1 values and metabolic disorders and insulin resistance variables. Moreover, our study showed a compromised inflammatory state in metabolically disordered COVID patients, through significant elevation in CRP, ferritin, and ESR levels. It is speculated that COVID-19 may trigger the change in characteristics of RBCs or plasma, resulting in increased ESR, which is an important inflammatory biomarker elevated in COVID-19 along with CRP (Ponti *et al.,* 2020), which acts as a valuable early marker for predicting the possibility of progression of disease (Liu *et al.,* 2020), (Gajendra, 2022). The research studies by Guo and Cai and their collegues also showed that COVID-19 patients with diabetes had considerably higher blood levels of inflammation-related biomarkers including IL-6 and CRP (Guo et al., 2020), (Cai *et al.,* 2020). The blood levels of interleukin-6 (IL-6), C-reactive protein, and ferritin were considerably greater in COVID-19 patients with DM than in those without DM. As T2DM is a pro-inflammatory condition characterized by an inappropriate and excessive cytokine response (Guo e*t al.,* 2020). Patients who do not have diabetes but experience hyperglycemia may experience worsened COVID-19 symptoms due to higher levels of serum proinflammatory cytokines. This condition is also linked to severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1) infection (Zhu *et al.,* 2020). Thus, we might suggest that the cytokine storm seen in Covid-19 patients with obesity and/or DM may also be aggravated by inflammation brought on by insulin resistance. According to this, those who have both insulin resistance and hyperglycemia are more likely to experience an inflammatory cytokine storm that ultimately causes a fast worsening of COVID-19 infection. Because of this, people with COVID-19 and pre-existing metabolic illnesses exhibit a variety of outcomes depending on their glucose control. Furthermore, when coupled with COVID-19 infection, metabolically associated inflammation and compromised immune function may lead to a more severe inflammatory response.

Highlighting the renin-angiotensin-aldosterone system (RAAS), we suggest that modulation of RAAS is another important mechanism of the effects of COVID-19 in metabolically disordered patients. Our findings indicated a significant reduction in ACE2 levels in all COVID-19-positive patients along with upregulation of angiotensin II. Our regression study suggests that ACE2 is significantly ($P<0.05$) and negatively predicted systolic blood pressure (SBP) and explains 45.6% of the variance in SBP ($R^2=0.456$) in metabolically disordered COVID-19 patients. ACE2 is a membrane-bound aminopeptidase that counteracts the RAAS pathway. Angiotensin 1-9 and angiotensin 1-7, which are inactive isoforms and possess vasodilator and antifibrotic properties, are produced when ACE2 breaks down angiotensins AI and AII. According to one theory, SARS-CoV binds to and suppresses the production of ACE2, which is linked to AII's unopposed activity, hypertension, and fibrotic states seen in COVID patients (Santos *et al.,* 2021) (Xu *et al.,* 2020). Previous studies confirmed human pancreatic beta cells are highly permissive to SARS-CoV-2 infection (Yang *et al.,* 2020). This might partially explain why individuals with hyperglycemia have a higher risk of developing serious infections. Additionally,

individuals with hyperglycemia had delayed SARS-CoV-2 clearance (Chen *et al.,* 2021). It is therefore conceivable that individuals with hyperglycemia are vulnerable to SARS-CoV-2 infection (Cai *et al.,* 2020). This is consistent with our findings suggesting a strong significant correlation between ACE2 levels and the metabolic markers of blood glucose, insulin, insulin resistance, and inflammatory markers. Thus highlighting the significant correlation between hypertension and the severity of COVID-19 infection with the co-existence of metabolic comorbidities.

In our study vitamin K status is evaluated by measuring dp-ucMGP in blood, and we reported that it is significantly lower in COVID-19 patients and significantly correlated with the co-existence of metabolic comorbidities in those patients. Previous studies indicated that Vitamin K deficiency is correlated with BMI, inflammatory markers, and comorbidities (metabolic, cardiovascular, pulmonary, and renal disorders) that increase the severity of COVID-19 (Linneberg *et al.,* 2021). Vitamin K deficiencies have been demonstrated in adults hospitalized with COVID-19 infection (Anastasi *et al.,* 2020) and might predict higher mortality rates (Desai et al., 2021). Poor vitamin K status has been associated with high body weight, BMI, and fat mass (Knapen et al., 2012). It has been hypothesized that adipose tissue can sequester fat-soluble nutrients, which lowers their bioavailability (Dam *et al.,* 2015), which explains the decrease in vitamin K status observed in obese subjects. Higher vitamin K1 ingestion was also correlated with higher insulin sensitivity and glycemic status in the 2-h oral glucose tolerance test, suggesting that vitamin K1 intake may have a beneficial effect on glucose homeostasis, and insulin level and is related to a lower risk of T2DM (Ho et al., 2020). This might be due to the anti-inflammatory and antioxidant activity of vitamin K (Nuszkiewicz *et al.,* 2023). A positive correlation between low hepatic vitamin K status and elevated interleukin 6 (IL-6), TNF-α, IL-1α, IL-1β (Pan *et al.,* 2016). This might be because vitamin K can inhibit the release of IkB from NF-kB leading to its inhibition (Ohsaki *et al.,* 2010). These observations suggest that the association between vitamin K, and COVID-19 severity outcome is likely partially due to vitamin K's anti-inflammatory effect, lower extrahepatic activation of the anticoagulant protein S, leading to accelerated thrombosis formation, and lower activation of MGP, reducing inhibition of mineralization of the elastic fibers in the lung, leading to accelerated elastic fiber damage (Desai *et al.,* 2021).

The findings of poor vitamin K status could be correlated with compromised hematological indices in our study. We found that COVID-19-positive patients with pre-existing obesity and T2DM have lower levels of blood hemoglobin, red blood cell count, and lymphocyte/monocyte ratio. Also, leukopenia is correlated to some extent with COVID-19 infection. The findings indicated significant anemic and worsened immunity and inflammatory states and are correlated with the existence of metabolic comorbidities, thus increasing the severity and mortality risk in COVID-19 patients especially when accompanied by obesity and T2DM. The most frequent laboratory finding in SARS-CoV-2 infection is lymphopenia, which may be seen in 25 to more than 80% of patients at the time of admission (Hu *et al.,* 2020). A possible cause of lymphopenia is the direct attachment of viral proteins to ACE2 receptors located on their surface, leading to the destruction of the cell (Xu *et al.,* 2020), or lymphocyte apoptosis caused by the viral-related cytokine storm (Liao *et al.,* 2002), This worsens the already impaired immunity and more severe inflammatory symptoms seen by COVID patients, which were already present in individuals with metabolic problems. Numerous lines of evidence point to obesity and diabetes as significant mortality hazards in COVID-19 patients (D. Wang *et al.,* 2020) and we suggest that COVID infection in those patients could have more worsened and adverse metabolic impacts alongside the co-existed metabolic burden. The underlying mechanism of adverse consequences of COVID-19 might include insulin resistance, hypertension, and inflammatory storm (Guo

et al., 2020), (Whyte *et al.,* 2020), (Cheng *et al.,* 2021).

The limitations of this study should be noted. First off, since our study only included one center and had limited samples of people with diabetes, obesity, and diabesity, the results need to be validated in a larger study. Second the small sample size due to that at the time of the study, COVID infection was somehow controlled by the worldwide hygienic applications. Third the effects of diabetic medical treatment couldn't be collected from all cases and is not considered in the study. Data from bigger populations and several centers are necessary to further corroborate the findings of the current study to prevent statistical bias.

Conclusion:

In the present study, the hospital confirmed COVID-19 positive patients with/without obesity and/or T2DM had adverse outcomes, which might be correlated to the consequences of coexisting poor vitamin K status, hyperglycemia, insulin resistance, deteriorated inflammation, impaired immunity, and vascular effects. Our study suggested that the downregulation of PKR by viral RNA is responsible for the subsequent downregulation of insulin signaling IRS/PI3K/AKT/mTOR pathway. Also, the regression study showed that IRS and systolic blood pressure were dependently and significantly affected by PKR and ACE2 levels respectively. Our study suggests a worsened status of vitamin K and viral infection in metabolically disordered patients along with their susceptibility to viral infection.

Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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