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Assessment of plasmin and inducible nitric oxide synthase in the patients with COVID-19

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***Abstract*---**Aims of study: The purpose of this study is to compare the levels of plasmin, inducible nitric oxide, and nitric oxide in COVID-19 infection patients to a healthy control group, with a scientific interpretation of the predicted results. Methods: Data was collected from ninety people, divided into two groups: patients and controls, and a demographic study was conducted, which included age, gender, oxygen saturation, and BMI, with diabetes and asthma eliminated. The plasmin and inducible nitric oxide levels are estimated using the enzyme-linked immune sorbent assay (ELISA) technique, and nitric oxide is measured using a spectrophotometer. Results: Patients with COVID-19 showed a substantial rise in the values of plasmin and inducible nitric oxide synthase enzyme, as well as a decrease in the value of nitric oxide, when compared to healthy controls.

***Keywords*---**COVID-19, plasmin, inducible nitric oxide, nitric oxide, griess reagent.

Introduction

The Coronavirus Disease 2019 (COVID-19), caused by a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has spread over the world by early October 2020, resulting in about 3.5 million confirmed cases and 1 million deaths. Although the majority of COVID-19 patients have minor, self-limiting symptoms, a considerable percentage of individuals develop a severe disease characterized by respiratory or multi-organ failure, as well as hyperinflammatory syndromes. Hypertension, diabetes, and chronic cardiac or renal disorders have all been associated to a higher risk of severe or fatal heart attacks. COVID-19 [1]. Plasmin is a crucial enzyme in the fibrinolysis process, which is responsible for

cleaving fibrin and dissolving clots during wound healing. Plasmin is a major fibrinolytic protease that is generated in the presence or absence of fibrin by fibrinolytic activators converting its zymogen plasminogen. Plasminogen is mostly produced in the liver and circulated throughout the body. at a concentration of 1.5 mol/L. Glu-plasminogen is a single-chain glycoprotein having an N-terminal activation peptide (NTP), five kringle domains (K1–K5), and a serine protease domain containing the catalytic triad, with glutamic acid (Glu) as the NH_2 residue [2] .

The inducible nitric oxide synthase enzyme (iNO) during viral infection, it is frequently increased in host cells, and it inhibits viral replication in SARS-CoV-1 infection. Its expression can be aided by bacterial lipopolysaccharide, cytokines, and other chemicals. Although the enzyme is mostly expressed in macrophages, it can be induced in almost any cell or tissue provided the appropriate inducing stimuli are found. The inducible nitric oxide synthase enzyme modulates vasomotor tone, inflammatory cell recruitment and function, and thrombosis regulation by acting as a vasoactive molecule. In acute respiratory distress syndrome (ARDS), the balance switches from pulmonary vasodilators to vasoconstrictors, resulting in increased pulmonary vascular resistance and pulmonary hypertension[3][4] .

Materials and Methods

Study Design

The study project is a case control study that began in October 2021 with (90) participants divided into two groups: (45) patients and (45) controls from the Merjan Medical City/Babylon City/Iraq respiratory care unit (RCU). Also, for the patients and control groups, demographic research criteria were taken (age, gender, and BMI).

Patients

Patients diagnosed with COVID-19 by a respiratory disease specialist based on CT scans and laboratory tests, who were mostly diagnosed with COVID-19 based on nasopharyngeal swab or sputum samples examined with a reverse transcriptase polymerase chain reaction (RT-PCR) for the presence of SARS-CoV-2 based on the study's criteria (age, gender, BMI, severity of disease, and oxygen saturation levels), and some cases that The information was gathered in Merjin Medical City in Babylon, Provence, notably at the respiratory care unit (RCU) and intensive care unit (ICU) (ICU).

Control

Collected data from control group depending on same criteria for control group matching with patients for (age, Gender and BMI).

Statistical Analysis

All data were reported as mean and standard deviation with a higher and lower 95 percent confidence interval for all variables. The T-test was used to determine if there was a significant difference between the two groups COVID 19 patients and controls and the probability values were less than 0.05. also results are analysis was performed using software package version 22 (SPSS)

Material and Methods

Samples Collection

The sample was obtained by taking whole blood with a disposable syringe and placing it in a gel tube for centrifugation at 4000 RPM to obtain serum, which was then frozen at -20°C until analysis. The plasmin standard curve was obtained using the ELISA technique. (sandwich method) (Figure 1).

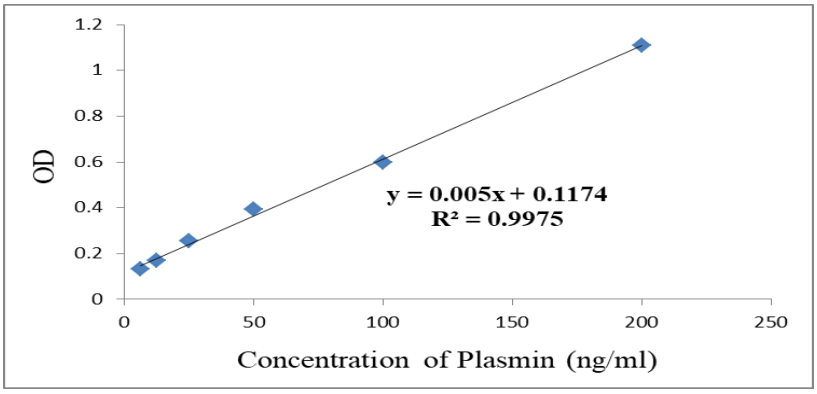

Fig. 1: Standard Curve of Plasmin Concentration

The standard curve of inducible nitric oxide synthase by ELISA (Figure 2).

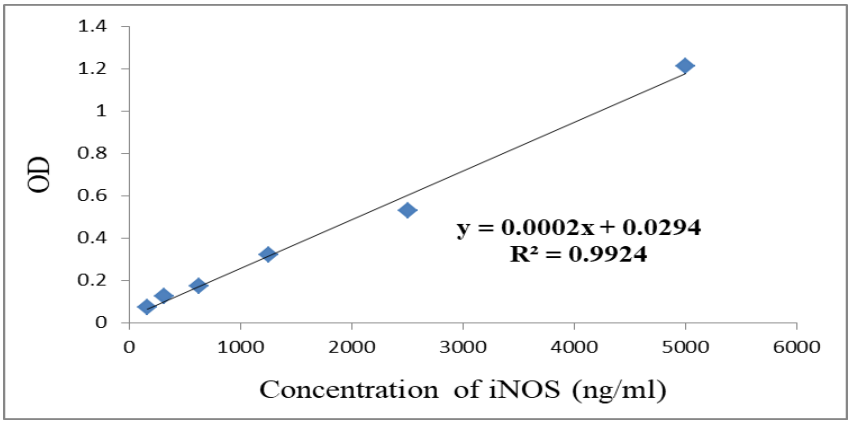

Fig. 2: Standard Curve of Inducible Nitric Oxide Synthase Enzyme Concentration

Nitric oxide (NO) can be determined by spectrophotometric method by using Griess reagent and the following the standard curve of NO [5](Figure 3):

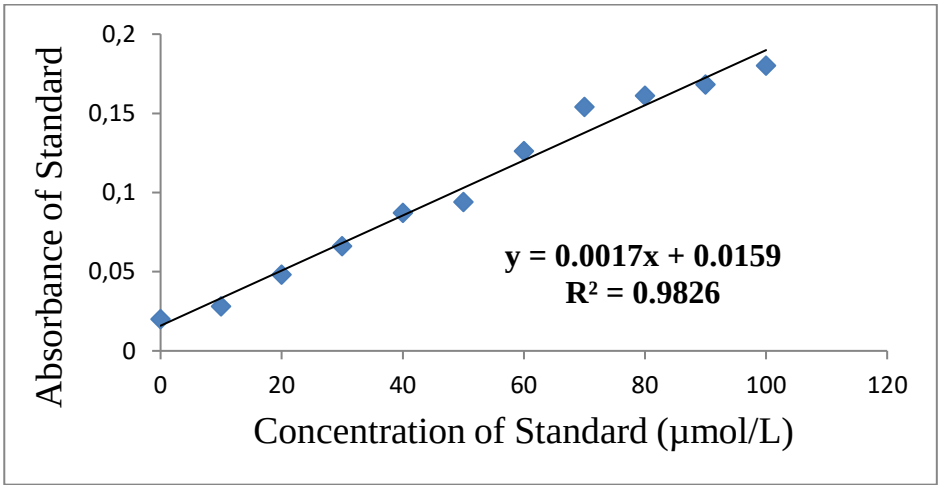

Fig. 3: Standard Curve of Nitric Oxide Concentration.

Results and Discussions

The medical information collected for the patients from whom samples were taken are shown in (Table 1), including age, BMI, gender, duration of hospitalization, oxygen saturation rate, disease severity, and lung damage rate through CT-SCAN results. It was noticed that there was a significant increase in the d-dimer value of the group of patients compared to the healthy controls. The severity of the disease was divided by specialized doctors into three stages, which are simple, medium and severe (Table 1).

Table 1: Some Medical Information for Patients and Controls

Medical Variables	Patients (mean ± SD)	Controls (mean ± SD)
1- Age	47.75±7.15	46.66±7.44
2- Gender	26 Male/19 Female	25 Male/20 Female
3- Percentage of Lungs damage by CT-SCAN	20.11±12.36	Not Found
4-Duration in Hospital for treatment /day	10.02±5.51	Not Found
5- Oxygen saturation percent %	89.13±3.98*	99.02±0.69
6- BMI	22.22±2.32	21.73±2.52
7- Severity of disease	13Severe/18Moderate/ 14 Simple	Not Found
8- D-dimer (mg/L)	617.33±217.75*	106.44±25.72

*The significance results when P-Value ≤ 0.05.

The results appear significant differences between the patient's group and control groups (Table 2) represent there is elevated in plasmin level in the COVID19 group (116.33±45.99) where the control group appears (72.70± 24.98).

Table 2: Result of plasmin levels

Biochemical Parameter	Type Groups	N	Means ± SD	SE	95%Confidence Interval		P-Value
					Lower	Upper	
Plasmin (ng/ml)	Patients	45	116.33±45.99	6.85	28.12	59.13	0.001
	Control	45	72.70± 24.98	3.72			

*The significance results when P-Value ≤ 0.05.

People who are susceptible to SARS often have elevated plasmin levels due to underlying medical disorders such as hypertension, diabetes, cardiovascular disease, cerebrovascular disease, and chronic renal illness. Infection with CoV-2 By cleaving the spike proteins of the SARS-CoV-2 virus, plasmin increases its virulence and infectivity.[6].

The S protein of coronaviruses can be broken by plasmin, trypsin, cathepsins, and elastase, and this cleavage may improve virus penetration into bronchial epithelial cells. In vitro, plasmin also cleaves the S proteins of SARS-CoV. [7]. The results of (Table 3) appeared significant elevated between the patients with COVID19 and control in levels of inducible nitric oxide synthase enzyme (3546±1111) This happened because viral infections frequently generate rapid and severe systemic inflammation, which causes active innate immune cells, monocytes, and macrophages to manufacture inducible nitric oxide synthase (iNOS) NO generated from iNOS was found to have anti-inflammatory properties in the study.

Excess production of NO (Table 4) occurs in inflammatory cascade and thromboembolism are caused by COVID-19. To lower this output, we intend to propose the usage of sildenafil. In addition, lung injury models have shown that higher levels of iNOS-derived NO are associated with positive benefits, but high bioavailability of NO from inducible NO synthase (iNOS) may aggravate lung injury. The development of harmful reactive nitrogen intermediates can result from persistently elevated (iNOs) levels..

Table 3: Result of Inducible Nitric Oxide Synthase Enzyme levels

Parameter	Groups	N	Means ±SD	SE	95%Confidence Interval		P-Value
					Lower	Upper	
iNOS (ng/ml)	Patients	45	3546±1111	165.68	210.123	1268.054	0.007
	Control	45	2807±1156	172.45			

*The significance results when P-Value ≤ 0.05.

Because inducible nitric oxide is created at 10 parts per million (ppm) in the human sinuses and diffuses into the bronchi and lungs to provide vasodilator and broncho dilatory effects, high levels of the enzyme operate as a preventive response against viral infection. It also aids in the activation of ciliary movement

and mucus secretion, which helps to prevent virus particles from entering the respiratory tract. The findings support prior research such as (Assessment of nitric oxide (NO) ability to attenuate COVID-19 severity)[8][9][10].

Table 4: Result of Nitric Oxide levels

Parameter	Groups	N	Means ±SD	SE	95%Confidence Interval		P-Value
					Lower	Upper	
NOS (µmol/L)	Patients	45	33.05±13.88	2.06	2.67	15.85	0.006
	Control	45	42.32±17.38	2.59			

*The significance results when P-Value ≤ 0.05.

From (Table 4) can notice a significant decrease in the concentrations of NO in patients with COVID-19 compared with controls may be consumed in by tissues lung to rise vasodilatations and also consumed by their activity to killed microorganisms and prevent viral replications, therefore there are some studies refer to the beneficial role of NO as a part of the protocol to treatment COVID-19.

Conclusion

A high levels of plasmin act as risk factor for morbidity in COVID-19 lead for elevated in D.Dimer and thrombus formation, also plasmin play role in cleavage the spike protein of COVID 19 infection . In contrast, the increment inducible nitric oxide synthase level consider a protective effectors in COVID 19 infection and inhibitory the viral replication cycle. But dramatically low levels of NO in patients with COVID-19 may be consumed in by tissues lung to rise vasodilatations and also consumed by their activity to killed microorganisms and prevent viral replications.

Ethical Approval: The Iraqi Ministry of Health gave their blessing to the research.
Study Conflict: No other studies are in conflict.

References

1. A. Zaremba, R. Kizys, D. Y. Aharon, and Z. Umar, “Term spreads and the COVID-19 pandemic: Evidence from international sovereign bond markets,” *Financ. Res. Lett.*, vol. 44, p. 102042, 2022.
2. A. G. Bharadwaj, R. W. Holloway, V. A. Miller, and D. M. Waisman, “Plasmin and plasminogen system in the tumor microenvironment: implications for cancer diagnosis, prognosis, and therapy,” *Cancers (Basel).*, vol. 13, no. 8, p. 1838, 2021.
3. K. Alkharfy, M. U. Rehman, and A. Ahmad, “Nitric oxide pathway as a potential therapeutic target in COVID-19,” *Farmacia*, vol. 68, no. 6, pp. 966–969, 2020.
4. K. M. Alkharfy, M. U. Rehman, and A. Ahmad, “Nitric oxide pathway as a potential therapeutic target in COVID-19,” *Farmacia*, vol. 68, no. 6, pp. 966–969, 2020, doi: 10.31925/farmacia.2020.6.2.

5. A. M. A. Al-Kufaishi, “Estimation the Scavenging Activities of Ascorbic Acid, Uric Acid, Gallic Acid and GSH to DPPH Radical,” *Jinu. M, Thankamma. P. Georg. NA Balaram, Sujisha. SS 2. Profile Burn Deaths A Study Based Postmortem Exam. Burn Cases RNT*, vol. 20, no. 3, p. 447, 2020.
6. M. W. Henderson *et al.*, “Plasmin-mediated cleavage of high-molecular-weight kininogen contributes to acetaminophen-induced acute liver failure,” *Blood, J. Am. Soc. Hematol.*, vol. 138, no. 3, pp. 259–272, 2021.
7. C. A. M. De Haan, L. Kuo, P. S. Masters, H. Vennema, and P. J. M. Rottier, “Coronavirus particle assembly: primary structure requirements of the membrane protein,” *J. Virol.*, vol. 72, no. 8, pp. 6838–6850, 1998.
8. U. Förstermann and H. Kleinert, “Nitric oxide synthase: expression and expressional control of the three isoforms,” *Naunyn. Schmiedebergs. Arch. Pharmacol.*, vol. 352, no. 4, pp. 351–364, 1995, doi: 10.1007/BF00172772.
9. P. Shree *et al.*, “Targeting COVID-19 (SARS-CoV-2) main protease through active phytochemicals of ayurvedic medicinal plants–Withania somnifera (Ashwagandha), Tinospora cordifolia (Giloy) and Ocimum sanctum (Tulsi)–a molecular docking study,” *J. Biomol. Struct. Dyn.*, vol. 40, no. 1, pp. 190–203, 2022.
10. K. Fehsel, A. Jalowy, S. Qi, V. Burkart, B. Hartmann, and H. Kolb, “Islet cell DNA is a target of inflammatory attack by nitric oxide,” *Diabetes*, vol. 42, no. 3, pp. 496–500, 1993.
11. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2021). The COVID-19 pandemic. *International Journal of Health Sciences*, *5*(2), vi-ix. https://doi.org/10.53730/ijhs.v5n2.2937
12. Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949
13. Khidoyatova, M. R., Kayumov, U. K., Inoyatova, F. K., Fozilov, K. G., Khamidullaeva, G. A., & Eshpulatov, A. S. (2022). Clinical status of patients with coronary artery disease post COVID-19. *International Journal of Health & Medical Sciences*, *5*(1), 137-144. https://doi.org/10.21744/ijhms.v5n1.1858