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The correlation between Bradykinin and Leukotreine with Glutathione in Patients with COVID19

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ABSTRACT

In Dec 2019 in Wuhan, China, several acute atypical respiratory diseases were brought on by a novel coronavirus that is currently known as SARS-CoV-2. A worldwide pandemic has been caused by the virus spreading from person to person. Numerous countries have been forced to enact public isolation and lockout as the death toll keeps mounting. There is still a need for therapeutic strategies, which is a problem. According to statistical data, senior people are more vulnerable to serious illnesses than children, who often experience minimal side effects. Assessment and study the correlation of bradykinin, leukotrienes B4 and D4 with glutathione for patients with COVID-19 compared to a healthy controlled study, with results that were interpreted scientifically. Ninety individuals, separated into a sick group and a healthy group, had blood samples taken. An analysis of demographic data, including gender, age, clinical severity, blood oxygen saturation, and body mass index, depending on the exclusion criteria for people with asthma, diabetics, pregnant women, and those with heart disease. Bradykinin and leukotriene B4 and D4 levels were estimated using enzyme-linked immunosorbent assay (ELISA), and glutathione measured by spectrophotometry method. The results manifest that the levels of bradykinin and leukotrienes B4, and D4 increased in significant values in the patients with COVID-19 compared with healthy control with a decrease in the values of glutathione the same comparison. Also, there is a negative correlation for glutathione with other parameters that were studied. Through the results shown in the study, it can be considered leukotrienes B4, D4, and bradykinin are very important for evaluating the functions of the lungs, as well as it is possible to give the specialist an important idea about the extent of the deterioration of the patient's condition, and thus facilitate the appropriate treatment strategy for him.

Keywords: COVID-19, Bradykinin, Leukotrienes, Glutathione, Oxidative stress

INTRODUCTION:

COVID-19 poses a threat to global health due to its rapid spread. In December 2019, a pneumonia infection with an undetermined etiology occurred for the first time in Wuhan, China. Ninety-five percent of those infected with the corona virus either have no clinical signs or have mild ones like high fever, headache, drowsiness, and loss of flavor and smell, while 15% of those infections caused have severe symptoms like shortness of breath, violent coughing, and oxygen deprivation that necessitates pulmonary rehabilitation(1). Bradykinin is a blood plasma protein formed in injured tissues, acts in the vasodilatation of small arteries, plays a part with inflammation processes. Bradykinin is the primary mediator of hereditary angioedema, which is a nine-amino acids peptide chain created by hydrolyzing the high-molecular- weight kininogen that serves as its precursor (HMWK). The negatively charged biomolecules bind to factor XII, causing a mechanical shift that activates the kallikrein-kinin system (KKS), which releases bradykinin(2).

Leukotrienes are chemical messengers that activate the immune response. Arachidonic acid is considered the precursor from which leukotriene elicits mediated by 5-lipoxygenase. Leukotriene A4 (LTA4) can be transformed to leukotriene B4 (LTB4) by the enzyme LTA4 hydrolase, or leukotriene synthase can couple it to reductive glutathione (LTA4). LTB4 and LTC4 are produced, respectively, by human neutrophils and eosinophils(3) . LTB4 and cysteinyl leukotrienes (cys-LTs) are both produced by monocytes and macrophages as well (4).The cells that produce this mediator metabolize LTC4 into LTD4 and LTE4. Additionally, the leukocytes' hypochlorous acid, which is produced during the respiratory burst, can convert the cysLTs into 6-trans-LTB4 (5). LTB4 is catabolically inactivated as a result of the interaction with PPAR, which in turn reduces inflammation caused by LTB4. It has also been noted that several LTB4 receptor antagonists possess PPAR antagonism action. As a result, reactions to LTB4 might be a combination including both anti-inflammatory and pro-inflammatory actions(6) .

Leukotriene B4 is significant in asthma, according to a lot of research. Asthmatic patients had elevated amounts of 5-lipoxygenase (5-LO) and leukotriene A4 hydrolase (LTA4H) in their bronchi and circulation neutrophils. Asthmatics have been shown to have elevated amounts of LTB4 in their blood, BAL fluid, and inhaled breath condensates(7) . LTB4 is a pro-inflammatory mediator with substantial functions in the recruiting, activation, and extension of the life of myeloid leukocytes, particularly neutrophils and eosinophils, as opposed to cys-LTs, which are powerful bronchoconstriction mediator (8).

The levels of reactive oxide species (ROS) are in dynamic equilibrium in healthy people, however, when exposure human to harmful factors such as radiation, inflammatory diseases, or malnutrition due to internal or external reasons, an abundance of free radicals are generated and accumulated in the body, leading to oxidative stress and antioxidant deficiency. Furthermore, the supplemental administration of antioxidants and nutrients could raise the body's levels of glutathione (GSH), which enhances the immunological activity and antioxidant activities(9).

The crucial of the organism's antioxidant systems is GSH, which is essential for preserving updates and improvements in redox status. Glutathione is a compound containing three amino acids that plays a key role in regulating the cell's response to redox associated with reactive oxygen species, detoxification of metabolic product, and regulating apoptosis and gene expression as well as the transport of solutes across the membrane(10) .

SUBJECTS AND METHODS

Study Design

A case-control study design started in September 2022 with (90) participants divided into two groups: (45) patients and (45) controls from the Merjan Medical City/Babylon City/Iraq respiratory care unit (RCU)for the patients and control groups, demographic research criteria were taken (age, gender, BMI, and severity of disease). The samples size is calculated as the following equation:

$$n = Z^2P(1 – P)/d^2$$

Ninety samples were collected, (n) represents the number of samples, Z-Score (1.96), (P) refers to the population, and (d) is an absolute marginal error (equivalent to 5%)(11).

Patients: By using laboratory tests, nasopharyngeal swabs or sputum sampling tested with a reverse transcriptase polymerase chain reaction (RT-PCR) the determine SARS-CoV-2 based on the study's inclusion criteria (age, sex, body mass index BMI, the severity of disease, and oxygen saturation levels(spo_2%), as well as in some cases, a respiratory disease specialist diagnosed patients as having COVID-19. The data was compiled at Merjin Medical hospital in Babylon (ICU).

Control: control group data were collected from the same gauge for the control group symmetry with patients for (Age, Gender, and body mass index (BMI)).

Samples Collection: Blood collection from the vein of approximately (5ml) was obtained from patients and control, let clotting for 15 minutes and centrifugation at (4500xg) for10 minutes to obtain serum stored at -20ºC.

Methods of Measurement Biochemical Parameters

Determination of Human Bradykinin (BK), LeukotrieneB4 (LTB4), and Leukotriene D4 (LTD4) by ELISA kit from Elab science company applies to the in vitro quantitative in serum. This assay depend on the competitive-ELISA principle.

Determination of reduced total glutathione (GSH):

5,-5-Dithionbis (2-nitrobenzoic acid)(DTNP) is a di chromogen that that is readily reduced by sulfhydryl groups of GSH to produce an intensely yellow compound .Reduced chromogen has maximum absorbance at 412nm and is directly proportional to GSH concentration(12).

Statistical Analysis: All statistical analyses were performed using software package version 22 (SPSS) data were presented as (mean ± standard deviation) with higher and lower 95% confidence intervals all variables were assessed by the T. test (P-Value <0.05) used to determine significant differences between the two groups of patients with COVID 19 and control and correlation test was performed to find an association between variables and the likelihood values less than P. value 0.05 were consider statistically significant. The diagnostic values of bradykinin, LTD4, LTB4 and GSH was assessed using a Receiver Operating characteristic (ROC)curve Fig. (1,2 and 3). By choosing the point that is closest to the top left corner of the ROC curve , one may determine the sensitivity and specificity of biochemical parameters and calculate the optimal cutoff according to the Youden Index(13).

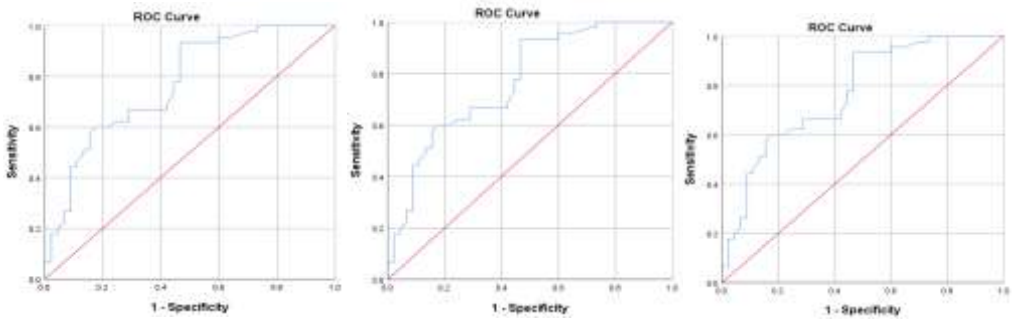

Fig.1, 2 and 3: ROC curve for bradykinin, LTD4, and LTB4 respectively.

RESULTS AND DISCUSSIONS

Some of the medical data that were statistically analyzed for the patients and the control group (Table 1) were obtained from the hospital and under the supervision of doctors specializing in respiratory diseases, such as age, body mass index, sex, hospitalization period, and oxygen level.

Table 1: Medical Data with Patients and Controls

Medical information	Patients (mean±SD)	Controls (mean±SD)
1-Age/Years	36.17±9.05	39.02±6.84
2-Gender	20 male/25 female	20 male/25 female
3- Body mass index (BMI)	22.97±1.69	20.77±0.89
4- Duration/day	5.08±2.00	Not found
5-SPO_2Oxygen saturation%	91.02±0.72	98.58±1.08*

***The result's significance at P-Value ≤ 0.05.**

(Table 2) appear significant differences between the patient group and the control group that represent an increase in the levels of bradykinin in the COVID-19 group (49.36 ± 11.24) while the control group (34.78 ± 7.15).

Table 2: Bradykinin levels in Control and Patients with COVID-19.

Parameter	Group	means ± SD	95%Confidence Interval		P-Value
			Lower	Upper	
Bradykinin (ng/mL)	Patients	49.36±11.24	46.61	53.53	0.036
	Control	34.78±7.15	32.01	37.23	

The main causes of increment in bradykinin concentrations for patients with COVID-19 may be due to inflammation of bronchioles and alveoli, and tissue-resident granulosa cells, also mast cells

can synthesize bradykinin by secreting heparin, activating clotting factor XII, and forming plasma kallikrein. Therefore, the increase in bradykinin may be due to the increased density of mast cells in the lungs of COVID-19 patients(14) . Furthermore, arginine-9-bradykinin (Arg9-BK) metabolites and bradykinin have a role in the inflammation of vascular permeability and vasodilation (15).

Table 3: Leukotriene B4 (LTB4) in Control and Patients with COVID-19

Parameters	Group	Mean ±SD	95%confidence interval		P-Value
			Lower	Upper	
LTB4 (pg/mL)	Patients	401.96±89.23	380.14	432.06	0.001
	Control	316.40±78.56	293.42	339.49	
LTD4 (ng/mL)	Patients	11.50±1.65	10.01	12.84	0.031
	Control	8.33±1.37	7.91	8.66	

The significant variations between the patients' and control groups (Table 3) representing there are an increase LTB4 (Leukotriene B4) levels for patients with COVID-19 (401.96 ±89.23), whereas the healthy control group (316.40±78.56). Also, (Leukotriene D4) level in the COVID-19 patients (was 11.50±1.65) whereas the control group (to 8.33±1.37). Serum and airway leukotriene levels are increased in COVID-19(16,17). Leukotrienes are potent granulocyte-chemotactic metabolites derived from arachidonic acid that cause vascular leakage, bronchoconstriction,and airwayremodeling (18). Resident and recruited macrophages in the lung produce high levels of leukotriene D4 (LTD4) and leukotriene B4 (LTB4),thereby promoting granulocyte infiltration, airway inflammation, and tissue remodeling (19).

The significant variation's between the patient and control groups appear in Table 4 representing there are a decrease glutathione (GSH) level in the COVID-19 patients (23.60 ± 4.00) whereas the control group appears (28.82±5.40). Conditions associated with decreased GSH concentration play a central role in the pathophysiology of human diseases(20). Numerous pathogenic problems, such as lung infections, HIV, diabetes, cancer, and diseases associated with aging, are associated with GSH imbalance. If we consider the conditions clinically associated with severe COVID-19 disease, we find evidence of a perturbed GSH replenishment (21).

Table 4: Reduced Total Glutathione (GSH) levels in Control and Patients with COVID19.

Parameters	Group	Mean±SD	95% Confidence Interval		P-Value
			Lower	Upper	
GSH (µmol/L)	Control	28.82±5.40	27.41	30.35	**<0.001**
	Patients	23.60 ± 4.00	22.59	24.82	

The results shown in Figures (4, 5, and 6) respectively, appear a negative correlation between glutathione with each of(BK,LTB4,and LTD4),where the values of the correlation coefficient (r = - 0.266*, -0.270*, -0.428 *) respectively. These results can be explained by the increased risk of developing severe cases of COVID-19, due to the weakening of the immune system and the lack of flexibility in the lungs also due to the high level of free radicals during the acute phase of the COVID-19 infection as a defense mechanism against viral infection(22).

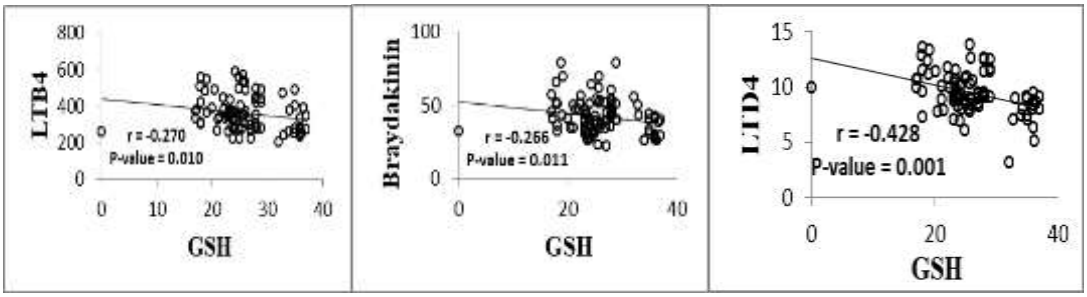

Fig. 4,5 and 6: The correlation of GSH with Bradykinin, LTB4 and LTD4 respectively.

CONCLUSION

Elevated levels of bradykinin leukotriene B4 and leukotriene D4 can be considered an indicator of the severity of COVID-19 disease and indicative of deterioration of the condition because their elevation is concomitant with bronchoconstriction, vascular leakage,airway remodeling, and vascular permeability. For this reason, there is a negative association with dilatation vessels with low glutathione levels are among the COVID-19 cases that are the worst.

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Ethical Approval: The study received approval from the Iraqi Ministry of Health.

Study Conflict: No other research is in disagreement.

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