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ORIGINAL ARTICLE

ESTIMATION OF OXIDATIVE STRESS AND ANTIOXIDANT CAPACITY IN COVID-19 PATIENTS, CURED PATIENTS AND PERSONS TAKING VACCINE

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Summary

The study was designed to evaluate the medical relevance of Malondialdehyde (MDA) (a marker of oxidative stress) and total antioxidant capacity (TAC) in coronavirus disease 2019 (COVID-19) groups, cured groups, and control groups; before and after taking the vaccine. Blood samples were taken from Oncology Unit in Al-Mahaweel hospital in Hilla city. Sixteen patients, sixteen cured patients, thirty control, sixteen subjects were taken one dose of Pfizer vaccine, sixteen subjects were taken two doses of Pfizer vaccine. We found that significantly increased lipid peroxidation, measured as MDA, was demonstrated in the serum of COVID-19 patients and TAC decreased in patients when compared with the control groups. Inversely, we found the mean MDA levels decrease and increase in TAC levels in cured patients when compare with COVID-19 patients. In addition, it is found that subjects were taken one dose or two doses of the Pfizer vaccine have less MDA levels and more TAC levels than the COVID-19 vaccine for that reason the Pfizer vaccines play the important role in the activity of immune systems.

Key words: COVID-19; Oxidative Stress; Malondialdehyde; Antioxidant

Introduction

The new coronavirus SARSCoV2 produces respiratory sickness in the majority of cases, needing no particular medical therapy, nevertheless, up to 20% of COVID-19 patients require hospitalization (1). Infection with COVID-19 causes an unbalanced and uncontrolled cytokine response (known as cytokine storm), exuberant endothelial inflammatory responses, and vascular thrombosis. Acute respiratory distress syndrome (ARDS), a leading cause of mortality in COVID-19 patients, may be caused by these and other unknown causes (2). SARSCoV2 causes viral pneumonia, which causes an overactive immune response in the lung tissues that are impacted by virus replication, alongside the presence of oxidative stress during this pathogenic process (3).

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The inability of the body to defend itself against ROS due to a disruption in the endogenous balance between them and oxidizing agents (OA) is known as oxidative stress (4). This imbalance has the potential to cause structural and functional problems. ROS are oxygenated chemical entities such as free radicals, oxygenated ions, and peroxides that have unpaired valence electrons in the outermost orbital, making them chemically extremely reactive. Either oxidation (loss of this free electron) or reduction (acquisition of another electron) restores the equilibrium (5).

Several variables impact oxidative stress, some of which increase ROS generation, such as increased O_2 consumption during high-energy sports exercise, and others which reduce antioxidant capacities, such as congenital G6PD enzyme deficiency (6), such as 4-hydroxynonenal (4-HNE) and Malondialdehyde (MDA) (7-9) were among the reactive metabolites produced by lipid peroxidation. MDA is a naturally occurring end product of lipid peroxidation that is widely employed as a lipid peroxidation indicator. MDA is produced when ROS destroy polyunsaturated lipids (10). This substance is a reactive aldehyde, one of the numerous reactive electrophile species that produce toxic stress in cells and create advanced glycation end products. A high quantity of aldehyde is utilized as a biomarker to assess an organism's level of oxidative stress. MDA is a carcinogen that has been linked to ageing, carcinogenesis, diabetic nephropathy, and radiation damage (11, 12).

The body uses a variety of enzymatic and non-enzymatic defensive mechanisms to protect itself against the creation and attack of these oxidants. Antioxidant species are chemicals capable of neutralizing active forms of oxygen and allowing non-cytotoxic amounts of free radicals to be maintained at the cell and organism levels. It decreases or prevents the oxidation of other chemical compounds, among other things (13, 14).

Antioxidants work in a variety of ways: they can scavenge radicals, block certain oxidizing enzymes, or react with oxidizing chemicals before they harm biological components. Primary defences and secondary defences are the two types of systems responsible for reversing the damaging effects of free radicals.

Materials and Methods

Study Design: This study is a controlled observational clinical study, in which the sample collection was conducted during the period from October-2021 to November-2021. The subjects enrolled in the study were classified into either one of the following classes:

- Group-1: 30 healthy group
- Group-2: 16 COVID-19 patients
- Group-3: 16 cured patients
- Group-4: 16 persons who get the Pfizer vaccination of one dose
- Group-5: 16 persons who get the Pfizer vaccination of two dose

Sample collection and preparation: Blood Sample Collection: Five millilitres of venous blood were withdrawn from each of the patients, control group, and cured patients. receiving a group of one dose Pfizer vaccine and receiving a group of one dose Pfizer vaccine by medical syringes. The blood samples were placed in gel tubes and then left at room temperature for (20) minutes for coagulation, then centrifuged (at 3000 X g) for 20 minutes for serum separation. The sera were divided into five Eppendorf tubes and stored at (-20 Cº) until the time of biochemical estimation.

The study patients in Al-Mahaweel hospital excluded patients with other chronic diseases which might affect the oxidant/antioxidant status, smoker, hyperlipidemia, diabetes, metabolic syndrome, vascular diseases, multiple sclerosis, systematic immune disease, gestational diabetes, thyroid gland diseases, and kidney diseases.

Assay principle of TAC and MDA: The principle of assay base on a sandwich kit for the accurate quantitative detection of human Total Antioxidant Capacity (TAC) and Human MDA (also known as T-AOC- MDA) in serum, an Enzyme-Linked Immunosorbent Assay (ELISA).

Statistical analysis: The result is represented as mean ± SD (1SD). Data analysis was performed using a student's t-test to obtain significance between the patient group and healthy group are considered as p values <0.05.

Results

Patients with COVID-19 had less TAC (17 ± 1.7) levels ($p<0.001$) than the healthy group (21 ± 2.9) U/ml. Conversely, the MDA levels of patients (19.6 ± 9.5) were more than the MDA levels in control groups (18.3 ± 5.8) nmol/ml.

A non-significant difference (P.0.05) exists between TAC and MDA levels of cured patients compared to the control group. In vaccinated groups, TAC was significantly higher and MDA was significantly lower than in the control group (Figure 1).

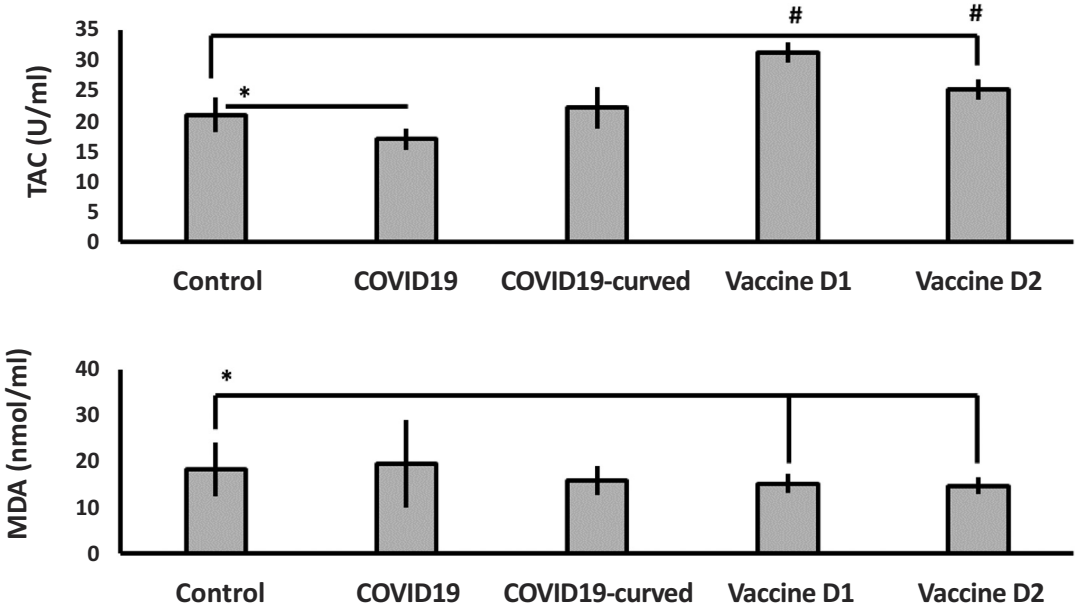

Figure 1. Oxidant-antioxidant status in COVID-19 patients, cured COVID-19 patients, and vaccinated individuals. Data expressed as mean±SD, *$p<0.05$ indicate significantly higher measured parameters in the control group compared to the other group, and #$p<0.05$ indicate significantly higher measured parameters in a specified group compared to the control group. D1= first dose vaccine, D2= second dose vaccine, MDA= Malondialdehyde and TAC=total antioxidant capacity.

Discussion

ROS are present in tolerable amounts in the cell, and their concentration is controlled by the balance between their rate of creation and rate of removal by antioxidant systems. In the quiescent state, the balance of antioxidants and pro-oxidants (balancing redox) is considered to be in equilibrium (15).

This redox balance, however, can be disrupted by either an increase in ROS generation (as seen in ageing or atherosclerosis) or a reduction in antioxidant capacity (as in people suffering from obesity and smokers). This is referred to as oxidative stress. The activation of ROS production systems can generate this imbalance in a regulated manner (16, 17).

The antioxidant response is then successful in balancing this production, and the imbalance is only temporary. On the other hand, in some pathological circumstances (such as cancer), ROS generation is greater and lasts longer, and the antioxidant response is inadequate (18, 19). Oxidative stress is a physiological condition in which the cellular antioxidant buffering capability is increased by systemic quantities of ROS, eventually causing damage to cellular macromolecules (20). During normal cellular metabolism, ROS and free radicals are produced. ROS get a favourable role in crucial signalling pathways that are necessary for basic cellular processes under normal physiological settings (21).

One of the underlying causes of organ damage in COVID-19 might be mitochondrial dysfunctions leading to increased ROS generation as a result of SARS CoV/SARS CoV 2 Spike Protein interactions with ACE2 (22, 23). ACE2 is a membrane-bound protein that is responsible for the conversion of Ang II, a vasoconstrictor, to Ang 1–7, a vasodilator, according to current understanding (24). As a result of the greater interaction with spike protein, Ang II creates RONS via activating membrane-bound NADPH oxidase, resulting in severe oxidative stress due to increased ROS. MDA levels in COVID-19 patients were higher in our research. In terms of the virus, patients' oxidative stress levels were found to be excessive. M. Martin-Fernandez *et al*. (2021) claimed that a change in peroxidant-antioxidant balance in COVID-19 patients caused an increase in LPO when compared to a healthy group (25). A recent study indicated that elevated oxidative stress in COVID-19 patients had a link to ROS-induced damage (26). Our findings are consistent with earlier research suggesting that CIVID patients may cause cell damage. The cells that contain arachidonic and linoleic acid) in their cellular biomembranes, in particular, become targets for excessive ROS (24, 26). As a result, we postulated that an excess of LPO causes an increase in MDA product movement in the circulatory system, resulting in higher MDA levels in COVID-19 patients.

MDA levels were lower in group 4 (people who received the first dose) and group 5 (those who received the second dose) than in COVID-19 patients in the current investigation, however, neither group 4 nor group 5 were statistically significant for the control. Various pre-existing environmental stressors and pressures linked to coronavirus illness 2019 increase the viral disease's consequences by causing oxidative stress (27). The oxidative stress causes cell membrane or DNA damage, which can lead to viral mutations and limit the efficiency of COVID-19 treatment, including vaccination (28). COVID-19 is vaccinated using the Pfizer BioNTech (BNT162b2) vaccine. The Pfizer-BioNTech COVID-19 mRNA vaccine is safe and efficient, and it is designed to increase the production of immunogens while avoiding the underlying innate immune response (29). Our findings corroborate earlier findings. Because the spike protein generated by these vaccinations has proline mutations, it loses its capacity to bind to ACE2 and is unable to adjust to its form, the MDA level remains unchanged. As a result, the possibility of the spike protein in the vaccine interacting with ACE2 receptors on platelets cannot be ruled out, and the immune response resulting in the production of neutralizing antibodies against the spike protein will also protect against any systemic effects of spike protein interactions with ACE2.

TAC in COVID-19 patients is lower than in healthy people in our study, and both groups get the vaccination. Respiratory virus infections are commonly connected with cytokine production, inflammation, and other pathophysiological processes resulting from a redox imbalance, disturbance of the thiol redox cycle, and other redox (3) circuits, according to (Delgado-Roche L *et al*, 2020). As a result, one of the important processes connected to viral replication and eventual virus-associated illness is the overproduction of ROS and the depletion of antioxidant mechanisms (30).

Thiol groups appear to be the principal regulators of oxidative stress, as they are among the most common and essential antioxidant molecules present in both cells and plasma. Organic molecules called thiols are found inside protein structures. To maintain dynamic thiol-disulfide homeostasis, thiol groups can create reversible disulfide bridges when exposed to oxidants (31).

The thiol-disulfide equilibrium, which is important for viral entrance, reactivity, and fusing with the host cell, can be disrupted by oxidative stress (32–35). When the disulfide linkages in both angiotensin-converting enzyme II (ACE2) and the SARS-CoV/CoV2 spike proteins were reduced to thiol groups, the binding affinity was dramatically decreased, according to the researchers. TAC levels in patients who received vaccination were higher than in COVID-19 patients in our study, which is consistent with earlier research. This is because the vaccine's spike protein does not bind to ACE2. These pathways of coronavirus utilization to invade the host has been extensively reviewed by Adnan *et al*. and the review has confirmed some antihypertensive drugs could be used whilst other should not be used, similarly antidiabetic and medication for various diseases has been reported to be safe or carrying deleterious impact on COVID-19 and associated illness; these could subsequently affect the course of vaccination or admission therapy (36).

The findings of this study refer to COVID-19 may be elevated production of ROS relying on inhibition of ACE2 and impaired antioxidation enzymic, while vaccination increases from the activity of antioxidation enzymic.

Conclusion

Our finding shows that COVID-19 is associated with oxidative stress and decline activity of antioxidation enzymic, thereby the MDA levels elevated. Pfizer vaccine helpful by the rising defence of immunosystem through elevated activity antioxidant of an enzyme. We also suggest that the formation of ROS decreased when taking the vaccine may be increased the activity of ACE2.

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Conflict of interest

The authors declare no conflict of interest concerned in the present study.

Adherence to Ethical Standards

The study was approved by the Research Ethical Committee and Scientific Committee in the College of Science for Women, the University of Babylon as a part of Master's student projects.

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