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Original Research Article

Evaluation and comparison of haematological and antioxidant profiles as a marker of severity in COVID-19 infection among children

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Abstract

Introduction: The COVID-19 pandemic caused relatively high mortality in patients, especially in those with concomitant diseases (i.e., diabetes, hypertension, and chronic obstructive pulmonary disease (COPD)). In most of aforementioned comorbidities, the oxidative stress appears to be an important player in their pathogenesis. The direct cause of death in critically ill patients with COVID-19 is still far from being elucidated. In this regard oxidative stress is one of the topics that need to be investigated. Therefore, the present research study was carried out to explore the relationship between the oxidant/antioxidant system and COVID- 19 exacerbation.

Materials and Methods: A total number of 60 children were involved in this study; they further equally divided into patient and control group. Blood Samples were collected from 30children confirmed diagnosed with COVID-19 infection and 30 healthy children volunteers as the control group. The patient group consisted of 22children with mild disease and 8 children severely ill patients.

Results: COVID-19 patients with mild and severe disease have shown the signs ofanaemia, leucocytosis and thrombocytopenia than control group. Serum levels of total antioxidant capacity (TAC) and nitric oxide (NO) were measured. TAC levels were considerably lower in patients compared with healthy individuals (control group). An increasing trend was found in NO concentration as well as MDA levels in patient group.

Conclusion: These findings suggest that COVID-19 patients may be susceptible to depleted total antioxidant capacity. Moreover, showing such variations in blood samples of infected individuals could be considered as a predictive marker of COVID-19 severity.

Keywords: COVID-19, nitric oxide, antioxidant enzyme, oxidative stress

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of a global serious pandemic, was first identified in late 2019 in Wuhan (China). The clinical manifestations of COVID-19 cover a broad range from asymptomatic to critically ill patients [1-3]. Recent studies have demonstrated that a wide variety of factors may be associated with the severity and outcomes of COVID-19 infection including genetic background, immune system defense against coronavirus, several comorbidities such as diabetes, hypertension and asthma, old age, BMI, lifestyle, and even gender discrepancies in health and risk behaviour [4-

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[7]. However, the rapid spread of the coronavirus throughout the world and its high mortality rates, have made it an increasingly urgent problem that needs particular attention by researchers of any profession. Despite growing evidence on SARS-CoV-2 pathogenesis, the puzzle is yet to be completed and used for developing therapeutic strategies.

The potential of the relationship between COVID-19 progression and various haematological and biochemical findings is possible breakthrough in management of the COVID-19 patient [7-10]. This study is aimed at examining the role of haematological and biochemical parameters among COVID-19 patients.

The complicated mechanisms behind COVID-19 infection include repression of host antiviral immunity, oxidative stress, and inflammation due to excessive cytokine secretion or cytokine storm which causes acute lung disease, tissue microscopic fibrosis, coagulopathy, and pneumonia [11]. Oxidative stress is basically characterized by a disruption in the balancebetween oxidant production and antioxidant protective responses that may be induced during natural metabolic processes or pathologic conditions. Respiratory viral infections in general, cause the imbalance of the oxidant antioxidant system by overproduction of reactive oxygen or nitrogen species (ROS or RNS) and particularly superoxide ions. Moreover, they may disturb the antioxidant defense potential against SARS-CoV-2 infection by a direct effect on the antioxidant molecules and enzymes [12-14].

Furthermore, an important degree of negative correlation between total antioxidant capacity (TAC) and total oxidant capacity (TOC) in many diseases that cause oxidative stress has been reported in earlier studies [11-14]. Oxidative defence mechanisms against ROS, although activated, might not be efficient enough and clinical symptoms of illness may occur [12].

Nitric oxide (NO) is generated from the terminal guanidine nitrogen atom of l-arginine by NO synthase, and is released from a variety of cells [15, 16]. NO is an important molecule involved in physiological and pathological processes in humans. It has been reported that NO has pro-inflammatory and injurious effects on several systems or organs due to the formation of the oxidant peroxynitrite [17-19]. NO is known to play a major role in the primary defence against several species of bacteria, viruses and parasites [17-20]. There is scarcity of data available in the scientific literature relating to oxidative stress in COVID-19 disease progression.

Therefore, the objective of this study was to determine whether haematological, biochemical parameters and important markers of oxidative stress, such as serum total antioxidant capacity (TAC), total oxidant capacity (TOC), total nitric oxide (NO) and malondialdehyde (MDA) can play important role in determining the severity of covid-19 infection among children.

Materials and Methods

Study Design: This study was a case control study conducted in Sri Sathya Sai Medical College and Research Institute in Chennai during the period of April, 2021 to December 2021.

Study Subjects: A total number of 30 patients, tested positive for COVID-19 confirmed by RT-PCR within 14 days were included in the study. Patients older than 18 years of were excluded from the study. Subjects with history of diabetes, hypertention, cancers, auto immune disorders, and smokers were excluded in both from control and patient group. The protocol of this study was approved by Institutional Ethical committee. All the patients had given their informed consent before included in the study. Same number of age and gender matched healthy children were also enrolled in this study as control group.

Blood sample collection: Venous blood samples were collected during the day time within 24 hours after admission in the hospital from patient group. The samples were collected under aseptic precautions by using vacutainers. The samples were centrifuged at 3000 rpm for 10 min; serum was separated, collected in aliquot and stored at -20 °C till it was analyzed. The collected samples were later used for haematological and biochemical parameter estimations.

Assays to measure oxidative stress markers: Commercial kits were used to analyze serum enzymes. All the tests were performed according to the manufactures' instructions.

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Malondialdehyde (MDA): The changes in MDA levels in serum samples were measured spectrophotometrically with a method modified by Placer *et al*. [21]. MDA is the end product of the peroxidation of fatty acids with three and more double bonds. It reacts with thiobarbituric acid (TBA) producing a pink-coloured complex which is measured photometrically at a wavelength of 532 nm.

Total antioxidant capacity (TAC): TAC values were determined using a novel automated colorimetric measurement method developed by Erel [22]. (TAC assay kit, Rel Assay Diagnostic).The absorption of the solution at 660 nm was measured 30 s (value A1) and 5 min (value A2) after mixing. The results are expressed as mmol Trolox equivalents/l (Eq/l) for serum.

Total oxidant capacity (TOC): TOC was determined using a novel automated measurement method, developed by Erel [23, 24] (TOC assay kit, Rel Assay Diagnostic).The absorption of the solution at 660 nm was measured 30 s (value A1) and 5 min (value A2) after mixing. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of µmol H2O2 equivalent/l for serum.

Nitric oxide (NO): The nitric oxide (total), detection kit (Enzo Life Science) is a complete kit for the quantitative determination of total NO in biological fluids [25]. The kit involves the enzymatic conversion of nitrate to nitrite, by the enzyme nitrate reductase, followed by the colorimetric detection of nitrite as a coloured azo dye product of the Griess reaction that absorbs visible light at 540 nm.

Statistical analysis: All analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 16.0. Mean for variables were compared across different groups independent sample t test.A $p<0.05$ was considered as statistically significant.

Result

The results presented in Table 1 shows that 14 (46.66%) of COVID-19 patients were boys and 16 (53.33%) were girls, while 17 (56.66%) of control group were boys and 13 (43.33%) were girls. The mean age of COVID-19 patients was 9.67±4.71 years old; the control group mean age was 8.51±3.64. Regarding the COVID-19 patients' situation during the study period, 18 (60%) recovered and were discharged from the hospital within three weeks after admission, 12 (40%) were in ICU patients at the end of the six weeks period.

Table 1: Comparison of demographics and baseline characteristics among Control and COVID-19 patients groups

Parameters		**Control Group (N=30)**	**COVID-19 patient group (N=30)**	**p Value**
Age (Years)		8.51±3.64	9.67±4.71	0.63
BMI		18.82±3.65	17.81±4.16	0.82
Pulse Beats/min		74.81±5.23	82.28±9.27	0.03
SBP mmHg		114.37±8.54	115.68±9.84	0.18
DBP mmHg		78.62±8.92	82.61±6.72	0.64
Gender	(M/F)	17/13	14/16	0.27
Clinical Symptoms	Cough, N (%)	1 (3.33)	12 (40)	0.61
	Expectoration, N (%)	0 (0)	8 (26.66)	-
	Headache, N (%)	1 (3.33)	4 (3.33)	0.52
	Muscle soreness, N (%)	0 (0)	6 (20)	-
	Fatigue, N (%)	1 (3.33)	7 (23.33)	0.37
	Fever, N (%)	2 (6.66)	16 (53.33)	0.18

All values were expressed as Mean±SD, N= indicates the number of subjects, $p< 0.05$ highly significant.

The results showed in Table 2 highlights the comparison of haematologicalprofiles among control group and COVID-19 patient group. The mean haemoglobin concentration, RBC count, platelet count, mean indices like MCH,MCHC, and MCV were all significantly lower in COVID-19 patients compared with control group, while the RDW and WBC count was significantly higher in COVID-19 patients compared with control group.

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Table 2: Comparison of haematological profiles among Control and COVID-19 patients groups

Parameters	Control Group (N=30)	COVID-19 patient group (N=30)	p Value
Hb (g/dL)	12.9±2.34	11.96±1.47	0.81
RBC (10^{12}/ L)	5.21±0.82	3.98±0.24	1.02
MCV (fL)	84.26±8.42	87.14±7.29	0.94
MCH (pg)	27.42±2.93	28.61±2.57	0.61
MCHC (g/ dL)	32.10±3.14	31.94±2.61	0.37
RDW (%)	16.21±2.64	15.97±3.16	0.96
WBC count (10^9/L)	9.64±2.67	10.62±3.26	0.39
Platelet (10^9/ L)	259.43±68.47	197.53±38.44	0.92

All values were expressed as Mean±SD, N= indicates the number of subjects, $p< 0.05$ highly significant.

Biochemical profiles of both control group and COVID-19 patient group were compared in Table 3. Random blood glucoselevels, serum urea, serum creatinine were determined for every participant. In our study, these variables were slightlyelevated in COVID-19 patient group but they were found not to be significant.

Serum alanine transaminase and aspartate transaminase levels were significantly higher in patient group than the control group, but this was also not statically significant.

Table 3: Comparison of biochemical profiles among Control and COVID-19 patients groups

Parameters	Control Group (N=30)	COVID-19 patient group (N=30)	p Value
RBG (mg/dl)	86.34±19.46	90.48±17.63	0.44
Serum Urea (mg/dl)	22.14±5.48	25.67±3.94	0.89
Serum Creatinine (mg/dl)	0.75±0.08	0.82±0.17	0.67
AST (IU/L)	25.64±9.46	44.37±11.94	1.06
ALT (IU/L)	18.37±9.62	39.64±14.53	0.57

RBG- Random blood glucose; AST- Aspartate transaminase; ALT- Alanine transaminase. All values were expressed as Mean±SD, N= indicates the number of subjects, $p< 0.05$ highly significant.

The result presented in Table 4 highlights that the serum Total Antioxidant Capacity (TAC) levels were found to be significantly lower in COVID-19 patient group compared with control group.

Table 4: Comparison of total nitric oxide (NO) and serum malondialdehyde (MDA) levels among Control and COVID-19 patients groups

Parameters	Control Group (N=30)	COVID-19 patient group (N=30)	p Value
NO (µmol/l)	47.82±7.63	71.26±8.62	0.37
MDA (nmol/ml)	32.61±4.82	48.86±5.12	0.49
TAC (mmol Trolox Equiv/l)	1.78±0.46	1.06±0.08	0.81
TOC (µmol H_2O_2 Equiv/l)	1.64±0.76	3.42±0.34	0.06

NO = Nitric Oxide, MDA = Malondialdehyde; TAC= Total Antioxidant Capacity, TOC = Total Oxidant Capacity. All values were expressed as Mean±SD, N= indicates the number of subjects, $p< 0.05$ highly significant.

In contrast, the serum Total Oxidant Capacity (TOC), Nitric Oxide(NO) and Malondialdehyde (MDA) levels were found to be significantly higher in in COVID-19 patient group compared with control group.

Discussion

We have witnessed the prevalence of COVID-19 cases throughout the globe. Assessing biochemical, clinical, haematological and demographic, indicators is important when

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investigating the COVID-19 outbreak [1-3]. This present study has investigated haematological and biochemical parameters associated with COVID-19 infection. We observed the reduced levels of haemoglobin concentration, RBC count, platelet count and increased leukocyte count in COVID-19 patients compared to control group. It thereby recognises the importance of these parameters in diagnosis of the disease. Identifying altered biochemical, haematological parameters and continuous observation can also be essential in the disease progression of COVID-19.

Recent studies have shown that free radicals and oxidative processes are involved in the onset and development of some of the complications associated with COVID-19 disease [11-13]. According to the oxidative hypothesis, there are many variables which are capable to generate free radicals that cause cell damage. Oxidative stress via lipid peroxidation products, such as MDA, plays a significant role in the pathogenesis of a large number of complications in different organs, including lung, colon, kidney, bladder, etc. [14-16]. MDA is one of the best-investigated products of lipid peroxidation. The MDA serum level reflects free-radical cell damage. The results of our study showed that the MDA serum level in COVID-19 patient group was significantly higher than the control group, a finding compatible with the results of several studies [17-21].

The difference in TAC levels over various groups of this study could be attributed to high ROS production, acute inflammatory condition and infiltration of inflammatory cells into the different organs, multiorgan involvement, and declined oxygen saturation [11-13, 15-17]. Although patients with COVID-19 had normal oxygen levels, their amount of TAC was lower than healthy subjects; therefore it might be a stronger and more reliable prognostic factor. Additionally, oxygen levels may be improved following the O_2 therapy for patients; in these COVID-19 patients, TAC could serve as a useful index to evaluate the patient's condition. Since the O_2 saturation may change fast and mislead the physicians, a combination of O_2 saturation and TAC may indicate the patient's condition more accurately. Moreover, the patient quickly enters the severe phase of the disease, so, TAC can be used for monitoring the status of the patients. Upon treatment and correction of TAC, the patient's condition may become more stable. Furthermore, prognostic clinical applications can be considered to prevent the severe phase of the disease.

TAC encompasses a wide spectrum of activities of the measurable exogenous and endogenous antioxidants [22]. Its value expresses the number of antioxidant molecules present in serum. An individual antioxidant level or activity indicates the antioxidant characteristics of only one antioxidant, whereas TAC may represent the total antioxidant characteristics of all antioxidants found in the sera [23].

When the oxidant/antioxidant balance is tilted towards oxidants and oxidative stress arises, there is a significant negative correlation between the TAC and TOC values [24]. In this study a negative correlation between TAC and TOC values was found, which corresponds to previously reported findings [22-24]. The low TAC level in the COVID-19 patient group can be explained with excessive depletion of the antioxidant capacity caused by free radical elimination.

Nitric oxide, which has several very different biological effects, is strongly involved in the cellular response to infections caused by a wide range of pathogenesis [25]. In this study we have determined the levels of nitrate and nitrite by the Griess assay from the blood samples of participants, which revealed that the production of NO was significantly higher in COVID-19 patient than that of healthy individuals. This may be compatible with macrophage activation, which is common during inflammatory immune responses. Inducible nitric oxide synthase (iNOS) in macrophages can be 2-3 fold higher following inflammation, which releases a large amount of NO leading to local and systemic increases of nitrate or nitrite [25-28]. Patients with moderate and sever COVID-19 often develop respiratory distress compensated by oxygen therapy that could cause oxidative stress and ADRS. It was shown that hyperoxia induces ROS generation in mitochondria. Mitochondria represent one of the crucial ROS sources in non-immune cells, particularly endothelial cells. Inhibiting oxidative phosphorylation and lowering ATP level. Thus, targeted protection of the pulmonary cell

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mitochondria represents a promising approach to prevent hyperoxia related lung tissue damage [28-30].

The present study concludes that oxidative stress may deteriorate COVID-19 disease whether it is induced by SARS-CoV-2 virusor existed before viral infection. This study program has raised important questions about the mechanisms behind redox imbalance as a leading factor in SARS-CoV-2 virus infection and severity. It also lays the groundwork for future research into the possible roles of other components of the oxidant/antioxidant system in COVID-19 exacerbation. The insights gained from this paper may be of assistance to develop diagnostic, prognostic, or therapeutic strategies for the SARS-CoV-2 virus.

Conclusion

The present study highlighted the comparison of haematological and biochemical parameters among control and COVID-19 patient group. Identifying abnormal haematological parameters and continuous observation can also be essential in the disease progression of COVID-19. It thereby recognises the importance of these parameters in diagnosis of the disease.

COVID-19 progression towardsmore complications and severity stagecan be accompanied by a significant increase in MDA levels, this may suggests a possible role of oxidative stress in the pathogenesis and progression of the COVID-19. The results of the present study suggest that MDA serum level might play a significant role as a biomarker in the progression of COVID-19, as well as in the monitoring of its severity. However, further research involving studies with higher sample size is required to determine the accuracy and widespread applicability of MDA as a biomarker in patients with COVID-19.

There is a clear association between oxidative stress and severity of several viral diseases. Our study suggest that elevated MDA and nitric oxide level in COVID-19infections and oxidative stress may be associated with increased hospitalization, complications and mortality. A significant decrease in TAC levels and increase in TOC and nitric oxide levels were observed in COVID-19 patients, which could explain in part the pathogenesis of the infection and may be used for diagnostic, prognostic, and therapeutic purposes.

Thus, the strategies for reducing or preventing of oxidative stress may act as an adjuvant therapy in management of COVID-19 patients. Although, in this study we had limitations of smaller sample size. A complete comprehensive study and further evaluation of role of antioxidant enzymes is necessary to understand the role of oxidative stress in COVID-19 patients.

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