Quest Journals
Journal of Medical and Dental Science Research
Volume 8~ Issue 8 (2021) pp: 35-38
ISSN(Online) : 2394-076X ISSN (Print):2394-0751
www.questjournals.org

Research Paper

Elevated levels of serum MDA and Nitric Oxide in covid-19 patients: A case control study

Aarti K Salve[1], Sarita A Basu [2], Irfan G Mulla[1*] ,Sharad B Kole[3],Sunil Kharge[4] Abhishek Talathi[5]

[1,1*]*Senior Lecturer, Department of Biochemistry Yogita Dental College and Hospital ,Khed, Maharashtra*
[2]*Reader , Department of Biochemistry Yogita Dental College and Hospital ,Khed, Maharashtra .*
[3]*Professor and Head, Department of Physiology and Biochemistry,Yogita Dental College and Hospital Khed, Maharashtra.*
[4]*Assistant Professor ,Department of Biochemistry ,PIMS , Urun Islampur ,Maharashtra.*
[5]*HOD, Department of Public Health Dentistry, Yogita Dental College and Hospital Khed, Maharashtra.*
**Corresponding Author : Irfan Gous Mulla , Senior Lecturer Department of Biochemistry ,Yogita Dental College and Hospital , Khed, Maharashtra.*

ABSTRACT
The interaction between oxidative stress and inflammatory cytokines can lead to several organ failures in Covid-19 patients who proceed to worsening condition. In view of this we planned our study to investigate the oxidative stress by measuring Nitric Oxide and MDA level in hospitalized Covid-19 patients and compared with healthy controls: A case control study.
Key Word: *MDA, NO, ROS ,RNS.*

Received 10August, 2021; Revised: 24 August, 2021; Accepted 26 August, 2021 © The author(s) 2021. Published with open access at www.questjournals.org

I. INTRODUCTION

Over the last few months, a new strain of human coronavirus, SARS-CoV 2(also known as 2019-nCoV), has caught the world's seven continents attention with its rapid global spread, affecting at least 200 countries and territories, infecting more than 3,000,000 and claiming more than 202,597 lives worldwide (1).

A WHO report from the 19th May 2020, confirmed 4,731,458 COVID -19 positive cases from 213 countries worldwide, of which 1,477,516 cases were reported in the United States of America, 231,606 cases in Spain, 225,886 cases in Italy, 246,410 in the United Kingdome, 84,500 in China (the origin of the pandemic) and 101,139 cases in India. (4)

Viral infections causing respiratory complications are known to change host cell redox homeostasis, which involves balancing ROS/ RNS as a critical event (5). In airway epithelial cells, virus induced ROS was found to originate from diverse oxidase activities, including NADPH oxidases, dual oxidase, and xanthine oxidase (5). RNA viruses were suggested to utilize oxidative stress during infection to control genome RNA capping and genome replication (6,7,8).NO is formed by the oxidative deamination of the amino acid L-arginine by nitric oxide synthases (NOS). (9) Biogenesis of higher levels of NO can suppress type 1 helper Tcell-dependent immune responses, which can impair type 2 helper T-cell-biased immunological host responses (10). eNOS is mostly present in endothelial cells and its functionality can be restored with renin- and angiotensin-converting enzyme-inhibitors or angiotensin receptor blockers, both commonly used to regulate blood pressure in hypertensive patients (11). The entry point of the coronavirus has been recognized to be Angiotensin Converting Enzyme 2 (ACE2) receptors, which are expressed in endothelial and epithelial cells (12). ACE2 pathway is known to modulate a cascade of events including vascular compliance and vasodialation (13). In primary lung epithelial cells validated by biopsies of COVID-19 patients, SARS-CoV-2 induced oxidative stress due to mitochondrial dysfunction The lungs are the preferred target of COVID-19 by the large area exposed to viruses, they are among the most oxygenated organs in the human body.However, during pathological events, such as viral infections, there may be an increase in the production of oxidant species not neutralized by the antioxidant system, resulting in oxidative stress that promotes cellular damage through protein denaturation, changes in the functions of nucleic acid, lipid peroxidation, and cell death.COVID-19 triggers inflammatory

reaction which releases pro-inflammatory cytokines . A great association between the proinflammatory elements and the reactive oxygen species (ROS) in the different lung disease including coronavirus infection which is associated with inflammation and oxidative stress.(14)

Oxidative stress is a typical for infection of human respiratory syncytial virus, rhinoviruses and many other viruses. Patients infected with influenza virus have a high level of oxidized biomolecules such as DNA, lipids, and proteins. Moreover, elevated ROS production, upregulated NO synthase-2 (NOS2) expression, and high level of nitrated proteins indicating developed oxidative and nitrosative stress were observed in the lung tissue samples from the deceased influenza patients.(15) A primary consequence of oxidative stress is lipid peroxidation, or the oxidative degeneration of lipids. Lipid peroxidation is caused by a free radical chain reaction mainly involving membrane polyunsaturated fatty acids. If not quenched, this reaction can permanently damage cell membranes, ultimately leading to cell death. Exposures to oxidant air pollutants cause lipid peroxidation in human beings and rodents. The end products of lipid peroxidation can lead to subsequent pathological consequences. Once formed, peroxyl radicals can be rearranged via a cyclisation reaction to endoperoxides (precursors of malondialdehyde) with the final product of the peroxidation process being malondialdehyde (MDA) (16) hence, It can be postulated that oxidative stress is associated with pathogenesis of SARS coV- 2 infection.

The rapidly progressing COVID -19 pandemic has led to difficult decision making regarding the treatment of critically ill patients with new viral infection.In this study we measured oxidative stress markers including MDA and nitric oxide levels in Covid-19 patients compared to healthy control subjects.

II. MATERIAL & METHOD

Study Design:

This study was a case control study conducted in Shivtej Aarogya Seva Sansth's Govt. Covid Care Center khed. Maharashtra.

Study Subjects:

50 patients, tested positive for Covid -19 confirmed by RT-PCR within 14 days were included in the study. Pregnant women and patients younger than 18 years of age or older than 60 years were excluded from the study. Subjects with history of diabetes, hypertention, cancers, auto immune disorders, and smokers were excluded in both from control and case 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.

Sample collection

Venous blood samples were collected during the day time within 24 hrs after admission. The samples were collected under aseptic precautions by using vacutainers. The samples were centrifuged at 3000 rpm for 10 min; serum was separated, collect aliquot and stored at -20 C till analyzed.

The collected samples were later used for biochemical estimations of Serum MDA assayed by standard Kei Satoh's method (17). And serum nitric oxide was estimated by Najwa Cortas and Nabil Wakid method (18).

III. RESULT

Table 1: Nitric oxide and serum MDA level in healthy individuals and Covid 19 patients

Sr. No.	**parameters**	**Covid-19 patients (n=50)**		**Healthy Controls (n=50)**		**significance**
		Mean±SD	**Std Errorof Mean**	**Mean±SD**	**Std Error of Mean**	
1	Nitic oxide µmol/L	100.67 ± 10.46	1.48	62.76 ± 13.44	1.90	< 0.001
2	MDA µmol/L	9.51 ± .79	.11	3.88 ± 1.39	.19	< 0.001

All values were expressed as Mean ± SD, n= indicates the number of subjects, $p < 0.001$ highly significant,$p<0.05$ Significant , $p> 0.05$ non significant.

IV. DISCUSSION

Several studies proposedthe association between oxidative stress and covid-19 pathogenesis. (19) Our results has shown increased oxidative stress markers MDA and Nitric oxide level with significant increase in covid-19 patients compared with healthy control(as shown in table above). Our report is an agreement with other two reports (19,20). Report (20)has shown that patients with severe illness had slightly elevated NO, whereas patients who died had significantly higher levels of NO compared to levels in patients who survived ($p<0.0001$) While other report (21) on MDA has shown lower ($p<0.001$)in covid-19 patients than control(21). There is a significant correlation between oxidative markers and respiratory viral infections particularly RNA viruses. The beginning of oxidative stress by virus infection is necessary for activation of Innate immunity by

cytokines production. Besides, oxidative stress induced by several viruses involved in facilitation of virus replication inside the cell Fereshteh Mehri, Amir Hossein et al observed high serum level of oxidative stress and inflammatory markers and low serum level of antioxidants in covid-19 patients they observed high serum level of MDA in case groups.It described the function of macrophage respiratory burst in reactive to covid-19 infection, which can lead to ROS production. Over production of ROS/ RNS have role in lung tissue injury and dysfunction of epithelial barrier induced by acute respiratory viral infections. NADPH oxidase has important role in ROS production, arterial dysfunction, and thrombosis (induced by platelet activation) . It is indicated that NADPH oxidase overaction in covid-19 patents.(19) Angel et al and Aninagyei et al documented that isoprostanes or produced from arachidonic acid metabolism bound to the cell membranes when attacked by free radical or ROS activity. Among the isoprostanes 8-iso-PGF2α is a very useful analytical tool for the assessment of endogenous lipid peroxidation. Elevated levels of MDA in covid-19 patients compared to control group reported in the present research, it suggest that overproduction of free radicals which in turn destroys lipid membranes with subsequent formation of MDA and iso-PGF2αas by products.(22)

Determination of nitrate and nitrite by the Griess assay in the blood of patients with severe covid-19 revealed that the production of NO was significantly higher 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 (23). In our study also NO level has been found to be significantly higher in covid-19 patients as compared to control group. Until now, however, it remains unclear whether COVID-19 triggers oxidative stress in the airway epithelium. 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 leve, Thus, targeted protection of the pulmonary cell mitochondria represents a promising approach to prevent hyperoxia related lung tissue damage.(24)

V. CONCLUSION

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-19 infections and oxidative stress may be associated with increased hospitalization, complications and mortality.it seems that strategies for reducing or preventing oxidative stress may help in covid-19 management although we had limitations of small sample size. A complete comprehensive study and further evaluation on antioxidant enzymes with clinical study is necessary to understand the role of oxidative stress in covid-19 patients. Thus strategies for reducing or preventing of oxidative stress may act as an adjuvant therapy in management of Covid-19 patients.

REFERENCES

[1]. World Health Organization (WHO), " Middle East respiratory syndrome(MERS),"2020, May 2020, https://www.emro.who.int/docs/default-source/coronaviruse/situation-reports/20200428-sitrep-99-covid-19.pdf?sfvrsn=119fc381_2.

[2]. Benvenuto D. Giovanetti M et al The 2019-new coronavirus epidemic: evidence for virus evalution. J Med Virol 2020;92(4);455-459.

[3]. World Health Organization. Novel Coronavirus (2019-nCoV).WHO Bulletin, 2020, http://Who.int/docs/default-source/coronaviruse/situation-reports/20200121-striep-1-2019-ncov.pdf

[4]. World Health Organization. Situation report-120;Coronavirus disease 2019(COVID-19). Available at, 2020.www.who.int;2020.Assessed May 20,2020.

[5]. Khomich OA, Kochetkov SN, Bartosch B, Ivanov AV. Redox Biology of Respiratory Viral Infections. Viruses (2018) 10(8):392. doi: 10.3390/ v10080392 23.

[6]. Gullberg RC, Jordan Steel J, Moon SL, Soltani E, Geiss BJ. Oxidative Stress Influences Positive Strand RNA Virus Genome Synthesis and Capping. Virology (2015) 475:219–29. doi: 10.1016/j.virol.2014.10.037 15

[7]. Hyodo K, Hashimoto K, Kuchitsu K, Suzuki N, Okuno T. Harnessing Host Ros Generating Machinery for the Robust Genome Replication of a Plant Rna Virus. Proc Natl Acad Sci U.S.A. (2017) 114(7):E1282–90:dot:10.1073/pnas.1610212114.

[8]. Nishikiori M, Ahlquist P. Organelle Luminal Dependence of (+)Strand RNA Virus Replication Reveals a Hidden Druggable Target. Sci Adv (2018) 4(1): eaap8258. doi: 10.1126/sciadv.aap8258 25

[9]. Nathan C, Xie QW. Nitric oxide synthases: roles, tolls, and controls. Cell 1994;78:915–8. 2 De Groote MA, Fang FC. NO inhibitions: antimicrobial

[10]. Akaike T, Maeda H. Nitric Oxide and Virus Infection. Immunology (2000) 101:300–8. doi: 10.1046/j.1365-2567.2000.00142.x 30.

[11]. Förstermann U, Sessa WC. Nitric Oxide Synthases: Regulation and Function. Eur Heart J (2012) 33(7):829–37, 837a-837d. doi: 10.1093/ eurheartj/ehr304 31.

[12]. Yan R, Zhang Y, Li Y, Xia L, Guo Y, et al (2020) Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 367: 1444-1448

[13]. Patel KP, Schultz HD (2013) Angiotensin peptides and nitric oxide in cardiovascular discase. Antioxide Redox Signal 19: 1121-1132

[14]. Di Paola Rosanna and Cuzzocrea Salvatore: Reactive oxygen species, Inflammation, and Lung Diseases; Department of clinical and Experimental Medicine and pharmacology,2012,18,3889-3900.

[15]. B. V. Cernyak, E. N. Popova, A. S. Prikhodka et al : covid-19 and oxidative stress: Biochemistry(Mascow) 2020,vol 85,Nov.12-19.pp 1543-1553.

[16]. Di paola Rosanna and Cuzzocrea Salvatare: Reactive oxygen species,Inflammation and lung Diseases; current pharmaceutical Design, 2012,18.3889-3900.
[17]. Estimation of Lipid peroxide(MDA) by kei satoh method. Clinical chemical Acta 1998:37-43.
[18]. Najwa K. Cortas and W. Wakid. Determination of inorganic nitrate in serum and urine by kinetic cadmium reduction method. Clin. Chem. Chem. 3618,1440-1443.
[19]. Fereshteh Mehri. Amir Hossein Rahbar, Elaheh Talehi Gbane et al : The comparison of oxidative markers between covid-19 patients and healthy subjects; Archives of Medical Reasearch. June 18,2021.
[20]. Paari Dominic, Javaria Ahmad, Ruchi Bhandari et al: Decreaed availability of Nitric oxide and hydrogen sulfide is a hallmark of covid-19; Redox Biology, 43(2021) 101982: https://doi.org/10.1016/j.redox.2021 101982
[21]. Yahaya M, Yamuna K, Sani I et al Deficiency of,antioxidant and increase oxidative stress in Covid-19 patients : A cross- sectional comparative study in Jigawa north western Nigeria; vol. 09: I-8 6th January 2021.
[22]. Angel BPL, Jadoon S and Malik A. A comprehensive review article on isoprostanes as biological markers. Biochem Pharmacol 2018; 7:2.
[23]. Wanyi Fang, Jingurui Jiang, Lei Su et al: The role of NO in Covid-19 and potential therapeutic strategies: free radical biology and medicine 163(2021) 153-162. http://doi.org/10.1016.j.freeradbiomed.2020.12.008
[24]. B.V. Chernyak, E. N. Popova, A.S. Prikhodko et al: Covid-19 and Oxidative stress; Biochemistry (Mascow),2020,vol.85,Nos.12-13,pp,1543-1553.