## Nitric Oxide Signalling & Oxidative Stress In Patients with Infectious Diseases

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**Abstract :**

The Nitric oxide ($NO^{\cdot}$) is an intermediate between molecular oxygen (O2) and nitrogen (N2). N2 has low solubility and readily diffuse through membranes as easily as through cytoplasm and is a strong oxidant. Molecular oxygen propagates free radical damage and has a central role in oxidative stress and in infectious diseases. In our study nitric oxide and lipid peroxide (MDA) level is measured in 100 healthy controls and in 60 infectious diseases patients. Both nitric oxide level ($P<0.001$) and lipid peroxide ($p<0.001$) was found significantly high in infectious diseases patient group than normal healthy control. The study concludes that $NO\cdot$ is the effector molecule that initiates the cytotoxic effects by increasing the oxidative stress.

**Key words :** Nitric oxide ($NO\cdot$), lipid peroxides (MDA), Infectious diseases, Nitric oxide synthase, Cytokines.

**Introduction:**

$NO^{\cdot}$ has stimulated an extraordinary thrust for scientific research in all the fields of medicine.[1] $NO^{\cdot}$ has emerged as fundamental signaling device regulating virtually every critical cellular function as well as a potent mediator of cellular damage in wide range of conditions.

In macrophages, nitric oxide synthase activity appears slowly after exposure of the cells to cytokines and bacterial products, is sustained, and functions independently of calcium and calmodulin. The cytokine-inducible nitric oxide synthase (iNOS) is activated by several immunological stimuli, leading to the production of large quantities of nitric oxide which can be cytotoxic.[2]

Immunity to various types of infections is complex and not properly understood till yet, a number of different effector mechanisms in addition to NO have been implicated.[3] It is suggested that a cascade of reactions leading to NO production are involved in various types of infectious processes.[4] Generated NO has cytotoxic properties against tumor cells, intracellular bacteria, protozoa, extra-cellular fungi and helminthes. Which majority of times causes infectious diseases. Thus causing overall increase in the oxidative stress level.

The cytotoxicity attributed to NO is due to per-oxynitrite interaction with lipids, DNA and proteins etc.[5] Cytokine-induced synthesis of NO from L-arginine, as intracellular functions as well as tumor necrosis factor (TNF)-α is also a potent second signal in the induction of high-output NO synthesis from L-arginine in IFN-α-treated macrophages. In addition microbial products, such as muramyl dipeptide and LPS, act by inducing TNF-α synthesis. TNF-α is the actual physiological co-signal induced by microbial products and the final intermediary that induces high-output NO synthesis by IFN-α-primed murine macrophages T.[6]

**Methodology :**

**Aims & Objectives :** The present work was planned to study role of nitric oxide in infectious diseases and its effect on oxidative stress:

1. To estimate the alteration in nitric serum oxide level in increased nitrosative stress.[7]
2. To find out the increased in conc. of MDA level in the infectious condition.[8]

The present study was conducted in the Department of Biochemistry of DVVPFs Medical College & Hospital, Ahmednagar. The patients selected for the present study were attending indoor/outdoor department of medicine of tertiary care hospital, Ahmednagar.

**Inclusion criteria:** 60 infectious disease patients in the age group of 20-60 years were included. As well as 100 healthy control subjects were included in the study. The disease group is then compared with 100 healthy control subjects. Informed consent was obtained from each participant in the present study.

**Exclusion criteria:** Smokers with Hypertension, Malignancy, Cardiac failure, Recent surgery, Severe endocrine, hepatic, renal diseases, HIV infected and with lung disorders were excluded.

The control subjects were completely healthy and showed no abnormality on clinical examinations and were completely symptom free. The study was cleared by institutional ethics committee.

**Study design :** Present study was analytical case control study. Sampling was done by using simple random sampling type. **Study protocol :** 5 ml blood was collected by using 20 G disposable needle from cubital vein with all aseptic precautions in plain bulb for serum and centrifuged at 3000 RPM for 10 minutes at room temperature. All samples were analysed on the same day of collection.

The serum nitric oxide level was estimated by Najwa Cortas and Nabil Wakid method[6] and lipid peroxide (MDA) level was assayed by Kei Satoh method.[7]

Statistical analysis was carried out using student unpaired 't' test. Probability values $<0.05$ was considered as significant. Also data were expressed in mean $\pm$ SD form.

**Results:**

**Table 1:** Nitric Oxide & Lipid peroxide level in Healthy & Infectious disease patients

| S. No. | Groups | Nitric Oxide level $\mu$mol/L | Lipid peroxide level $\mu$mol/L |
|---|---|---|---|
| 1 | Healthy Control (n =100) | 33.15± 6.13 | 1.66±0.289 |
| 2 | Infectious Disease patients (n=60) | 133.58±16.19 | 4.5±2.76 |

All values are expressed as Mean $\pm$ SD, n = Indicates the number of subjects, $p<0.001$ highly significant, $p>0.05$ non-significant.

In this study, we studied different parameters to understand role of nitric oxide and lipid peroxide levels in infectious disease patients the level was found to significantly increased ($P<0.001$) than healthy control subjects **(Table -1).**

**Discussion:**

Nitric oxide level in the infectious disease was found to be significantly high ($p<0.001$) than healthy control subjects. This is due to cytokines induced macrophage cell lines, which synthesize NO· from L-arginine with high concentration (Interleukin-1 was found to be a potent second signal for high-output NO).[8] Cytokine-induced NO·-mediated autotoxicity (i.e., high-output NO· synthesis by macrophage). Don Granger[9] demonstrated that the L-arginine-dependent effector mechanism has potent cytostatic effects for the facultative intracellular pathogen. The high-output nitrogen oxide synthesis by cytokine-activated macrophages that induced cytotoxic and biochemical lesions in target cells **Fig-1**.[10] Our identification of the enzymatic activity that synthesizes nitrogen oxides (the precursor molecule L-arginine; L-citrulline, the other product of the reaction; and Nw-monomethyl—L-arginine, an inhibitor of all three NOS isoforms.[11]

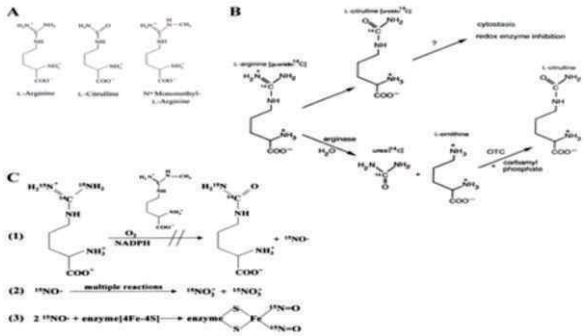

**Fig 1 :**The cytokines & microbial products induce the activated macrophage effector mechanism

**MDA :** Oxidative stress plays a dual role in infections. Free radicals protect against invading microorganisms, and they can also cause tissue damage during the resulting inflammation. In the process of infection, there is generation of reactive species by myeloperoxidase, NADPH oxidase, and nitric oxide synthase. On the other hand, reactive species can be generated among others, by cytochrome P450, some metals, and xanthine oxidase. Some pathologies arising during infection can be attributed to oxidative stress and generation of reactive species in infection.[12] Lipid peroxide level (MDA) ($P<0.001$) is increased significantly in the infectious disease patients than healthy controls.

**Conclusion:**

From the above study, it is been concluded that the NO· is the effector molecule that initiates the cytotoxic effects. Elevated production of nitric oxide and increased indices of NO dependent oxidative stress are due to the formation of the oxidant per-oxynitrite in infectious disease patient.

**References :**

1. Poole RK. Nitric oxide and nitrosative stress tolerance in bacteria. Biochem. Soc. Trans 2005; 33:176-180.
2. H. Nahrevanian , Nitric Oxide Functions in Infections Iran J Basic Med Sci, Winter 2009,Vol. 11, No. 4,,197.
3. Refik M, Mehmet N, Durmaz R, Ersoy Y. Cytokine profile and nitric oxide levels in sera from patients with brucellosis. Braz J Med Biol Res 2004; 37:1659-1663.
4. John B .Hibbs, Infection and nitric oxide. The Journal of Infectious Diseases, Volume 185, Issue Supplement,1, 15 February 2002, Pages S9–S17.
5. Chiwakata CB, Hemmer CJ, Dietrich M. High levels of inducible nitric oxide synthase mRNA are associated with increased monocyte counts in blood and have a beneficial role in Plasmodium falciparum malaria. Infect Immun 2000; 68:394-399.
6. Nahrevanian H. Nitric oxide involvement during malaria infection; Immunological concepts, mechanisms & complexities; A novel review. J Trop Med Parasitol 2004; 27:93-101.
7. 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.
8. Estimation of lipid peroxide (MDA) by Kei satoh method. Clinical chemical Acta 1998: 37-43.
9. Nahrevanian H, Dascombe MJ. Nitric oxide and reactive nitrogen intermediates in lethal and nonlethal strains of murine malaria. Parasite Immunol 2001; 23:491-501.
10. Munzel T, Heitzer T, Harrison DG. The physiology and pathophysiology of the nitric oxide/superoxide system. Herz 1997; 22:158-172.
11. Vazquez-Torres A, Stevanin T, Jones-Carson J, Castor M, Read RC, Fang FC. Analysis of nitric oxide dependent antimicrobial actions in macrophages & mice. Methods Enzymol 2008; 437:521-538.
12. Selçuk Kaya, Recep Sütçü, Emel Sesli, Cetin Cicioglu Arıdogan, Namık Delibaş Mustafa Demirci. Lipid peroxidation level & antioxidant enzyme activities in the blood of patients with acute & chronic fascioliasis. Int j of Infectious Disease. Vol. 11, issue 3 ,May 2007,251-255.