

## Study of Serum Nitric Oxide Levels in Senile Cataract Patients and in Normal Individuals



### Medical Science

**KEYWORDS :** Nitric oxide, nitric oxide synthase, Senile cataract.

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| <b>Puneet Parasurampuria</b> | Department of Ophthalmology, J.N.M.C.H., Aligarh Muslim University, A.M.U. Aligarh, U.P., India-202002           |
| <b>Adeeb Alam Khan</b>       | Assistant Professor, Institute of Ophthalmology, J.N.M.C.H., Aligarh Muslim University, U.P., India-202002       |
| <b>Mohammad Ashraf</b>       | Professor, Institute of Ophthalmology, J.N.M.C.H., Aligarh Muslim University, U.P., India-202002                 |
| <b>Shazia Aman</b>           | Research Scholar, Department of Biochemistry, J.N.M.C.H., Aligarh Muslim University, Aligarh, U.P., India-202002 |
| <b>Shagufta Moin</b>         | Associate Professor, Department of Biochemistry, J.N.M.C.H., Aligarh Muslim University, U.P., India-202002       |

### ABSTRACT

*Cataract is a phenomenon in which the eye becomes opaque resulting in severe visual impairment, and senile cataract is the most common cause of blindness in the world. Levels of nitrite, a metabolite of nitric oxide (NO) are higher in the lens of human senile cataract patients than in normal lenses. There is induction of inducible nitric oxide synthase (iNOS) protein in the lenses, and administration of an iNOS inhibitor can prevent the lens opacification in the Shumiya cataract rat (SCR), UPL rat and selenite-induced cataract rat. The aim of this study was to determine the serum NO levels in senile cataract patients. Using the method of Griess L et al, the levels of NO in sera obtained from 120 senile cataract patients and 100 senile normal volunteers (age and sex matched) were determined. The mean serum NO level in the test group was  $25.01 \pm 10.35$  and  $16.33 \pm 6.34$   $\mu\text{M/L}$  in the control group ( $p < 0.01$ ). The levels of NO in serum of senile cataract group were significantly higher than those of control group. This study revealed that serum NO level in cataract patients was higher than in normal individuals. These findings provide significant information that levels of NO are related to the pathogenesis of cataract. iNOS inhibitors can help with the prevention of cataracts which is a major health burden in many countries.*

### Introduction

Cataract, the opacification of the lens of the eye, is the leading cause of blindness worldwide – it accounts for approximately 42% of all blindness. More than 17 million people are blind because of cataract, and 28000 new cases are reported daily worldwide. Approximately 25% of the populations over 65 and about 50% over 80 have serious loss of vision because of cataract. In developing countries, there is simply no sufficient number of surgeons to perform cataract operations. Besides possible complications, an artificial lens just does not have the overall optical qualities of a normal lens (1). This is the reason for highly required biochemical solutions or pharmacological intervention that will maintain the transparency of the lens; it is estimated that a delay in cataract formation of about 10 years would reduce the prevalence of visually disabling cataract by about 45% (2). Such a delay would enhance the quality of life for much of the world's older and diabetic populations and substantially diminish both the economic burden due to disability and surgery related to cataract.

NO is a readily diffusible, short-lived molecule i.e., produced by the action of nitric oxide synthase (NOS) on L-arginine. One of three known cytoplasmic isoforms of NOS, inducible NOS (iNOS/ NOS2), is generally expressed in response to immunological challenge or some other pathophysiological stimulus (3,4). iNOS activity can produce much larger amounts of NO over several days (5,6). Excessive NO production can result in cellular damage by a various mechanisms, which include the formation of highly reactive free radicals such as peroxynitrite (6). Induction of iNOS and abnormal production of NO occur in certain animal models of cataract (7,8,9). Moreover, the concentration of NO in the aqueous humor is known to be elevated in traumatic cataract (10) and to increase with age in senile cataract patients (11). A role for NO in the etiology of cataract has been proposed because of its ability to modify lens proteins and/or cause or exacerbate oxidative damage to lens cells or predispose them to such damage (12). Levels of nitrite, a metabolite of nitric oxide (NO) are higher in the lens of human senile cataract patients than in normal lenses (13). There is induction of inducible nitric oxide synthase (iNOS) protein

in the lenses, and administration of an iNOS inhibitor can prevent the lens opacification in the Shumiya cataract rat (SCR), UPL rat and selenite-induced cataract rat. Here we report our data on the levels of NO in serum of patients with senile cataract.

### Material and Methods

The study comprised 120 patients of cataract (30 PSC, 30 Nuclear, 30 Cortical and 30 of Mixed type) and 100 normal individuals as controls, of same age. After careful clinical examination venous blood was collected after informed consent from patients and controls with a disposable syringe and needle under all aseptic conditions. Three ml of blood was collected in a gel vacutainer. Blood collected in gel vacutainer was allowed to stand undisturbed till clot retraction occurred. Serum was separated by centrifuging at 2500 rpm for 5 minutes. These samples were stored at  $-20^{\circ}\text{C}$  and used for estimation of nitric.

Nitric Oxide (NO) in serum was determined indirectly, by the measurement of stable decomposition products nitrite and nitrate, employing the Griess reaction according to the method of Griess L. et al, 1982.

The following solutions were prepared for the assay of Nitric Oxide in serum:

Griess reagent is a mixture of equal volume (1:1) ratio of

- 1) 0.1% (w/v) naphthylendiamine dihydrochloride
- 2) 1% (w/v) Sulfanilamide prepared in 2.5% (v/v) metaphosphoric acid.

The reagent was prepared freshly each time before use.

Reagents (1) and (2) were stored for a maximum period of two weeks at  $4^{\circ}\text{C}$ .

Nitrite ( $\mu\text{M/L}$ ) was determined from the standard curve, constructed from the known standard concentration, and their corresponding absorbance values at 540nm.

### Statistical analysis

All the data have been expressed as mean  $\pm$  Standard deviation (SD) for  $n=3$ . Statistical significance of the data was determined by student's  $t$ -test. The probability of occurrence was selected at  $p$ -value  $\leq 0.01$ .

### Results

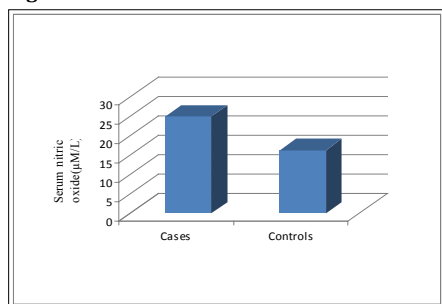
The mean serum Nitric Oxide level  $\pm$ S.D ( $\mu$ M/L) of cataract patients was  $25.01 \pm 10.35$  compared to  $16.33 \pm 6.34$  in the controls ( $p < 0.01$ ). The mean level of nitric oxide was also statistically significant among different subgroups as compared to the control ( $p < 0.01$ ) (Table 1, Figure 1). The mean serum NO level was highest ( $25.70 \pm 8.60$ ) in the posterior subcapsular subgroup among the cases though there was no statistically significant difference in NO levels between the posterior subcapsular and nuclear subgroup, posterior subcapsular and cortical subgroup and posterior subcapsular and mixed subgroup. Also, there was no significant difference in serum NO levels between the nuclear and cortical subgroup, or nuclear and mixed subgroup and between cortical and mixed subgroup (Table 2, Figure 2) shows the mean NO levels in the serum in the subgroups (PSC, Nuclear, Cortical, Mixed and Controls).

**Table 1 about here**

| Parameter                       |          | No. | Mean  | Std. Deviation | Significance |
|---------------------------------|----------|-----|-------|----------------|--------------|
| Serum Nitric Oxide ( $\mu$ M/L) | Cases    | 120 | 25.01 | 10.35          | $P < 0.01$   |
|                                 | Controls | 100 | 16.33 | 6.34           |              |

**Table 1: Serum Nitric Oxide Level in cases and controls.**

**Figure 1 about here**



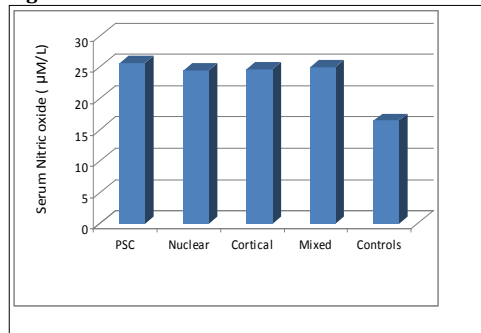
**Figure 1: Serum Nitric Oxide level in cases and Controls.**

**Table 2 about here**

| Parameter                       | Subgroups | No. | Mean  | Standard Deviation |
|---------------------------------|-----------|-----|-------|--------------------|
| Serum Nitric Oxide ( $\mu$ M/L) | PSC       | 30  | 25.7  | 8.6                |
|                                 | Nuclear   | 30  | 24.56 | 11.83              |
|                                 | Cortical  | 30  | 24.71 | 10.83              |
|                                 | Mixed     | 30  | 25.06 | 11.06              |
|                                 | Control   | 100 | 16.53 | 6.14               |
|                                 | Total     | 220 | 21.15 | 9.65               |

**Table 2: Serum Nitric Oxide level in Subgroups.**

**Figure 2 about here**



**Figure 2: Serum Nitric Oxide level in Subgroups.**

### Discussion

NO exert either harmful or beneficial effects in cellular systems via a variety of mechanisms (6). These include both pro-apoptotic and anti-apoptotic effects. The outcome depends on the concentration of NO and factors such as oxidative stress and free radicals in the environment. At high concentrations and under conditions that lead to the generation of large amounts of peroxynitrate, NO is cytotoxic. Oxidative damage to the lens, especially the lens epithelium, is thought to be a triggering factor in the etiology of several forms of cataract, and NO is regarded as an agent capable of contributing to such damage (12). Changes that are indicative of oxidative stress and potentially catarctogenic were noted in a study in which rat lenses were exposed in vitro to an NO donor at a high concentration (14). However, NO is known to be present in the lens environment under physiological concentrations. A low concentration of NO is present in aqueous that bathes the lens anterior. Furthermore, the lens epithelium and neighbouring ocular tissues express NOS in situ and therefore represent the potential sources of NO (7). The result of our present study is in harmony with the results of the above studies. The mean NO level ( $\mu$ M/L) in serum of cataract patients was  $25.01 \pm 10.35$  compared to  $16.33 \pm 6.34$  in controls ( $p < 0.01$ ) in our study. This suggest that oxidative damage to the lens, especially lens epithelium, and protein alterations in the lens, which are the key factor in cataract formation, are related to increased Nitric oxide level in serum. The mean serum NO level ( $\mu$ M/L) was highest ( $25.70 \pm 8.60$ ) in the subcapsular subgroup among the cases though there was no statistically significant difference in serum level within the different subgroup. The serum level in different subgroups was higher than control and the difference was statistically significant. Ornek et al., has compared Nitrite levels among lenses with various types of cataracts and revealed higher levels in lenses with posterior subcapsular cataracts. The underlying mechanisms are yet to be known and more studies are needed to find a relevant explanation to this fact.

### Conclusion

The present study revealed that serum NO level in cataract patients was higher than in normal individuals. These findings provide significant information that levels of NO are related to the pathogenesis of cataract. iNOS inhibitors can help with the prevention of cataracts which is a major health burden in many countries.

### Acknowledgement

The authors are thankful to Department of Biochemistry, Faculty of Medicine, J.N.Medical College, A.M.U., Aligarh for providing the instrument facilities created with the financial help.

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