

Effect Of Panretinal Laser Photocoagulation On The Concentration Of Enzymatic Antioxidants In The Serum Of Diabetic Patients Single Vs Multiple Sitzings.

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Citation

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Abstract

The present study was carried on 60 eyes of 60 patients who attended the "Retina Centre" of Institute of Ophthalmology Aligarh Muslim University. The patients included in the study were divided into two groups; one group (Group A) comprised of 30 patients who received panretinal laser photocoagulation (PRP) in single sitting and the other group (Group B) also comprised of 30 patients who received PRP in four sittings. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee of the centre. Estimation of enzyme levels of superoxide dismutase, catalase and glutathione peroxidase was done in serum prior to laser photocoagulation, 24 hours after photocoagulation and 6 weeks after the last sitting of laser photocoagulation. In our study the mean enzyme level 24 hours after each sitting of laser photocoagulation in both the groups were significantly higher than the pre laser levels. This increase in the mean antioxidant enzyme level could be due to tissue response to increase in reactive oxygen species. The increase in mean enzyme level decreases after each sitting of laser photocoagulation in Group B corroborates with the fact that the defense mechanism against oxidative stress gradually becomes more efficacious after each laser sitting. The increase in mean level of enzymatic antioxidants remained significant even after 6 weeks of laser photocoagulation, may help to explain the mechanism where local laser treatment causes clinical improvement through out the retina and also explains why successful panretinal photocoagulation often prevents further retinal microvascular change despite the continued metabolic derangement. In the present study the mean serum change in the levels of catalase, superoxide dismutase and glutathione peroxidase after 24 hours was greater in Group A as compared to mean of the enzyme changes in all the sittings of laser photocoagulation in Group B. This difference in the change in antioxidant level between Group A and Group B was statistically significant at 24 hours after laser photocoagulation. At 6 weeks after the last laser sitting the mean change in serum catalase, superoxide dismutase and glutathione peroxidase levels in Group A were higher than the levels of these enzymes in Group B. This difference in the level of enzymatic antioxidants was statistically significant (p value < 0.05). The fact that the change in mean of enzymatic antioxidants is greater in Group A as compared to the change in the mean of the enzyme levels in all the laser sittings in Group B as well as, at 6 weeks could be due to inducement of antioxidant enzymes at each laser sitting there by producing lesser changes in subsequent laser sittings. Therefore, it can be concluded that the oxidative stress produced by panretinal photocoagulation in single sitting is greater than that produced in multiple sittings.

INTRODUCTION

Oxidative stress is the disturbance in the equilibrium status of pro-oxidant and antioxidant systems in intact cells. In tissues where glucose uptake is independent of insulin, including retina exposure to elevated glucose levels causes an increase in intracellular sorbitol and fructose levels due to increased activity of aldose reductase and sorbitol dehydrogenase¹. These two enzymes constitute the polyol pathway. Increased substrate flux through the polyol pathway not only increases cellular levels of sorbitol and

fructose but also decreases the ratio of NADPH to NADP⁺ and increases the cytosolic NADH to NAD⁺ ratio. The depletion of NADPH cell stores by aldose reductase may inhibit the activity of other NADPH – requiring enzymes².

An increased production of malondialdehyde has been found in erythrocyte membranes of diabetic patients, together with a depressed erythrocyte content of reduced glutathione³. Studies carried by Nishigaki et al⁴, (1991) and Altomare⁵ (1992) showed that the melondialdehyde is also higher in plasma of diabetic subjects as compared to non-

diabetic subjects. The free radicals may spontaneously decay but there are several defense systems that contribute to the termination of free radical reactions. They include endogenous and exogenous non enzymatic antioxidant mediated protection and enzymatic antioxidant mediated protection. In physiological conditions these defense mechanisms maintain a low steady state concentration of free radicals in the cell and their activities are precisely regulated.⁶

The enzymatic antioxidants that protect the cells from oxidative stress are superoxide dismutase, catalase and glutathione peroxidase.⁷⁻⁹ Superoxide dismutase is the first line of defense against oxygen derived free radicals. It dismutates superoxide radical ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2).⁷ Metabolism of H_2O_2 produced by univalent reductions of superoxide anion is carried by catalase.⁸ Glutathione peroxidase reduces lipid and non lipidic hydroperoxides as well as H_2O_2 while oxidizing two molecules of glutathione.⁹ Laser retinal photocoagulation causes a temporary increase in free radical activity leading to oxidative stress.¹⁰ It is plausible that in the acute phase both thermal and photochemical effects of laser energy directly cause generation of reactive oxygen species. Furthermore, inflammatory cell infiltration may contribute to additional generation of reactive oxygen species. The acute increase in oxidative stress causes an increased redox effect within the cells inducing formation of enzymatic antioxidants and causes prolonged changes in their level.

No previous report in literature has evaluated the change in catalase, superoxide dismutase and glutathione peroxidase levels in serum following laser photocoagulation. Increased oxidative stress following laser photocoagulation in the form of increase in reactive oxygen species have been reported by Stefansson et al.,¹¹ Molnar,¹² Alder,¹³ Novack,¹⁴ Stefansson et al.,¹⁵ and Taguchi.¹⁶

In this backdrop we carried out the present study; 1) To find out the level of superoxide dismutase, glutathione peroxidase and catalase in the serum of diabetic patients. 2) To estimate the changes in serum levels of superoxide dismutase, glutathione peroxidase and catalase produced by single sitting laser photocoagulation and after multiple sittings of laser photocoagulation. 3) To compare the changes produced in superoxide dismutase, glutathione peroxidase and catalase after single sitting laser photocoagulation with changes produced in these enzymes after multiple sittings of laser photocoagulation.

MATERIALS AND METHODS

The present study was carried on 60 eyes of 60 patients who attended the "Retina centre" of Institute of Ophthalmology Aligarh Muslim University. The patients included in the study were divided into two groups; one group (Group A) comprised of 30 patients who received panretinal laser photocoagulation (PRP) in single sitting and the other group (Group B) also comprised of 30 patients who received PRP in four sittings. Informed consent was obtained from all the participants of the study and the protocol was approved by the ethical committee of the centre. Estimation of enzyme levels of superoxide dismutase, catalase and glutathione peroxidase was done in serum prior to laser photocoagulation, 24 hours after photocoagulation and 6 weeks after the last sitting of laser photocoagulation.

ENZYME ESTIMATION

Estimation of Glutathione peroxide was done by technique adapted by Paglia and Valentine¹⁷ and modified by Bilgihan et al. Estimation of Catalase was done by technique adapted by Aebi.¹⁸ Estimation of Superoxide Dismutase was done by technique adapted by Oberly and Spitz.¹⁹

STATISTICAL ANALYSIS

All statistics were analyzed by using SPSS for windows 11 software.

The data obtained was analysed using students "t" test. Correlations between the variables were estimated by Pearson's correlation coefficients.

RESULTS

The mean age of the patents in Group A was 54.16 ± 8.79 years and in Group B the mean age of patients was 51.61 ± 6.67 years. Mean serum antioxidant levels of catalase, glutathione peroxidase and superoxide dismutase increased 24 hours after laser photocoagulation and remained higher than the base value after 6 weeks in Group A (Table 1). There was a increase in the mean serum levels of CAT, SOD and GPx 24 hours after each sitting of laser photocoagulation in Group B. There was a progressively increasing trend in the mean prelaser enzyme levels before each sitting in Group B The levels of CAT, SOD and GPx 6 weeks after last sitting of laser photocoagulation remains significantly raised though the level is lower than the level after 24 hours (Table 2). Statistical analysis of change in serum antioxidant level showed that the levels were significant in both the groups (Table 3&4).

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Figure 1

TABLE – 1 MEAN SERUM ANTIOXIDANT ENZYME LEVELS IN GROUP A

Time of Estimation	Catalase*	Superoxide** Dismutase	Glutathione*** Peroxidase
Pre laser	6.62 ± 0.63	7.54 ± 0.77	8.31 ± 0.77
24 hour post laser	12.00 ± 1.06	15.21 ± 1.36	20.46 ± 2.34
6 week post laser	7.24 ± 0.95	8.24 ± 0.95	9.09 ± 1.06

* $\mu\text{mol H}_2\text{O}_2/\text{min mg serum protein}$ ** unit/mg protein. *** $\mu\text{mol oxidized}$

NADPH/min/mg protein.

Figure 2

TABLE – 2 MEAN SERUM ANTIOXIDANT ENZYME LEVELS IN GROUP B

Estimation	Catalase*	Catalase*	SOD	SOD	GPx***	GPx***
	Pre laser	Post laser	Pre laser	Post laser	Pre laser	Post laser
1st sitting	6.66 ± 1.32	12.69 ± 1.54	7.36 ± 1.40	15.99 ± 1.51	8.64 ± 1.28	20.99 ± 2.84
2 nd sitting	8.03 ± 1.14	10.78 ± 1.53	8.55 ± 1.40	13.00 ± 2.12	9.84 ± 1.40	16.15 ± 2.53
3 rd sitting	8.30 ± 0.91	10.02 ± 1.15	8.95 ± 1.02	11.47 ± 1.30	10.01 ± 1.56	13.33 ± 1.76
6week later	7.21 ± 1.23	7.96 ± 1.31	9.06 ± 0.87			

* $\mu\text{mol H}_2\text{O}_2/\text{min mg serum protein}$. # Time interval in between each laser sitting was 1 week.

** unit/mg serum protein. *** $\mu\text{mol oxidized NADPH/min/mg serum protein}$.

Figure 3

TABLE – 3 STATISTICAL ANALYSIS OF CHANGE IN SERUM ANTIOXIDANT LEVELS IN GROUP A

Time of Estimation	Catalase	Superoxide Dismutase	Glutathione Peroxidase
24 hours post laser	t = 31.118 p < 0.001	t = 25.801 p < 0.001	t = 26.769 p < 0.001
6 weeks post laser	t = 8.416 p < 0.001	t = 12.351 p < 0.001	t = 10.683 p < 0.001

p = 0.05 is significant.

Figure 4

TABLE – 4 STATISTICAL ANALYSIS OF MEAN CHANGE IN SERUM ANTIOXIDANT LEVELS IN GROUP B

Time of Estimation	Catalase	Superoxide Dismutase	Glutathione Peroxidase
After 1st Sitting	t = 25.271 p < 0.001	t = 32.702 p < 0.001	t = 29.437 p < 0.001
After IInd Sitting	t = 13.249 p < 0.001	T = 18.925 p < 0.001	t = 16.674 p < 0.001
After IIIrd Sitting	t = 10.132 p < 0.001	T = 15.099 p < 0.001	t = 9.797 p < 0.001
After IVth Sitting	t = 5.130 p < 0.001	t = 7.883 p < 0.001	t = 12.903 p < 0.001

p = 0.05 is significant.

DISCUSSION

We have estimated and compared the change in the levels of enzymatic antioxidants produced by retinal laser photocoagulation in single sitting (Group A) and with changes produced in multiple sittings (Group B). The purpose was to observe the response to acute oxidative stress produced by laser photocoagulation by the estimation of serum enzymatic antioxidants. Panretinal photocoagulation is known to cause a temporary increase in free radical activity resulting directly in an increase in lipid peroxidation. The acute oxidative stress induced protective mechanism in the form of increased redox level produces more prolonged changes in the level of antioxidant enzymes. Increase in superoxide dismutase level in diabetic subjects was also obtained by Kakkar et al.,²⁰ and Yadav et al.²¹ Increase in catalase level in diabetic subjects was also obtained by Tatsuki et al.,²²; and Stefek et al.²³

In our study the mean enzyme level 24 hours after each sitting of laser photocoagulation in both the groups was significantly higher than the pre laser level. This increase in the mean antioxidant enzyme level could be due to tissue response to increase in reactive oxygen species. The change induced in the redox status of the tissue may initiate intracellular signal transduction process that could trigger expression of different proteins including enzymatic antioxidants. Therefore in retinal photocoagulation as a consequence of an oxidative stress, there could be a compensatory increase in catalase, superoxide dismutase and

glutathione peroxidase. Adamo et al.,²⁴ Gonzalez et al.,²⁵ have demonstrated a parallel change in enzymatic antioxidants in case of oxidative stress in various tissues.

The fact that increase in mean enzyme level decreases after each sitting of laser photocoagulation in Group B corroborates with the fact that the defense mechanism against oxidative stress gradually becomes more efficacious after each laser sitting. This is due to pre existing increase in serum levels of the enzymatic antioxidants induced by the previous laser sittings which can tackle the oxidative stress. As a consequence net increase in antioxidant enzyme levels is less in subsequent sittings.

The increase in mean level of enzymatic antioxidants remained significant even after 6 weeks of laser photocoagulation, may help to explain the mechanism where local laser treatment causes clinical improvement throughout the retina and also explains why successful panretinal photocoagulation often prevents further retinal microvascular change despite the continued metabolic derangement. In the present study the mean serum change in the levels of catalase, superoxide dismutase and glutathione peroxidase after 24 hours was greater in Group A as compared to mean of the enzyme changes in all the sittings of laser photocoagulation in Group B. This difference in the change in antioxidant level between Group A and Group B was statistically significant at 24 hours after laser photocoagulation. At 6 weeks after the last laser sitting the mean change in serum catalase, superoxide dismutase and glutathione peroxidase levels in Group A were higher than the levels of these enzymes in Group B. This difference in the level of enzymatic antioxidants was statistically significant (p value < 0.05). The fact that the change in mean of enzymatic antioxidants is greater in Group A as compared to the change in the mean of the enzyme levels in all the laser sittings in Group B as well as, at 6 weeks could be due to inducement of antioxidant enzymes at each laser sitting there by producing lesser changes in subsequent laser sittings. Therefore, it can be concluded that the oxidative stress produced by panretinal photocoagulation in single sitting is greater than that produced in multiple sittings.

COMMENTS

Laser photocoagulation is a successful treatment, which has been demonstrated to induce sudden temporary increase in free radical activity. This may occur either by direct thermal damage or by oxygen reperfusion. The acute increase in oxidative stress induces protective mechanism in the form of

increased redox level, which produces pronounced changes in the level of serum enzymatic antioxidants. Increased antioxidant level as a response to oxygen-derived free radicals can occur in some conditions where cells and organisms are exposed to oxidative stress. Recovery capacity of cells exposed to an oxidative stress depends on the intensity of the stress, higher the stress the lower the recovery. These studies suggest the presence of a threshold of oxidative damage that cannot be totally repaired and that impair cell division. It is hypothesized that this threshold of oxidative damage, below which recovery is still possible, can be modulated by the antioxidant enzyme activities of the cells; the idea is that if the antioxidant enzyme activities are increased, the cell could undergo a more severe oxidative stress and still be able to recover. The demonstration of increased enzymatic antioxidants in serum following photocoagulation helps to explain the mechanism by which local laser treatment causes demonstratable clinical improvement throughout the retina in untreated areas and also explains why successful panretinal photocoagulation often prevents further retinal microvascular changes despite the continued metabolic derangement.

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