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Research Article

Evaluation of Lipid Peroxide and Superoxide Dismutase in Burn Patients

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Abstract: In the present study an attempt has been made to estimate serum lipid peroxide and serum superoxide dismutase enzyme in the burn patients as burn is not a normal condition in which above balance remains same as in health. The present study has been conducted on 50 burn subjects (22 males and 28 females) of either sex or varying ages having varying degree of burn injury, admitted in the Surgical/Burn ward of J.L.N. Hospital, Ajmer. The results were compared with 25 normal age matched healthy subject of either sex (10 males and 15 females). In the present study significant rise in the level of SOD activity was observed in burn subjects as compared to control subjects. Similarly significant rise in the MDA concentration "a marker of lipid peroxidation" was also observed in burn subjects as compared to control subjects. There was no statistically significant variation in the SOD activity and MDA concentration when the results were compared sex wise in control group and burn subjects. The male subjects of burn group had significantly high SOD activity and MDA concentration as compared as compared to male subjects of control group. Similar trend was observed in female burn subjects and female control subjects. Serum SOD activity and MDA concentration increases in burn subjects as the percentage degree of burn injury increases.
Keywords: Lipid Peroxide, Superoxide Dismutase, Burn Patients, Malondialdehyde

INTRODUCTION

Electromagnetic radiations from the environment and manmade sources lead to generation of various free radicals for example hydroxy radical, superoxide radical, reactive oxygen species etc. These fearsomely reactive radicals, once generated, attacks whatever it is next to and usually leaving behind a legacy in the form of propagating free radical chain reactions. Free radicals are responsible for widespread and indiscriminate oxidation and peroxidation of lipids, denaturation of proteins, depolymerization of polysaccharides, break and modify DNA or any other cell causing cell death or organ damage [1].

Burn is associated with free radical production. In fact inflammatory, degenerative and neoplastic diseases, including the natural phenomenon of aging itself are now considered to be mediated by free radicals. Lipids are the most susceptible by free radicals attack. Increase free radicals cause lipid peroxidation of PUFA. One of the markers for increased lipid peroxidation is the level of malondialdehyde. There is considerable interest in the role played by free radical reactions and lipid peroxidation in human diseases. Mammalian cells have evolved an interrelated antioxidant defence mechanism which minimizes, injurious events that results from chemicals and normal oxidative products of cellular metabolism.

Cotgreave *et al*. [2] reported the antioxidant defense of two main categories, one whose role is to prevent the generation of free radicals and the other that intercept only that are generated and which exist both in aqueous and membrane compartments of cells and may be enzymes or nonenzymes [3].

In healthy condition at cellular level exists a balance between free radicals generation and disposal by antioxidant defence. Thus in the present study an attempt has been made to estimate serum lipid peroxide and serum superoxide dismutase enzyme in the burn patients as burn is not a normal condition in which above balance remains same as in health.

MATERIALS AND METHODS

The present study has been conducted on 50 burn subjects (22 males and 28 females) of either sex or varying ages having varying degree of burn injury, admitted in the Surgical/Burn ward of J.L.N. Hospital, Ajmer. The results were compared with 25 normal age matched healthy subject of either sex (10 males and 15 females). Samples were collected from patients when

admitted. Random blood samples were collected by aseptic treatment from antecubetal vein of each patient in EDTA and plain bulbs. Plasma samples were analyzed for superoxide dismutase activity on the same day and serum samples were analyzed for lipid peroxides in terms of malondialdehydes.

Malondialdehyde (Satoh K [4])

Principle

Trichloroacetic acid and thiobarbutric acid were added to serum and the mixture was heated in a boiling water bath for 30 minutes. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of organic phase was determined at the wave length of 530nm. The determined values are expressed in terms of malondialdehyde (nmol/ml) which is used as reference standard.

The absorbance of test sample was read from the standard curve and concentration of malondialdehyde was found.

Superoxide Dismutase (Nandi and Chatterjee [5])

Principle

This method utilizes the inhibition of auto oxidation of pyrogallol by superoxide dismutase enzyme.

Definition of Unit: One unit of superoxide dismutase is described as the amount of enzyme required to cause 50% inhibition of pyrogallol auto oxidation per 3 ml of assay mixture**.**

Calculation

$$\textit{Serum MDA Conc.} = \frac{\textit{Absorbance of test sample x Concentration of Standard}}{\textit{Absorbance of standard}}$$

$$\textit{Units of SOD / 3 ml of assay mixture} = \frac{\textit{Absorbance of control - Absorbance of sample x 100}}{\textit{Absorbance of control x 50}}$$

$$\textit{Units x 10 = Units/ ml of sample plasma}$$

RESULTS

The present study was conducted on 50 burn subjects of either sex or varying ages having variable degree of burn injury. The results were compared with 25 age matched normal healthy subjects of either sex or varying ages.

In the present study significant rise in the level of SOD activity was observed in burn subjects as compared to control subjects. Similarly significant rise in the MDA concentration "a marker of lipid peroxidation" was also observed in burn subjects as compared to control subjects.

There was no statistically significant variation in the SOD activity and MDA concentration when the results were compared sex wise in control group and born subjects. The male subjects of burn group had significantly high SOD activity and MDA concentration as compared as compared to male subjects of control group.

Similar trend was observed in female burn subjects and female control subjects. Serum SOD activity and MDA concentration raises in burn subjects as the percentage degree of burn injury increases.

Table 1: Serum SOD Activity (units/ml) and Serum MDA concentration (nmol/ml) in control subjects in relation to sex

S.N.	Subject	Number	SOD Activity (units/ml)	MDA concentration (nmol/ml)
1	Control	25	3.34±0.63	4.21±0.83
2	Male	10	3.48±0.61	4.30±0.94
3	Female	15	3.25±0.64	4.15±0.76

Table 2: Serum SOD Activity (units/ml) and Serum MDA concentration (nmol/ml) in Burn Patients in relation to sex

S.N.	Subject	Number	SOD Activity (units/ml)	MDA concentration (nmol/ml)
1	Burn Patient	50	5.53±1.14	5.70±1.50
2	Male	22	5.72±1.09	5.97±1.57
3	Female	28	5.39±1.17	5.48±1.45

Table 3: Serum SOD Activity (units/ml) and Serum MDA concentration (nmol/ml) in Control and Burn subjects

S.N.	Subject	Number	SOD Activity (units/ml)	MDA concentration (nmol/ml)
1	Control Subjects	25	3.34±0.63	4.21±0.83
2	Burn Subjects	50	5.53±1.14*	5.70±1.50**

*p< 0.001; **p< 0.0001

Table 4: Serum SOD Activity (units/ml) and Serum MDA concentration (nmol/ml) in Control and Burn Male subjects

S.N.	Subject	Number	SOD Activity (units/ml)	MDA concentration (nmol/ml)
1	Control Subjects	10	3.48±0.61	4.30±0.94
2	Burn Subjects	22	5.72±1.09*	5.97±1.57**

*p< 0.001; **p< 0.0001

Table 5: Serum SOD Activity (units/ml) and Serum MDA concentration (nmol/ml) in Control and Burn Female subjects

S.N.	Subject	Number	SOD Activity (units/ml)	MDA concentration (nmol/ml)
1	Control Subjects	15	3.25±0.64	4.15±0.76
2	Burn Subjects	28	5.39±1.17*	5.48±1.45**

*p< 0.001; **p< 0.0001

Table 6: Serum SOD Activity (units/ml) and Serum MDA concentration (nmol/ml) in Burn Patient in relation to degree of Burn Percentage

S.N.	Percentage (%)	SOD Activity (units/ml)	MDA concentration (nmol/ml)
1	<25%	4.73±1.09	5.25±1.06
2	<50%	5.18±1.34	5.48±0.86
3	<75%	5.74±1.18	5.89±1.46
4	>75%	6.20±1.39	6.65±0.98

DISCUSSION

Oxygen derived free radicals have been implicated as a general mechanism of cell injury in inflammation, irradiation injury, oxygen toxicity and ischemia. Antioxidant enzymes are of vital importance in an organism's defence against oxidative stress. The most important is the superoxide dismutase which converts superoxide to hydrogen peroxide and oxygen.

$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow > H_2O_2 + O_2$

Thus SOD protects against the deleterious effects of superoxide free radical [6]. A variety of indirect and direct methods have been published for the assay of SOD. Marklund S [7] described direct spectrophotometric method which is sensitive and specific. Nandi and Chatterjee [5] observed the reliable and reproducible results in assay of SOD in crude tissue homogenates by the pyragallol auto oxidation method of Marklund and Marklund [8], after making minor modifications.

In the present study mean serum superoxide dismutase (SOD) activity in control subjects was found to be 3.34±0.63 units/ml. The mean serum SOD activity in control male subjects was found to be 3.48 ± 0.61 units/ml and in female subjects 3.25±0.64 units/ml showing that there is no significant alteration in male and female subjects (Table 1). The results are in accordance with Guemouri *et al*. [9].

In the present study significantly increased serum SOD activity was observed in burn cases. In burn patients the mean serum SOD activity was found to be 5.53±1.14 units/ml which is significantly high as compared to the control subjects (Table 1, 2). In burn male subjects the mean SOD activity was 5.72±1.09 units/ml and in female subjects 5.39±1.17 units/ml which shows that there is no significant alteration in male and female subjects. But on comparison between male subjects of control group and burn group, a significantly higher value was observed in burn subjects. A similar finding has also been found in the female subjects of both the groups (Table 1 and 2).

Lipid peroxidation, a general mechanism of tissue damage by free radicals is known to be responsible for cell damage and may induce pathological events [10]. Clinical chemistry tests to assess lipid peroxidation include the various techniques but the most widely used method is the thiobarbutric acid reaction with malondialdehyde because of its procedural simplicity and nanomolar sensitivity [11-14].

In the present study, the serum mean MDA concentration in 25 healthy controls were found to be 4.21±0.83 nmol/ml with 4.30±0.94 nmol/ml in male group and 4.15±0.76 nmol/ml in female group and no statistical difference was observed between male and female groups (Table 1). In burn patients the mean MDA concentration was found to be 5.70±1.50 nmol/ml as compared to the control subjects. In burn male subjects the mean serum MDA concentration was 5.97±1.57 nmol/ml and in female subjects 5.48±1.45 nmol/ml shows that there is no significant alteration in male and female subjects (Table 2).

CONCLUSION

In the present study significant rise in the level of Superoxide Dismutase (SOD) activity and Malondialdehyde (MDA) concentration was observed in burn subjects as compared to control subjects. This is supported by the fact that burn lead to increase formation of lipid peroxide in skin and serum which is measured by the level of MDA and enzyme SOD scavenges superoxide ($O_2^{\cdot-}$) formed by accelerating spontaneous dismutation of superoxide to H_2O_2 and molecular O_2.

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