**Original Article**

# Antioxidant Enzyme Activity and Malondialdehyde Concentration in Patients with Urticaria Induced By Non Steroidal Anti-Inflammatory Drugs

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**Abstract**

**Objective:** The Urticaria caused by NSAIDs was studied for their antioxidant enzyme activity and malondialdehyde (MDA) concentration. **Methodology:** Patients were divided into two groups; group one act as a control and the remaining group was urticaria patients. The levels of the Thiobarbituric acid reactive substances (TBARS, as a marker of lipid peroxidation), superoxide dismutase (SOD), glutathione (GSH) and catalase (CAT) in the serum were estimated. The enzymatic activity was estimated and compared with different categories of NSAIDs. **Results:** In present study CAT, SOD and lipid peroxidation product (MDA) were statistically different from that of healthy control and showed significant changes ($p< 0.05$) while GSH showed a non-significant ($p>0.05$) value. **Conclusion:** It is concluded that antioxidative system of the body is accelerated and shows variations in the patient of Urticaria. Moreover, present study shows the co-ordinated enzymatic mechanism and the interrelationship between enzymatic activities in allergy. **Key Words:** Urticaria, NSAIDs, Antioxidant enzymes activity, Malondialdehyde (MDA).

## INTRODUCTION

Urticaria's first depiction has been introduced by Herberden in 1772 but it has been known since Hippocrates [1]. Urticaria is one the common disarray of skin affecting one in four and one in six people sometimes throughout their lives. It can be classified into two groups i-e acute and chronic based on urticarial episodes, urticarial attack less than 6 weeks' are called acute those lasting longer are consider chronic [2]. Pathophysiological mechanism also classified the urticaria into groups either it is IgE-dependent or complement-mediated or nonimmunologic which show direct effect on mast cell or on arachidonic acid metabolism and those whose condition is idiopathic [3].

Immunological or non- immunological mechanism is initiated by various triggered factors as result histamine and large number of newly synthesized mediators are released which give a stiff effect on the microvasculature. Cytokines, enzymes, neuropeptides and various chemotactic factors release from triggered mast cell which recruit and activate various inflammatory cells such as lymphocytes and polymorph nuclear cells (e.g neutrophils and eosinophils). Increased level of inflammatory mediators and/or factors in different type of urticarial has been found by many researchers [4]. One of the most prescribed drugs class in the world are Nonsteroidal anti-inflammatory drugs (NSAIDs). In many countries their enlarge use, further increased by the fact that some very popular compounds such as acetylsalicylic acid (ASA) propionic acid derivatives, or paracetamol (Acetaminophen) are in over the counterdrug, indubitably the main reason for the increasing number of inauspicious reaction caused by these drugs that have been recognized all over the world. NSAIDs may induce a large spectrum of adverse reaction, few of which are potentially tragic although they are generally well endured. The most severe reaction associated to their inhibitory effect on cyclooxygenase-1 (COX-1) enzyme is gastritis and peptic ulcers [5]. It has been figured out the average consumption as high as 80 tablets per person per year [6]. NSAIDs are one the

foremost cause of adverse reaction among pharmaceutical products due to the magnitude of its exposure in population [7]. Susceptible to NSAIDs, including aspirin, can cause exaggerate pre-existing chronic urticaria or acute urticaria [2].

There are several types of antioxidants such as Glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and various peroxidases [8]. The unpaired electronic atoms or atomic group, molecules or ion are refers to as free radicals (FR) [9]. The perspective studied of free radical showed that, almost all the phenomenon of life and pathological processes such as aging, shock, inflammation, autoimmune diseases, stress and cancer enabling them to have awareness of many illnesses that causes pathogenesis and provide a new basis for the treatment of some diseases in the field of biology and medicine [10]. There are many free radicals, referred to as reactive oxygen species (ROS) that play an imperative role in the pathogenesis of several diseases [11] which also comprise some skin diseases [12].

Free radical mediated damage and oxidative stress is a good marker of lipid peroxidation end product i-e MDA [13]. Moreover, the initiation of cytotoxic processes leading to adverse effect induced by non-steroidal anti-inflammatory drugs (NSAIDs) has been hypothesized due to oxidative stress [14]. Catalase (CAT) and superoxide dismutase (SOD) are different antioxidants enzymes [15].

The fabrication of free radicals (FR) through enzymatic and non-enzymatic system is the most lively and damaging under disease condition. Polyunsaturated fatty acids (PUFA) is present in membranes, the free radicals enhance lipid peroxidation which lead to the formation of lipid peroxides such as malondialdehyde (MDA), therefore as a result tissue or cells damage [16]. The evacuation of free radicals is reliant on the prevention or interrupted regulations of antioxidant defense system [17].

## MATERIAL AND METHODS

In this study 50 normal subjects taken as control & 40 patients having urticaria induced by NSAIDs were enrolled. Among the 40 patients of urticaria 23 were male & 17 female. From each subject 05 ml blood was drawn from anticubital vein. Blood was allowed to clot for 01 hour & then centrifuged at 3000 rpm for 05 minutes & serum was separated for biochemical analysis. All chemical reagents of analytical grades were purchased from Sigma Chemical Co. (St. Louis, Mo, USA) & analytical work was done at the institute of Molecular Biology & Biotechnology, The University of Lahore.

## BIOCHEMICAL PROCEDURE

- The serum level of MDA (malondialdehyde), a marker of lipid peroxidation (LPO) was measured by the method of Ohkawa [18].
- Antioxidant activity of enzymes Superoxide dismutase (SOD) was determined by the method of Kakkar [19].
- Catalase (CAT) was determined by method of Aebi [20].
- Glutathione (GSH) was estimated according to the method of Moron [21].

## STATISTICAL ANALYSIS

Statistical analysis was performed on a PC using SPSS, V.16. Data was presented as arithmetic mean ± S.D., The difference among means has been analyzed by one-way ANOVA. A value of $P < 0.05$ was considered as statistically significant.

## RESULTS

The levels (Mean±S.D) of serum MDA in nmol/ml among control & patients were 5.37±1.56 & 14.92±6.98 respectively. The patients group had significantly high levels ($P < 0.01$) of MDA than the control subjects. The activities of SOD & Catalase were also significantly low in patient population than the control subject. There was no significant difference in the levels of GSH among the both groups.

While the levels of MDA among the patient taking selective COX-2 inibitors and enolic acid were not significantly different than the control subjects.

**Table-1**

**Parameters of antioxidative system in control and urticaria group**

| Parameters | Normal | Patients | P value |
|---|---|---|---|
| MDA nmol/ml | 5.37±1.56 | 14.92±6.98 | <0.001* |
| SOD U/ml | 1.83±0.73 | 1.23±1.23 | <0.01* |
| GSH µg/ml | 21.09±4.01 | 24.6±9.10 | <0.154 |
| CAT U/ml | 3.31±0.85 | 2.35±0.00 | <0.01** |

Abbreviation: CAT, catalase; SOD, superoxide dismutase; GSH, glutathione; MDA, malondialdehyde.

**Table-2**
**Pearson Correlation between CAT, GSH, SOD and MDA**

| | CAT | GSH | SOD | MDA |
|---|---|---|---|---|
| CAT | 1 | 0.030<br>0.834 | 0.318*<br>0.025 | -0.267*<br>0.041 |
| GSH | | 1 | -0.189<br>0.189 | 0.020<br>0.891 |
| SOD | | | 1 | -0.211<br>0.142 |
| MDA | | | | 1 |

*Correlation is significant at the 0.05 level (2-tailed).

**Table-3**
**Parameters of antioxidative system in control and patients with various NSAIDs group**

| Groups | GSH µg/ml | SOD µg/ml | MDA nmol/ml | CAT nmol/mol of protein |
|---|---|---|---|---|
| Control | 21.0±4.01 | 1.83±0.73 | 5.37±1.56 | 3.31±0.85 |
| DC-1<br>Sig. | 23.4±5.67<br>0.24 | 0.79±0.55<br>0.00** | 12.9±6.70<br>0.00** | 1.83±0.79<br>0.00** |
| DC-2<br>Sig. | 18.3±4.87<br>0.22 | 1.22±0.77<br>0.04* | 10.7±5.07<br>0.02* | 1.88±0.96<br>0.00** |
| DC-3<br>Sig. | 24.6±9.10<br>0.36 | 0.48±0.19<br>0.01* | 12.1±0.12<br>0.10 | 2.35±0.00<br>0.13 |
| DC-4<br>Sig. | 18.2±0.32<br>0.401 | 0.42±0.23<br>0.00** | 5.93±2.75<br>0.86 | 0.88±0.59<br>0.00** |
| DC-5<br>Sig. | 19.4±5.26<br>0.57 | 0.69±0.51<br>0.00** | 14.9±6.98<br>0.00** | 1.39±0.65<br>0.00** |
| DC-6<br>Sig. | 18.5±2.07<br>0.52 | 1.23±1.23<br>0.24 | 5.87±3.59<br>0.90 | 2.17±0.26<br>0.07 |

DC, Drug Category; DC-1, Salicylates; DC- 2, Proponic acid derivative; DC 3, Acetic acid derivatives; DC-4, Enolic acid (Oxicam) derivatives; DC-5, Fenamic acid derivatives (Fenamates); DC-6 Selective COX-2 inhibitors (Coxibs); DC-7, Sulphonanilides

**Figure-1**
**Graph A: MDA conc (nmol/ml) along with drugs**

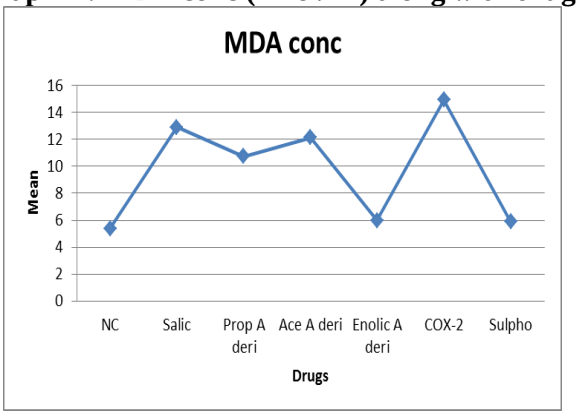



**Figure-2**
**Graph B: SOD conc (µg/ml) along with drugs**

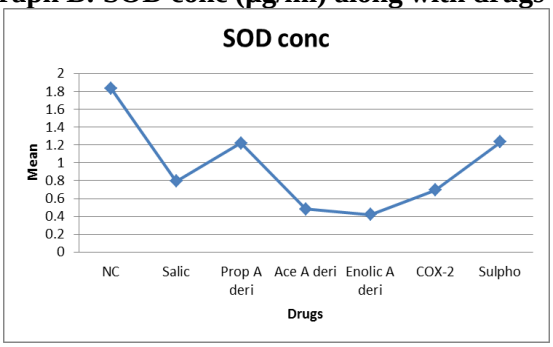



**Figure-3**
**Graph C: GSH conc (µg/ml) along with drugs**

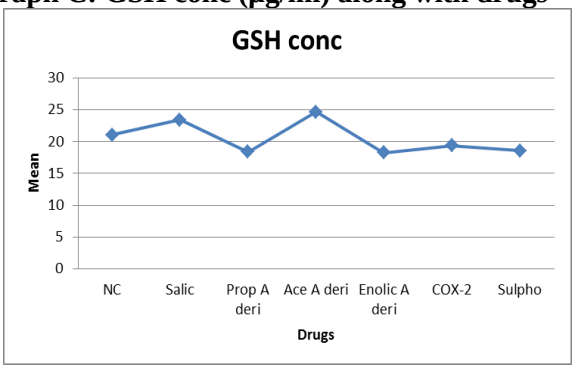
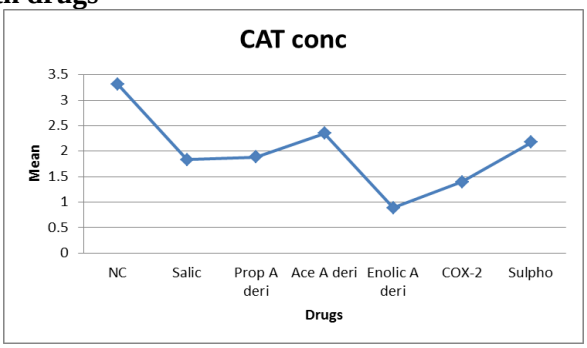



**Figure-4**
**Graph D: CAT conc (nmol/mol of protein) along with drugs**




## DISCUSSION

Reactive oxygen species (ROS) are continuously generated in response to both external and internal stimuli in all aerobic organisms at the molecular level. Radicals may be beneficial or even indispensible at low concentration in intracellular messaging and defense against microorganisms, contributing to phagocytic bactericidal activity In contrast, the oxidative stress occurs in response to high dose and/or inadequate

removal of active oxygen which may have a responsibility to cause severe metabolic malfunctions. There are many reactive oxygen intermediates including superoxide anions ($O_{2.}^-$), hydroxyl radicals (.OH), superocide anions ($O_2^-$), hydrogen peroxide ($H_2O_2$), nitric oxide (NO), lead to lipid peroxidation and specific oxidation of some enzymes and protein oxidation and degradation [22]. Recently, anti-inflammatory and/or immunosuppressive properties have been definitely linked to antioxidant functions [23]. An increase of peroxide availability caused by the imbalance of SOD/GSHPx ratio and thus, consequence of higher ROS production by of Fenton's and Haber-Weiss reactions occur [24]. Degree of unsaturation of fatty acids present in membranes mainly reflects a degree of lipid peroxidation potential this is a general conclusion from recent studies [25]. Therefore, patient suffering from allergy due to NSAIDS exposure showed a similar global stress. In fact, it is also established that, in asthma, inflammatory cells release oxygen radicals that cause tissue damage [26]. In present research, the enzymatic antioxidant activity and malondialdehyde (MDA) concentration in patients of urticaria induced by nonsteroidal anti-inflammatory drugs (NSAIDs) was observed. It has been suggested that NSAIDs have different influence on oxidative stress and antioxidant related parameters in vivo [27]. We observed a significant change ($p< 0.05$) in lipid peroxidation (LPO) expressed as MDA level in patients suffering from urticaria caused by different category of NSAIDs as compared with healthy control. Malondialdehyde (MDA) show negative correlation with catalase (CAT) if the MDA level is increasing the CAT level was observed to be lessening and vice versa. The CAT vs. MDA, r = -0.267*. Moreover, significant changes were observed in enzymatic antioxidant defense measured by the activity of Cu/Zn SOD and catalase (CAT) both showed a positive co relationship according to statistical analysis. There was no direct production of $OH^-$ ion so SOD and CAT work together and this reaction occured due to singlet oxygen species. This singlet oxygen was converted into molecular oxygen ($O_2$) and ($H_2O_2$) hydrogen peroxide. The superoxide dismutase (SOD) is the enzyme that catalyzes this reaction. After that hydrogen peroxide ($H_2O_2$) was converted into water ($H_2O$) and molecular oxygen ($O_2$) with the help of enzyme catalase. The correlation of CAT vs SOD, was found statistically significant was (r = 0.318**). The different in the levels of serum reduced glutathione (GSH) measured among both patients of urticaria and the controls were not statistically significant ($p<0.5$). The GSH was statistically non-significant in all drug categories, while the catalase, superoxide dismutase and MDA were significant (Table 3).

## CONCLUSION

In conclusion, when antioxidant, free radical scavenging systems are accelerated, inflammatory, hypersensitivity and autoimmune condition may result. The observed difference in the defense system against free radicals in allergic patients is a part of main role of antioxidant enzyme in blood cells detoxification, showing the co-ordinated enzymatic mechanism and the interrelationship between all enzymatic activities in allergy.

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