# THE STUDY OF SERUM SUPEROXIDE DISMUTASE ACTIVITY IN CHILDREN WITH RENAL DISEASES

HARUNOBU HIRANO

*Department of Pediatrics, School of Medicine, Niigata University*

## ABSTRACT

Superoxide dismutase (SOD) is known to be a specific enzyme that scavenges superoxide and protects from tissue injury. The author investigated serum SOD activity in children with renal diseases. Serum SOD activity in MPGN was low (6.7 $\pm$4.9 $\mu$g/ml, P<0.01), as it also in PSAGN at the acute phase (8.0 $\pm$5.7 $\mu$g/ml, P<0.05), in comparison with the controls. In the cases of CGN, the more serious the histological diagnosis was, the lower the tendency of serum SOD activity showed. Low SOD activity is considered to be closely associated with the active phase of renal disease. Superoxide can be a vital activator in glomerular injury. SOD can be a healing factor in renal disease.

## INTRODUCTION

In vivo studies have shown considerable evidence that oxygen-free radicals [hydrogen peroxidase ($H_2O_2$), hydroxyl radical ($\cdot$OH), singlet oxygen ($^1O_2$) and superoxide ($O_2^-$)] play vital roles in an organism. Such radicals were recently clarified as also being significant factors in causing inflammation.[1)2)3)] In addition, numerous studies have revealed that the immunological mechanism is closely associated with the onset and progress of renal disease. However, the role of oxygen-free radicals, such as superoxide, in association with the onset and the progress of renal disease has yet to be studied. Superoxide dismutase (SOD), identified by McCord and Fridovich in 1969, is a specific enzyme acting as a potent activator in scavenging superoxide.[4)] To study the role of superoxide at the onset and during the progress of renal disease, the author investigated serum levels of SOD in children with renal diseases.

## PATIENTS AND METHODS

Among patients examined at our pediatrics department, the cases below were selected for experiment, with 54 healthy children ranging in age from 3 to 15 serving as controls.

Blood samples were collected between 8:00 a.m. and 11:00 a.m. and the patients were classified according to generally accepted clinco-histological criteria as follows: idiopathic nephrotic syndrome (INS), onset stage (before administration of corticosteroids): 3 cases: relapse stage: 8 cases; remission stage: 26 cases; congenital nephrotic syndrome (cong NS): 2 cases; poststreptococcal acute glomerulonephritis (PSAGN), acute phase (within 4 weeks): 9 cases; healing phase (after 4 weeks): 8 cases; membranoproliferative glomerulonephritis (MPGN): 7 cases; focal glomerular sclerosis (FGS): 2 cases; purpura nephritis (HSPN), nephritic type: 11 cases, nephrotic type: 3 cases; IgA nephropathy (IgA neph): 7 cases; other chronic glomerulonephritis (other CGN): 43 cases.

The NBT-xanthine oxidase method was used to examine serum SOD activity, and measurement was done using a device of the author's construction.[5]
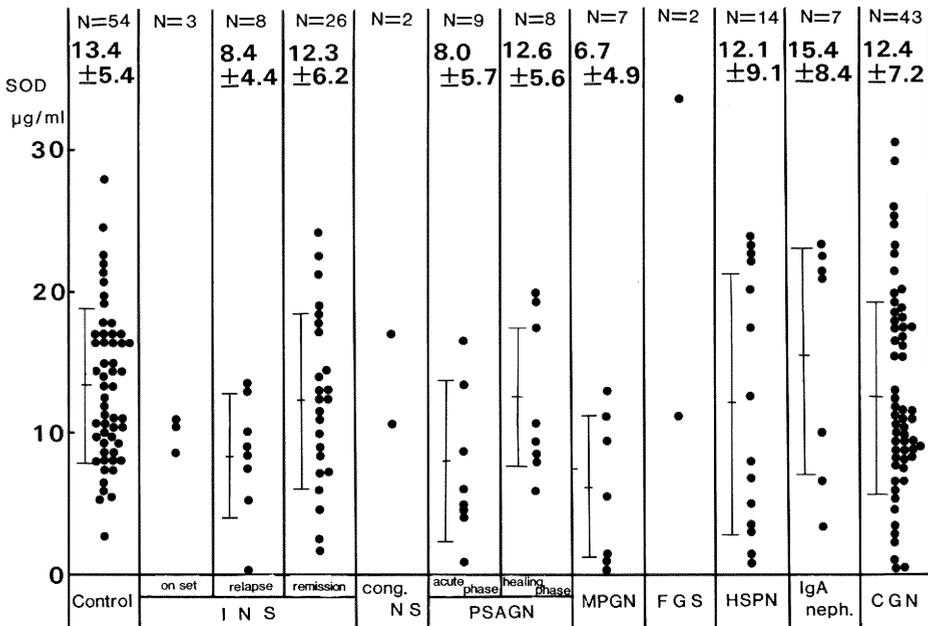
## RESULTS (Fig. 1)



Fig. 1. Serum SOD activity in children with renal diseases

The figure shows mean±SD.

1) Control group

Mean serum SOD activity in the 54 healthy children was found to be 13.4±5.4 $\mu$g/ml, which figure was designated as normal. No significant differences were observed in relation to age (Table 1).

**Table 1** Serum SOD activity in normal children

| Age (y. o.) | 3~5 | 6~9 | 10~12 | 13~15 |
|---|---|---|---|---|
| Number | 8 | 20 | 14 | 12 |
| SOD ($\mu$g/ml) | 11.5±4.2 | 13.5±5.8 | 14.1±4.4 | 13.5±6.9 |

2) Idiopathic nephrotic syndrome (INS)

Serum SOD activities in the 3 cases at the onset of INS were 8.4, 11.1 and 10.3 $\mu$g/ml (mean=9.9 $\mu$g/ml). In case of INS relapse, the mean was 8.4±4.4 $\mu$g/ml; that of INS remission stage was 12.3±6.2 $\mu$g/ml, showing no significant difference in comparison with the controls. However, the serum SOD activity in INS relapse showed to be lower than that in the INS remission. As for the influence of corticosteroids, no significant difference in remission was evidenced between their administration and non-administration (Fig. 2).
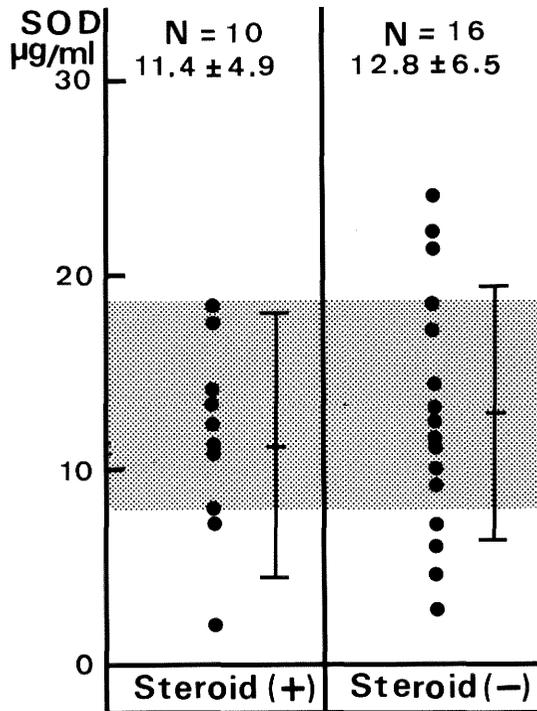


Fig. 2. Influence of Corticosteroids on serum SOD activity (INS remission stage)

3)  Focal glomerular sclerosis (FGS)

Serum SOD activities in these two cases were 11.0 and 33.8 $\mu$g/ml.   BUN and serum creatinine levels in the latter case were 30mg/dl and 2.4mg/dl, respectively; high serum SOD activity in this case was associated with the deterioration of renal function.

4)  Congenital nephrotic syndrome (Cong NS)

Serum SOD activities in these two cases were 10.5 and 16.9 $\mu$g/ml, both figures being within the normal limits.

5)  Poststreptococcal acute glomerulonephritis (PSAGN)

Mean serum SOD activities at the acute and healing phases were 8.0±5.7 $\mu$g/ml and 12.6±5.6$\mu$g/ml, respectively.   No significant difference was evidenced between these two phases (0.1<P).   However, in comparison with the controls, the acute phase value was low (P<00.5) while serum SOD activity at the healing phase showed no significant difference from the controls (0.1<P).

Serum SOD activity was usually low at the acute phase, and gradually normalized. It is suggested that the activity of PSAGN is associated with serum SOD activity change (Fig. 3).

6)  Membranoproliferative glomerulonephritis (MPGN)

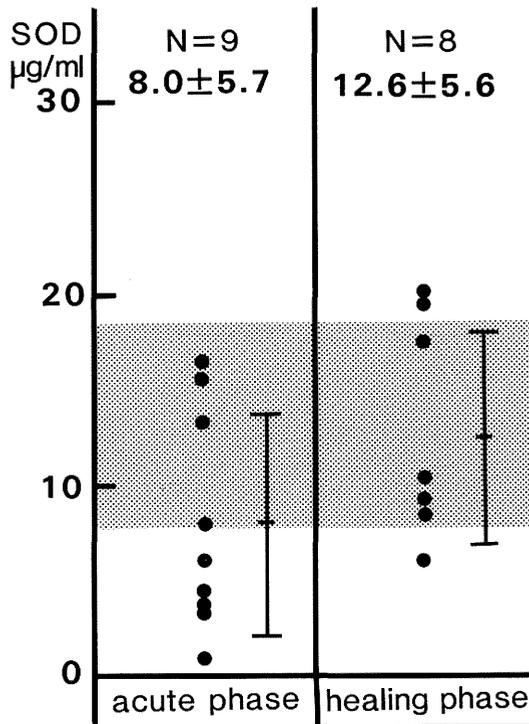Mean serum SOD activity in this group was low, 6.7±4.9 $\mu$g/ml, in comparison with



Fig. 3.   Serum SOD activity in PSAGN

the controls (P<0.01). In this group, low SOD activity was evidenced which was not related to either proteinuria or renal function.

Serum SOD activity was compared between histologically diagnosed MPGN and hypocomplementemic glomerulonephritis, which is clinically similar to MPGN. Differential diagnosis from MPGN is often quite difficult in these diseases because of the low complement value. All 6 cases of hypocomplementemic glomerulonephritis were diagnosed by renal biopsy as being mild or moderate proliferative glomerulonephritis without having the features of MPGN. Experiment showed serum SOD activity of the hypocomplementemic glomerulonephritis cases to be within the normal limits, while many MPGN cases showed low figures (Fig. 4).

7) Purpura nephritis (HSPN)

Mean serum SOD activity in the HSPN group was $12.1 \pm 9.1 \mu g/ml$. This activity was investigated to find differences between clinical types (renal insufficiency type: no cases). Mean serum SOD activities in nephritic and nephrotic types were $11.6 \pm 9.4 \mu g/ml$ and $14.1 \mu g/ml$ (6.4, 12.6, 23.3), respectively (Fig. 5).

8) IgA nephropathy (IgA neph)

Mean serum SOD activity was $15.4 \pm 8.4 \mu g/ml$. No specific tendency was evidenced, as these cases showed both low and high figures.

9) Other chronic glomerulonephritis (other CGN)

Mean serum SOD activity in other CGN, not histologically diagnosed and categorized commonly as CGN (abnormal urinalysis over one year)[6], was $12.4 \pm 7.2 \mu g/ml$.
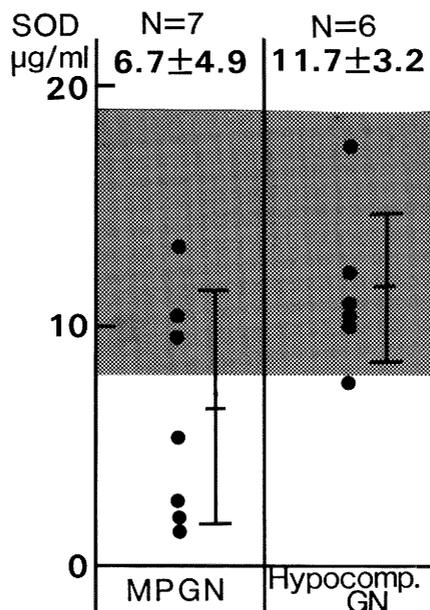


Fig. 4. Serum SOD activity in MPGN and hypocomplementemic GN

*H. HIRANO*


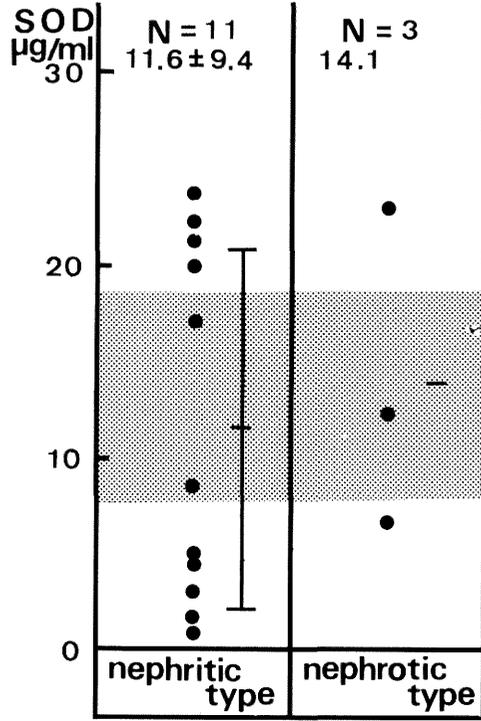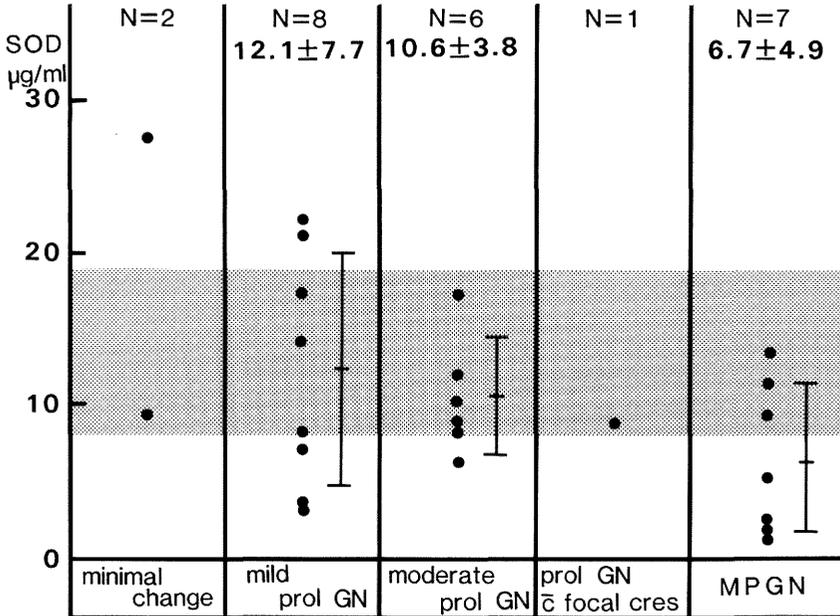
Fig. 5.  Serum SOD activity in HSPN



Fig. 6.  Serum SOD activity in chronic glomerulonephritis

**Table 2**  Classification of renal glomerular lesions

| | |
|---|---|
| 1 | normal or minimal change GN |
| 2–A-a | mild proliferative GN |
| 2–A-b | moderate proliferative GN |
| 2–A-c | severe proliferative GN |
| 2B | proliferative GN with focal crescents |
| 2C | proliferative GN with generalized crescents |
| 3 | membranous nephropathy |
| 4 | membranoproliferative GN (MPGN) |
| 5 | focal GN |
| 6 | too advanced to be classified |
| 7 | unclassified |

GN : glomrulonephritis

10)  Relation between serum SOD activity and kidney histological findings (Fig. 6)

As for the 24 histologically diagnosed cases of CGN, a comparative study of serum SOD activity was undertaken in which cases were classified in accordance with the classification of their renal glomerular lesions (Table 2): minimal change: 2 cases; mild proliferative glomerulonephritis (mild prol GN): 8 cases; moderate proliferative glomerulonephritis (moderate prol GN): 6 cases; proliferative glomerulonephritis with focal crescents (prol GN c̄ focal cres): 1 case; MPGN: 7 cases.  Mean serum SOD activity in the mild prol GN was $12.1 \pm 7.7$ $\mu$g/ml; that in moderate prol GN was $10.6 \pm 3.8$ $\mu$g/ml······ showing no significant difference from the controls ($0.1 < P$, $0.1 < P$); however, serum SOD activity in moderate prol GN showed a tendency to be lower than that in mild prol GN. As for the minimal change cases and prol GN c̄ focal cres, comparison was rather difficult, since the cases were of insufficient number.  As a whole, however, serum SOD activity showed a gradual lowering tendency, in the following order: minimal change→ mild prol GN→moderate prol GN→prol GN c̄ focal cres→MPGN.

### DISCUSSION

The onset and progress of renal disease in many cases are closely associated with the immunological mechanism.  Various antigens (Ag) generate immune-complex formation (in-situ immune-complex formation)[7)8)] in the glomeruli, in some cases generating circulating immune-complex trapping.[9)10)]  These immune-complexes lead to glomerular injury, directly or indirectly, through complement activation, neutrophils and macrophages (monocytes).  Also, Ag causes direct glomerular injury through T-cells or macrophages.  On the other hand, in case of neutrophils[11)12)] and macrophages,[13)14)] glomerular injury is caused by release of protease and/or oxygen-free radicals.  It is reported that mesangial cells not only possess a phagocytic function, such as the removal of Ag, but also produce inflammatory mediators such as oxygen-free radicals;[15)16)] it is therefore suggested that glomerular injury can be induced by mesangial cells.

Superoxide, while functioning in an organism as a beneficial radical to remove foreign objects, disinfect, destroy cancer cells and metabolize drugs, also has harmful effects such as lipid peroxide generation, cell membrane destruction, enzyme inactivation, etc. Specifically, as for inflammation, it directly attacks tissues, generates platelet agglutination or releases the lysosomal enzyme of leukocytes. It also affects cell membranes, which leads to the production of prostaglandin and lipid peroxide, with resultant acceleration of vascular permeability and chemotaxis of leukocytes. Thus, superoxide is a potent activator of inflammation. However, there have been no reports clearly mentioning the relationship between SOD and glomerulonephritis.

The author noted the roles of superoxide and its scavenger, SOD, in association with renal disease. As an initial step, the author launched a study on SOD serum levels.

The results of this study revealed high serum activity in patients suffering from deterioration of the renal function and a tendency toward low serum activity in patients with INS at the onset and relapse stages. Such results must be considered related to the fact that the SOD molecule weight, being from 30,000 to 40,000,[4] is likely to be affected by glomerular filtration. Shimosaka and Sawaki have reported that serum SOD activity increased in patients with renal insufficiecy. In case of patients with deteriorated renal function, although SOD excretion from the glomeruli decreases because of the lower glomerular filtration rate, serum SOD activity increases. When considerable proteinuria is observed, such as at the onset and relapse stages of INS, the SOD excretion level increases along with the proteinuria; this explains the lowering tendency of serum SOD activity. There was only one case available for the study, as children rarely suffer from deterioration or insufficiency of renal function. this single case revealed high serum SOD activity, a result compatible with the report by Shimosaka and Sawaki.[17][18]

In the cases of MPGN serum SOD activity was low regardless of the degree of proteinuria, and in the cases of PSAGN at the acute or active phase, serum SOD activity was low in comparison with the controls. In the cases of CGN, the more serious the histological diagnosis was, the lower the tendency of serum SOD activity showed. This result cannot be explained by SOD excretion from the glomeruli. It is considered that low serum SOD activity is closely associated with the active phase of renal disease at its acute and critical stages. Whether low serum SOD activity in patients with renal disease at the clinically and histologically active stage is associated with the increase of SOD digestion in the kidney or whether it has an adverse effect at the healing stage of renal disease owing to low production of SOD is yet to be studied. However, as for glomerulonephritis, it is clear that oxygen-free radicals such as superoxide can be vital activators in glomerular injury and that SOD, as a scavenger of superoxide, can be a healing factor in glomerular injury. Reports noting the improvement of proteinuria and kidney histological findings evidenced in experimental renal disease induced by the administration of SOD and catalase support the author's theory.[11][19][20] The major role played by oxygen-free radicals such as superoxide at the onset and during the progressive

stage of glomerulonephritis seems to be closely associated with serum SOD activity change.  Furthermore, it is suggested that SOD has a part in the restraint and healing of glomerulonephritis.

In HSPN, however, no clear results were obtained, these cases showing both low and high figures.  It has been reported, however, that specifiic clinical types of HSPN, such as the nephrotic type, tend toward poor prognosis.[21)22)]  Therefore, the author compared serum SOD activity between the nephritic and nephrotic types, but no clear tendency was evidenced.

IgA nephropathy comprises the greater part of chronic glomerulonephritis in children and has various clinical and histological features.  Serum SOD activity in IgA nephropathy shows no clear tendency.  The reason for this seems to hinge on the question of whether superoxide has little connection with the onset and progress of HSPN and IgA nephropathy or whether these diseases are not pathologically homogeneous diseases. Judging from the results of the present experiment and other reports of the benefical effects achieved by administering SOD in experimental glomerulonephritis, serum SOD activity can be useful as an index of glomerulonephritis and can be used effectively in treating the disease.

In conclusion, the author has shown that serum SOD activity in children with renal diseases at the clinically and histologically active stage is low.  SOD seems to have a part in the prevention and healing of renal disease, as indicated above.  There have been no studies dealing with the relation between SOD and renal disease.  This study suggests that superoxide can be a vital activator in glomerular injury and SOD can be a healing factor in renal disease.

### REFERENCES

1) Petron, W. F., English D. K., Wrong K. and McCord J. M.:  Free radicals and inflammation.  *Proc. Natl. Acad. Sci.* 77: 1159-1163, 1980.
2) Fantone, J. C., Ward, P. A.:  Role of oxygen derived free radicals and metabolites in leukocytes dependent inflammatory reaction.  *Am J Pathol* 107: 397-418, 1982.
3) McCord, J. M.:  Free radical and inflammation: Protection of synovial fluid by superoxide dismutase.  *Science,* 185: 529-531, 1974.
4) McCord, J. M. and Fridovich, I.:  Superoxide dimutase.  *J. Biol. Chem.,* 244: 6049-6055, 1969.
5) Hirano, H.:  A preliminary study on serum superoxide dismutase activity.  *Niigata Med. J.,* 100: 284-292, 1986.
6) West, C. D., et al.:  The chronic glomerulonephritis of childhood.  *J. Pediatr.* 93: 1-12, 1978.
7) Couser, W. G., Salant, D. J.:  In situ immune complex formation and glomerular injury.  *Kidney Int.* 17: 1-13, 1980.
8) Vogt, A., et al.:  Interaction of cationized antigen with rat glomerular basement membrane: In situ immune complex formation.  *Kidney Int.,* 22: 27-35, 1982.

9) Gallo, G. R., et al.: Charge of circulating immune complexes as a factor in glomerular basement membrane localization in mice. *J. Clin. Invest.,* **67**: 1305-1313, 1981.

10) Ooi, Y. M., et al.: Serum immune complexes in membranoproliferative and other glomerulonephritis. *Kidney Int.,* **11**: 275-283, 1977.

11) Rehan, A., et al.: Role of oxygen radicals in phorbol myristate acetate-induced glomerular injury. *Kidney Int.,* **27**: 503-511, 1985.

12) Davies, M., et al.: The degradation of human glomerular basement membrane with purified lysosomal proteinases. Evidence for the pathogenic role of the polymorphonuclear leucocyte in glomerulonephritis. *Clin. Sci. Mol. Med.,* **54**: 233-240, 1978.

13) Franco, F., et al.: The detection monocytes in human glomerulonephritis. *Kidney Int.,* **28**: 513 -519, 1985.

14) Holdworth, S. R., et al.: Abrogation of macrophage-dependent injury in experimental glomerulonephritis in the rabbit. Use of anti-macrophage serum. *J. Clin. Invest.,* **68**: 686-696, 1981.

15) Baul, L., et al.: Reactive oxygen production by cultured rat glomerular mesangial cells during phagocytosis is associated with stimulation of lipoxygenase activity. *J. Exp. Med.,* **158**: 1836-1852, 1983.

16) Sedor, J. R., Abboud, H. E.: Platelet activating factor stimulates oxygen radical release by cultured mesangial cells. *Kidney Int.,* **27**: 222, 1985.

17) Simosaka, H., et al.: Biological and immunological activities of superoxide dismutase in human serum with uremic patients. *Medicine and Biology.* **106**: 181-184, 1983.

18) Sawaki, S., et.: Change in urinary and serum Cu Zn-superoxide dismutase levels in renal failure. *J. Aichi Med. Assoc.,* **10**: 16-20, 1982.

19) Stokes, S. H., McCord, J. M.: Prevention of immune complex induced glomerulonephritis by superoxide dismutase. *Ala J Ked Sci.* **16**: 33, 1979.

20) Rehan A., et al.: Evedence for the role of oxygen radical in acute nephrotoxic nephritis. *Laboratory Investigation.,* **51**: 396-403, 1984.

21) Counahan, R., et al.: Prognosis of Henoch-Schonlein nephritis in children. *Brit. Med. J.,* **2**: 11-16, 1977.

22) Meadow, S. R.: The prognosis of Henoch-Scholein nephritis. *Clin. Nephrol.,* **9**: 87-93, 1978.