



Comparative Study of Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase in Urolithiasis Patients: A Case Control Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Urolithiasis i.e. stone forming disease in the urinary passages is one of the frequently encountered diseases in man. Perhaps the disease is as ancient as the man himself as has been revealed by the archaeological excavation done in different parts of the world such as in Egypt.

Aim: Comparative Study of Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase in Urolithiasis Patients: A Case Control Study

Materials and Methods: The study was carried out in the Department of Biochemistry, Datta Meghe Medical College Nagpur in collaboration with Department of Surgery, Division of Urology and Department of Pharmacology.

Results: The urolithiasis patients have shown a marked increase in plasma MDA levels. There was a significant increase in the values of superoxide dismutase in patients suffering from urolithiasis ($6.26 \pm 0.86 \mu\text{mol/l}$ RBC lysate) as compared to the normal control values ($3.40 \pm 1.09 \mu\text{mol/l}$ RBC

lysate) in human volunteers ($p < 0.01$). A significantly decreased value of glutathione peroxidase has been observed in patients suffering from urolithiasis as compared to the control group.

Conclusion: Enhanced SOD can reduce the formation of Calcium Oxalate crystals and reduce the damage of renal tubular epithelial cell.

Keywords: Urolithiasis; SOD; MDA; Glutathione Peroxidase and Calcium Oxalate.

1. INTRODUCTION

Urolithiasis or stone disease is perhaps as old as the history of the mankind. The archaeological survey has revealed this truth very well that ancient man was afflicted with stone disease [1]. One of the earliest examples of urinary calculus was discovered by Sir Grafton-Elliott Smith in 1901 among the pelvic bones of a young men of pre-dynastic (7000-3100 BC) era in upper Egypt. There are number of such examples from the excavated skeletons, thousands of mummies and similar archaeological findings. The incidence of urinary calculi has increase during this century [1]. It is third most common disease in northern Italy [2].

The incidence of stone is rising due to many reasons e.g. change in the life style which includes change in the diet [3], use of a large number of chemicals in the form of drugs and other preservatives, pollution and similar other factors.

2. EPIDEMIOLOGY OF UROLITHIASIS

Stone disease has been described to occur in the literature in different times and the data is based on the archaeological excavations. However, the incidence of the stone disease has increased with the improvement of the socioeconomic status in different communities all over the world. This point has been substantiated by a fact at during the world war-II when there was more of starvation and short supply of food material, not only the incidence of the cardiovascular diseases like MI decreased but there was also a definite decrease in the incidence of the urinary calculi [4].

3. SITES OF STONE FORM TION IN URINARY TRACT [5-8]

1. Urinary bladder where urine is collected.
2. The ureters which are the passages through which the urine flows from kidney to urinary bladder.
3. Pelvic-ureteric junction where the ureter meets with the pelvis of the kidney from

where the stones once formed are difficult to dislodge.

4. Pelvis of the kidney from where again the stones are difficult to pass down.
5. In the cortical or medullary portions of the kidney. Since it is within the substance of the kidney, this is known as nephrolithiasis.
6. Sometimes stone may lodge in the terminal lower most part of urinary tract i.e. urethra.

Increased oxidative stress on one hand and the reduced anti-oxidants on the other hand bring about changes in the composition of lipoproteins and increased oxidation of low density lipoproteins along with accelerated development of atherosclerosis in cases of renal failure [9]. Many of the cells present in the arterial wall such as endothelial cells, vascular smooth muscle cells and macrophages can oxidise LDL which can also be initiated by lipooxygenases [10-11]. Thus cardiovascular disease is the main cause of death in a case of renal failure. Oxidised LDL is chemotactic for circulating monocytes, cytotoxic to the cells, produces number of cytokines and other growth factor and enhances platelet aggregation and interferes with EDRF [12]. Oxidised LDL is also immunogenic and is capable of forming autoantibodies and can destroy the cells. Such oxidative species have been described in chronic renal failure which are of low molecular weight (less than 3000 Daltons), and are removable with dialysis.

AIM: Comparative Study of Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase in Urolithiasis Patients: A Case Control Study.

4. MATERIALS AND METHODS

The study was carried out in the Department of Biochemistry, Datta Meghe Medical College Nagpur in collaboration with Department of Surgery, Division of Urology and Department of Pharmacology.

The present study was conducted on 50 patients of urolithiasis admitted in the indoor surgical

urology ward as well as on the patients attending the outpatient department of as well as on 50 normal human volunteers, after obtaining their informed consent. The total number of 100 human subjects included belonged to either sex and of the age ranging from 1 year to 72 years.

Control group: Control group consisted of normal healthy volunteers of either sex chosen from amongst the staff members and their family members residing at Nagpur.

Study Group: Study group consisting of patients suffering from urolithiasis.

Diagnosis: The patients were diagnosed on the basis of history, clinical presentation with signs and symptoms and differential diagnosis and investigations like X-ray/ultrasound/IVP or urine examination [12].

Sample Collection: 5 ml of blood from e control group as well as from patients was drawn from the antecubital vein and was collected in sterile vials for the estimation of MDA, SOD and GPx. These samples were kept in ice (4 °C) till processing.

4.1 Biochemical Investigation

MDA was estimated by the method of Buege and Aust, 1978 [13]

SOD was estimated by the method of Misra and Fridovich, 1972 [14]

GPx was estimated by the method of Flohe L and Gunzler WA, 1985 [15]

4.2 Statistical Analysis

For analysis of the data the mean values were calculated in each of the groups along with the standard error/deviations for the different parameters.

The students 't' tests was employed for finding out the statistical significance (p-value) of the results between different groups.

5. RESULTS

The urolithiasis patients have shown a marked increase in plasma MDA levels. The different values of the malondialdehyde levels in plasma along with their SD values have been given in Table 1. Malondialdehyde plasma levels have been found to be significantly raised ($p < 0.0001$)

in patients suffering from urolithiasis (11.73 ± 1.82 nmoles/ml of plasma) as compared to the normal control group of volunteers (9.19 ± 1.27 nmoles/ml of plasma). Table 1 also shows that there was a significant increase in the values of superoxide dismutase in patients suffering from urolithiasis (6.26 ± 0.86 $\mu\text{mol/l}$ RBC lysate) as compared to the normal control values (3.40 ± 1.09 $\mu\text{mol/l}$ RBC lysate) in human volunteers ($p < 0.01$). A significantly decreased value of glutathione peroxidase has been observed in patients suffering from urolithiasis (3.9 ± 0.91 $\mu\text{mol/ml}$ of haemolysate) as compared to the control group of normal human volunteers (6.0 ± 0.80 $\mu\text{mol/ml}$ of haemolysate) as has been depicted in Table 1.

6. DISCUSSION

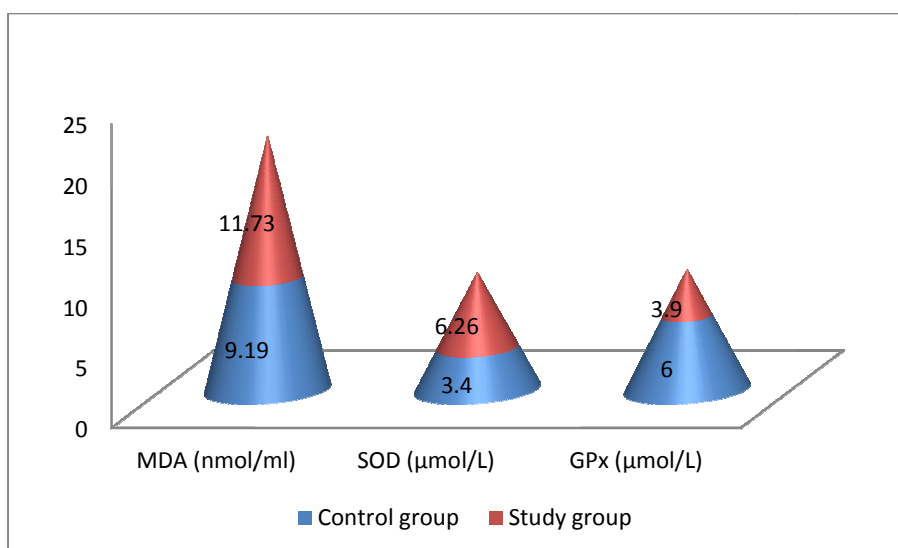
There are reports that renal tissue cells are much more susceptible to the damaging effects of free radicals as compared to hepatocytes and other cells [16,17]. These observations are based on the estimations of 8-OHdG (ROS induced damaged DNA product [16,18] in vivo as well as in-vitro studies using renal tubular epithelial cell culture line (OK CRL-1840 cells) [19]. In our present study we have observed a rise in levels of free radicals as seen by the increased values of oxidative toxic metabolite viz. MDA and decrease in the protective anti-oxidant enzyme like Glutathione Peroxidase, the first line of defence [20]. Therefore, it is logical to think that in urolithiasis free radicals are generated which are likely to damage the endothelial cell lining of the tubules forming a "nidus" for the settlement of the amorphous or crystalline form of insoluble calcium oxalate salt or other such substances and for their subsequent growth.

It is evident from the Table 1, Graph 1, that there is a significant rise in the levels of malondialdehyde in cases of urolithiasis as compared to the normal human volunteers.

The increase in the levels of MDA is due to the formation of more OH^\bullet free radicals which causes the damage of lipid biomembrane mainly polyunsaturated fatty acids (PUFA) forming lipid hydroperoxide further leading to formation of MDA. Decrease in the levels of MDA shows that there is decreased generation of OH^\bullet free radicals and further less damage to biomembrane forming less hydroperoxides, toxic metabolites. This OH^\bullet free radicals are generated from the superoxide anion, O_2^\bullet free radicals.

Table 1. Comparison of MDA, SOD and GPx levels in control and study group

Parameters	Control group	Study group	P-Value
MDA (nmol/ml)	9.19±1.27	11.73±1.82	P < 0.0001
SOD (µmol/L)	3.40±1.09	6.26±0.86	P < 0.0001
GPx (µmol/L)	6.0±0.80	3.9±0.91	P < 0.0001



Graph 1. Comparison of MDA, SOD and GPx Levels in control and study group

Kato J et al. [21] conducted a study in 100 subjects and found raised MDA level in urolithiasis patients. All untreated patients of urolithiasis operated or un-operated (Table 1. Graph 1) showed an increase in the values of superoxide dismutase (6.26 ± 0.86 $\mu\text{mol/L}$ RBC lysate) as compared to their respective control values. This indicates that there has been a need based rise in the values of superoxide dismutase to deal with the increase of free radicals produced as a result of operative surgical trauma or due to the presence of calcium oxalate crystals in the urinary passages producing mechanical or chemical injury in patients suffering from urolithiasis.

SOD is a key enzyme in the elimination of superoxide radicals. It is used to scavenge ROS and speed up the mutation of superoxide anion radicals, shielding cells from toxic by-products of aerobic metabolites and serving as the first line of protection toward ROS. Previous research increased SOD activity to quickly eliminate excess ROS for normal cell function and host survival in order to strengthen and cope with ROS disruption and preserve redox homeostasis [22]. Kang J et al. 2020 [23]. Compared with the control group, the stone model group showed a

significantly higher renal / body mass index with a higher expression of autophagy-ERS- and apoptosis-related proteins LC3B, BECN1, GRP78, CHOP, Bax and Caspase-3; and low levels of p62, bcl-2 protein, and SOD.

Glutathione peroxidase values have been found to be decreased in all the patients of urolithiasis (Table 1) as compared to the normal human subjects. This reflects that there has been a better utilisation or decreased need of the enzyme in the diseased condition.

Mahmoud RH et al. [24] found in patients with urolithiasis, the mean serum MDA level increased as compared to the control group, and there was a substantial gap between serum MDA in the control group [25-29].

7. CONCLUSION

It appears that the role of lipid peroxidation exists in the pathogenesis of urolithiasis as observed from the positive correlation between the MDA and SOD and negative correlation between MDA and GPx. Enhanced SOD can reduce the formation of Calcium Oxalate crystals and reduce the damage of renal tubular epithelial cell.

ETHICAL CLEARANCE

Taken from institutional ethics committee

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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