Antioxidant status in polycystic end-staged renal diseased patients and antihemolytic effect of *Boerhaavia diffusa*

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Chronic renal failure (CRF) induces anaemia by shortening the lifespan of erythrocytes, due to an increase in oxidative stress, which is considered to be one of the major risk factors in CRF patients undergoing hemodialysis. In the present study, the antioxidant status of the end-staged renal disease (ESRD) patients was investigated. The antihemolytic activity of *Boerhaavia diffusa* on the erythrocytes of the patients was also studied. Protein, lipid peroxides (LPO), reduced glutathione (GSH) levels and glutathione peroxidase (GPX) and glutathione-S-transferase (GST) activities were measured in the hemolysate from 55 polycystic ESRD patients (Group II) and compared with normal subjects (Group I). The antioxidant status was found to be significantly reduced in the patients as compared to normal healthy volunteers, due to increased oxidative stress. Also, aqueous extract of *B. diffusa* showed a significant antihemolytic activity on the erythrocytes of the polycystic ESRD patients.

**Keywords**: Hemodialysis, Erythrocytes, *Boerhaavia diffusa*, End-staged renal disease, Antioxidant status, Antihemolytic activity

Chronic renal failure (CRF) requiring dialysis or transplantation is known as end-staged renal disease (ESRD)\(^1\). Patients in renal failure are prone to all the complications of any underlying condition, such as diabetes and hypertension. In addition, renal failure causes a variety of metabolic and physiologic derangements. Anemia is inevitable in CRF because of loss of erythropoietin production. Hemodialysis can worsen the anemia, due to the procedure associated blood loss and mild effect on oxygen transporting function. Hypersplenism may rarely be associated with dialysis, leading to a sequestration of erythrocytes and further destruction of circulating red cells\(^2\). Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the appearance and slow growth of fluid-filled cysts within the parenchyma of the kidney\(^3\). In the autosomal dominant form of disease, the genetic mutations do not appear to interfere with the normal development of the kidney.

*Boerhaavia diffusa*, an herbaceous member of the Nyctaginaceae family has been widely used for the treatment of dyspepsia, jaundice, abdominal pain etc\(^4\) and is very effective in nephrotic syndrome and other kidney ailments\(^5\). In our previous study, we have found that the overall antioxidant status of the ESRD patients is significantly reduced as compared to the normal group\(^6\). As polycystic kidney disease is chiefly genetic in origin, and alterations in antioxidant status is expected, hence in the present study, the antioxidant status of the erythrocytes isolated from the blood of polycystic ESRD patients has been analyzed. The antihemolytic activity of *B. diffusa* on the erythrocytes of the patients has also been investigated.

**Materials and Methods**

The blood samples were collected from 55 polycystic end-staged renal diseased (ESRD) patients in pre-dialysis condition from the Department of Nephrology, Stanley Medical College Hospital, Chennai. The healthy volunteers served as control (Group I). The subjects were male ESRD patients in the age group of 20-50 suffering from polycystic kidney disease (PKD) with creatinine level greater than 5 mg/dl and served as (Group II). The patients with diabetes, fever, infection, liver disorders, smoking and alcoholism were excluded for the study.

**Preparation of hemolysate**

The blood sample obtained was treated as described earlier\(^7,8\). Plasma and red cells were separated from blood collected from the chronic renal failure patients with EDTA as an anticoagulant by centrifugation at 1,500 \(\times\) g for 15 min. The sedimented packed cells were washed thrice with isotonic saline and sedimented to remove the buffy coat.

**Estimation of protein**

Protein content in the erythrocytes was determined by the method of Lowry et al\(^9\). Briefly, to 0.1 ml of
the hemolysate, 3.0 ml of distilled water was added. 0.1-0.5 ml of standard bovine serum albumin solution was also made up to the same volume and 3.0 ml of water was taken as blank. Thereafter, 4.5 ml of alkaline copper sulphate solution was added, followed by 0.5 ml of Folin’s phenol reagent and incubated for 10 min. The colour developed was read at 640 nm in a UV visible spectrophotometer. Protein content was expressed in mg/dl.

**Estimation of lipid peroxide**

The lipid peroxides in the hemolysate were estimated by as described previously\(^\text{10}\). Briefly, to 0.2 ml of the hemolysate, 4.0 ml of 0.86 N H\(_2\)SO\(_4\) was added and mixed gently with 0.5 ml of phosphotungstic acid (PTA) for 10 min. It was centrifuged for 10 min and to the pellet added 2.0 ml of N/12 H\(_2\)SO\(_4\) and 0.3 ml of 10% PTA. The mixture was suspended in 4.0 ml of water and 1.0 ml of thiobarbituric acid (TBA). The tubes were kept in boiling water bath for 1 h. After cooling 5.0 ml of n-butanol was added and the colour present in butanol phase was read at 532 nm in a UV spectrophotometer. The amount of peroxide was expressed in nmoles of TBA formed/mg protein.

**Estimation of reduced glutathione (GSH)**

The level of GSH in the hemolysate was estimated as described previously\(^\text{11}\). Briefly, to 0.5 ml of hemolysate 1.0 ml of trichloroacetic acid (TCA) was added and centrifuged for 10 min at 1500 rpm. About 2.0 ml of dithionitrobenzoic acid (DTNB, 0.6 mM, pH 8.0) was added to the 0.5 ml of the supernatant and total volume made up to 3.0 ml with 0.2 M phosphate buffer. The colour developed was read at 412 nm in a UV spectrophotometer. The activity was expressed in nmoles of GSH oxidized/min/mgHb.

**Estimation of antioxidant enzymes**

The activity of glutathione peroxidase (GPx) was estimated using the method described earlier\(^\text{12}\). Briefly, to 0.2 ml of hemolysate, added 0.2 ml of EDTA, 0.1 ml of 10 mM sodium azide and 0.2 ml of 0.4 M phosphate buffer was added and mixed well. To the mixture, added 0.2 M of glutathione and 0.1 ml of 1 mM hydrogen peroxide and incubated at 37°C for 10 min. The reaction was arrested by adding 0.5 ml of 10% TCA. The contents were centrifuged and supernatants were assayed for glutathione content by DTNB. The blank was treated similarly to which 0.2 ml of the enzyme was added after incubation. The colour developed was read at 412 nm and the activity expressed in nmoles of GSH oxidized/min/mgHb.

The activity of GST was estimated as described earlier\(^\text{13}\). The following solutions were taken in a quarts cuvette in the given proportion: 1.0 ml of 0.3 M phosphate buffer, 0.1 ml of 1-chloro, 2, 4-dintrobenzene (CDNB) and 0.1 ml of hemolysate. The volume was adjusted to 2.9 ml with distilled water. The mixture was incubated at 37°C for 5 min. Then 0.1 ml of glutathione was added and change in O.D. was measured at 340 nm in a UV spectrophotometer at 15 s interval for 3 min. The enzyme activity was expressed in nmoles of CDNB conjugated/min/mgHb.

**Preparation of the plant extract**

*Boerhaavia diffusa* was obtained from Anna Hospital and Siddha Research Centre, Chennai, India. The authenticity of the plant was certified by the Department of Botany, Bharathi Women’s College, Chennai. Aqueous extract of the plant was prepared by grinding 1 g of shade-dried whole plant with mortar and pestle. The plant material was soaked in 5 ml of distilled water, kept overnight, then homogenized and filtered, and 0.5 ml of the filtered aqueous extract was used for the study. The dosage was fixed by repeated trial and error method.

**Osmotic fragility test (OFT)**

OFT was performed to determine the percentage of hemolysis by the method described elsewhere\(^\text{14}\). About 5 ml of the whole blood was centrifuged at 2000 rpm for 5 min and the supernatant was discarded and washed thrice with 1% buffered saline. About 0.5 ml of the cells was incubated with 0.5 ml of the plant extract for 15 min and the percentage of hemolysis was calculated.

**Statistical analysis**

Data were presented as mean ± standard deviation. Statistical analysis was performed using SPSS software. The statistical significance was evaluated by One-way-ANOVA.

**Results and Discussion**

CRF is usually the end stage of variety of renal diseases and accompanied by complex pathology. The occurrence of CRF in the study group patients was confirmed by an increased serum urea, creatinine and uric acid levels. Table 1 shows the level of protein, lipid peroxides, reduced glutathione and the activity of GPx and GST in groups I and II. The level of
protein in the polycystic ESRD patients was increased when compared to the control (p<0.01), suggestive of a highly impaired condition. The mechanisms and machinery for the export of proteins to the erythrocyte membrane are largely unknown. In eukaryotic cells, cholesterol-rich membrane microdomains or “rafts” have been shown to play an important role in the export of proteins to the cell surface.\(^\text{15}\)

Lipid peroxidation is reported to cell injury by altering the basic physical properties and structural organization of membrane components. Oxidative modification of polyunsaturated phospholipids has been shown, in particular to alter the intermolecular packing, thermodynamic, and phase parameters of the membrane bilayer.\(^\text{16}\) The levels of lipid peroxides were increased (p<0.01), indicating a rise in the level of peroxidation in the membrane structure of the erythrocytes.

The extent to which a cell withstands the potentially damaging effects of oxidative stress is determined by the balance between the rate at which oxidant species are generated and the capacity of metabolic processes to produce antioxidants. The antioxidant defense systems include a complex of interrelated functions, each of which tends to buffer the effects of the others. The glutathione (GSH) system is a major component of overall antioxidant defenses.\(^\text{17}\) In the present investigation, the decreased level of the GSH in polycystic ESRD patients (p<0.01) was related to the increase in RBC susceptibility to oxidative stress.\(^\text{18}\)

The activity of enzymatic antioxidant defense system is reduced in the RBC of CRF patients. The activities of GSH-Px and GSSG-Rd are severely impaired in dialysis patientsboth in erythrocytes and in plasma.\(^\text{19}\) GSTs detoxify commonly encountered products generated by oxidative damage, and reduced GST activity has been reported in several diseased states. In the present study, the activities of the antioxidant enzymes GPx and GST in the hemolysate were significantly reduced (p<0.05), suggesting increased free radical production and antioxidant depletion and was in agreement with earlier report.\(^\text{20}\)

**Table 1**—Level of protein, lipid peroxide, reduced glutathione and the activity of GPx and GST in the hemolysate of group I and II

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Protein (mg/dl)</th>
<th>Lipid peroxide (nmoles of TBA formed/mg protein)</th>
<th>Reduced glutathione (µg/mg Hb)</th>
<th>GPx (nmoles of glutathione oxidized/min/mg Hb)</th>
<th>GST (nmoles of CDNB conjugated/min/mg Hb)</th>
</tr>
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<tbody>
<tr>
<td>Group I</td>
<td>8.87 ± 0.08</td>
<td>4.88 ± 0.007</td>
<td>12.50 ± 0.13</td>
<td>5.0 ± 0.12</td>
<td>4.26 ± 0.01</td>
</tr>
<tr>
<td>Group II</td>
<td>14.21 ± 0.07**</td>
<td>13.23 ± 0.02**</td>
<td>5.11 ± 0.057**</td>
<td>1.96 ± 0.01*</td>
<td>0.35 ± 0.08*</td>
</tr>
</tbody>
</table>

The statistical significance provided by comparing group II with group I. *p< 0.05 * *p<0.01

Figure 1 represents the percentage of hemolysis in the control and patient groups with and without the aqueous extract of *Boerhaavia diffusa*.

In conclusion, the preliminary biochemical study in the hemolysate of polycystic ESRD patients indicated the altered protein, increased level of lipid peroxide with lowered glutathione levels and reduced activities of GPx and GST, suggesting depletion in the antioxidant status and increase in free radical production. Also, the aqueous extract of *B. diffusa* showed significant antihemolytic activity.

**References**

10 Okhawa H, Ohishi N & Yagi M (1979) Anal Biochem 95, 351-358