Amrita Bindu therapy in diabetic retinopathy: Effect on antioxidant defenses and the disease process

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Summary
Amrita Bindu is a salt-spice herbal mixture, evolved according to the Indian system of Medicine for the combating of degenerative and inflammatory changes during ageing and exposure to environmental stress. Amrita Bindu was administered as a diet supplement to 25 cases of non-insulin-dependent diabetes mellitus (NIDDM) with mild or borderline retinopathy for a period of six months. An equal number of cases were followed up on conventional therapy alone. Age and sex matched non-diabetics provided the controls.

Plasma, RBC and membrane lipid peroxidation and antioxidant levels are measured before and after Amrita Bindu administration. The patients with diabetic retinopathy were subjected to fundoscopic examination to assess the retinal lesions before and after the follow up period.

17 out of the 25 cases of Amrita Bindu therapy and 18 out of 25 in the conventional therapy groups were available for the study at the end of 6 months. 8 out of the 25 cases on Amrita Bindu therapy showed regression (disappearance of microaneurysms) while none in the other group showed regression.

Amrita Bindu therapy led to a significant reduction in lipid peroxidation in plasma, RBCs and cell membrane and to an increase of the antioxidant levels in blood, confirming that this Indian medicinal formula is able to protect cells from antioxidant damage and the resultant tissue injury.

Keywords: Amrita Bindu, non-insulin-dependent diabetes mellitus (NIDDM), diabetic retinopathy, lipid peroxidation

Zusammenfassung


17 der 25 Patienten der Verumgruppe und 18 der 25 Patienten der Kontrollgruppe konnten zur Auswertung herangezogen werden. 8 Verum-Patienten zeigten eine Regression (Verschwinden der Mikroanorysmen), während dies bei keinem der Patienten der Kontrollgruppe der Fall war.

AB führte ferner zu einer signifikanten Reduktion der Lipidperoxidation im Plasma und in Erythrozyten und zur Erhöhung der Antioxidantien im Blut. Die Daten bestätigen, daß dieses indische Mittel Zellen und Gewebe vor oxidativen Schäden bewahrt.

Schlüsselwörter: Amrita Bindu, Typ-II-Diabetes, diabetische Retinopathie, Lipidperoxidation

Diabetic patients have a reduced capacity to compensate the oxidative stress due to decreased antioxidant status. Antioxidant deficiency and lipid peroxidation are important risk factors in the development of retinopathy (19).

Increased oxidative stress may result from overproduction of precursors for reactive oxygen species and/or decreased efficiency of inhibitory and scavenging systems. The stress may then be amplified and propagated by an autocatalytic cycle of metabolic stress, tissue damage and cell death, leading to further increase in free radical production and depletion of antioxidants (2). Initiation of lipid peroxidation (LPO) is a process solely carried out by free radicals.

The primary enzymatic defence mechanism against the damage caused by oxygen free radicals are superoxide dismutase (SOD), glutathione peroxidase (GP) and catalase (CAT) (24), while the non-enzymatic antioxidants are vitamins A, E and C and reduced glutathione (GSH).

Amrita Bindu is a salt-spice herbal mixture based on Ayurved and Siddha medicine, for combating morbidity due to degenerative and inflammatory disorders and slow down the ageing process. Indian medicine advocates the intake of Jivanya (life sustaining) groups of herbal and salts as food additives. According to Charaka (1st century A.D.) Jivanya includes invigorators, nourishing drugs, laxatives, wound healers and digestive stimulants. A second group Baiya are producers of strength, complexion, voice and cardiac resilience to stress. The third are Soudhanyas or cleansers of the organ systems. These prevents skin diseases haemorrhoids, inflammation and infection. The pharmacological action of the drugs in the Ji-
vanya groups are
a) cleaning the passages or ducts
b) improve excretion of unwanted or toxic materials by inducing the secretion of the eyes and nose
c) decoction of which can be used for enema to induce focal removal of toxins from the body
d) those which controls thirst,correct dizziness, fever and fatigue, diuretics and antidiuretics and
e) pain relievers, laemomatines, sedative and rejuvenators.

Amrita Bindu is a formula developed using Jivanye groups of herbs (some of which are also used as spices) for a long healthy life. In addition to common salt, Indian medicine advocates a variety of water like Rogh salt, Sandhak and Bantoo salt which provides chloride, phosphates and sulphates of potassium, magnesium and Black salt with sulphides and trace elements. Earlier studies have shown that dietary supplementation of Amrita Bindu to rats exposed to the carcinogenic microamine derivates N-methyl-N'-Nitro-N-nitroso guanidine (MNNNG) prevented depletion of antioxidant enzymes and scavengers, thus providing protection against free radical attack (23).

Materials and Methods

Studies were made on blood samples obtained from 50 cases of non-insulin dependent diabetes mellitus (NIDDM) with mild diabetic retinopathy who were regular out patients at M. V. Diabetes Specialities Centre, Madras, and were broadly classified according to their period of treatment.
- **Group I**: 25 patients with mild diabetic background retinopathy treated with Amrita Bindu. Amrita Bindu was administered as 500 mg capsules twice daily after food, together with all other medication. 17 subjects were followed up after 6 months treatment. 8 patients could not be followed up. All the patients in the group continued with their diets and medication as before.
- **Group II**: 25 patients with mild diabetic background retinopathy served as controls. They were followed up for a period of 6 months.
- **Group III**: The healthy control population comprised of the employees of Madras University and were age and sex matched with the patient population studied. They were free from diabetes mellitus and other diseases.

A detailed case history, which included physical measurements, duration and family history of diabetes, we obtained from each individual in all the above mentioned groups.

A detailed fundoscopic examination was done for all the cases of NIDDM before and after 6 months of treatment and the condition was documented by the Hammarstenh Hospital grading system (17). Patients with mild background retinopathy and moderate background retinopathy, but without maculopathy were included into the study. Mild background retinopathy was defined as the presence of microaneurysms only or dot and blot haemorrhages or soft exudates in more than 3 fields. The criteria for classification as moderate background retinopathy without maculopathy are the presence of microaneurysms, dot and blot haemorrhages, soft exudates and hard exudates in any field of the retina except the macula. Regression or progression of retinopathy is documented with the disappearance of microaneurysms in field detailed earlier, and the appearance of a new microaneurysm respectively. The patients were examined at the end of six months therapy in the case of group I and six months after the initial investigation in group II. Group III provided the data on healthy non-diabetic subjects. They had no retinopathy.

Blood samples were drawn by vein puncture from the subjects after a 12 hour overnight fast into tubes containing EDTA (1 mg/mL). Fasting blood sugar was estimated by the O-toluidine method of Dubowski (6) as modified by Sasaki and Matsu (21). Glycosylated haemoglobin (HbA1C) was measured by the method of Wang and Yang (28). The product of lipid peroxidation (LPO) was assayed as the thiobarbituric acid reactive species (TBA RS) and expressed in terms of malondialdehyde (MDA) according to the method of Yagi (30) in the plasma and by the method of Cynamon et al. (4) in the erythrocyte and erythrocyte membrane. The erythrocyte membrane was isolated according to the method of Dodge et al. (5) with modifications reported earlier by Sharmagustardram et al. (23). Superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich (16) and glutathione peroxide (GPO), according to Rotruck et al. (20) in the haemolysate. Catalase activity was determined in the cell membrane by the method of Sinha (25).

Vitamin A (1), Vitamin E (10) and Vitamin C (18) were assayed in the plasma. Reduced glutathione (GSH) was assayed in whole blood (3).

Statistical analysis

The values are expressed as mean ± S.D. for the patients and healthy population separately. Statistically significant difference between the values before and after Amrita Bindu therapy were arrived at using two tailed students t test.

Results

Table 1 shows the general data of the population investigated. It can be seen that the population were age matched. The body mass index of patients with diabetic retinopathy and the controls were similar suggesting they were not obese. The incidence of family history of diabetes are significantly higher in NIDDM groups (Table 1), when compared to healthy controls. 17 patients

<table>
<thead>
<tr>
<th>SL No</th>
<th>Parameter</th>
<th>Group I (n = 25)</th>
<th>Group II (n = 25)</th>
<th>Group III (n = 56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Age (Years)</td>
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<td>46±4</td>
<td>45±4</td>
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<td>2.</td>
<td>Height (cm)</td>
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<td>139±6</td>
<td>160±4</td>
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<td>3.</td>
<td>Weight (kg)</td>
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<td>59±9</td>
<td>60±2</td>
</tr>
<tr>
<td>4.</td>
<td>Body mass index (BMI)</td>
<td>24±4</td>
<td>24±4</td>
<td>23±5</td>
</tr>
<tr>
<td>5.</td>
<td>Family history of diabetes</td>
<td>86</td>
<td>76</td>
<td>8</td>
</tr>
<tr>
<td>6.</td>
<td>NIDDM %</td>
<td>100</td>
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<td>0</td>
</tr>
</tbody>
</table>

Table 1: General data on the subjects studied.
(out of 25) in group I were reviewed after 6 months. One case had discontinued the drug due to gastritis, 7 cases showed poor adherence to diet, medication including hypoglycaemic drugs and routine check up. 5 out of the 7 had left the city, and did not come for the evaluation. Seventeen patients of the group I were followed up for 6 months and the retina was graded. In 8 patients, the microaneurysms disappeared in more than 2 fields of retina and this was taken as regression of retinopathy. In eight others the retinopathy remained the same in both eyes whereas one patient developed changes in the macula at the end of 6 months.

In group II, 18 patients were followed up and the retina was graded. In 10 cases, the retinopathy remained the same in both the eyes, while in 8 others, additional microaneurysms appeared in one or more fields.

Lipid peroxidation product malondialdehyde (MDA) in blood is given in Table 2. MDA in plasma is elevated by 70% in subjects with diabetic retinopathy. A two fold and three fold increase were observed in erythrocyte and erythrocyte membrane MDA levels, respectively. Supplementation of Amritha Bindu significantly reduced the lipid peroxidation product (MDA) levels of the plasma, erythrocyte and erythrocyte membrane. In the patients without Amritha Bindu supplementation, lipid peroxidation levels did not show any reduction.

The blood antioxidant status is given in Table 3. SOD and CAT activity is greatly reduced in diabetic retinopathy. Amritha Bindu supplementation showed changes in the reverse direction. GP activity in red cells is higher in NIDDM, when compared to non-diabetic and during Amritha Bindu therapy, this is partially reversed.

Table 4 shows the blood levels of the non-enzymatic antioxidants (also known as scavengers) vitamin A, E, C and GSH. Vitamin A levels in diabetic retinopathy is slightly reduced while a 40% reduction in Vitamin C and 30% reduction in Vitamin E are seen. GSH is lowered by about 20% in diabetic retinopathy, when compared to healthy controls. Amritha Bindu supplementation improves all the four antioxidants in the cases following treatment.

Statistically significant changes from the initial levels are indicated as * for p < 0.001.
Discussion and Conclusions

Antioxidant deficiency in the blood cells is acute in diabetic retinopathy and this results in higher levels of lipid peroxidation products in the plasma and cells (Table 2). LPO induces membrane fragmentation and is one of the causative factors for capillary damage. Amrita Bindu supplementation produces a significant reduction in lipid peroxidation in both plasma, cells and cell membrane, and appears to reduce membrane fragmentation, as can be seen by the regression of retinopathy in 8 out of the 17 patients followed up for 6 months. In group II, with conventional therapy alone, no regression is reported.

Alteration in superoxide dismutase, peroxidase, and catalase activities and tissue glutathione concentrations have been reported in diabetes (12). Loven et al. (11) showed that Cu-Zn SOD activity decreased as a result of inactivation of the enzyme due to the inability of the cells to metabolise hydrogen peroxide completely. Matkovic et al. (15) reported that in NIDDM, glutathione peroxidase activity was increased together with increase in the endogenous production of H$_2$O$_2$. Diabetes may affect other aspects of the glutathione-peroxide interaction. Glutathione reacts with peroxide to form glutathione disulphide in a reaction catalysed by GPx. Oxidised glutathione is then regenerated by an NADPH-linked reduction, catalysed by glutathione reductase. NADPH is generated by glucose catabolism using hexose monophosphate (HMP) shunt. Because, HMP shunt activity is abnormal in diabetes, NADPH availability is reduced and the ability to recycle glutathione disulphide to glutathione is altered in diabetes (13).

Vitamin A, E and C, the three essential nutrients that can scavenge free radicals directly, are also very much lowered in diabetes mellitus (Table 4). Supplementation of ascorbic acid in GSH deficient rats lessened the severity of signs of GSH deficiency and decreased mortality (14). Amrita Bindu supplementation led to remarkable increase in GSH after 6 months. The values obtained were nearly normal, comparable to that of the healthy controls. Vitamin E was also found to be raised significantly. An increase in the levels of SOD and CAT and a decrease in GPx activity were also encountered suggesting that Amrita Bindu supplementation helps in striking a balance between production of lipid peroxides and ability of the antioxidants to combat the oxidative stress.

We also followed up a group of patients with BDR as controls, without the supplementation of Amrita Bindu. Results show that lipid peroxidation remained high and the antioxidant levels remained low with conventional therapy.

Free radical reduced lipid peroxidation is associated with diabetes mellitus and in a number of secondary complications (12). Due to insulin insufficiency glycolytic pathway is inhibited leading to elevated levels of triosephosphate resulting in the formation of methylglyoxal (27) which is accumulated in the erythrocyte (28). The formation of methylglyoxal proceeds with the generation of H$_2$O$_2$ (9) and is the initiator for peroxidative damage to the cell membrane lipids, proteins and cellular enzymes. Auto-oxidation of carbohydrates and glycosylated protein also lead to free radical intermediates (8) which inactivate protein by cross-linking and is a basis for the development of secondary complications (7).

The positive effect of Amrita Bindu supplementation in augmenting antioxidant levels, lowering lipid peroxidation and the associated regression of retinopathy (in 8 out of 25 cases studied) suggest the effectiveness of the supplementation in controlling the development of secondary complications. Longer treatment periods and larger number of cases are needed for assessing the usefulness of the formula in reversing diabetic retinopathy.

Acknowledgement

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References

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Behebung sexueller Funktionstörungen bei Peyronie-Krankheit


Diese Befunde scheinen mehrere tierexperimentelle Studien zu bestätigen, die für die Bedeutung von Kalzium beim Metabolismus der Fibroblasten und einer möglichen Beeinflussung durch Kalziumantagonisten auf die Fibroblastenproliferation und die Synthese von Matrixkollagen sprechen. Nach Meinung der Autoren sollte diese kostengünstige, nichtoperative Behandlung der Peyronie-Krankheit, eventuell kombiniert mit einer oralen Therapie, weiter untersucht werden, um den physisch stark belasteten Patienten eine nützliche Alternative zum operativen Eingriff anbieten zu können.

Reinhard Seib, Mannheim


Vorhofflimmern: Hohe Konversionsrate mit Propafenon


Dr. Angelika Bischoff, Graefelfing