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RESEARCH ARTICLE

EFFECT OF BENFOTIAMINE ON MARKERS OF OXIDATIVE STRESS IN CONTROLLED TYPE 2 DIABETES MELLITUS PATIENTS

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ABSTRACT

Background: Diabetes is a chronic disease associated with various micro and macro complications. Increased state of oxidative stress associated with these complications. Benfotiamine, a lipid soluble derivative of thiamine, thought to reduce oxidative stress and hence complications associated with diabetes.
Objectives: To study the effect of benfotiamine on markers of oxidative stress in type 2 diabetes mellitus patients with controlled blood sugar levels.
Methods: 50 patients of type 2 diabetes mellitus with controlled levels of blood sugar were enrolled from diabetes clinic of Pt BDS PGIMS, Rohtak. All patients were evaluated for markers of oxidative stress and other routine tests at the start of the study. All patients were then started on 200 mg of benfotiamine and followed up weekly in person or telephonically for a month. After 30 days, patients were again evaluated for same markers of oxidative stress and other routine tests. Results were compared at the end of the study.
Results: In present study 50 type 2 diabetes mellitus patients were studied for oxidative stress at baseline and after 30 days of benfotiamine therapy. All patients had controlled diabetic status at start of study (Fasting blood sugar 119.28 ± 24.40 mg/dl and post prandial blood sugar 177.80 ± 37.84) with glycosylated HbA1C of 6.81 ± 0.13 and the controlled state was maintained throughout the study period (Fasting blood sugar 102.82 ± 16.93 mg/dl and post prandial blood sugar 171.32 ± 30.38). All patients also had a controlled blood pressure (Systolic blood pressure 124.64 ± 5.25 mm hg and Diastolic blood pressure 79.16 ± 3.40 mm hg). Oxidative stress at baseline was elevated despite control of hyperglycemia as evidence by high MDA levels (2.59 ± 0.85 µmol/l vs 0.69 ± 0.24 µmol/l, $p < 0.001$), low vitamin E levels (7.47 ± 1.07 mg/dl vs 13.33 ± 2.02 mg/dl, $p < 0.001$) and low reduced glutathione levels (10.38 ± 0.67 mg/dl vs 22.28 ± 2.22 mg/dl, $p < 0.001$). All these markers improved after 30 days of benfotiamine therapy MDA levels decreased from 2.59 ± 0.85 µmol/l to 1.14 ± 0.40 µmol/l, $p < 0.001$, vitamin E levels increased from 7.47 ± 1.07 mg/dl to 9.76 ± 1.19 mg/dl, $p < 0.001$ and reduced glutathione levels increased from 10.38 ± 0.67 mg/dl to 13.54 ± 0.68 mg/dl, $p < 0.001$.
Conclusion: The study showed that patients with type 2 diabetes mellitus have elevated level of oxidative stress despite adequate control of hyperglycemia. Benfotiamine, a lipid soluble derivative of thiamine effectively reduces oxidative stress and thus reduces various complications associated with diabetes.

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INTRODUCTION

Diabetes is a chronic disease which is associated with various micro-and macro-vascular complications. Increase in oxidative stress has been implicated as culprit for these complications (Baynes and Thorpe, 1999). Thiamine plays an important role in intracellular glucose metabolism and it has been shown that diabetic subjects tend to have lower blood thiamine concentrations than healthy controls, together with a reduced erythrocyte transketolase activity (Jermendy, 2006). Benfotiamine, a lipid soluble derivative of thiamine, decreases accumulation of detrimental glucose metabolites by acting in various pathways thereby preventing formation of advanced glycation end products (AGEs). Benfotiamine was demonstrated to normalizes protein kinase C (PKC) activity and prevents nuclear factor-kB (NF-kB) activation in the retina of diabetics (Hammes *et al*., 2003). Benfotiamine was demonstrated to inhibit the accumulation of triose phosphates through their conversion to ribose 5- Phosphate, increase transketolase expression in renal glomeruli, decrease Protein kinase C activation, oxidative stress and protein glycation, and finally inhibit the development of micro albuminuria (Babaei-Jadidi *et al*., 2003). Several studies have evaluated the role of benfotiamine in diabetic patients and have shown its positive effect in reducing diabetes related complications (Stirban *et al*., 2014). However, no study was done in controlled diabetic patient population which can clarify its role in reducing oxidative stress over and above control of hyperglycemia. Thus, this study was designed to evaluate effect of benfotiamine on established markers of oxidative stress in controlled type 2 diabetic population.

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MATERIALS AND METHODS

Study Population

The study was conducted on 50 patients with controlled type 2 diabetes mellitus, enrolled from diabetes clinic of Pt. B.D. Sharma PGIMS, Rohtak, Haryana, India.

Inclusion criteria

Controlled type 2 diabetes mellitus patients of more than one year duration irrespective of age, sex and their antidiabetic treatment and associated drugs

Exclusion criteria

- HbA_{1c}>7%
- Pregnancy
- Renal failure (serum creatinine >1.2mg%)
- Chronic Smoker
- Hypertension (resting blood pressure >180/110mmHg)

Study Protocol

Study design

It was an open, comparative and non-crossover clinical study

Duration of Study

Total duration of study was 30 days. All patients were advised to adhere to diabetic diet. An informed consent was taken and patients were put on 200mg of benfotiamine per day and asked to continue antidiabetic therapy already being taken. Patients were regularly evaluated at each visit (during the study) for drug compliance and any adverse effect of benfotiamine.

Details of visits

The study comprised of 5 visits.

Visit 1 (Day 0) –	To adopt inclusion and exclusion criteria.
Visit 2 –	Day 8 (follow up visit)
Visit 3 –	Day 15 (follow up visit)
Visit 4 –	Day 22 (follow up visit)
Visit 5 (Day 30) –	Final visit

Visit 1 (Day 0)

Each patient was subjected to a detailed history and physical examination including general and systemic examination with special reference to complications of diabetes.

The following baselines investigations were performed and recorded on a specially designed proforma.

- Hb, TLC, DLC, hematocrit. Routine urine analysis
- Blood sugar - Fasting and postprandial.
- HbA_{1c}
- Serum Lipid profile
- Serum creatinine
- Fundus examination
- ECG

Patients were enrolled for the study on the basis of inclusion and exclusion criteria. After enrollment, patients were evaluated for the target parameters.

Target Parameters

- Reduced glutathione (GSH) (done by method of beutler *et al*) (Srivastava and Beutler, 1967)
- α-Tocopherol (Vit. E) (done by method of Duggan *et al*) (Baker and frank, 2002)
- Serum malonyldialdehyde (MDA) (done by method of baker *et al*) (Placer *et al*., 1966)

Benfotiamine Therapy

All selected patients were then put on 200mg/day benfotiamine therapy which was continued along with antidiabetic medications and diabetic diet.

Follow up visits

The patients were followed up at 8^{th}, 15^{th} and 22^{nd} day (visit 2, 3 and 4) as detailed below:

1. General physical and systemic examination was performed
2. Compliance of anti-diabetic drugs was ensured.
3. Compliance of benfotiamine 200mg/day was ensured.
4. Side effects of benfotiamine, if any, was asked.
5. Compliance of strict diabetic diet was ensured.
6. Fasting and postprandial blood sugar estimation.

After taking fasting blood sample, patient took breakfast with antidiabetic medication. 2 hr later sample was collected for postprandial blood sugar.

Final visit (5^{th}) at 30 days

On this visit, reevaluation was done as follows:

1. Compliance of benfotiamine 200mg/day, by enquiry.
2. Side effects of benfotiamine, if any, by enquiry.
3. Compliance of strict diabetic diet and drugs, by enquiry.
4. Fasting blood sample was taken before breakfast for blood sugar estimation.
5. Patient took regular antidiabetic medication
6. 2 hours after breakfast blood sample was taken to estimate postprandial blood sugar.
7. Levels of reduced glutathione, alpha tocopherol, serum MDA were reassessed.

RESULTS

The present study included 50 patients of both sexes with controlled type 2 diabetes mellitus (i.e. HbA1c < 7%). The study excluded patients with hypertension, renal failure, chronic smoker and pregnancy. Baseline characteristics of the patients is shown in Table 1. Patients included in the study were in the age group of 31-80 years. The mean age of the patients was 56.24 ± 10.82 years. The study consisted of 30 males and 20 females. Mean body mass index (BMI) of the patients was 25.05 ± 1.73 kg/m^2 irrespective of sex. The mean fasting and postprandial blood sugar levels of the patients were 119.28 ± 24.40 mg/dl and 177.80 ± 37.84 mg/dl respectively at

the start of study and HbA1c level was 6.81 ± 0.13% which indicated the controlled status of the patients at start of therapy. Serial blood sugar (fasting and postprandial) estimations were done on each visit to ascertain the controlled status of diabetes. At the end of study (i.e. at 30 days), the mean values of fasting and postprandial blood sugar were 102.82 ± 16.93 mg/dl and 171.32 ± 30.38 mg/dl respectively (table 2). These findings indicated that the controlled status of diabetes was maintained throughout the course of study with antidiabetic treatment (Fig 1). Blood pressure of all the patients was measured before the start of study to exclude patients with hypertension (shown in Table 1).

Effect of Benfotiamine on Malonyldialdehyde (MDA) levels

Malonyldialdehyde (MDA) levels were measured in all patients at the start of the study and at 30 days of benfotiamine therapy. Serum Malonyldialdehyde (MDA) levels were high as compared to normal indicating oxidative stress even in controlled state of diabetes (Table 3). The levels declined from 2.59 ± 0.85 µmol/l at the beginning of the study to 1.14 ± 0.40 µmol/l at 30 days of benfotiamine 200 mg/day therapy (Table 3 and Fig. 2). The difference was statistically significant ($p<0.0001$). This finding indicated reduction of lipid peroxidation at 30 days of therapy with benfotiamine in patients with controlled type 2 diabetes mellitus.

Effect of benfotiamine therapy on Vitamin E

Vitamin E levels were measured in all patients at the start of the study and at 30 days of benfotiamine therapy. Vitamin E levels were low as compared to normal suggesting the presence of oxidative stress even in controlled state of diabetes (Table 4). The levels increased from 7.47 ± 1.07 mg% at the beginning of the study to 9.76 ± 1.19 mg% at 30 days of therapy with benfotiamine (Table 4 and Figure 3). The difference was statistically significant ($p<0.0001$). This indicated that vitamin E, an antioxidant, levels improved at 30 days of therapy with benfotiamine.

Effect of benfotiamine on reduced glutathione

Reduced glutathione levels were measured in all patients at the start of the study and at 30 days of benfotiamine therapy. Reduced glutathione levels were lower than normal pointing to the existence of oxidative stress even in controlled state of diabetes (Table 5). The levels increased from 10.38 ± 0.67 at the beginning of the study to 13.54 ± 0.68 at 30 days of therapy with benfotiamine (table 5 and fig 4). The difference was statistically significant ($p<0.0001$). Reduced glutathione, a marker of antioxidant activity, improved at 30 days of therapy.

DISCUSSION

The study was an open, comparative and non-crossover clinical study consisting of 50 patients of controlled type 2 diabetes mellitus. The total duration of study was 30 days with a total of 5 visits during which patient received benfotiamine in a dose of 200 mg/day along with their usual antidiabetic medications and diabetic diet. The controlled status of diabetes was judged by measuring fasting and postprandial blood sugar and glycosylated hemoglobin (HbA1c) at the time of enrollment of patient and was maintained throughout the study. We measured 3 established markers of oxidative stress i.e. Malonyldialdehyde (MDA), Vitamin E, and reduced glutathione (GSH) in the beginning and at the end (30 days) so

Table 1. Baseline characteristics of patients

Mean age (years)	56.24 ± 10.82
Sex (M:F)	30:20
Blood sugar fasting (mg/dl)	119.28 ± 24.40
Blood sugar postprandial (mg/dl)	177.80 ± 37.84
Glycosylated hemoglobin (%)	6.81 ± 0.13
Systolic blood pressure (mmHg)	124.64±5.25
Diastolic blood pressure (mmHg)	79.16±3.40

Table 2. Controlled diabetic status of patients at the end of the study

Parameters	At 30 day (Mean ± S.D)
Blood sugar fasting (mg/dl)	102.82 ± 16.93
Blood sugar postprandial (mg/dl)	171.32 ± 30.38

Table 3. Serum Malonyldialdehyde (MDA) levels before and after Benfotiamine therapy

Normal value (µmol/l)	0 Day (first visit) (Mean ± S.D.) (µmol/l)	At 30 days (5^{th} visit) (Mean ± S.D.) (µmol/l)	P value
0.69 ± 0.24	2.59 ± 0.85 P < 0.001	1.14 ± 0.40 P < 0.001	<0.0001

Table 4. Vitamin E levels before and after benfotiamine therapy

Normal value (mg%)	0 Day (first visit) (Mean ± S.D.) (mg%)	At 30 Days (5^{th} visit) (Mean ± S.D.) (mg%)	P value
13.33 ± 2.02	7.47 ±1.07 P < 0.001	9.76 ± 1.19 P < 0.001	< 0.0001

Table 5. Reduced glutathione before and after benfotiamine therapy

Normal value (mg%)	0 Day (first visit) (Mean ± S.D.) (mg %)	At 30 Days (5^{th} visit) (Mean ± S.D.) (mg%)	P value
22.28 ± 2.22	10.38 ± 0.67 P < 0.001	13.54 ± 0.68 P < 0.001	< 0.0001

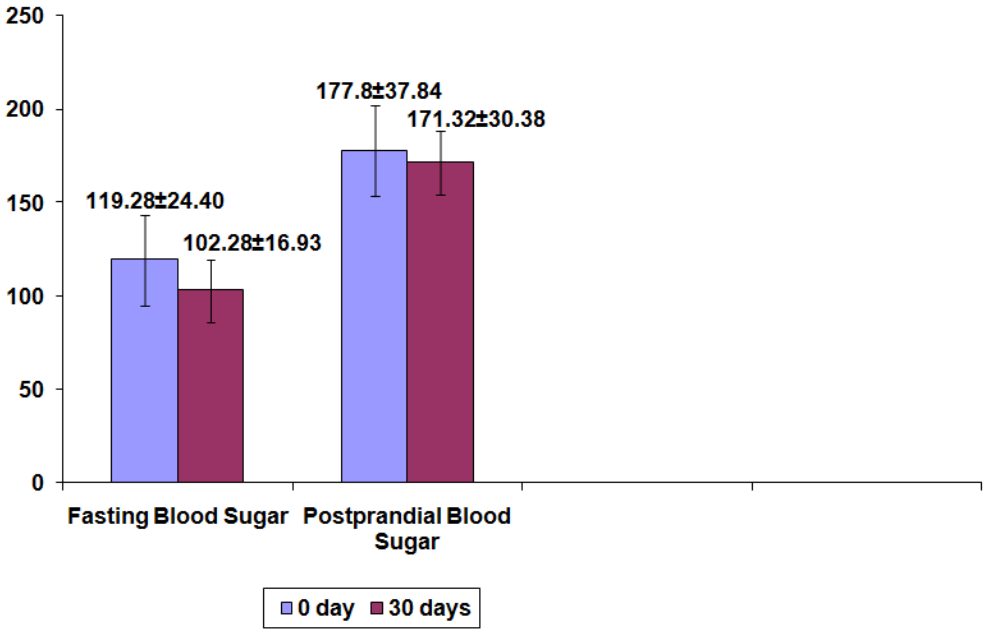

Figure 1. Bar Graph showing Controlled diabetic status of patients at 30 days

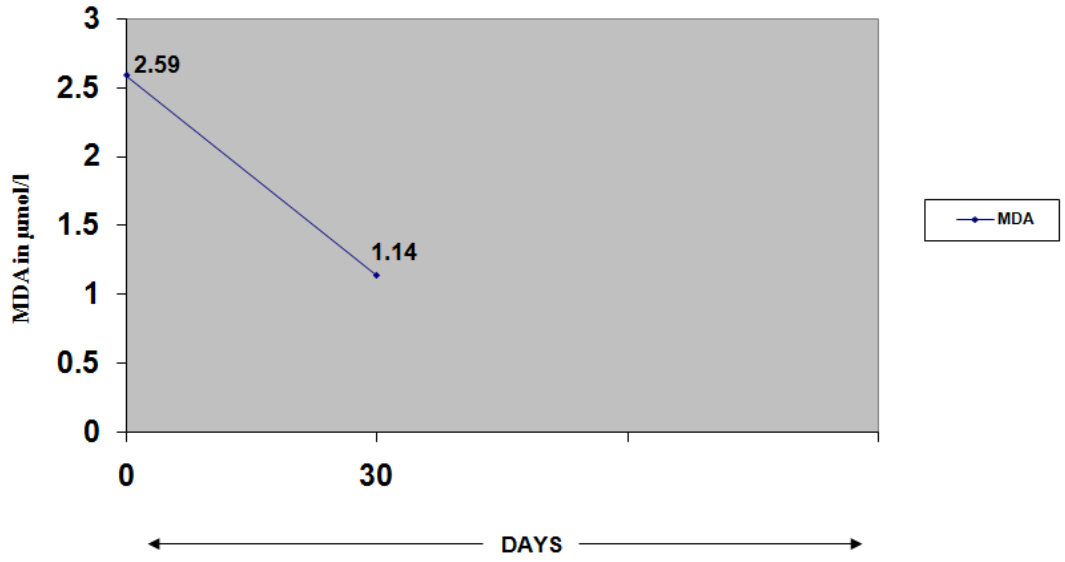

Figure 2. Effect of Benfotiamine therapy on Malonylaldehyde (MDA)

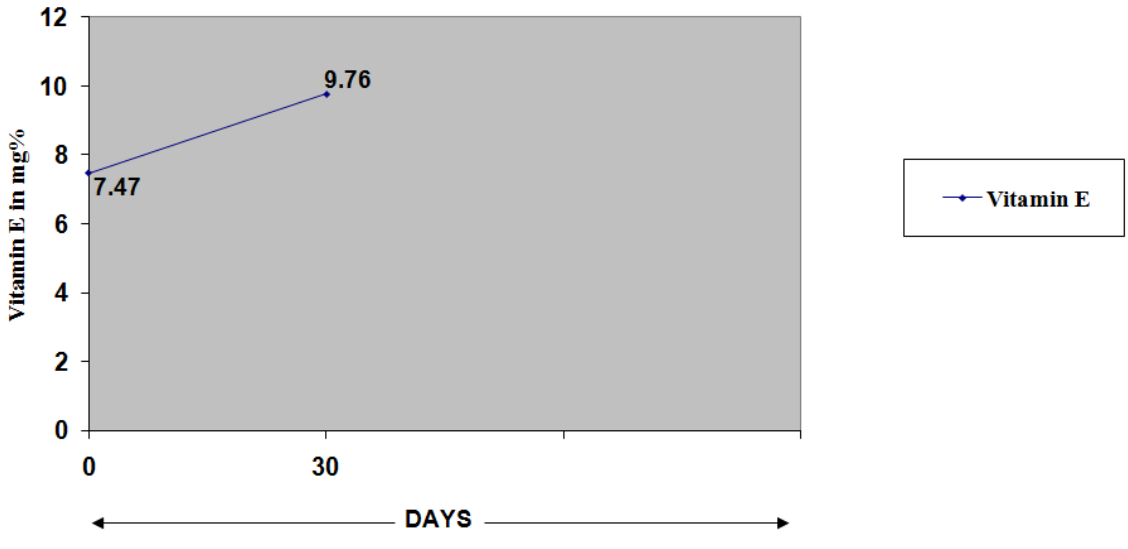

Figure 3. Effect of Benfotiamine therapy on Vitamin E

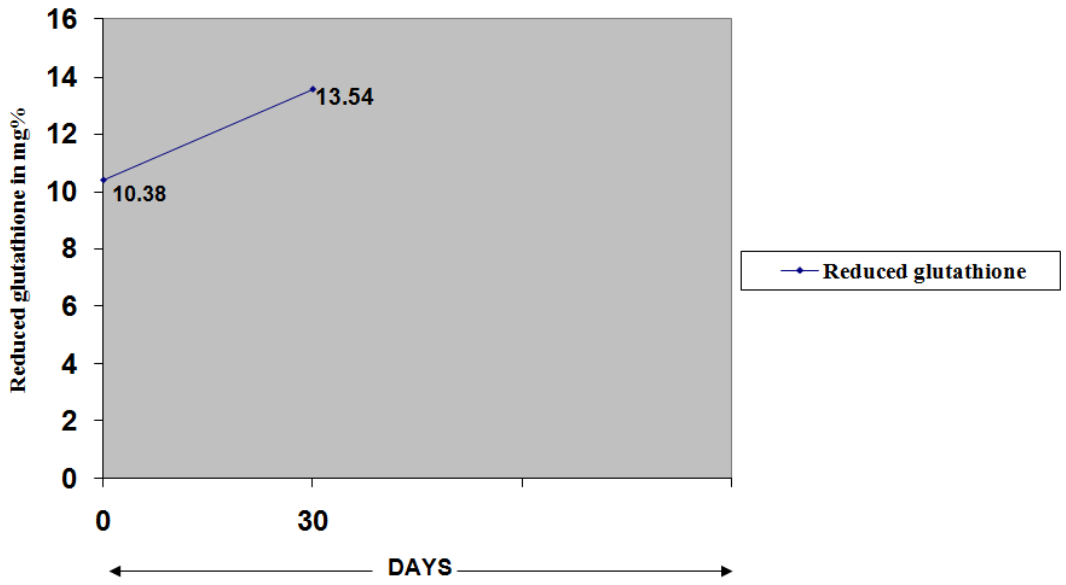

Figure 4. Effect of Benfotiamine therapy on reduced glutathione

as to know the effect of benfotiamine therapy. The study made some interesting observations which are discussed further. The study consisted of 50 patients in the age group of 31-80 years, out of which 30 were males and 20 females, with a mean age of 56.24 ± 10.82 years. The mean fasting and postprandial blood sugar levels of the patients were 119.28 ± 24.40 mg/dl and 177.8 ± 37.84 mg/dl respectively at the start of the study. The mean glycosylated hemoglobin (HbA1c) level of the patients at the start of the study was 6.81 ± 0.13 % (Table 1). These findings indicated the controlled status of the patients at the start of the therapy. At 30 days (visit 5), the mean values of fasting and postprandial blood sugar were 102.82 ± 16.93 mg/dl and 171.32 ± 30.38 mg/dl respectively (Table 2). These observations indicated continued controlled status of diabetes throughout the course of study with antidiabetic treatment. The already controlled status (i.e. HbA1c <7%) of our patients and its maintenance throughout the course of the study confirmed exclusion of the effect of control of hyperglycemia on oxidative stress, as it was pre-requisite, for benfotiamine therapy. Hypertension is known to influence endothelial function in diabetics (Dandona et al., 2004), that is why, we excluded the patients with hypertension. Blood pressure of all patients was measured at each visit. The mean blood pressure was 124.65 ± 5.25/79.16 ± 3.40 mmHg (Table 1) at the start of study, which confirmed that all patients included in the study were non hypertensive. Endothelial function is also affected by certain drugs like statins, insulin, fibrates, thiazolidinediones (TZD), ACE inhibitors etc. During our study, patients who were already on any of these drugs were allowed to continue these medications during the whole study, so as to neutralize their effect during benfotiamine therapy.

Glucose is a direct source of free radicals, hence, hyperglycemia is associated with oxidative stress. In addition to this, a number of other mechanisms like changes in energy metabolism, alterations in sorbitol pathway activity, changes in the level of inflammatory mediators, altered antioxidant defense systems and localized tissue hypoxia and reperfusion may lead to oxidative stress (Baynes and Thorpe, 1999). Oxidative stress means production of oxygen free radicals overwhelming antioxidant defenses (both enzymatic and non-enzymatic). In diabetes, oxidative stress causes the levels of antioxidants to fall below normal and contribute to the development of diabetic complications. Lipid peroxidation and protein denaturation are accepted parameters of oxidative stress. Lipid peroxidation is peroxidation of membrane polyunsaturated fatty acids leading to loss of cellular integrity. The parameters of cellular lipid peroxidation are malonyldialdehyde (MDA) and diene conjugates which are easily measurable. Reduced glutathione (GSH) is oxidized to oxidized glutathione (GSSG) with the help of glutathione peroxidase enzyme, thus, acts as a potent free radical scavenger. Similarly Vitamin E also gets consumed and its level fall in oxidative stress. Hence, reduced levels of reduced glutathione and vitamin E will indicate oxidative stress. The normal values of MDA, vitamin E and reduced glutathione in healthy adults are 0.69 ±0.24 µmol/l, 13.33 ±2.02 mg% and 22.28 ± 2.22 mg% respectively (Chugh *et al*., 1999). These parameters were measured at the start of the study. In our study, the MDA levels were high i.e. 2.59 ± 0.85 µmol/l (Table 3), vitamin E levels were low i.e. 7.47 ± 1.07 mg% (Table 4) and reduced glutathione levels were also low i.e. 10.38 ± 0.67 mg% (Table 5) at the beginning of the study. These observations clearly demonstrated the presence of oxidative stress in controlled type 2 diabetic subjects. Benfotiamine is a lipid soluble derivative of thiamine, which is converted to biologically active thiamine after absorption. Thiamine is converted to TDP which acts as a coenzyme for transketolase and other enzymes of glycolysis. Benfotiamine increases transketolase activity and as a result, blocks all the major pathways of hyperglycemia induced oxidative stress (Brownlee, 2003). Beneficial effect of benfotiamine therapy on oxidative stress can be verified by measuring Malonyldialdehyde and other antioxidant levels like Vitamin E and reduced glutathione. In our study, the MDA levels decreased from 2.59 ± 0.85 µmol/l at day 0 to 1.14 ± 0.40 µmol/l at 30 days of therapy (Table 3), Vitamin E levels increased from 7.47 ± 1.07 at day 0 to 9.76 ± 1.19 mg/dl at 30 days of therapy (Table 4) and reduced glutathione levels also increased from 10.38 ± 0.67 mg/dl at day 0 to 13.54 ± 0.68 mg/dl (Table 5) at 30 days of therapy. These findings demonstrated that benfotiamine reduced oxidative stress in controlled type 2 diabetic patients. Earlier it was reported that higher is the dose of benfotiamine, more is the beneficial effect (Winkler *et al*., 1999). It is also a known fact that higher doses are costly and prone to side effects. Therefore, keeping these

facts in mind, we tried a dose of 200 mg/day of benfotiamine therapy which is cost – effective. We evaluated our patients at each and every visit regarding any side effect. None of our patient complained of any side effect, hence, this dose was found to be safe and effective.

To summarize, our observations suggest that oxidative stress occur in diabetics, despite the control of hyperglycemia. Presence of oxidative stress is likely responsible for endothelial dysfunction frequently seen in diabetics. Presence of endothelial dysfunction can lead to various micro and macrovascular complications, hence, to be combated as early as possible. Benfotiamine was found to be a safe and well tolerated drug when used in moderate dose (i.e. 200 mg/day). Benfotiamine therapy (200 mg/day) resulted in a decrease in oxidative stress over and above the control of diabetes. Various studies, done so far, evaluated the effect of benfotiamine during uncontrolled state. In those studies it was not possible to say whether the beneficial effect was due to benfotiamine or as a result of control of hyperglycemia. Based on our findings, we hereby suggest that every diabetic should receive benfotiamine to delay or retard the complication. We further advocate that a large population based multicentric study may be undertaken to confirm our observations and to establish the definite role of benfotiamine so that it become a regular drug therapy in the management of diabetes.

Conclusion

Diabetes is associated with oxidative stress which persists even in the controlled state. Benfotiamine therapy had been found useful in relieving oxidative stress over and above the control of diabetes which may retard development of various micro and macrovascular complications associated with diabetes. It was thus found to be safe and effective therapy.

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