Research Article

Effect of Vitamin E and C plus Reduced Glutathione in Treatment of Diabetic Nephropathy

V. G. Kuchake[1], *C. D. Upasani[2]

1. R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Dhule, Maharashtra, India.
2. Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad, Nashik, Maharashtra, India.

ABSTRACT

Diabetes has describes, a group of metabolic diseases due to high blood sugar. Their uncontrolled lead to prolonged hyperglycemia resulting in long-term microvascular/macrovascular complications; and nephropathy is one of them. Objective: To evaluate the combine effect of antioxidant vitamins E and C plus reduced glutathione in diabetic nephropathy. Method: A randomized, open controlled clinical trial was conducted on 216 diabetic nephropathy patients who have had diabetes for at least 5 years. Result: 216 patients were selected and divided in to four groups such as positive control, vitamin, reduced glutathione and combination of vitamin E and C plus reduced glutathione. After 4 months supplementation of vitamin E and C plus reduced glutathione, it was found that the, vitamins and reduced glutathione treated groups shows significant ($p<0.009$ and $p<0.002$) decreased microalbuminuria levels from 33.2±2 to 30.5±2 and 31.4±2 to 30.1±1 as compared to control group. Interestingly, treatment of glutathione plus vitamins exhibited significant decreased ($p<0.001$) values from 33.3±2 to 27.2±2. Similarly, its effect on total protein shows significant difference ($p<0.01$ and $p<0.05$) decline in level from 8±0.4 to 7.6±0.3; 7.8±0.3 to 7.5±0.3 in vitamin and glutathione plus vitamin combination as compared to control and glutathione group alone. Conclusion: Diabetes is a chronic illness that requires a combine effect of treatment regimen for better glycemic control and to prevent its complications. So it has been suggested that initiating therapy with combination of antioxidants vitamin E and C plus reduced glutathione significantly lowers urinary albumin excretion rate and total protein in diabetic nephropathy.

Keywords: Diabetic Nephropathy, vitamin E, Vitamin C, reduced glutathione

Received 28 Oct 2013 Received in revised form 12 Nov 2013 Accepted 15 Nov 2013

***Address for correspondence:**
Dr. C. D. Upasani
Principal,
Shriman Sureshdada Jain College of Pharmacy, Neminagar, Chandwad, Nashik, Maharashtra, India.
E-mail: cdupasani@rediffmail.com

INTRODUCTION

The term diabetes has been used to describe a group of metabolic diseases in which a person has high blood sugar, either because the pancreas does not produce enough insulin, or because cells do not respond to the insulin that is produced. According to the Diabetes Atlas 2011 published by the International Diabetes Federation (IDF), the number of people living with diabetes is expected to rise from 366 million in 2011 to 552 million by 2030, unless urgent preventive steps are taken [1]. The manifestations of the disorder cause considerable human sufferings and massive economic cost, even though, the enormous facilities available to control its growth rate. The management of disorder and controlling its associated complications require correct diagnosis, self-care, exercise and necessity of ideal pharmacologic therapy to achieve optimal glycemic control because the progression of diabetes cause prolonged exposure of vascular tissues to hyperglycemia leads to long-term microvascular/macrovascular complications as cardiovascular diseases, renal disease, cerebro-vascular diseases, etc.; these are the prime causes of morbidity, disability and premature death [2]. Any irregularities in dosage regimen can

definitely invite severe complications in the health; and nephropathy is one of them. Diabetic nephropathy is a common complication of diabetes associated with oxidative stress and reduced levels of antioxidants because oxidative stress is hypothesized to play an important part in the development of late diabetes complications because chronic hyper-glycemia increases oxidative stress and considerably modifies the structure and function of proteins and lipids due to glycoxidation and peroxidation. These modified products could contribute to the morphological and functional abnormalities seen in the kidney of patients with diabetes as a result of development of proteinuria, culminating in end-stage renal disease with a particular high risk of cardiovascular morbidity and mortality in diabetic patients [2].

By convention, diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria (>300 mg/24 hours), on at least two occasions separated by 3 - 6 months [3]**.** This process is often associated with rising blood pressure [4]. Conventionally, nephropathy is divided into two types based on the urinary albumin excretion rate (UAER): incipient and overt incipient nephropathy is manifested as microalbuminuria (UAER 20 - 200 mg/24 hours) and usually occurs after 6-15 years of diabetes [5]**,** although microalbuminuria is an indicator of nephropathy [6]**.** Roughly 25 % of patients will regress to normal albumin excretion, and 40 % will remain microalbuminuric [7]. For these reasons, there has been need in the use of externally administered antioxidants to attenuate diabetic nephropathy as well as to control the worsen condition of the patients because supplementation of antioxidants when it comes to treat patients with diabetes which is claimed to be increases the effectiveness of main therapies and devoid of side-effects.

MATERIALS AND METHODS

This study was undertaken to further substantiate the effects of externally administered antioxidants on the renal function of the patients who have been diagnosed to have macroalbuminuria. This research study protocol were obtains an approval from Institutional Human Ethics Committee, of R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, Dhule, Maharashtra, India.

A total of 216 Indian diabetic patients were enrolled in the study on the basis of inclusion & exclusion criteria. Both men and women patients were enrolled after signing the informed consent form from inpatient as well as outpatient departments of Indira Gandhi memorial hospital, Shirpur, Maharashtra. The present study was a randomized, open controlled clinical trial conducted on type 2 diabetic patients aged 35–60 years who have had diabetes for at least 5 years with renal function of the patients who have been diagnosed to have microalbuminuria (Urine albumin excretion >20 mg/dL). Each patient was interviewed for their past medical as well as medication history for diabetes before participation in the study.

Study design: After the run-in period, patients entered a randomized open controlled trial and were randomly assigned to one of four treatment groups., first group considered as positive control group (n=54), second group considered as vitamins groups (n=54) in which patients received combination of antioxidant vitamin E plus vitamin C. Third group considered as reduced glutathione group (n=47) and fourth group was combination of antioxidant vitamin E and vitamin C plus reduced glutathione (n=61). All four groups received treatment for diabetes and ACE, ARB therapies. During study, patient's urine and serum samples were collected for evaluation of various renal as well as hematological parameters were done at the beginning of the run-in phase at randomization and at the end of each treatment period.

RESULTS AND DISCUSSION

It is important to note that diabetic nephropathy is a multistage condition that takes several years to become clinically overt. At the onset of diabetes, there are usually changes in renal function such as glomerular hyperfiltration, increased renal blood flow and hypertrophy of the kidney [8]. Most of these changes can be reversed at an early stage by good glycemic control but they persist in many patients and may

be important in the later development of clinical nephropathy [6]. Traditionally, nephropathy is divided into two types based on microalbuminuria, incipient and overt nephropathy usually occurs after 6 - 15 years of diabetes [5]. Persistent microalbuminuria is universally considered as being the first clinical sign of diabetic nephropathy [9]. Although microalbuminuria may progress to overt nephropathy, it has been shown that at these state glomerular changes can be modified and restoration of impaired glomerular filtration can be achieved. Several mechanisms have been proposed as being involved in the onset and progression of diabetic-related nephropathy [10]. Among them, increased oxidative stress associated with diabetic-related chronic hyperglycemia seems to play the most important role. Decreased antioxidant status has an important role in development of diabetic nephropathy, so antioxidant treatment could become a key element in prevention and reversal of diabetic nephropathy. Costagliola et.al, 1992 has demonstrate that, the therapy on patients undergoing treatment with reduced glutathione (1200 mg/day) could represent it to be a useful drug in the treatment and management of anemia in patients with chronic renal failure [11] but in our study we have used low dose of vitamin E, 400 mg and vitamin C, 500 mg, glutathione 50 mg per day for a period of 4 months and it was observed that, four months vitamins and reduced glutathione treated groups shows significant ($p<0.009$ and $p<0.002$) decreased microalbuminuria levels from 33.2±2 to 30.5±2 and 31.4±2 to 30.1±1 respectively as compared to control group. Interestingly, treatment of glutathione plus vitamins exhibited signi-ficant decreased ($p<0.001$) values from 33.3±2 to 27.2±2. Because glutathione is an extremely important cell protectant, it directly quenches reactive hydroxyl free radicals, oxygen-centered free radical, radical centers on DNA and other biomolecules which shows protective effects in diabetic nephropathy (**Figure 1**).

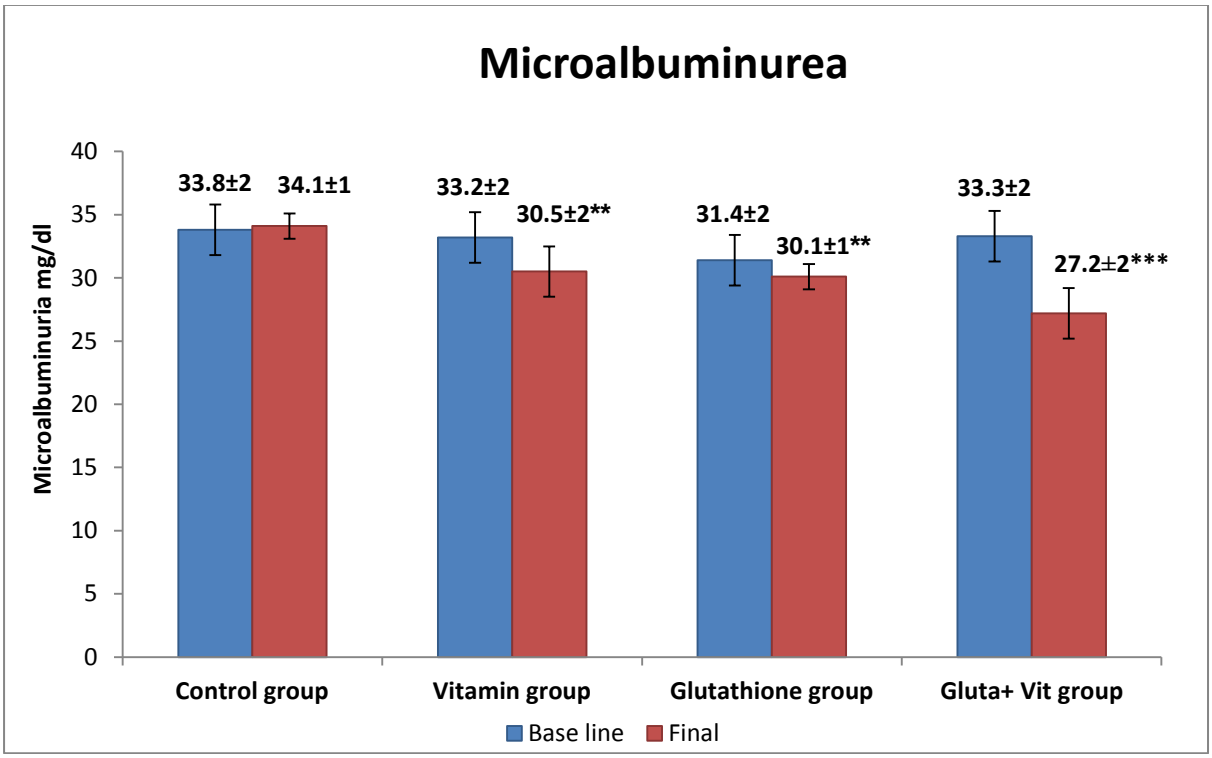

Figure 1: Effect of Antioxidants on Microalbuminuria

Values are expressed as mean ± SEM. Significance of difference in the means was determined by Paired t- Test. (*p< 0.05; **p< 0.01;*** p< 0.001)

Similarly, the total protein test is a rough measure of all the proteins found in the fluid portion of blood. It is commonly used for diagnosing and monitoring various primary and secondary nephropathies. Increased protein level associated with systemic and metabolic disorders such as hypertension, diabetes, the kidney may undergo slow but progressive deterioration, leading eventually to renal failure [12]. Renal involvement in these disorders is often first manifested by gradual increase in proteinuria, for which sensitive assays are therefore desirable [13, 14]. The current study shows significant difference ($p<0.001$ and $p<0.05$) decline in level from 8±0.4 to 7.6±0.3; 7.8±0.3 to 7.5±0.3 in patients treated with vitamin and glutathione plus vitamin combination as compared to control and glutathione group alone (**Figure 2**).

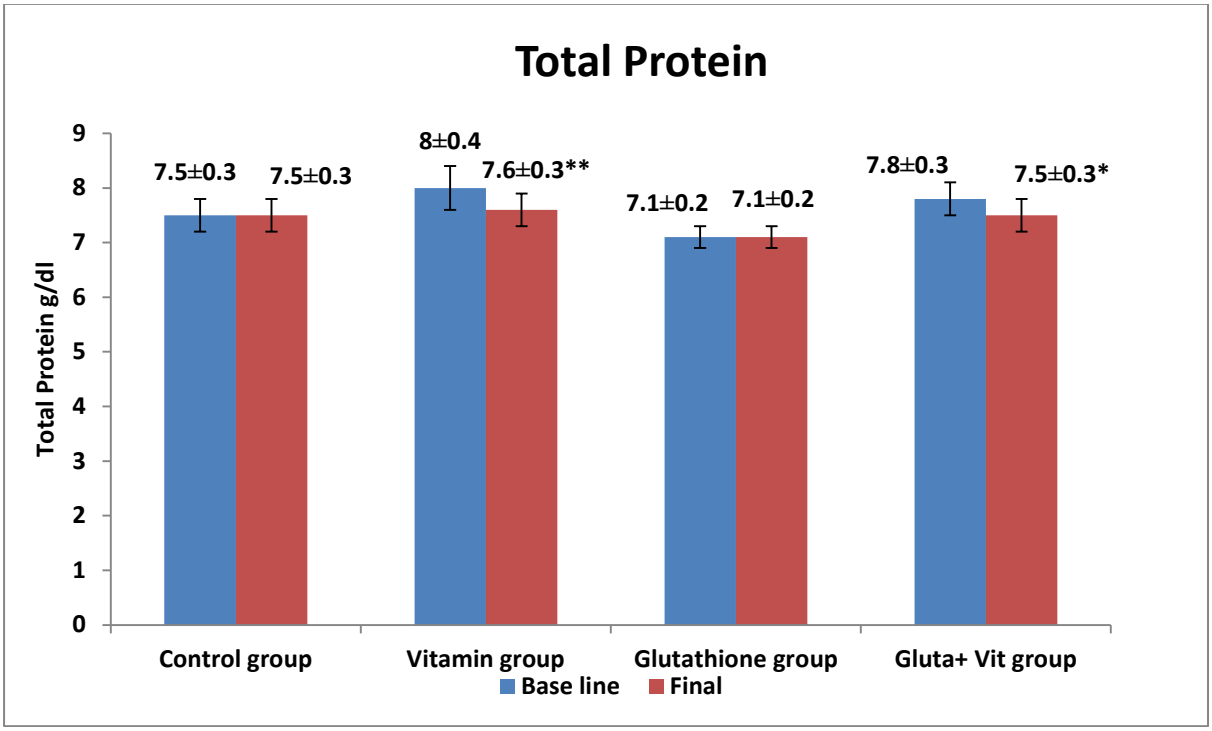

Figure2: Effect of Antioxidants on Total Protein

Values are expressed as mean ± SEM. Significance of difference in the means was determined by Paired t- Test. (*p< 0.05; **p< 0.01;*** p< 0.001)

CONCLUSION

The most important predictor of diabetic nephropathy is microalbuminuria which predicts the onset of renal disease in diabetic patients. Albuminuria reflects glomerular dysfunction in patients with diabetic nephropathy. The main marker of evaluation of diabetic patients is long term glycemic control and prediction of risk for development and progression of diabetic complication. The present study shows 4 months treatment of combination of reduced glutathione plus vitamins C and E supplementations significantly shows significant changes or shows positive results on urinary albumin excretion and total protein, which serves as a marker for glomerular renal function and blood glucose level. It shows that combination therapy of reduced glutathione plus vitamin E plus vitamin C gives synergistic antioxidant activity effect compare to alone administration of either vitamin E or vitamin C or reduced glutathione . So it has been suggested that initiating therapy with combination of antioxidants vitamins have complementary effects can increase the overall efficacy. The early use of combination of antioxidants with oral hypoglycemic agents is expected to improve both acute and long-term outcomes in patients with type 2 diabetes. A once daily

dose of antioxidants has been shown to effectively with a more rapid onset of action coupled with a longer duration of action compared with other oral hypoglycemic agents alone. These findings also concluded that receiving 400 mg vitamin C, 500 mg vitamin E and 50 mg reduced glutathione decreased the urinary albumin excretion ratio in diabetic patients. So it is important to highlight here that such antioxidant therapy, if started at the earlier stage of disease may help in reduce the harm to the development of diabetic nephropathy as well other complications. In this study, patients did not report any side-effects during the course because supplementation of antioxidants when it comes to treat patients with diabetes which is claimed to be devoid of side-effects.

REFERENCES

1. Diabetes Atlas, published by the International Diabetes Federation. 2011, 14.
2. Amy S, Darren K. McGuire, et al. Preventing Macrovascular Complications in Type 2 diabetes Mellitus: Glucose control and beyond. The American Journal Cardiology. 2007; 99 (IIA):5-11.
3. Ossman S, et al. Diabetic Nephropathy: Where We Have Been and Where We Are Going? Diabetes Spectrum. 19:153-157.
4. Thomas S, Viberti G, et al. Diabetic Nephropathy, Medicine Publishing Company Ltd. 2003;91-94.
5. Gross J, et al. Diabetic Nephropathy: Diagnosis, Prevention, and Treatment, Diabetes Care. 2005;28:176–188.
6. Viswanathan V, et al. Prevention of diabetic nephropathy: A diabetologist's perspective. Indian J Nephrol. 2004;14:157-162.
7. Fraser D, Phillips A, et al. Diabetic nephropathy. Medicine Publishing Company, 2007;35(9):501- 502.
8. Fraser DJ, Phillips AO, et al. Diabetic nephropathy. Medicine Publishing Company. 2007; 35(9):501- 502.
9. Deferrari G, et al. Diabetic nephropathy: from microalbuminuria to macroalbunuria. Nephrol Dial Transplant. 1998;13:11-15.
10. Giannini C, Lombardo F, et al. Effects of high-dose vitamin E supplementation on oxidative stress and microalbuminuria in young adult patients with childhood onset type 1 diabetes mellitus. Diabetes Metab Res Rev. 2007;23:539–546.
11. Costagliola L, Romano C, Scibelli G, Vincentiis A, Sorice P, Benedetto A, et al. Anemia and Chronic Renal Failure: A Therapeutically Approach by Reduced Glutathione Parenteral Administration. Nephron. 1992;61:404-408.
12. Shihabi ZK, Kone JC, Connor ML, et al. Albuminuria VS Urinary total protein for detecting chronic renal disease. Clinical chemistry. 1991;37(5): 620-624.
13. Arneson W, Brickell J, et al. Clinical Chemistry A Laboratory Perspective. F. A. Davis Company publication. 2007;1- 233.
14. Meloni C, et al. Adequate Protein Dietary Restriction in Diabetic and Nondiabetic Patients with Chronic Renal Failure. Journal of Renal Nutrition. 2004; 208-213.