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EVALUATION OF EFFECT OF ANTIOXIDANT VITAMIN IN PATIENTS OF DIABETIC NEPHROPATHY

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ABSTRACT

As diabetes mellitus progresses patients are at risk of developing specific complications, such as neuropathy, retinopathy and nephropathy. Where Diabetic nephropathy is a life-threatening condition associated with diabetes mellitus and is the leading cause of end-stage renal failure for patients in many countries. The study was designed to evaluate the effect of vitamins as an antioxidant in diabetic nephropathy patient. And to evaluate the effect of antioxidants treatment on renal parameters like, microalbuminuria and creatinine along with glycaemic parameter like HbA1c. In a randomized, open controlled clinical trial 40 type 1 and type 2 diabetic patients (29 men and 11 women) aged 30–80 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 >30 mg/dl) divided in 2 groups control and vitamin. Where the patients in vitamin group was receiving daily supplement of antioxidant vitamin (400 mg). Observation indicate that after 3 months of supplementation, levels of UAE decreased in, Vitamins group compare to control group ($p = 0.001$, $p = 0.05$ respectively). HbA1c decreased in, Vitamins group ($p = 0.01$). Level of serum creatinine significantly decreased in vitamin group ($p = 0.004$). Blood sugar level, systolic BP, diastolic BP did not significantly change in any group. The data demonstrate that treatment with vitamins E is significantly lowers UAER, Glycosylated hemoglobin and serum creatinine in diabetic nephropathy patients.

KEYWORDS: Diabetic nephropathy; microalbuminuria; vitamin E; oxidative stress and antioxidants.

INTRODUCTION

Diabetic nephropathy (DN), defined by persistent proteinuria, a progressive loss of the glomerular filtration rate (GFR) over time, arterial hypertension (Giancarlo Viberti 1991). And is the medical term for kidney disease. Recent research indicates that nephropathy can even affect people with prediabetes. The high glucose levels in the blood can damage the membranes within the kidney's nephrons that are responsible for filtering the blood and forming urine (Priyanka Tilak, 2007).

There are two different criteria of diabetic nephropathy in the medical literature. First one defines that persistent albuminuria (urinary albumin excretion rate, UAER >300 mg/24hours or 200 g/minute) is the hall-mark of diabetic nephropathy. Another one defines that persistently raised UAER already above arbitrary established normal range, so-called microalbuminuria (UAER>30mg/24hoursor20g/min (Czekalski 2005). The peak onset of nephropathy in type 1 diabetes is between 10 and 15 years after the onset of the disease. Those patients who have no proteinuria after 20 to 25 years have a risk of developing overt renal disease of only about one percent per year (Amalkumar Bhattacharya 2005).

Oxidative stress has a critical role in the pathogenesis of diabetic nephropathy. There are a number of sources for the formation of reactive oxygen species in diabetes, including the auto-oxidation of glucose, lipid per oxidation, AGEs, mitochondrial respiratory chain deficiencies, xanthine oxidase activity, peroxidases, nitric oxide synthase (NOS)(KARIN JANDELEIT et al 2005). Anti-oxidants are substances capable to mop up free radicals and prevent them from causing cell damage. The body produces different antioxidants (endogenous antioxidants) to neutralize free radicals and protect the body from different disease leads by the tissue injury. Exogenous antioxidants are externally supply to the body through food also plays important role to protect the body (Y. S. R. Reddy). Key to the development of diabetic nephropathy is the hyperglycemic state. Its affects directly or indirectly Glucose in sustained high concentration may be directly toxic to cells. Altering cell growth and protein expression and increasing extracellular matrix and growth factor production. Glucose may induce its effect indirectly through the formation of metabolic derivatives such as oxidants, AGE's. AGE's may damage the cells by modification to extracellular matrix protein. The sustained production of such metabolites may results in

continuous activation of different pathways, involving phospholipids kinase (Ralph Rabkin 2003).Antioxidants counter the action of free radicals by several mechanisms. the total antioxidant capacity in plasma of type 1 diabetics was shown to be 16% lower than that of normal subjects. The vitamin C and E combination can also be safely used in high doses to help prevent diabetes and cardiovascular disease. Numerous studies have demonstrated that antioxidant vitamins and supplements can help lower the markers indicative of oxidant stress and lipid per oxidation in diabetic subjects(RojaRahimi et al 2005). Vitamin E may prevent glycation modifications to proteins that are likely to occur in diabetes. Vitamin E has been shown to inhibit the glycation of hemoglobin, which serves as a biomarker for the diagnosis of diabetes in a clinical setting. Vitamin E appears to protect against macromolecule damage especially lipid per oxidation in experimental diabetes (Robert Pazdro 2010). Oral vitamin E treatment appears to be effective in normalizing retinal hemodynamic abnormalities and improving renal function in type 1 diabetic patients of short disease duration without inducing a significant change in glycemic control. This suggests that vitamin E supplementation may provide an additional benefit in reducing the risks for developing diabetic retinopathy or nephropathy (Brusell et al 1999). Some studies demonstrate that high-dose vitamin E supplementation reduces markers of oxidative stress and improves antioxidant defense in young patients with T1DM. However, although it positively affects the oxidant/antioxidant status, vitamin E supplementation does not reduce AER in patients with T1DM and persistent MA.High-dose vitamin E supplementation reduces markers of oxidative stress and improves antioxidant defense in young patients with T1DM (Giannini et al 2007).

MATERIALS AND METHODS

A randomized, open controlled clinical trial was conducted on 40 type 1 and type 2 patients (29 men and 11 women) aged 30–80 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 >30 mg/dl). The calculated sample size is 40 patients in two groups (control group and vitamin group) in order to have 100% power to detect urine albumin excretion. At institute of urology, Dhule.

Each subject received one tablets per day for a period of 4 months. Tablet contents vitamin E (400 mg). During study patients Urine and Serum sample measurements were done at the start of the run-in phase, at randomization, and at the end of each treatment period.

Biochemical estimations

The urine and serum samples were analyzed for biochemical parameters like serum creatinine, and microalbuminuria, BSL-fasting/pp, blood pressure and Glycosylated haemoglobin (Lin *et al.* 2003).

1) Micral (URINE ALBUMIN AND SERUM ALBUMIN)

Urine albumin

This test is most often done to detect diabetic nephropathy in a person who has had diabetes for several years. Detectable levels of the protein albumin in the urine signal the beginning of a condition called microalbuminuria, and are typical in disorders such as diabetic nephropathy. The test may show whether you are at risk for developing kidney disease (Brickell *et al.* 2007).

Normal values

Albumin >20 mg/dl or 30-300 mg of albumin in two different 24-hour urine samples is considered microalbuminuria.

Serum creatinine

Procedure

50µl. of serum sample is taken in one test tube and 1 ml of working reagent is been added. Mixed it well and labelled as Test. Similarly 50µl. of standard solution is taken in another test tube and 1 ml of working reagent is been added and mixed well and labelled as Standard. Both the test tubes are incubated at room temperature and the absorbance (A1) is measured after 30 sec. of incubation at 505 nm (490 – 520 nm). The absorbance (A2) is measured after 120 sec. from A1. Determine $\Delta A = A2 - A1$.

Normal values

A normal (usual) value is 0.8 to 1.4 mg/dl. Normal value ranges may vary slightly among different laboratories. Females have a lower creatinine than males, because they have less muscle mass (Kaplan *et al.* 2003).

HbA1c

Principle of HbA1c

The Micromat II HbA1c test uses boronate affinity chromatography to separate the glycated hemoglobin fraction from the non-glycated fraction. After a test cartridge has been placed

into the instrument, a small sample of blood is added to the first sample tube. The blood is instantly lysed to release the hemoglobin and the boronate affinity resin binds the glycated hemoglobin. After a short incubation step, the liquid is poured into the central funnel of the test cartridge, and non-glycated fraction is collected in an optical chamber where the hemoglobin concentration is photometrically measured. The glycated hemoglobin remains bound to the boronate affinity resin, which sits at the bottom of test cartridge funnel. The boronate affinity resin is then washed with content of second tube. The final step is elution of the glycated hemoglobin off the boronate affinity resin using the third tube. The glycated hemoglobin concentration is measured and the HbA1c concentration in the sample is calculated by the instrument (Dandona *et al.* 1979).

Normal values

5 – 6%. Note that normal values may vary among different laboratories.

Blood pressure estimation

Blood pressure is a measurement of the force applied to the walls of the arteries as the heart pumps blood through the body. The pressure is determined by the force and amount of blood pumped, and the size and flexibility of the arteries. Blood pressure is continually changing depending on activity, temperature, diet; emotional state, posture, physical state, and medication use (Giunti*et al.* 2006).

Measurements

Measurements were done at the start of the run-in phase, at randomization, and at the end of each treatment period of the open controlled phase. All the Urine and Serum sample were measured by Automated Biochemistry Analyzer VITROS Fusion 5 FS1 (JHONSON and JHONSON company), and the average of these measurements was used.

RESULTS

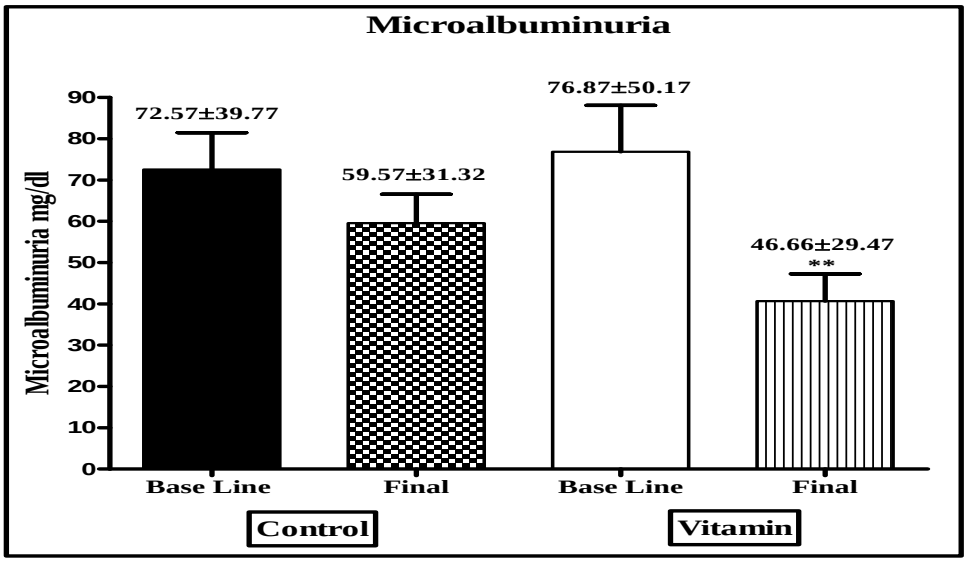
Distribution of patients in two groups, group depends upon age, sex, weight, height, body mass index (BMI). We recruited 40 patients with diabetic nephropathy (29 males and 11 females. The baseline demographic characteristics of the whole group of enrolled subjects are shown in Table.

Table 01: Effect of Antioxidants on the Microalbuminuria in the patients with Diabetic.

Sr no.	Baseline characteristics of patients		Control group (Standard Treatment)	Vitamin group (Antioxidant supplement)
1	Age	30-40 Years	01	00
		41-50 Years	04	06
		51-60 Years	11	07
		61-70 Years	04	05
		71-80 Years	00	02
2	Sex	Male	15	14
		Female	05	06
3	BMI	Below 18.5	01	01
		18.5 - 24.9	17	12
		25 - 29.9	01	06
		30.0& Above	01	01

Nephropath

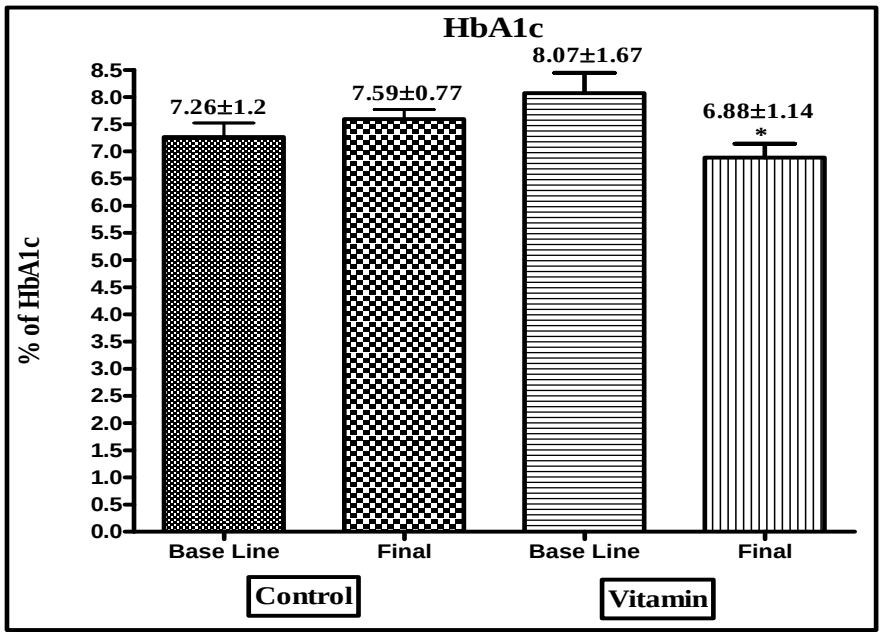
The difference between baseline values and final values of microalbuminuria values in the patients with control group there was not significant decline in microalbuminuria level. While in vitamin group there was significant difference (p=0.001) decline in microalbuminuria level from 76.87±50.17 to 40.66±29.47. The statistical comparison between the microalbuminuria level in the control and vitamin groups at the end of three months revealed a significant difference (p<0.05) decrease in the vitamin group patients.



Statistical analysis using paired t- test is performed and values are present in Mean ± SD, ** p<0.01.

Fig 1: Effect of Antioxidants on the Microalbuminuria in the patients of Diabetic Nephropathy.

The difference between baseline values and final values of HbA1c values in the patients with control group there was not significant decline in HbA1c level. While in vitamin group there was significant difference (p=0.01) decline in HbA1c level from 8.07±1.67 to 6.8±1.14. The statistical comparison between the HbA1c level in the control and vitamin treated groups at the end of three month revealed a significant difference (p<0.05) decrease in the Vitamin group patients.

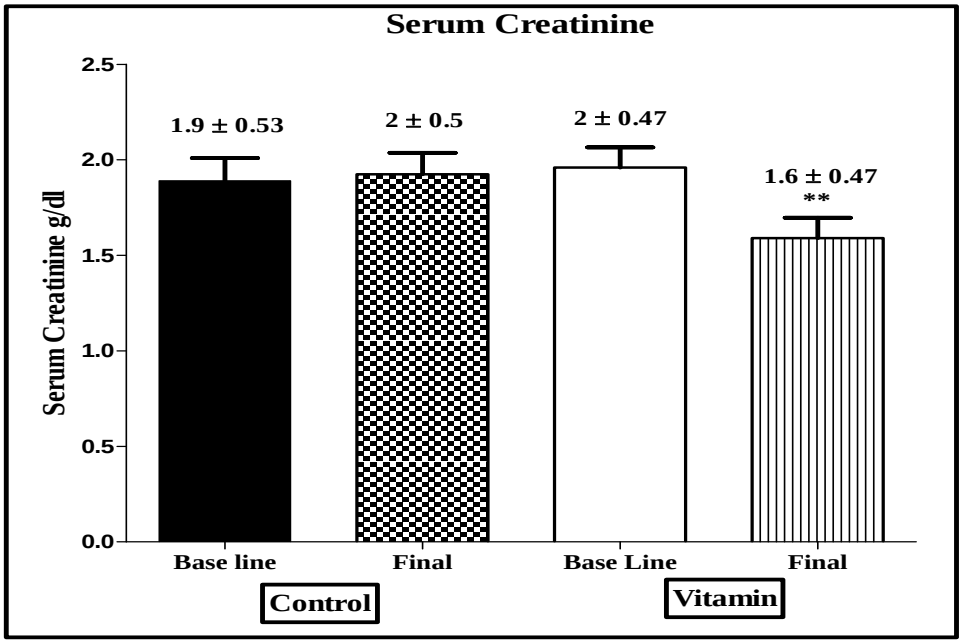


Statistical analysis using paired t- test is performed and values are present in Mean ± SD, * p<0.05.

Fig 2: Effect of Antioxidants on the HbA1c in the patients of Diabetic Nephropathy.

Serum creatinine

The difference between baseline values and final values of Serum creatinine values in the patients with control group was not significant result obtain. But in vitamin group there was a significant difference (P=0.004) decline in creatinine level from 2±0.47 to 1.6±0.47. The statistical comparison between the creatinine level in the control and vitamin treated groups at the end of three month revealed a significant difference (p<0.05) decrease in the Vitamin group patients.



Statistical analysis using paired t- test is performed and values are present in Mean ± SD, ** p<0.01.

Fig 3: Effect of Antioxidants on the serum creatinine in the patients of Diabetic Nephropathy.

Table 02: Effect of antioxidants on the biochemical parameter of Diabetic nephropathy patients.

Parameters	Control Group		Vitamin Group	
	Baseline	Final	Baseline	Final
Microalbuminuria	72.57 ± 39.77	59.57 ±31.32	76.87 ±50.17	40.66 ±29.47**
Serum creatinine	1.9 ± 0.53	2 ± 0.50	2 ± 0.47	1.6 ± 0.47**
HbA1c%	7.26 ± 1.20	7.59 ± 0.77	8.07 ± 1.67	6.88 ± 1.14*
Fasting	166.1 ± 52.46	170.5 ± 26.9	164.0 ± 60.8	144 ± 21.80
PP1	213.4 ± 50.41	218.2 ± 30.67	218.1 ±72.49	187.8 ± 39.57
Systolic	133.5 ± 15.9	136 ± 9.7	131.5 ± 15.3	131 ± 13.57
Diastolic	83.5 ± 8.7	88.10 ± 4.3**	84 ± 9.94	84 ± 7.5

DISCUSSION

The present study shows that 3 months of treatment with vitamin E supplementations significantly lowered urinary albumin excretion, which serves as a marker for renal function. Microalbuminuria predicts the onset of renal disease in diabetic patients. Long term vitamin E supplementation in patients with diabetes and vascular disease had no significant effect on micro vascular outcomes including nephropathy. But some studies show that antioxidants such as vitamin E and probucol retard the progression of renal disease(Cooper et al 2005). 'Giannini' has shown that there is no significant decrease in HbA1c and no significant results

were obtained about creatinine in vitamin vs control groups. So like this some studies reported that there is no significant effect of antioxidant vitamin E on glycemic / microalbuminuria control. But here it shows the whole duration of current study show or demonstrating that antioxidant supplement is able to reduce the microalbuminuria, HbA1c% and creatinine in terms of development of diabetic nephropathy. Our study results shows that the difference between the baseline values and the final values of microalbuminuria in the control group patients show no significant decline in the microalbuminuria. While in the vitamin group significant decline in the microalbuminuria was observed. At the end of three months revealed the significant decrease in the vitamin group patients. In this study decrease in the serum creatinine level was observed in the vitamin group and it was statistically significant. The positive results were obtained in serum creatinine level in vitamin and negative in control group, when it was compared with baseline with final readings. There is no significant decline was observed in systolic Blood pressure as compare to diastolic in control group. And no any significant change in vitamin group about Blood pressure. Decrease in the diastolic blood pressure was observed in the control group was not statistically significant as compared to the baseline readings; only vitamin treated group showed statistically significant results. Present study demonstrates that vitamin E receiving group shows significant change i.e. improved renal function and glycemic that is HbA1c status as compared to control group. It shows that therapy of antioxidant vitamin E (Evion 400) gives synergistic antioxidant effect as compared to control group. And significantly reduces urinary albumin excretion rate and oxidative stress.

CONCLUSION

The current study results shows that short duration of dose of vitamin E treatment in type 1 and type 2 diabetic patients with < 15 years duration of diabetes and there analysis of renal and Glycemic parameters. The one of most important predictor of diabetic nephropathy is microalbuminuria. Where albuminuria reflects the glomerular dysfunction in patients with diabetic nephropathy. The present study shows that 3 months treatment of vitamin E supplementation significantly lowered the urine albumin excretion rate, which serves as a marker for glomerular renal function. Also there is significant decreased in the level of serum creatinine comparatively control group. The marker of evaluation of long term glycemic control in diabetic patients and predicts the risk for development and progression of diabetic complication. In the current study there was significant decline in the final readings of glycosylated hemoglobin of vitamin group.So in current study we concluded that 3 months

treatment of vitamin E (Evion 400) significantly lowered the microalbuminuria, serum creatinine and HbA1c%, without showing any side effect. Which are serve as markers of glomerular renal function and blood glucose level? In contrast to systolic and diastolic blood pressure, Blood sugar level BSL fasting and PP1 were not significantly lowered in vitamin group. So control group was more prone than that of the vitamin group. It is important to highlight here that such antioxidant therapy, if started at the earlier state of disease may help in reduce the harm to the development of diabetic nephropathy.

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