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THE EFFECT OF α-TOCOPHEROL AND ASCORBIC ACID IN REDUCING THE INSULIN RESISTANCE OF EARLY TYPE 2 DIABETES MELLITUS PATIENT: AN OPEN LABEL RANDOMISED CONTROLLED STUDY

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Keywords:

Anti-oxidants, Type 2 Diabetes, Vitamin C, Vitamin E, Insulin Resistance

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ABSTRACT

OBJECTIVE:

To study the efficacy of α-tocopherol(Vitamin E) and Ascorbic acid(Vitamin C) in reducing the Insulin resistance in early diagnosed type II Diabetes Mellitus patients.

METHODOLOGY:

The study was conducted in adult patients with Type 2 Diabetes attending outpatient department, Diabetology, Madras medical college & Rajiv Gandhi government general hospital, Chennai, between May 2013 – April 2014 after ethical committee approval obtained. Trail no.09042013. Treatment period is 4 weeks+4 weeks follow-up per patient. Sample size is 60(30 patients-study drug+ standard treatment, 30 patients-standard treatment). After giving detailed note about the study, patient's consents were obtained and enrolled. The enrolled patients were randomized into either Control group (Metformin 500 mg OD) or Study group(Vitamin C 500 mg OD ,Vitamin E 400 mg OD +Metformin 500 mg OD) and received the respective therapy by simple randomization. Assessment of Insulin Resistance was done by Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) along with Baseline lab investigations before and after treatment.

RESULTS:

124 patients were screened out of which 60 patients were included in the study and all patients completed the study and were included in the analysis. A significant reductions in; Fasting plasma insulin(μU/ml) in study group -10.12 than control group -5.91(p-0.037); Insulin resistance(mass units) in study group -4.95 than control group -2.54 (p-0.030); Fasting Plasma Glucose(mg/dl) in study group -38 than control group -34 (p-0.020) ; Post-Prandial glucose(mg/dl) in study group -75 than control group -55 (p-0.038) noted. Commonest adverse effect found to be GI disturbances which is almost similar in study group(33.3%) and control group (36.6%).

CONCLUSION:

Anti oxidants along with standard therapy (Metformin) is highly effective in reducing the Insulin Resistance in newly detected Type 2 Diabetes mellitus patients than standard therapy alone.

1.0 INTRODUCTION

Type II Diabetes mellitus is characterised by normal or increased insulin level in blood with hyperglycemia due to insulin resistance[1].Chronic stress has been proved to be the most important factor in the pathogenesis of type 2 diabetes[2] followed by oxidative stress which causes hyperglycemia and insulin resistance respectively. Hyperglycemia is caused by neoglucogenesis induced by stress hormones like adrenaline, corticosteroids, glucagon, growth hormone, ACTH, somatostatin, released due to chronic stress[3]. When insulin binds with the extracellular α subunit of insulin receptor, the cytosolic domain of β subunit of insulin receptor is a tyrosine kinase gets activated. As a result tyrosine residue gets autophosphorylated and this inturn helps in the phosphorylation of other proteins like Insulin receptor substrate(IRS). Phosphorylated IRS activates other protein kinases, phosphatases, and insulin mediated glucose transporter (GLUT 4) present in the intracellular pool leading to biological action of insulin[4].

The insulin receptor complex undergoes internalisation. Once inside the cell insulin is degraded in the lysosomes and the receptor recycled to the cell surface. Oxidative stress increase the phosphorylation of serine / threonine sites on β subunit of insulin receptor and intracellular IRS protein which inhibit the extent of insulin stimulated tyrosine phosphorylation. This attentuates the insulin signalling via effects on IRS proteins causing insulin resistance. In addition the serine / threonine phosphorylated forms of IRS become susceptible to proteosome mediated degradation and this promotes the degradation of insulin receptor thus decreasing the number of surface receptors. This causes down regulation of insulin receptors[5]. Chronic oxidative stress and hyperglycemia is the cause for microvascular and macrovascular complications of Diabetes mellitus[6] . In tissues like renal tubules , hepatocytes, erythrocytes, neurons, retina, cornea, lens etc. glucose enters the cell by non insulin dependent glucose transport. Excess glucose is converted to sorbital by enzyme aldose reductase. Sorbital dehydrogenase oxidize sorbital to produce fructose. In tissues like retina, lens, kidney and nerve cell the enzyme sorbital dehydrogenase is low or absent as a result sorbital gets trapped in cells causing water retention due to strong osmotic effect. This causes various microvascular complications like retinopathy, nephropathy, cataract, neuropathy, hemolysis etc[7]. The macrovascular complications of diabetes like coronary artery disease (CAD), cerebro vascular accident (CVA), and peripheral vascular disease are caused by sudden vasospasm induced by depletion of EDRF (nitric oxide) in the blood vessel due to increased presence of reactive oxygen species (ROS). Nitric oxide (NO) gets inactivated by

ROS and converted into nitrates and nitrites like peroxynitrates (O=NOO) [reactive nitrogen species(RNS)][8]. Free radicals are highly reactive molecular species with an unpaired electron either donate or extract an electron with other molecules in order to achieve stability. They cause damage to nucleic acids , proteins and lipids in cell membrane and plasma lipoproteins. The most damaging radicals are oxygen radicals called as reactive oxygen species (ROS) like superoxide ($O_2^{-)}$, hydroxyl (OH^-), perhydroxyl ($O_2H^{\cdot}$)[9].

These are formed continuously as by-products of aerobic metabolism, through reactions with drugs, environmental toxins, infections, chronic hyperglycemia, radiation etc[9]. Tissue damage caused by free radicals are called as oxidative damage and the factors that protect against damage are called as antioxidants[9]. In normal cells there is a balance between prooxidants and antioxidants. This balance can be shifted towards prooxidants, when the production of oxygen species increased greatly or when the levels of antioxidants are diminished. This state is called oxidative damage if the stress is massive and prolonged[9].

Antioxidants like Ascorbic acid, α- tocopherol and β-carotene, help in scavenging , suppressing the formation or opposing the action of ROS[9]. In type II diabetes mellitus antioxidants act by scavenging free radicals decreases the phosphorylation of serine / threonine and thus facilitates the phosphorylation of tyrosine in the β subunit of insulin receptor which activates multiple signalling pathways leading to biological actions of Insulin[5]. Glucagon along with epinephrine, cortisol and growth regulatory hormones opposes many action of Insulin, especially glucagon helps to maintain blood glucose level. Under normal condition hypoglycemia is the potential signal for α- cell activation and secretion of glucagon[4]. Elevated levels of epinephrine or norepinephrine produced by sympathetic activation due to chronic stress stimulate the release of glucagon irrespective of blood glucose level , in anticipation of increased glucose use. In contrast insulin levels are decreased. Oxidative stress which causes sympathovagal imbalance[10], increases the sympathetic nervous system (SNS) activity. This in turn increases the release of stress hormones like glucagon. Antioxidants like Vitamin E & C decrease the insulin resistance by scavenging ROS and by modulating the SNS, decrease the release of stress hormone, thus improves glycaemic control in type II diabetes. Antioxidants also protects the pancreatic cells from free radical injury and arrest the disease progress[5].Therefore in this study α-tocopherol (vitamin E) and Ascorbic acid (vitamin C) are given in combination due to their synergistic action[11,] as an add on therapy for good glycaemic control, prevent the progression and complication in newly diagnosed Type II Diabetes mellitus.

2.0 OBJECTIVE

To study the efficacy of α-tocopherol and ascorbic acid in reducing the insulin resistance in early diagnosed type II Diabetes Mellitus

3.0 METHODOLOGY

3.1 Study design:

A randomized open label, prospective, parallel group ,two arms, comparative study.

3.2 Study population:

Recently diagnosed type 2 diabetic patient attending diabetic outpatient department.

3.3 Study Centre:

Institute of diabetology , Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai.

3.4 Study duration:

4 weeks of treatment + 4 weeks follow-up per patient.

This study was carried from May 2013 – April 2014

3.5 Sample size:

60(30 patients- Control group, 30 patients- Study group)

3.6 Eligibility criteria

Inclusion criteria:

- Age - 18 to 70 years
- Sex - both genders
- Patients diagnosed with type 2 diabetes within 6 months
- Subject willing to give written informed consent
- Subject capable and willing to comply with all study procedures

Exclusion criteria

- Patient with severe medical comorbidities
- H/o Hypersensitivity to any component of study medication
- Subject with diabetic complications
- Subject who participated in an investigational drug or device study within 30 days prior to study screening
- Pregnant or lactating women

3.7 Study procedure:

The study conducted after obtaining the approval from Instituitional Ethics Committee. Patients recently diagnosed with type 2 diabetes and undergoing regular treatment as

outpatient in ,Institute of Diabetology, Madras Medical College and Rajiv Gandhi Government General Hospital were briefed about the study purpose and procedures..

Written informed consent obtained from subjects willing to participate in the study, in the prescribed format in regional language. If the patient was illiterate, left thumb impression was sought. This was done in the presence of an impartial witness.

3.8 Screening

The subjects screened by complete medical history, clinical examination and blood investigations. The demographic details of the patients obtained and recorded. Subjects who fulfilled the inclusion and exclusion criteria were enrolled in the study and randomized to receive either the standard therapy alone or study drug along with standard therapy. Improvement measured by estimating insulin resistance using Homeostatic model assessment (HOMA)

3.9 Recruitment

124 patients were screened and total of 60 patient recruited .30 patients in each group.

3.10 Randomization

The enrolled patients randomized by simple randomization into either Control group or Study group and received the respective therapy.

3.11 Treatment plan:

Control Group (n=30 patients):

Standard treatment**:**

- T.Metformin 500 mg BD

Study Group (n=30 patients):

- Standard treatment

  Along with

- C.Vitamin E 400mg and & T.Vitamin C 500mg/day/patient respectively.
- Patients received above therapy for four weeks in each group
- Patients asked to return empty strips to check the compliance

3.12 Follow up:

Routine follow-ups were done every 2 weeks for a period of 4 weeks during the study period.

3.13 Compliance :

Patient compliance checked by empty strips collection and daily drug remainder chart.

3.14 Adverse drug effects :

Patients were advised to report as soon as possible in case of any adverse drug effects or occurrence of other illness or consumption of concomitant medications. Any adverse event observed or reported by the patient was recorded.

3.15 Withdrawal from Study

During the trial the subject was free to withdraw his/her voluntary consent. In case of any adverse effect either observed by the physician or reported during the study period, need of any additional anti diabetic drugs the patient was withdrawn from the study and appropriate medical care was given.

3.16 Statistical analysis:

The obtained data was analyzed statistically. Distribution of age was analysed using ANOVA and Sex distribution was analyzed by Chi square test. The insulin resistance status were performed on Day 0 and at the end of 4 weeks. The difference within the groups before and after treatment were analyzed using student's paired t-test. Whereas the difference between the Control and Test groups were analyzed using One Way ANOVA. . $p < 0.05$ is considered to be statistically significant.

4.0 RESULTS

124 patients were screened, of which 60 patients who met the inclusion criteria were recruited. 64 patients were excluded from the study based on exclusion criteria. Patients were randomised to either of two groups, control group or study group. Medications are dispensed as per protocol and patients were assessed for Glycaemic control and reduction of Insulin Resistance. Adverse drug reactions was monitored throughout the study period and follow up period. All the patients completed the study and results were analysed statistically.

TABLE 1 : AGE DISTRIBUTION

Table -1 shows the Age distribution of patients among the control and Study group.

GROUPS	n (No of patients)	MEAN AGE (in years)	SD	p VALUE
CONTROL	30	48	10.06	0.266
STUDY	30	46	11.05	

Table -2: GENDER DISTRIBUTION

Table -2 shows the distribution of male and female patients of two groups

SEX DISTRIBUTION	GROUPS			
	CONTROL		STUDY	
	N	%	n	%
MALE	15	50%	13	43%
FEMALE	15	50%	17	57%
TOTAL NO. OF PATIENTS	30		30	

TABLE 3 : BMI

Table -3 shows the BMI distribution of patients among the control and Study groups

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN (kg/m^2)	SD	MEAN (kg/m^2)	SD	
CONTROL	29.7	4.478	29.5	4.469	0.430
STUDY	30.2	4.777	30.3	4.706	0.421
p value	0.357		0.370		

Table –4 : FASTING BLOOD GLUCOSE

Table - 4 shows the mean Fasting blood glucose levels in control and study groups.

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	**SD**	**MEAN (mg/dl)**	**SD**	
CONTROL	153	37.478	119	31.369	0.030
STUDY	142	26.777	104	11.406	0.004
p value	0.357		0.020		

Fig –1 : FASTING BLOOD GLUCOSE

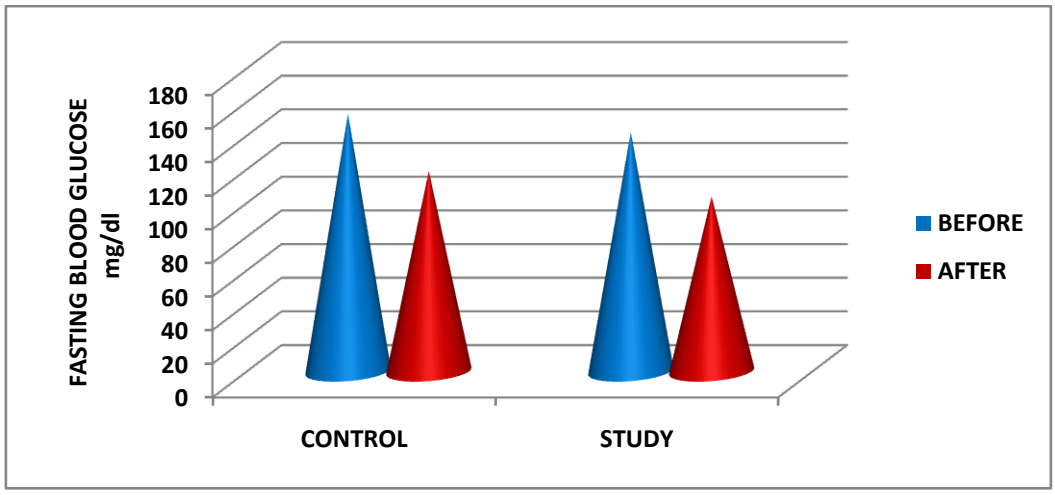

Fig 1 is the diagrammatic representation of Table 4

Table –5 : POSTPRANDIAL BLOOD GLUCOSE

Table - 5 shows the mean Postprandial blood glucose levels in control and study groups.

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN (mg/dl)	SD	MEAN (mg/dl)	SD	
CONTROL	213	47.168	158	22.289	0.042
STUDY	220	35.727	145	17.427	0.032
p value	0.357		0.038		

Fig –2 : POSTPRANDIAL BLOOD GLUCOSE

Fig 2 is the diagrammatic representation of Table 5

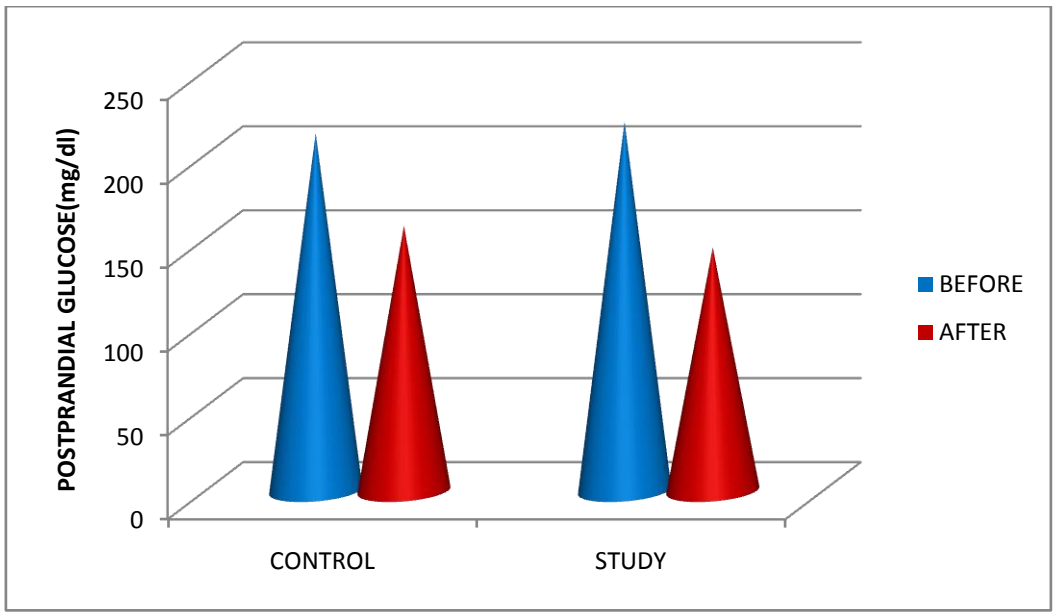

Table –6 : FASTING PLASMA INSULIN

Table - 6 shows the mean Fasting Plasma Insulin levels in control and study groups.

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN (µg/ml)	SD	MEAN (µg/ml)	SD	
CONTROL	19.57	17.924	13.66	9.382	0.115
STUDY	22.33	12.473	12.21	4.658	0.004
p value	0.491		0.037		

FIG –3 : FASTING PLASMA INSULIN

Fig 3 represents Fasting Plasma Insulin levels.

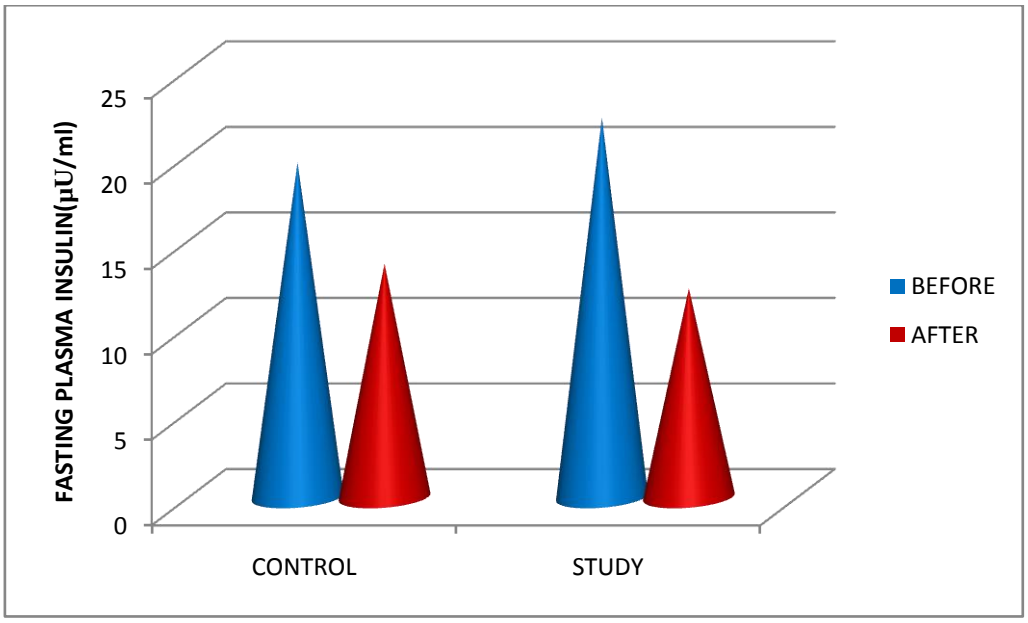

Table –7 : INSULIN RESISTANCE

Table - 7 shows the mean Insulin Resistance in control and Study group

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	7.15	6.669	4.61	3.229	0.066
STUDY	8.49	6.086	3.54	2.501	0.002
p value	0.421		0.030		

FIG –4 : INSULIN RESISTANCE

Fig 4. Insulin Resistance is represented

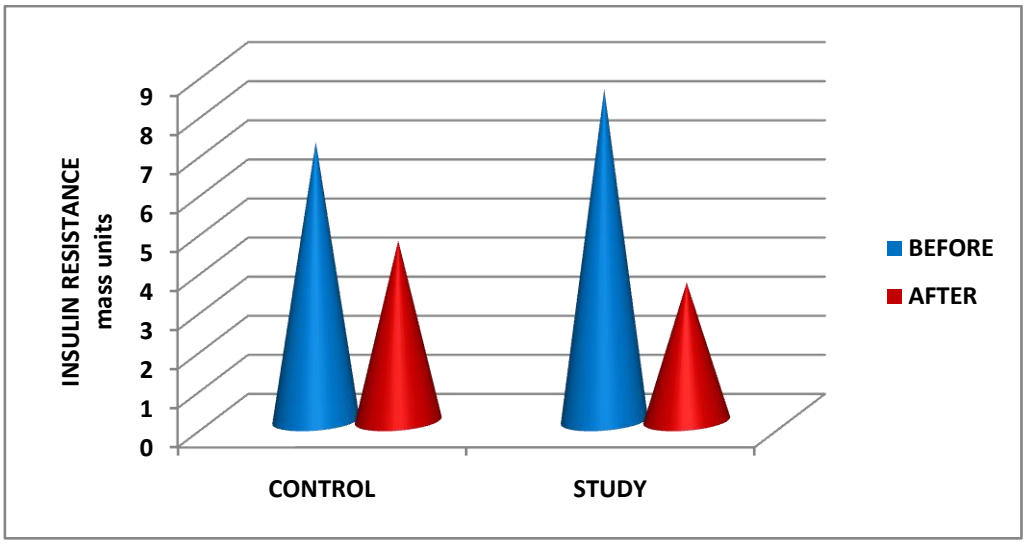

Table –8 : SYSTOLIC BLOOD PRESSURE

Table - 8 shows the mean Systolic Blood pressure in control and Study groups.

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN Mm Hg	SD	MEAN Mm Hg	SD	
CONTROL	133	8.947	134	8.169	0.697
STUDY	132	7.869	124	6.253	0.010
p value	0.760		0.020		

Fig –5 : SYSTOLIC BLOOD PRESSURE

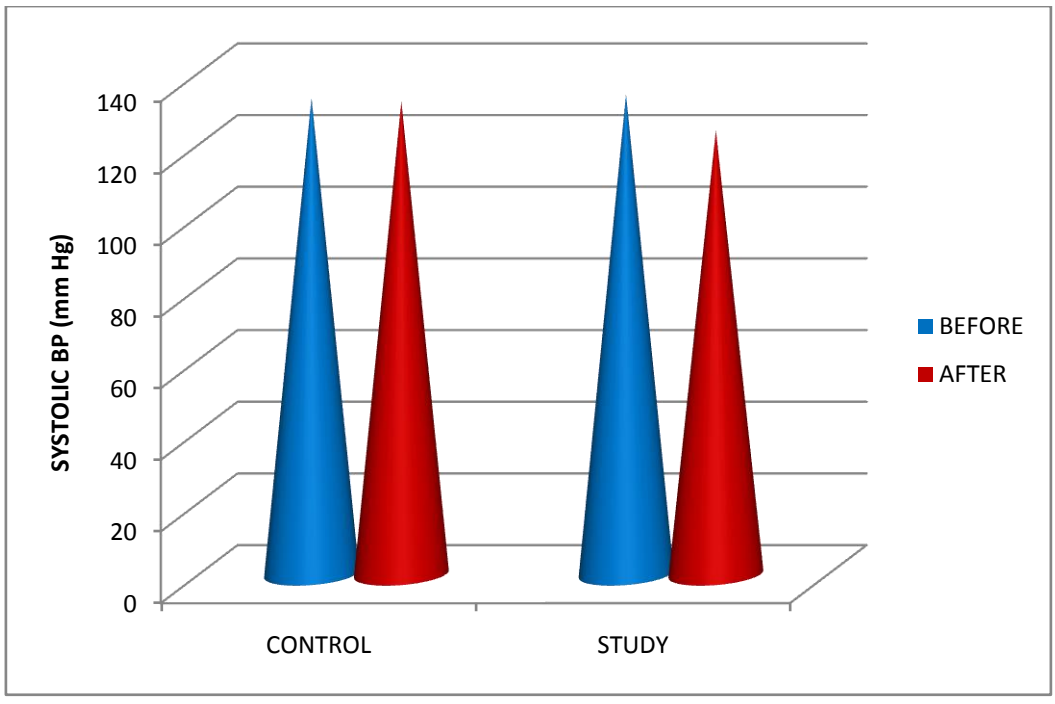

Fig 5 is the graphical representation of Table 8

TABLE 9 : DIASTOLIC BLOOD PRESSURE

Table - 9 shows The mean Diastolic Blood pressure in control and Study groups.

GROUPS	DAY 0		AT THE END OF 8 WEEKS		p value
	MEAN BP (mm hg)	SD	MEAN BP (mm hg)	SD	
CONTROL	86	4.966	85	4.180	0.434
STUDY	85	7.093	79	6.119	0.043
p value	0.451		0.032		

FIG 6: DIASTOLIC BLOOD PRESSURE

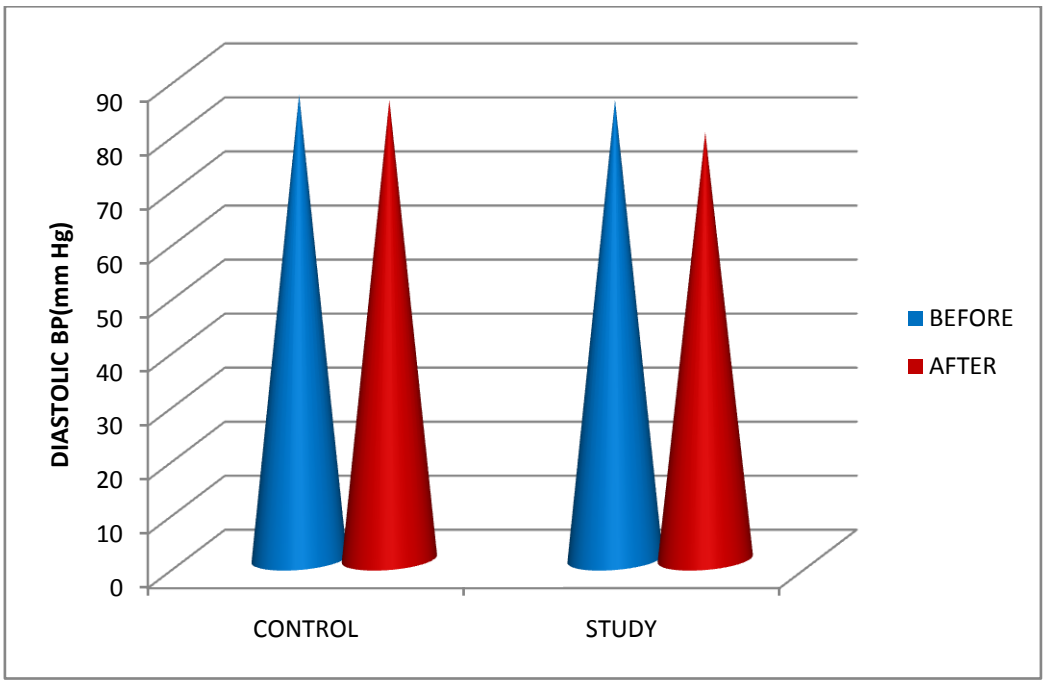

Fig 6 is the graphical representation of Mean Diastolic Blood Pressure

Table10.ADVERSE EVENT PROFILE

ADVERSE EVENT	CONTROL GROUP(30)	STUDY GROUP(30)
	NUMBER OF PATIENT	NUMBER OF PATIENT
NAUSEA	5	6
VOMITING	1	1
ABDOMINAL PAIN	4	3
HYPOGLYCEMIA	9	7
METALLIC TASTE	1	0

FIG.7 ADVERSE DRUG REACTION PATTERN

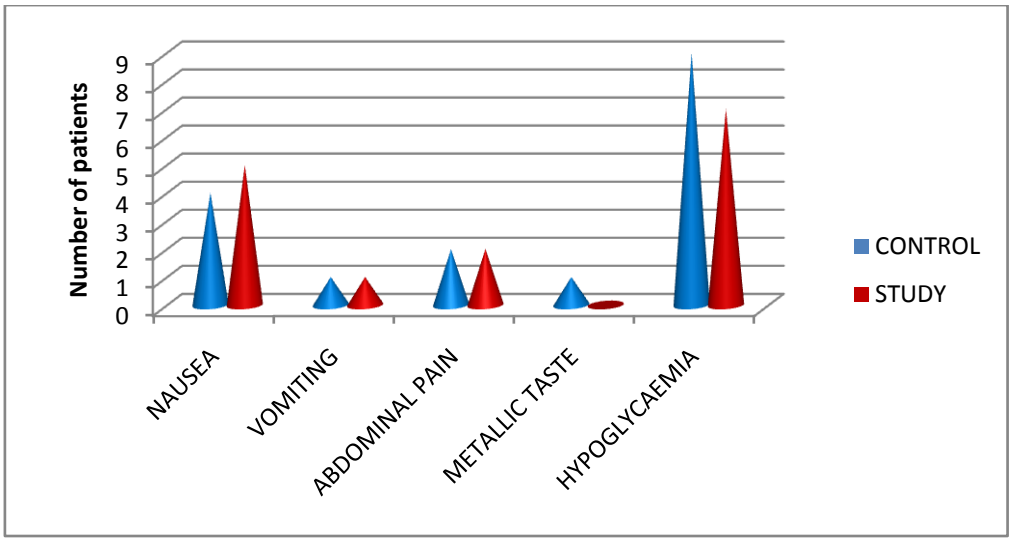

TABLE 11. COMPARATIVE DESCRIPTION OF ADR PROFILE

ADVERSE EFFECT	CONTROL(30) Number of patients	STUDY(30) Number of patients
GI DISTURBANCES	11(36.6%)	10(33.3%)
HYPOGLYCAEMIA	9(30%)	7(23.3%)

5.0 DISCUSSION

Insulin resistance is the most important etiological factor in Type II DM , characterised by hyperglycemia with increased secretion of insulin which fails to compensate.[7] Insulin resistance can be measured by Homeostatic model assessment of insulin resistance (HOMA-IR) using fasting blood glucose level and fasting insulin level.[12]

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{405}$$

Factors responsible for insulin resistance includes chronic stress which leads to increased level of anti insulin hormones and oxidative stress caused by increased production of free radicals.[3] Antioxidants scavenge the free radicals in the cells prevents the abnormal serine phosphorylation and allow the normal tyrosine phosphorylation, intermediate substrates like insulin receptor substrate(IRS) phosphorylation. This results in increased translocation of glucose receptor (GLUT 4) in target tissues and increases the insulin sensitivity.[5]

α-tocopherol and Ascorbic acid are potent antioxidants and also have synergistic action when given in combination[11]. In this clinical study therapeutic effect of combination of VitaminE

400mg and Vitamin C 500mg once daily was given for 4 weeks in recently diagnosed with Type II DM along with metformin 500 mg once daily. Control group patients took Metformin .Study patient took Vitamin E and C along with Metformin .Both the groups were counselled about their life style modification like diet and exercise. The important parameter assessed were fasting blood glucose , fasting plasma insulin and insulin resistance by HOMA method. The patients were assessed by clinical improvement and by measuring insulin sensitivity after 4 weeks of treatment. They were followed for further 4 weeks to assess residual efficacy.

Among the 60 patients who completed the study, the mean age was 48 years and 46 years in the study and control group respectively. This study showed most of the patient were middle aged. No sex difference noted. At the end of the study there was significant reduction in fasting plasma glucose (p-0.004), fasting plasma insulin (p-0.004) and insulin resistance (p-0.002) among the study group. In the control group there was significant reduction in fasting plasma glucose (p-0.030), but no statistically significant changes noted in fasting plasma insulin (p-0.115) and insulin resistance (p-0.066) at the end of the study.

The fasting plasma insulin and insulin resistance was reduced significantly in study group than control group. Stress plays a major role in role in the pathophysiology of Diabetes and cardiovascular diseases especially Hypertension. In our study we observed in routine blood pressure monitoring ,BP was slightly above normal in the range of 140/90 mm hg in both the groups and at the end of the study there was statistically significant reduction of systolic (p-0.010) and diastolic (p-0.043) BP noted among study group.There were no changes noted in systolic and diastolic BP in control group. This reduction in Blood pressure is probably due to the regulatory effect of antioxidants, especially vitamin C on the SNS activity and also prevention of degradation of NO (EDRF) caused by ROS[10].

Adverse effects like G.I disturbances (nausea, vomiting ,abdominal pain) and hypoglycemia present in both the groups. Where as in the study group there was a significant reduction in hypoglycemic episodes. This is probably due to decrease in fasting plasma insulin level due to internalisation of insulin receptor complex followed by degradation of insulin in lysosomes.[4] Hypoglycemia is one of the most important adverse effect of anti-diabetic drugs. Recurrent attacks of hypoglycemia causes severe morbidity even sudden death.

From this study we attribute that free radical injury is one of the important factor in the pathogenesis of Type II DM, insulin resistance and its complications. Antioxidants like alpha-tocopherol and ascorbic acid when given in combination has synergistic action and reduces insulin resistance significantly. In addition antioxidants prevent the oxidative damage

of beta cells and the complication of Diabetes Mellitus like nephropathy, retinopathy, neuropathy etc due to their cytoprotective and anti inflammatory action. In a newly diagnosed Type 2 DM patients, antioxidants can arrest the disease process and act as a disease modifying agent . Therefore antioxidants are novel agent in the treatment of newly diagnosed Type II DM.

6.0 CONCLUSION

In this study we conclude Type 2 DM is a stress induced disease and free radical injury is a major etiological factor in the development of insulin resistance in Type II DM patient.

The combination of α-tocopherol and ascorbic acid in these patients reduced the insulin resistance causing good glycaemic control along with reduction of blood pressure. Incidence of hypoglycemic attacks was decreased. This improves the sense of well being in the patients. Therefore stress management and antioxidants like α-tocopherol and ascorbic acid can be considered as novel treatment in newly diagnosed type 2 DM patients as intial therapy.

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