

RESEARCH ARTICLE

Effect of Insulin Therapy on Endothelial Dysfunction in Type II Diabetic Subjects without Any Complications

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ABSTRACT

Introduction: As we know, endothelial dysfunction (ED) is an initial stage of vascular complications in type II diabetes mellitus. Hypoglycemic drugs have shown beneficial effect on endothelial function. Insulin is a regulatory hormone for endothelial function in vessels. But, there was a controversial report on the effect of exogenous insulin on endothelial function in type II diabetes subjects.

Aim and objective: The study has been designed to observe the effect of insulin therapy on ED in type II diabetic subjects without any complications.

Result: Significantly high level of body mass index (BMI) was identified in insulin-treated subjects than subjects with hypoglycemic drugs and healthy controls. Nitric oxide (NO) has been shown to be significantly lower in insulin-treated subjects compared to subjects with hypoglycemic drugs and healthy controls. There was no significant change in the levels of lipid profile and oxidative stress between two treatment groups.

Conclusion: Significant effect of insulin therapy was observed on ED in type II diabetes mellitus. Exogenous insulin itself may cause ED by hyperinsulinemia due to high-fat diet or high dosage of insulin.

Keywords: Dyslipidemia, Endothelial Dysfunction, Hyperinsulinemia, Type II Diabetes Mellitus.

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INTRODUCTION

Diabetes is a highly complicated disease in worldwide population and the prevalence of diabetic complications is more in number. Antidiabetic treatment was targeted to reduce the level of blood glucose and its complications.¹ However, the prevalence of diabetic complication

is increasing day by day in worldwide population. There are many factors that contribute to the development of complications in diabetes, such as hyperglycemia, dyslipidemia, obesity, ED, oxidative stress, inflammation, and insulin resistance. Here, ED may play a major role in the development of pathogenesis and progression toward vascular complications.^{2,3}

Endothelial dysfunction is an imbalance between vasoconstriction and vasodilatation in vascular tissues. Endothelial function is mainly regulated by endothelial-1, NO, and prostacyclin.⁴ The most common soluble markers to analyze endothelial function include NO, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, E-selectin, and Von Willebrand factor, etc.^{5,6} Among these, NO is an important marker to assess vascular endothelial function and its impairment. Reduced availability of NO indicates impaired endothelial function and is also an initiator for the development and progression of atherosclerosis.⁷

Metformin improves endothelial function in non-diabetic subjects with metabolic syndrome. Addition of metformin to insulin therapy has shown significant improvement of endothelial function, but not chronic in type II diabetic subjects.⁸ Troglitazone improves endothelial function in recently diagnosed type II diabetic subjects and this improvement was strongly associated with reduction in fasting plasma insulin. However, the improvement of endothelial function was not seen in long-term diabetic subjects.⁹ Earlier studies have shown contradictory result on the effect of insulin on both beneficial and inhibitory effects on endothelial function.^{10,11}

Since ED is an initial stage in the development of atherosclerosis, the study has been designed to assess the effect of insulin therapy on ED in type II diabetes mellitus for earlier prediction of cardiovascular risk.

MATERIALS AND METHODS

The study was conducted on 120 subjects with the age of 38 to 59 years; among these, 60 subjects were type II diabetics and remaining 60 were healthy controls. Study subjects were divided into three groups: Healthy controls (n = 60), group I contained subjects with oral hypoglycemic drugs [OHD (metformin, glibenclamide, or glipizide)] (n = 27), group II had subjects with insulin in addition to OHD (OHDI) (n=33).

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Selection of Subjects

Sixty type II diabetic subjects who undergo regular antidiabetic treatment were selected from Government Hospital and Vinayaka Mission's Kirupananda Variyar (VMKV) Medical College & Hospital at Salem. Among these, 27 subjects were treated with OHD alone and remaining 33 subjects were treated with insulin in addition to OHD drugs.

Inclusion and Exclusion Criteria

Type II diabetic subjects with regular treatment and without any diabetic complications were included in the study. Subjects with smoking, alcohol, hypertension, and thyroid disorders were excluded from the study. Ethical clearance was obtained from VMKV Medical College & Hospital to conduct this study.

Sample Collection

A volume of 5 mL of venous blood sample was collected after obtaining informed consent from each subject. Serum and plasma were separated from blood sample after centrifugation at 3000 rpm. Fasting and postprandial sugar, glycosylated hemoglobin (HbA1c), and lipid profile were analyzed on the same day of sample collection in an auto-analyzer. Remaining sample was stored at -20°C in freezer until further analysis. Malondialdehyde (MDA), ferric reducing ability of plasma (FRAP), and NO were done by manual method.

Methods

Estimation of sugar was done by using glucose oxidase peroxidase method;¹² HbA1c was estimated by turbidimetric immunoassay method.¹³ Total cholesterol (TC) was determined by cholesterol esterase peroxidase method;¹⁴ triglyceride (TGL) was measured by glycerol phosphate oxidase and peroxidase method,¹⁵ and high-density lipoprotein (HDL) by immune inhibition 2 reagent method.¹⁶ Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated by using standard

Friedwald's equation.¹⁷ The MDA was manually estimated by thiobarbituric acid reactive substances method¹⁸ and antioxidant capacity was estimated as FRAP.¹⁹ The NO was measured by using kinetic cadmium reduction method.²⁰

Statistical Analysis

Statistical Package for the Social Sciences software, version 21.0, was used to evaluate the statistical significance among the study groups. Mean, standard deviation, and graphs were determined by using Microsoft excel. Analysis of variance "Bonferroni" test was performed for variable in parameters; p-value < 0.05 was considered as statistically significant.

RESULTS

The study has three groups: Healthy controls (n = 60), diabetic subjects with OHD (group I) (n = 27), and diabetic subjects with OHDI (group II) (n = 33).

Table 1 shows significantly high levels of BMI, fasting and postprandial sugars and HbA1c in both groups I and II diabetic-treated subjects compared with healthy controls. But, there was no significant difference among the diabetic-treated groups except fasting sugar, which shows significantly high level in subjects with OHD than in subjects with OHDI.

Significantly high level of TC, TGL, LDL-cholesterol (LDL-c) and VLDL-cholesterol (VLDL-c) were observed in both diabetic groups than in healthy control. But, no significant difference in the level of lipid profile was identified between diabetic-treated groups. We also found no significant difference in the level of HDL-cholesterol (HDL-c) among the three groups (Table 2).

The MDA was significantly high in both diabetic-treated groups compared with healthy controls. But there was no significant difference among the diabetic-treated groups.

Significantly lower level of FRAP was observed in both diabetic-treated groups than in healthy controls (Graph 1).

Significantly lower level of NO was identified in the OHDI group than in the OHD group and in healthy

Table 1: Difference between basic characteristics of study subjects

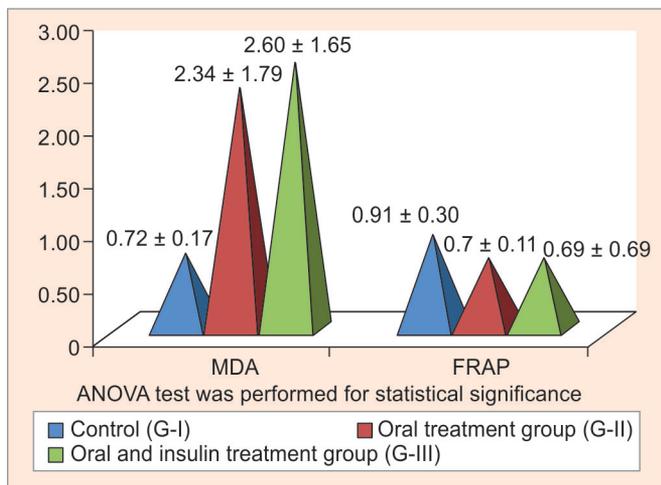
Parameters	Control (n = 60)	T2DM with OHD (group I = 27)	T2DM with OHDI (group II = 33)	p-value
Age	49.3 ± 10.27	48.96 ± 10.65	52.39 ± 9.76	0.310
BMI (kg/m ²)	20.48 ± 0.81	23.41 ± 3.07*	25.30 ± 2.91* [@]	0.000
FBS (mg/dL)	87.62 ± 9.95	171.25 ± 81.54* [§]	147.84 ± 50.89*	0.000
PPBS (mg/dL)	119.65 ± 6.07	280.71 ± 87.44*	274.95 ± 86.45*	0.000
HbA1c (%)	5.21 ± 0.29	9.24 ± 2.64*	8.96 ± 2.20*	0.000

p-value < 0.05 is statistically significant; *Diabetic treated groups are significantly higher than healthy control; [@]Insulin treated group is significantly higher than noninsulin treated group; [§]Insulin treated group is significantly lower than noninsulin treated group; FBS: Fasting blood sugar; PPBS: Postprandial blood sugar; T2DM: Type 2 diabetes mellitus

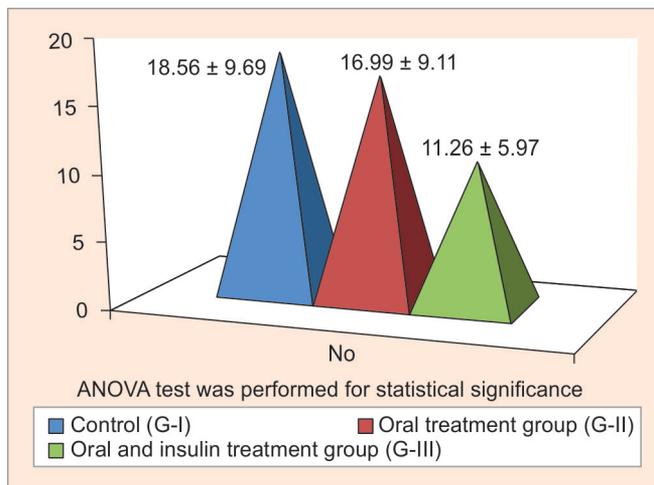
Table 2: Level of lipid profile between three study subjects

Parameters	Control (n = 60)	T2DM with OHD (group I = 27)	T2DM with OHDI (group II = 33)	p-value
TC	158.98 ± 17.91	203.26 ± 39.43*	187.59 ± 40.24*	0.000
TGL	105.07 ± 35.01	156.74 ± 66.99*	166.19 ± 67.53*	0.000
HDL	41.65 ± 9.28	40.41 ± 7.72	41.59 ± 8.40	0.814
LDL	96.32 ± 21.25	194.22 ± 37.28*	179.44 ± 45.43*	0.000
VLDL	21.01±7.00	31.30 ± 13.49*	33.28 ± 13.55*	0.000

T2DM: Type 2 diabetes mellitus; p-value at <0.05 is significant; *Diabetic treated groups have significantly higher than healthy controls



Graph 1: Status of oxidative stress between the study groups



Graph 2: Status of nitric oxide between three groups

controls. The OHD group has shown slightly lower level of NO when compared with healthy controls, but statistically not significant (Graph 2).

DISCUSSION

Type II diabetic subjects are more prone to cardiovascular diseases in worldwide population. Even though the subjects undergo antidiabetic treatment, the complications are more in these cases. Insulin is not a treatment for type II diabetic subjects, but in uncontrolled condition, insulin is required. Earlier studies have reported that hyperinsulinemia itself is a risk factor to cause vascular complications.²¹⁻²³ However, ED is an initial stage of developing vascular complications. The following factors are evaluated to see the effect of insulin treatment on ED in type II diabetic subjects.

Body Mass Index

As we know, obesity is a major factor to cause cardiovascular risk in type II diabetes subjects. The present study has shown significantly high level of BMI in two diabetic-treated groups (groups I and II) than in healthy controls. Earlier studies have reported that metformin can reduce the level of BMI compared with sulfonylurea drugs and insulin.^{24,25} Another study showed that type I and II diabetes have significantly high level of BMI in

intensively treated subjects compared with subjects with conventional treatment.²⁶

The present study also found significantly high level of BMI in insulin-treated subjects compared to subjects with hypoglycemic drugs alone. Type II diabetic subjects, who are treated with insulin, have high calorie intake to prevent nocturnal hypoglycemia. It was also shown that increased BMI might be due to enhanced conservation of ingested calorie. Another reason might be overinsulinized periphery by exogenous insulin when compared with insulinized liver. This causes accumulation of fat mass in insulin-treated subjects.^{26,27} Nevertheless, insulin is an effective treatment for hyperglycemic subjects, but it also has adverse effect on cardiovascular risk by increasing BMI.

Glycosylated Hemoglobin

Significantly high level of HbA1c was observed in both diabetic-treated groups than in healthy controls. But there was no significant difference among diabetic-treated groups. An earlier study found that hypoglycemic drugs have similar effect on lowering HbA1c; nevertheless, metformin was more effective for long-term glycemic control.²⁸ Another study has stated that insulin glargine with metformin and NPH with metformin have shown good glycemic control in diabetes.²⁹ In our study, we have found significantly high level of fasting sugar in the OHDI group than in the OHD group. But, there was no



significant difference in the level of postprandial sugar between two diabetic-treated groups. All diabetic subjects in this study were treated with hypoglycemic drugs alone or in addition to insulin. Here, poor glycemic control and treatment might be the reason for insignificant difference in the level of HbA1c between diabetic-treated groups.

Lipid Profile

Dyslipidemia is one of the foremost recognized factors to cause complications in diabetes mellitus.³⁰ The present study has shown significantly high levels of cholesterol, triglyceride, LDL-c, and VLDL-c in two diabetic-treated groups compared with healthy controls. But, there was no significant difference in the level of HDL-c among the groups. The study also has identified no significant difference in the levels of lipid profile among the diabetic-treated groups. Earlier studies have identified significantly high levels of cholesterol, triglyceride, LDL-c, VLDL-c, and significantly lower level of HDL-c in diabetic subjects than in healthy controls.^{31,32} Most of the studies on type II diabetic subjects have recognized that glycemic control has a major role in maintaining LDL level.³³⁻³⁵

The main target of antidiabetic treatment in type II diabetic subjects is to maintain the glycemic status in blood circulation and reduce the complications. An earlier study has shown the beneficial effect of antidiabetic treatment on lipid metabolism. It also stated that lipid profile can be improved along with development in glycemic control.³⁴ Among hypoglycemic drug-treated subjects, metformin-treated subjects have low LDL level than subjects with sulfonylurea.³⁶ As we know, diabetic dyslipidemia is primarily associated with insulin resistance and poor glycemic control. In our study, poor glycemic control might be the reason for insignificant difference in the level of lipid profile among diabetic-treated groups.

Oxidative Stress

In our study, significantly high level of MDA was found in two diabetic-treated groups compared with healthy controls. High level of MDA was identified in OHDI subjects compared with OHD, but statistically insignificant. Significantly lower level of FRAP was observed in OHDI- and OHD-treated subjects than in healthy controls. But, there was no significant difference in the level of FRAP between two diabetic-treated groups. Earlier studies have reported increased level of MDA and reduced antioxidant in type II diabetes mellitus than in healthy controls.^{31,32,37} Glucose oxidation may be the reason for increase of free radicals. Experimental studies have shown the alteration of proteins and lipids in diabetic subjects. Poor glycemic control might be the reason to stimulate platelet aggregation and

auto-oxidation of glucose and finally, to produce free radicals.^{38,39} Lipids are the primary targets of reactive oxygen species and produce highly reactive aldehydes which include MDA, acrolin, 4-hydroxynonenal, 4-oxononenal, and isolevuglandins.⁴⁰ Poor glycemic control might be the reason for high level of MDA in both diabetic-treated groups.

Endothelial Dysfunction

The present study has shown significant lower level of NO in the OHDI group than in the OHD group and healthy controls. Slightly lower level of NO was observed in subjects with hypoglycemic drugs alone (OHD) compared with healthy controls, but statistically insignificant. Earlier studies have reported that increased availability of NO by metformin therapy in diabetic subjects.^{41,42} This might be the reason for insignificant difference in the level of NO between subjects with hypoglycemic drugs alone and healthy controls.

Clinical studies have reported that insulin sensitizers have beneficial effect on vascular endothelium in diabetic subjects.^{43,44} Chronic insulin treatment improves the vascular relaxation in diabetes, but acute insulin treatment does not show any improvement in vascular relaxation even in diabetic subject in glycemic control.⁴⁵ Insulin is an important promoter for activation of endothelial nitric oxide synthase (eNOS) to produce NO in endothelium. Insulin binds to endothelial receptor and leads to phosphorylation of insulin receptor substance-I (IRS-I) and activates eNOS through phosphoinositide 3 (PI3) kinase/Akt pathway. This mechanism is further substantiated by the fact that mutation of IRS-I and inhibitors of PI3 kinase/Akt can block the activation of eNOS by insulin.^{42,43} Shanik et al⁴⁶ had observed that continuous exposure to high level of insulin or administration of high dosage of insulin can aggravate the extent of insulin resistance and its complication. This might be the reason for lower level of NO in insulin-treated diabetic subjects than in subjects with hypoglycemic drugs alone.

Obesity is another known factor to cause insulin resistance. In obese subjects, adipose tissue releases high amount of non-essential fatty acids, glycerol, hormones, proinflammatory cytokines, and other factors which are responsible for the development of insulin resistance.⁴⁷ Cao et al⁴⁸ had reported that increased basal insulin signaling (Akt-dependent) causes insulin resistance and hyperinsulinemia through the promotion of fat accumulation and oxidative stress in liver and skeletal muscle. This was identified in high-fat diet experimental mice. In our study, we have found high level of BMI in insulin-treated subjects. Hence, this might be another reason for lower level of NO in insulin-treated subjects.

Since, exogenous insulin is a common treatment for type I diabetes mellitus, but for type II diabetes only in uncontrolled condition. Instead of changing medicine or increasing dosages of insulin, physician can advice the patients to go for physical exercises and low-fat diet to control glycemic status. Further follow-up study is required to focus on dosage of insulin to get a precise result on the effect of insulin on ED in type II diabetes mellitus.

CONCLUSION

We have found significant effect of insulin therapy on ED in type II diabetic subjects. We also found that without dyslipidemia and oxidative stress, there will be a chance of ED in insulin-treated type II diabetic subjects. Hence, frequent examination of NO level is also necessary in type II diabetic subjects to predict early onset of complications.

Limitation

The present study has low sample size due to inclusion and exclusion criteria of subjects. Further follow-up study is required on type II diabetes mellitus with different dosages of insulin to give better result on the effect of insulin therapy on ED in type II diabetes mellitus.

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