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RESEARCH ARTICLE

Effect of Insulin Therapy on Endothelial Dysfunction in Type 2 Diabetic Subjects without any Complications

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ABSTRACT

Introduction: As we know endothelial dysfunction is an initial stage of vascular complications in type 2 diabetes mellitus (DM). Hypoglycaemic drugs have shown a beneficial effect on endothelial function. Insulin is a regulator hormone for endothelial function in vessels. But, there was a controversial report on the effect of exogenous insulin on endothelial function in type 2 diabetes subjects.

Aim and objectives: The study has been designed to observe the effect of insulin therapy on endothelial dysfunction in type 2 diabetic subjects without any complications.

Materials and methods: This Study include 120 Subjects (60-Diabetics and 60-Healthy Controls). MDA was manually estimated by thiobarbituric acid reactive substances (TBARS) method and anti-oxidant capacity was estimated as FRAP.[19] NO was measured by using kinetic cadmium reduction method.

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 shown significantly lower in insulin-treated subjects compared to subjects with hypoglycemic drugs and healthy controls. There was no sign in the levels of lipid profile and oxidative stress in between two treatment groups.

Conclusion: Significant effect of insulin therapy was observed on endothelial dysfunction in type 2 DM. Exogenous insulin itself may cause endothelial dysfunction by hyperinsulinemia because of a high-fat diet or high dosage of insulin.

Keywords: Clinical biochemistry, DM, Endothelial dysfunction.

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INTRODUCTION

Diabetes is a highly complicated disease in the worldwide population, and the prevalence of diabetic complications are 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 contribute for the development of complications in diabetes such as hyperglycemia, dyslipidemia, obesity, endothelial dysfunction, oxidative stress, inflammation, insulin resistance, etc. Here, endothelial dysfunction may play a significant role in the development of pathogenesis and progression towards vascular complications.[2,3]

Endothelial dysfunction (ED) 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 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin and von Willebrand factor (vWF) etc.[5,6] Among these, NO is an important marker to assess vascular endothelial function and its impairment. Reduced availability of nitric oxide indicates impaired endothelial function and 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 2 diabetic subjects.[8] Troglitazone improves endothelial function in recently diagnosed type 2 diabetic subjects and this improvement was strongly associated with a reduction in fasting plasma insulin. However, the improvement of endothelial function was not seen in long-term diabetic subjects.[9] Earlier studies have shown a contradictory result on the effect of insulin on both beneficial and inhibitory e ffects on endothelial function.[10,11]

Since ED is an initial stage in the development of atherosclerosis. The study has been designed to assess the impact of insulin therapy on ED in type 2 DM 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 2 diabetic and remaining 60 were healthy controls. Study subjects were divided into three groups; healthy controls

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(n–60), group-I contains subjects with [oral hypoglycemic drugs (OHD) Metformin, glibenclamide or glipizide] (n–27), group-II has subjects with insulin in addition to OHD (OHDI) (n–33).

Selection of subjects

Total 60 type 2 diabetic subjects who undergo regular anti-diabetic treatment were selected from government hospital and Vinayaka Mission's Kirupananda Variyar Medical College at Salem. Among this 27 subjects were treated with oral hypoglycemic drugs alone and remaining 33 subjects were treated with insulin in addition to oral hypoglycemic drugs.

Inclusion and Exclusion Criteria

Type 2 diabetic subjects with regular treatment and without any diabetic complications were included in the study. Subjects with smoking, alcohol, hypertension, thyroid disorders were excluded from the study. Ethical clearance was obtained from Vinayaka Mission's Kirupananda Variyar Medical College to conduct this study.

Sample Collection

Five ml of venous blood sample was collected after obtaining inform consent from each subject. Serum and plasma were separated from the blood sample after centrifugation at 3000 rpm. Fasting and postprandial sugar, hemoglobin A1c (HbA1c) and Lipid profile were analyzed on the same day of sample collection in auto-analyzer. The remaining sample was stored at –20° C freezer until further analyze. Malondialdehyde (MDA), ferric reducing ability of plasma (FRAP) and NO were done by the manual method.

METHODS

Estimation of sugar was done by using glucose oxidase-peroxidase (GOD-POD) methodm[12] HbA1c was estimated by turbidimetric immunoassay method.[13] Total cholesterol was done by cholesterol esterase peroxidase method,[14] triglyceride was measured by GPO-POD method,[15] high-density lipoproteins (HDL) by immune inhibition two reagent method.[16] low-density lipoproteins (LDL) and very low density lipoprotein (VLDL) were calculated by using standard Friedwald's equation.[17] MDA was manually estimated by thiobarbituric acid reactive substances (TBARS) method[18] and antioxidant capacity was estimated as FRAP.[19] NO was measured by using kinetic cadmium reduction method.[20]

STATISTICAL ANALYSIS

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

RESULTS

The study has three groups; healthy controls (n–60), diabetic subjects with OHD group 1 (n–27) and diabetic subjects with OHDI group 2 (n–33).

Table 1 shows significantly high levels of body mass index (BMI), fasting and postprandial sugars and HbA1c were observed in both group I and group II diabetic treated subjects compared to healthy controls. But, there was no significant difference among the diabetic treated groups except fasting sugar, which shows significantly high in subjects with OHD than subjects with OHDI.

Significantly high level of total cholesterol (TC), triglyceride (TGL), LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) were observed in both diabetic groups than 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-C among the three groups (Table 2).

Malondialdehyde (MDA) has shown significantly high in both diabetic treated groups compared to healthy

Table 1: Difference between basic characteristics of study subjects

Parameters	*Control (n = 60)*	*T2DM with OHD (Group 1 = 27)*	*T2DM with OHDI (Group 2 = 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

OHD: Oral hypoglycaemic drugs, OHDI: Insulin in addition to hypoglycaemic drugs

'p' value <0.05 is statistically significant.

'*' Diabetic treated groups are significantly higher than healthy control

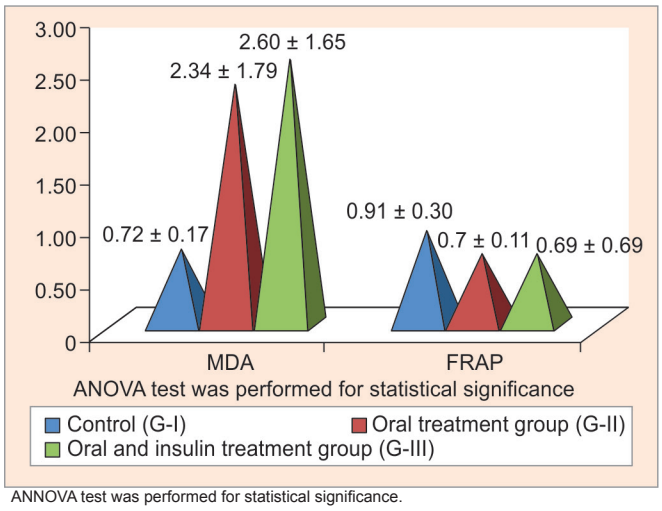
'@' Insulin treated group has significantly higher than non-insulin treated group

'$' Insulin treated group has significantly lower than non-insulin treated group

Table 2: Level of lipid profile between three study subjects

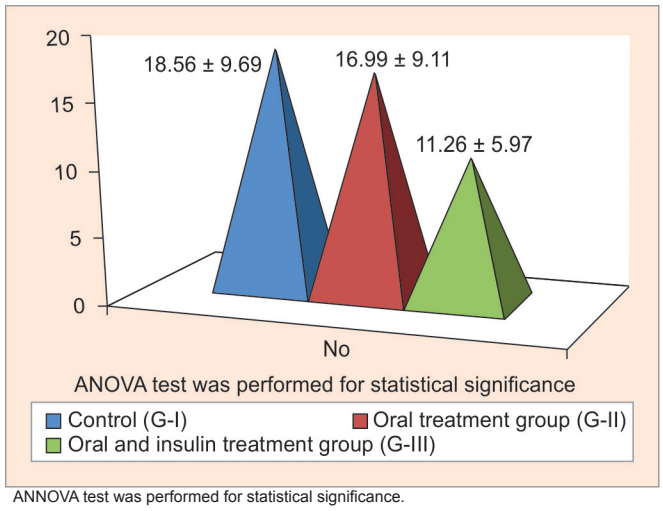
Parameters	*Control (n = 60)*	*T2DM with OHD (Group 1 = 27)*	*T2DM with OHDI (Group 2 = 33)*	*p-value*
TC	158.98 ± 17.91	203.26 ± 39.43*	187.59 ± 40.24*	0.000
TGL	105.07 ± 35.01	156.74 ± 66.99*	166.19 ± 67.53*	0.000
HDL	41.65 ± 9.28	40.41 ± 7.72	41.59 ± 8.40	0.814
LDL	96.32 ± 21.25	194.22 ± 37.28*	179.44 ± 45.43*	0.000
VLDL	21.01 ± 7.00	31.30 ± 13.49*	33.28 ± 13.55*	0.000

OHD: Oral hypoglycaemic drugs, OHDI: Insulin with hypoglycaemic drugs



ANNOVA test was performed for statistical significance.

Graph 1: Status of oxidative stress between the study groups



ANNOVA test was performed for statistical significance.

Graph 2: Status of Nitric oxide between three groups

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 healthy controls (Graph 1).

Significantly lower level of NO was identified in OHDI group than OHD group and healthy controls. OHD group has shown a slightly lower level of NO when compared to healthy controls, but statistically not significant (Graph 2).

DISCUSSION

Type 2 diabetic subjects are more prone to cardiovascular diseases in the worldwide population. Even though the subjects undergo anti-diabetic treatment, the complications are more in these cases. Insulin is not a treatment for type 2 diabetic subjects, but in uncontrolled condition, insulin is required. Earlier studies have been reported that hyperinsulin emia itself is a risk factor in caus ing vascular complications.[21-23] However, endothelial dysfunction is an initial stage of developing vascular complications. The following factors are evaluated to see the effect of insulin treatment on endothelial dysfunction in type 2 diabetic subjects.

Body Mass Index

As we know, obesity is a major factor in causing cardiovascular risk in type 2 diabetes subjects. The present study has shown a significantly high level of BMI in two diabetic treated groups (groups 1 and 2) than healthy controls. Earlier studies have been reported that metformin can reduce the level of BMI compared to sulfonylurea drugs and insulin.[24,25] Another study had shown that type 1 and 2 diabetes have a significantly high level of BMI in intensively treated subjects compared to subjects with conventional treatment.[26]

The present study also found a significantly high level of BMI in insulin-treated subjects compared to subjects with hypoglycemic drugs alone. Type 2 diabetic subjects, who are treated with insulin, they use to have a 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 due to "overinsulinized periphery by exogenous insulin when compared to insulinized liver". This causes accumulation of fat mass in insulin-treated subjects.[26,27] Nevertheless, insulin is awn effective treatment for hyperglycaemic subjects, but it also has an adverse effect on cardiovascular risk by increasing BMI.

Glycosylated Hemoglobin

Significantly high level of HbA1c was observed in both diabetic treated groups than healthy controls. But, there was no significant difference among diabetic treated groups. An earlier study had found that hypoglycemic

drugs have a similar effect on lowering HbA1c, nevertheless; metformin had more effective for long-term glycaemic control.[28] Another study has stated that insulin glargine with metformin and neutral protamine hagedorn (NPH) with metformin have shown good glycaemic control in diabetes.[29] In our study, we have found a significantly high level of fasting sugar in OHDI group than OHD group. But, there was the 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 glycaemic control and treatment might be the reason for the insignificant difference in the level of HbA1c between diabetic treated groups.

Lipid Profile

Dyslipidaemia is one of the foremost recognized factors to cause complications in DM.[30] The present study had shown significantly high levels of cholesterol, triglyceride, LDL-C, and VLDL-C in two diabetic treated groups compared to 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 been 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 2 diabetic subjects have recognized that glycaemic control have a major role in maintaining LDL level.[33-35]

The main target of antidiabetic treatment in type 2 diabetic subjects is to maintain the glycaemic status in blood circulation and reduce the complications. An earlier study has shown the beneficial effect of anti-diabetic treatment on lipid metabolism. It also stated that lipid profile can be improved along with development in glycaemic control.[34] Among hypoglycaemic drugs 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 glycaemic control. In our study, poor glycaemic control might be the reason for the insignificant difference in the level of lipid profile among diabetic treated groups.

Oxidative Stress

In our study, a significantly high level of MDA was found in two diabetic treated groups compared to healthy controls. High level of MDA was identified in OHDI subjects compared to OHD, but statistically insignificant. Significantly lower level of FRAP was observed in OHDI, and OHD treated subjects than healthy controls. But, there was no significant difference in the level of FRAP between two diabetic treated groups. Earlier studies have been reported that increased level of MDA and reduced anti-oxidant in T2DM than healthy controls.[31,32,37] Glucose oxidation may be the reason for increase free radicals. Experimental studies have been shown the alteration of proteins and lipids in diabetic subjects. Poor glycaemic control might be the reason to stimulate platelet aggregation and auto-oxidation of glucose and finally produce free radicals.[38,39] Lipids are the primary targets of ROS and produce highly reactive aldehydes includes MDA, acrolein, 4-hydroxynonenal (4-HNE), 4-oxononenal (4-ONE) and isolevuglandins (IsoLGs).[40] Poor glycaemic control might be the reason for the high level of MDA in both diabetic treated groups.

Endothelial Dysfunction

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

Clinical studies have been reported that insulin sensitizers have a beneficial effect on vascular en dothelium in diabetic subjects.[43,44] Chronic insulin treatment improves the vascular relaxation in diabetes, but in acute insulin treatment does not show any improvement in vascular relaxation even diabetic subject in glycaemic control.[45] Insulin is an important promoter for activation of eNOS to produce nitric oxide in endothelium. Insulin binds to endothelial receptor leads to phosphorylation of Insulin receptor substance-I (IRS-I) and activates eNOS through 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 al. 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.[46] This might be the reason for the lower level of NO in insulin-treated diabetic subjects than subjects with hypoglycemic drugs alone.

Obesity is another known factor in causing insulin resistance. In obese subjects, adipose tissue releases a high amount of non-essential fatty acids, glycerol, hormones, pro-inflammatory cytokines and other factors which are

responsible for the development of insulin resistance.[47] Cao et al. 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 a high-fat diet experimental mice.[48] In our study, we have found a high level of BMI in insulin-treated subjects. Hence, this might be another reason for the lower level of NO in insulin-treated subjects.

Since exogenous insulin is a common treatment for Type 1 diabetes mellitus, but for type 2 diabetes only in an uncontrolled condition. Instead of changing medicine or increasing dosages of insulin, the physician can advise the patients to go for physical exercises and low-fat diet to control glycaemic status. Further follow-up study is required to focus on the dosage of insulin to get a precise result on the effect of insulin on endothelial dysfunction in type 2 DM.

CONCLUSION

We have found a significant effect of insulin therapy on endothelial dysfunction in type 2 diabetic subjects. We also found that without dyslipidemia and oxidative stress there will be a chance of endothelial dysfunction in insulin-treated type 2 diabetic subjects. Hence, frequent examination of NO level is also necessary for type 2 diabetic subjects to predict early onset of complications.

Limitation

The present study has a low sample size due to the inclusion and exclusion criteria of subjects. Further follow-up study is required on T2DM with different dosage of insulin to give better result on the effect of insulin therapy on endothelial dysfunction in T2DM.

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