Original Article

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A Study on the Level of Oxidative Stress and Inflammatory Markers in Type 2 Diabetes Mellitus Patients with Different Treatment Modalities

Biochemistry Section

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ABSTRACT

Introduction: The prevalence of type 2 diabetes mellitus is increasing worldwide in all the age group. UKPDS study had shown that good glycaemic control is maintained by the administration of insulin in addition to hypoglycaemic drugs. But, hyperinsulinemia might cause vascular complications in T2DM. Oxidative stress and inflammation are common in diabetes and plays an important role in vascular complications.

Aim: The study has been designed to estimate and compare the level of oxidative stress and inflammation in type 2 diabetic patients under different treatment modalities.

Materials and Methods: Sixty Type 2 diabetic subjects undergoing treatment were selected from Government Hospital and VMKV Medical College & Hospital at Salem. The subjects were divided into two groups based on treatment modalities, hypoglycaemic drugs subjects as Group-I (30) and hypoglycaemic drugs & Insulin subjects in Group-II (30). BMI was calculated by standard formula and Fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated haemoglobin (HbA1c), Lipid profile, oxidative stress (MDA) and inflammatory markers were measured by well established methods. SPSS 16.0 version was used for statistical analysis.

Result: In our study we have found significantly high levels of BMI, MDA and hsCRP (25.5±2.79, 2.73±1.65, 1.98±0.85) in Group II subjects when compared to Group I subjects (23.4±3.09, 2.23±1.76, 1.168±1.124).

Conclusion: Since risk factors like BMI, MDA and hsCRP were high in Diabetes mellitus patients on both oral hypoglycaemic drugs and insulin, they are more susceptible to cardiovascular diseases. Evaluation of these markers at regular interval can reduce the incidence of vascular complications in Type 2 DM patients.

Keywords: Body Mass Index (BMI), Glycosylated Haemoglobin (HbA1c), High sensitive C-reactive protein (hsCRP), Hypoglycaemic drugs & Insulin (HGDI), Hypoglycaemic drugs (HGD), Malondialdehyde (MDA)

INTRODUCTION

Diabetes mellitus is known to be a serious and expensive disease in adults worldwide [1]. The prevalence of type 2 diabetes mellitus (T2DM) is drastically increasing in all the ages of people [2]. The diabetic patient who depends on oral hypoglycaemic drugs finds it very difficult to maintain their HbA1c level below 7%. But the patients who rely on insulin in addition to the oral therapy, according to UK prospective diabetes study (UKPDS) are able to have good glycaemic control and are able to maintain their glycated haemoglobin near to the WHO recommended level [3]. This intensive therapy is found to be effective and can reduce the incidence and progression of microvascular complications in Type 1 DM patients. But in Type 2 DM, this therapy usually results in hyperinsulinemia, which might initiate various complications [1].

It is a well-established fact that lipid peroxidation plays a major role in inflammation [4] and pathogenesis of vascular complications in diabetic patients [5]. Inflammatory events occurred due to chronic alteration related to insulin resistance predisposes people to atherosclerosis [6]. Previous studies have shown an elevated level of high sensitive C-reactive protein (hsCRP) and fibrinogen in chronic inflammatory condition [7,8]. Inflammatory markers like hsCRP and fibrinogen are evolved as novel biomarkers to analyse the extend and severity of atherosclerotic lesions. CRP is also considered as an effective marker to track progress of cardiovascular disease [6].

As it is well known that both hyperglycaemia and hyperinsulinemia can increase the vascular complications, we have designed a study to compare the risk factors of cardiovascular diseases like BMI, HbA1c, Lipid profile, Oxidative stress and Inflammatory markers in Type 2 Diabetes mellitus patients undergoing different modalities of treatment.

MATERIALS AND METHODS

Sixty type 2 diabetic subjects of 30-60 years undergoing regular treatment at Government Hospital and VMKV Medical College and Hospital at Salem, in Tamil Nadu, India were selected for this study. The subjects were grouped into two: diabetic subjects with hypoglycaemic drugs alone as HGD or Group-I (n=30) and diabetic subjects who were treated with both hypoglycaemic drugs and insulin as HGDI or Group-II (n=30). Type 2 diabetic subjects in our study group were using metformin and sulfonylurea as oral hypoglycaemic drugs.

The study was approved by the institutional ethics committee from each patient, an informed consent was obtained. A 3ml venous blood sample was collected after an overnight fast of 12 hours. Fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycosylated haemoglobin (HbA1c), Total cholesterol (TC), Triglyceride (TG), High density lipoprotein cholesterol (HDL-C), Oxidative stress as thiobarbituric acid reactive substances (TBARS), total antioxidant capacity was estimated as the ferric reducing ability of plasma (FRAP) were analysed. FBS, PPBS, HbA1c, Fibrinogen, hsCRP and lipid profile were estimated using commercially available standard kits. LDL-C and VLDL-C were calculated using the Friedewald's formula (Friedewald W T, et.al.,1972). Body Mass Index (BMI) was calculated by using the formula=weight (kg)/height^2 (meters).

STATISTICAL ANALYSIS

Data analysis has been done by using SPSS version16 software; Non-Parametric analysis, Kruskal Wallis has been used to analyse the statistical significance between two groups.

RESULTS

The clinical characteristics of the present study group are shown in [Table/Fig-1]. The study groups were age and sex matched. BMI was found to be significantly high ($p<0.05$) in subjects of HGDI group. No significant difference in the levels of blood glucose, HbA1c and lipid profile (total cholesterol, triglyceride, HDL, VLDL and LDL) was observed between two treatment groups.

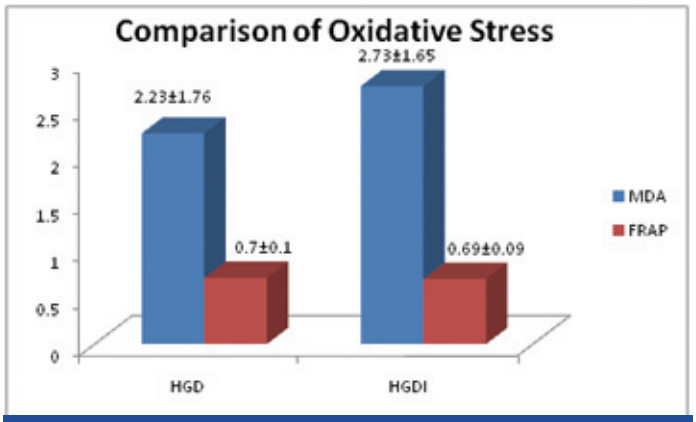
Status of Malondialdehyde and antioxidant capacity in the present study groups are depicted in the [Table/Fig-2]. A significantly high level of Malondialdehyde was observed in type 2 diabetic subjects with insulin in addition to hypoglycaemic drugs (HGDI) when compared to subjects with hypoglycaemic drugs alone (HGD) ($p<0.05$). No significant difference in the level of FRAP (ferric reducing ability of plasma) was identified between two treatment groups.

Comparison on the level of fibrinogen between two treatment groups is represented in the [Table/Fig-3]. Slightly high level (statistically insignificant) of fibrinogen was noticed in subjects of HGDI group when compared to HGD group.

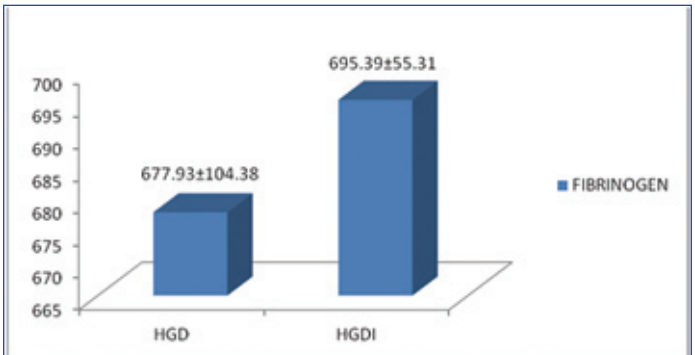
Parameters	Type-II DM with HGD (Mean ± SD)	Type-II DM with HGDI (Mean ± SD)	'p' values
AGE	48.33±10.99	53.37±8.88	>0.05
FBS	176.04±82.46	140.94±42.25	>0.05
PPBS	286.64±89.74	267.39±82.25	>0.05
HbA1c	9.44±2.73	8.71±1.97	>0.05
BMI	23.4±3.09	25.5±2.79	<0.05*
T CHOL	199.93±39.02	198.41±41.59	>0.05
TGL	156.53±65.57	167.38±68.91	>0.05
HDL	39.93±7.46	42.21±8.59	>0.05
VLDL	31.27±13.18	33.52±13.85	>0.05
LDL	191.37±37.2	180.86±46.89	>0.05

[Table/Fig-1]: Compares the level of FBS, PPBS, BMI, HbA1c and lipid profile between two treatment groups

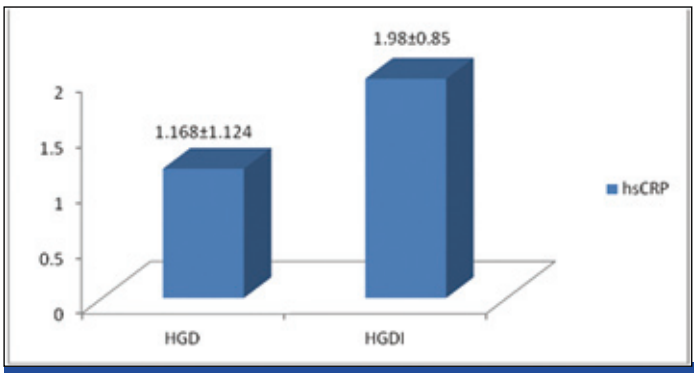
Hypoglycaemic drugs (HGD), Hypoglycaemic drugs with Insulin (HGDI)
*Significant = <0.05, Non Significant = >0.05



[Table/Fig-2]: Compares the level of oxidative stress between two groups
Malondialdehyde (MDA), Ferric reducing ability of plasma (FRAP)
Hypoglycaemic drugs (HGD), Hypoglycaemic drugs with Insulin (HGDI)



[Table/Fig-3]: Represents the level of fibrinogen in the two groups of our study
Hypoglycaemic drugs (HGD), Hypoglycaemic drugs with Insulin (HGDI)



[Table/Fig-4]: Depicts the level of hsCRP of two groups
Hypoglycaemic drugs (HGD), Hypoglycaemic drugs with Insulin (HGDI)

Level of high sensitive C-reactive protein (hsCRP) in the present study groups is described [Table/Fig-4]. Type 2 diabetic subjects with insulin and hypoglycaemic drugs had a higher level of hsCRP than HGD group ($p<0.05$).

DISCUSSION

Cardiovascular disease is the major cause of morbidity and mortality in Type 2 Diabetes mellitus patients and is found to be 2 to 4 times more than the general population. Treatments to control hyperglycaemia can improve microvascular outcome, but not on macrovascular complications. So a study was designed to compare the risk factors of CVD among the diabetic patients with different modes of treatment.

Hypoglycaemic treatment targets the reduction of high levels of blood glucose to the normal level or glycaemic control in diabetic patients. Metformin is widely preferred as an initial drug of choice to control blood sugar in T2DM. If this drug fails to attain the target, physicians relay on sulfonylurea or insulin therapy [9]. In our study no significant difference was noticed between two treatment groups in the levels of fasting and postprandial blood sugar.

Metformin predominantly is involved in the reduction of hepatic glucose production. The mechanism of action of metformin has been shown to inhibit the gene expression of rate-limiting enzymes for hepatic gluconeogenesis, such as phosphenolpyruvate carboxykinase and glucose-6-phosphatase. The primary effect of metformin is mediated by adenosine monophosphate activated protein kinase (AMPK) which inhibits hepatic gluconeogenesis and lipogenesis. Metformin can also decrease the absorption of glucose in intestine and increase the insulin-regulated glucose transporter (GLUT-4) in striated muscle and adipose tissue. It has been reported that through AMPK activation metformin can also improve endothelial function. Thus it is proved that metformin has not only the hypoglycaemic effect, but also has the efficacy to reduce cardiovascular complications [10]. Adverse effects of metformin include minor gastrointestinal problem, lactic acidosis, folate malabsorption and vitamin B deficiency [11].

The additional hypoglycaemic agent, sulfonylureas, inhibits the opening of ATP sensitive potassium channels in beta cells of pancreas [9] and cause an increase in intracellular free levels of calcium by activating the calcium influx. These two processes promote the release of insulin [10]. Even though, Sulfonylurea has beneficial effects in controlling blood glucose levels, it has several adverse effects like weight gain and increased risk of hypoglycaemia [9].

Glycosylated Haemoglobin (HbA1c) is a key indicator of long term glycaemic control and its level is proportional to the Glucose concentration in the blood. It is commonly considered as the average mean glucose level of the past 3 months (time period detected by 120 days life span of the erythrocyte) [12]. In our study, no significant difference in the levels of HbA1c was noticed

between two treatment groups. Early studies had shown that both hypoglycaemic agents (metformin and sulfonylurea) have a similar efficacy in lowering HbA1c level. However, metformin have a better long-term maintenance of glycaemic control [2]. UKPDS study had showed that a decrease of HbA1c by 1%, can reduce the risk of macrovascular complications by 37%, risk of microvascular complications by 14% and the rate of mortality by 14%. Hence, frequent estimation and analysis of HbA1c level and changes in dietary habits may be helpful to reduce the risk of morbidity and mortality [12]. HbA1c level in both treatment groups was found to be more than the normal range (<6.0%). This underlines the fact that our study subjects were not under good glycaemic control as shown from FBS and PPBS levels.

Our study had found a significantly high level of BMI in subjects of HGDI group when compared to HGD subjects. Some of the previous studies had reported a significant increase in the level of BMI in T2D patients on treatment with sulfonylurea drugs and insulin, whereas maintenance of steady body weight and BMI in the patients on metformin therapy [9,13]. A 24 week study by Mathew et al., also had an increase in BMI in Type 2 Diabetes Mellitus patients on addition of insulin to oral therapy [3]. Patients, who are on treatment with insulin, always have a high calorie intake, as a part of "defensive snacking", to prevent nocturnal hypoglycaemia. Studies had shown that observed increase in BMI might be due to improved conservation of this ingested calorie. A phenomenon "Overinsulinized periphery due to exogenous insulin when compared to under insulinized liver" might be another reason for the uneven accumulation of fat mass in insulin treated patients [14]. So even though insulin is regarded as the most effective treatment for hyperglycaemia, it has a negative impact on the cardiovascular profile due to disproportionate weight gain.

Dyslipidemia is found to be a well-recognized risk factor for cardiovascular diseases. Since it is a modifiable one, dyslipidemia is considered as a key factor for cardiovascular preventive management and is regularly assessed [15]. Our study didn't show any significant difference in the levels of lipid profile (cholesterol, TGL, VLDL, LDL and HDL) between the two treatment groups. Most common pattern observed in diabetic patients when compared to normal subjects is a low level of HDL and moderately high levels of Triglyceride and LDL cholesterol [16]. This typical diabetic dyslipidemia pattern is mainly associated with insulin resistance and poor glycaemic control [17]. It has been reported that anti- diabetic treatment, regardless of its type has a beneficial effect on Lipid metabolism. Lipid values are found to be improved along with the improvement in glycaemic control [15,18]. This might be the reason for the observed insignificant difference in the lipid levels between the two treatment groups.

In our analysis, significantly high level of MDA was spotted in HGDI group when compared to the HGD group. But no significant difference in the levels of total antioxidant (FRAP) was observed. Previous studies had shown an increased level of MDA and reduced anti-oxidant levels in T2DM when compared to control subjects [19]. Fangfang Song et al., also had described an increased level of oxidative stress in newly diagnosed T2D patients [20]. Earlier studies had identified that hyperinsulinemia can cause the activation of NADPH oxidase enzyme and thus increase the production of ROS [21]. This might be the reason for observed high levels of MDA in HGDI group.

Type 2 Diabetes mellitus is now considered as an inflammatory disease and inflammatory process seems to play an important role in the development of diabetes and its late complications like CVD [6]. On comparison on the levels of fibrinogen, no significant difference was observed between the groups. Earlier studies have reported a significantly high level of fibrinogen in Type 2 Diabetes mellitus patients and a positive correlation with glycaemic level [22,23]. Glycaemic control is found to have a major role in the synthesis and degradation of fibrinogen [24]. In our study both groups were found to have similar glycaemic control. This might be the reason for the observed insignificant difference in the level of fibrinogen between the groups.

But in our study, a significantly high level of hsCRP was observed in HGDI group when compared to HGD group. Previous studies have shown a significantly high level of hsCRP in T2DM [25,26]. Tomoya et al., study had described the existence of a strong positive correlation between hsCRP and insulin resistance [27]. Noako Nishitani and Yasuki Hayashino also had shown a significant proportional association between BMI and hsCRP [28,29]. Hence, insulin resistance and high BMI observed might be the reason for the increased levels of hsCRP in HGDI group. High level of hsCRP is considered as a sensitive marker for systemic inflammation [25,27]. Inflammation plays a major role in all the steps of atherogenesis. Studies on CAD patients had shown that level of hsCRP increase with the severity of the disease [6]. Evidences had proved that hsCRP is not only an inflammatory marker but also an atherogenic molecule [30,31]. So, our study discloses the fact that the diabetic patients on insulin therapy are more susceptible to cardiovascular complications than diabetic patients on hypoglycaemic drugs alone.

CONCLUSION

It was observed that in diabetes mellitus patients who are treated with both oral hypoglycaemic drugs and insulin, levels of BMI, inflammatory marker- hsCRP and oxidative stress are significantly high. Since these are high risk factors of CVD, these patients can be considered as high risk group for cardiovascular disease. According to these findings, it is suggested that during regular checkup, along with blood glucose and traditional risk factors, these parameters should also be included.

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