EFFECT OF SHORT TERM INSULIN REGIMEN ON LIPID PEROXIDATION IN TYPE-2 DIABETES MELLITUS

P.K. Mohanty¹, Bhavana Bai²

ABSTRACT
Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of either secretion or action of endogenous Insulin. The disease is better known for its complications affecting the vascular system, kidney, retina, lens, peripheral nerves and skin, which are extremely costly in terms of longevity and quality of life. Increased oxidative stress is an widely accepted contributing factor towards development and progression of diabetes and its complications. Diabetes is usually accompanied by increased production of free radicals or reactive oxygen species (ROS) and impaired antioxidant defenses that leads to damage of all bio-molecules like lipids, proteins, carbohydrates, DNA etc. and initiation of micro and macro vascular complications. MDA (Malondialdehyde) is used as an index of oxidative damage and has the ability to interact with lipoproteins. The present study examines the extent of oxidative damage and alteration of lipid profile in 60 NIDDM cases and 25 age and gender matched control subjects. It is observed that diabetics reveal increased oxidative damage as reflected by their higher MDA level in comparison to normal controls (5.30±2.8 vs 4.6±0.83). Among the diabetics hypercholesterolemia (21%), Triglyceridemia (48.3%) and higher BMI (66.6% > 22.9) are frequent. The study reveals that patients on short term insulin regimen show considerably low levels of MDA (3.41±1.4 mmol/L), lowered serum triglycerides (143±99) and eventually a better lipid profile as compared to cases on oral hypoglycemic agents and cases adhering to other regimes for glycemic control. Therefore short term insulin regimen should often be recommended to NIDDM cases not only as an efficient means of glycemic control, but as an active agent for improving anti-oxidant defense status and reducing lipid per-oxidation. In conclusion short term insulin regimen is advocated for long term health benefits in NIDDM.

INTRODUCTION
Diabetes in all its heterogeneity has taken the centre stage as one of the ultimate medical challenges. The term diabetes mellitus, describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism, resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and various organ failures. These complications are major cause of mortality and morbidity in patients with type 2 DM. The long–term effects of diabetes mellitus include progressive development of the specific complications of

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retinopathy with potential blindness, nephropathy that may lead to renal failure, neuropathy with risk of foot ulcers, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease.

(WHO*)

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "Diabetes Capital of the World". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of major causes of morbidity and mortality affecting the youth and middle aged people. Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the more common form of diabetes, namely type 2 diabetes, which accounts for more than 90 per cent of all diabetes cases. World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2002. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025.

Diabetes is better known for its complications. Numerous complications affecting the vascular system, kidney, retina, lens, peripheral nerves, and skin are common and are extremely costly in terms of longevity and quality of life.

Macroangiopathic complications such as coronary artery disease (CAD), peripheral vascular disease(PVD) and cerebro-vascular disease (CVD) are important causes of morbidity and mortality in type 2 diabetes mellitus (DM). It has been suggested that hyperglycaemia can increase free radical activity through various pathways strictly associated with hyperglycaemia(polyol pathway, protein glycation, glucose autooxidation and prostanoid synthesis). (Singhania*)

Patients with type 2 diabetes mellitus are at increased risk of developing vascular and other complications. This excess is only partially explained by traditional risk factors including smoking, hypertension and dyslipidaemia. Therefore it has been proposed that oxidative stress is an explanation for accelerated complications in type 2 DM. (Nourooz Zadeh)

Although the complications surface late in the disease course the real cause starts very early at molecular level. Diabetes mellitus is considered to be rank one of free radical diseases which propagates complications with increased free radical formation. (E) Lipid hydroperoxides are non-radical intermediates derived from unsaturated fatty acids, phospholipids, glycolipids, cholesterol esters and cholesterol itself. Their formation occurs in enzymatic or non-enzymatic reactions involving activated
chemical species known as "reactive oxygen species" (ROS) which are responsible for toxic effects in the body via various tissue damages. These ROS include among others hydroxyl radicals, lipid oxy or peroxy radicals, singlet oxygen, and peroxinitrite formed from nitrogen oxide (NO), all these groups of atoms behave as a unit and are now named "free radical".

Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids, and eventually cell death. Various mechanisms have been suggested to contribute to the formation of these reactive oxygen-free radicals. Glucose oxidation is believed to be the main source of free radicals. In its enediol form, glucose is oxidized in a transition-metal-dependent reaction to an enediol radical anion that is converted into reactive ketoaddehydes and to superoxideanion radicals. The superoxide anion radicals undergo dismutation to hydrogen peroxide, which if not degraded by catalase or glutathione peroxidase, and in the presence of transition metals, can lead to production of extremely reactive hydroxyl radicals. Superoxide anion radicals can also react with nitric oxide to form reactive peroxynitrite radicals. Hyperglycemia is also found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals. Another important source of free radicals in diabetes is the interaction of glucose with proteins leading to the formation of an Amadori product and then advanced glycation endproducts (AGEs). These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions, promote free radical formation, and quench and block antiproliferative effects of nitric oxide. By increasing intracellular oxidative stress, AGES activate the transcription factor NF-κB, thus promoting up-regulation of various NF-κB controlled target genes. NF-κB enhances production of nitric oxide, which is believed to be a mediator of islet beta cell damage.

The imbalance between protective antioxidants (antioxidant defense) and increased free radical production, leading to oxidative damage, is known as oxidative stress (Hanachi et al., 2004). Oxidative stress is caused by a relative overload of oxidants, i.e., reactive oxygen species and the evidence suggests complications of diabetes partially are mediated by oxidative stress (Hayoz et al., 1998, Rosen et al., 1998, Szaleczky et al., 1999).

Oxidative stress can be measured by monitoring the changes in blood Malondialdehyde (MDA) which is a marker of lipid oxidation. Evidence suggests that oxidative damage is increased in diabetes (West, 2000). Some of studies have implied that Lipid peroxidation acts on fatty acid and begin to change the lipids structure (Murray, 1996). As a result of this peroxidation, substances such as MDA is produced which attacks the lysine amino acid in protein which results in proteolysis (Buege, 1987). Malondialdehyde (MDA), which is widely used as an index of oxidative damage, has received particular attention in pharmacological studies for its ability to interact with lipoproteins.
MATERIALS AND METHODS

The present study was designed to study the extent of lipid peroxidation and lipid profile abnormalities in type2 diabetic individuals. Malondialdehyde(MDA) was estimated for all study subjects and the controls as measure of lipid peroxidation and Lipid profile parameters such as serum total cholesterol, serum triglycerides, and lipoprotein fractions like HDLc, LDLc, and VLDLc are also estimated to diagnose lipid profile abnormalities.

The subjects for the present study were selected from the diabetic patients attending the OPD of VMMC & H Karaikal, who were uncomplicated and ambulatory. 60 Type 2 diabetics comprising of 33 male and 27 females within the age range of 30-69 years were included in the study. 25 age and gender matched non diabetic apparently healthy individuals were included in the study as controls.

METHODS

Blood samples were collected under aseptic condition in vaccutainers (plain and EDTA) for separation of serum and plasma from the study subjects in fasting state. 2 hr. prandial blood sample in EDTA was collected for post-prandial blood glucose estimation. The following parameters were analysed as per standard methods.

1. Plasma Malondialdehyde (MDA) - method of Estrerbauer and Steinberg 1989
2. Serum Total Cholesterol – CHOD-POD METHOD (Young DS, 1973)
4. Serum HDL cholesterol – Precipitation method (Denahcherp NM, 1980)
6. Serum LDLc and VLDLc were calculated using Friedwald formula.

OBSERVATION AND DISCUSSION

Out of these 60 cases, 22 were on OHA only, 18 were on insulin only (2 to 4 weeks) and the rest 20 were either on diet and exercise or no therapeutic regimen. Diabetes patients were also grouped as per severity of hyperglycaemia. The cases having FBG < 119.9 mg/dl were considered to have good metabolic control (WHO 1985). Those patients having FBG between 120 – 150 mg/dl were fairly controlled and cases with FBG > 150 mg/dl were poorly controlled.

The study shows that mean BMI of cases is 24.56 ± 4.50 with 66.7 % being obese (WHO experts’ asian BMI > 23kg/sq m) and that of controls mean BMI is only 22.24 ± 4.19 with 56% being less than 22.9 kg/ sqm indicating that diabetes is a factor for obesity.

The metabolic parameters (lipid profile) such as total cholesterol, triglycerides, LDL-C were well above the normal range with mean values of 167.4 ± 45.3 mg/dl, 168.4 ± 98.1 mg/dl, 101.6 ±
38.6 mg/dl respectively in diabetics as compared to controls, which were only 151 ± 26 mg/dl, 115 ± 35 mg/dl, 89 ± 29 mg/dl in the same order, clearly indicating the dyslipidaemia in diabetes. (Suryavanshi et al). 21.6 % of the cases have total cholesterol more than 200 mg %, where as in controls only 1 % have cholesterol more than 200 mg%. Considering triglyceridaemia, 48.3 % cases have serum triglyceride more than 150 mg%, whereas only 20% of the control cases had more than 150 gm%.

Within the cases the metabolic parameters (lipid profile) varied with the therapeutic regimen with the cases only on OHA having mean total cholesterol, mean triglycerides, mean LDL-C levels as 181.18 ± 48.3 mg/dl, 167.4 ± 88.1 mg/dl, 113 ± 43.7 mg/dl, the cases not on any therapeutic regimen having levels as 167.8 ± 32.4 mg/dl, 176.4 ± 74.9 mg/dl, 97.86 ± 31.6 mg/dl and those cases on Insulin having levels as 152.3 ± 33.17 mg/dl, 130.1 ± 59.5 mg/dl and 93.7 ± 26.3 respectively indicating that insulin plays a role in correction of dyslipidaemia to near normal reference levels apart from reducing the glucose level which is not seen in treatment with OHA's alone.

<table>
<thead>
<tr>
<th>TREATMENT REGIMEN</th>
<th>TOTAL Cholesterol (mg%)</th>
<th>TRIGLYCERIDES (mg%)</th>
<th>HDL-Cholesterol (mg%)</th>
<th>LDL-C (mg%)</th>
<th>VLDL-C (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON INSULIN</td>
<td>152.8±33.17</td>
<td>130±59.5</td>
<td>32.9±6.7</td>
<td>99.8±26.3</td>
<td>26±11.9</td>
</tr>
<tr>
<td>ON O.H.A</td>
<td>181.1±48.3</td>
<td>167.4±88.1</td>
<td>34.3±17.1</td>
<td>113±43.7</td>
<td>33±17.6</td>
</tr>
<tr>
<td>OTHERS</td>
<td>172.8±39.1</td>
<td>192.2±106.5</td>
<td>34.5±6.4</td>
<td>100.1±32</td>
<td>33±4±21.3</td>
</tr>
<tr>
<td>ALL CASES</td>
<td>167.4±45.3</td>
<td>168.4±98.9</td>
<td>32.1±11.8</td>
<td>101.6±38.6</td>
<td>33±19.7</td>
</tr>
<tr>
<td>CONTROL</td>
<td>153±32</td>
<td>109±56</td>
<td>39±13</td>
<td>97±36</td>
<td>22±11</td>
</tr>
</tbody>
</table>

The lipid profile of cases doesn't show much variation with the categorization done by glycaemic status. The study indicates that the lipid peroxidation in diabetes goes on at an increased rate as evidenced by the increased MDA levels in cases as compared to controls. MDA being the well accepted indicator of lipid per oxidation also indicates that oxidative stress is more in diabetics than non diabetics. It is also seen that age and gender can be modifying factors on MDA levels with MDA being higher in younger age group and females. Mean MDA levels are higher in female cases as compared to male diabetics but in control subjects its vice-versa males have higher mean MDA levels hence more oxidative stress than female control subjects. This association is reversed in diabetic cases.

The present study showed that cases on Insulin as therapeutic regime had lower mean MDA level (3.41 ± 1.48 mmol/l) as compared those on OHA (6.54 ± 3.23 mmol/l) indicating lesser level of oxidative stress in diabetic subjects on insulin. Considering MDA levels among cases on the basis of their glycaemic status showed no
significant variation in mean MDA levels of good and fair glycaemic control cases. But uncontrolled fasting hyperglycaemic cases with FBG > 151 mg/dl had very high mean MDA levels (6.41 ± 3.1 mmol/l).

<table>
<thead>
<tr>
<th>THERAPEUTIC REGIMEN</th>
<th>3.41±1.48</th>
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<tbody>
<tr>
<td>ON INSULIN</td>
<td>3.41±1.48</td>
</tr>
<tr>
<td>ON O.H.A</td>
<td>6.54±3.23</td>
</tr>
<tr>
<td>OTHERS</td>
<td>5.6±2.57</td>
</tr>
<tr>
<td>CONTROL</td>
<td>4.60±0.83</td>
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<tr>
<th>GROUPS</th>
<th>Glycaemic status</th>
<th>M.D.A</th>
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<tbody>
<tr>
<td>A</td>
<td>FBG &lt; 119.9</td>
<td>4.63±2.19</td>
</tr>
<tr>
<td>B</td>
<td>FBG 120-150</td>
<td>4.11±2.21</td>
</tr>
<tr>
<td>C</td>
<td>FBG &gt;151</td>
<td>6.41±3.1</td>
</tr>
<tr>
<td>CONTROL</td>
<td>FBG&lt;110</td>
<td>4.60±0.83</td>
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</table>

CONCLUSION

It stands out clearly in this study that the various therapeutic regimes for the management of type - 2 diabetes can potentially bring about glycaemic control in an individual. Regarding other metabolic parameters like lipid profile and lipid peroxidation varying responses have been observed in various therapeutic regimes. Insulin as a therapeutic regime can efficiently bring about glycaemic control as well as improve other metabolic parameters where as other regimen's are not that efficient in improving the metabolic parameters other than glycaemic control. New therapies will continue to evolve as insights in to molecular mechanisms further expand our therapeutic horizon. However we must now actively try to diagnose all type – 2 diabetic individuals at an earlier stage and begin early and aggressive initiation of insulin therapy in an attempt to prevent disease progression, long term complications and thus minimize the burden of diabetes associated complications.

REFERENCES

2. ADA (American Diabetes Association), Diagnosis and classification of Diabetes Mellitus, Diabetes Care, 2005, 28(suppl. 1), S37–S43.


16. HIRAMATSU, K., S. ARIMORI, Increased superoxide production by mononuclear cells of patients with hypertrygliceridemia and diabetes, Diabetes, 1988, 37, 832–837.


22. Rama Srivatsan MSc, Sujata Das MSc, Ranjita Gadde MSc, Antioxidants and Lipid Peroxidation Status in Diabetic Patients with and without Complications Archives of Iranian Medicine, Volume 12, Number 2, : 121 – 127 March 2009


