Estimation of lipid peroxidation and lipid profile levels in Type II Diabetes Mellitus patients of Kashmir valley

Irfan Majeed*, Rabia Farooq**, Rawoof Malik**, Devendra Singh Rawath***, Shajrul Amin****, Nida Hasan***** and Sabhiya Majid**

*Department of Biochemistry, BFIT Dehradun, India, **Department of Biochemistry, Government Medical College Srinagar, India, ***Department of Zoology, BFIT Dehradun, India, ****Department of Biochemistry, University of Kashmir, India, *****Department of Biotechnology, University of Kashmir srinagar, India.

E-mail: Sabumajid@yahoo.com

Abstract

The lipid profile levels were found to be elevated in T2DM diabetic patients. The study was designed to find out the correlation between lipid peroxidation, lipid profile and T2DM. Degree of lipid peroxidation was measured in terms of malondialdehyde (MDA) along with lipid profile and blood glucose in diabetes mellitus. Totally 50 known diabetic patients and 50 non-diabetic controls were studied. Significant increase in Malondialdehyde (MDA) and lipid profile, except HDL cholesterol, which is decreased, has been found in Type 2 diabetes mellitus (T2DM) cases as compared to controls. It has also been observed that the level of lipid peroxide increased as per the increase in concentration of blood glucose. The increase in lipid peroxidation in the hyperglycemic condition may be explained, as the superoxide dismutase enzyme an antioxidant becomes inactive due the formation of superoxide radical within the cell. The lipid peroxidation leads to the damage of the tissues and organs which results in complications in diabetic patients. So in chronic diabetic cases, secondary complications are well marked. High levels of total cholesterol, TGs and LDL may appear may be due to increased cholesterol synthesis. It may be concluded that good metabolic control of hyperglycemia will prevent in the peroxidation and the lipid metabolism, which may help in good prognosis and preventing the manifestation of vascular and secondary complications in diabetes mellitus.

Keywords: Malondialdehyde, lipid Peroxide, diabetes mellitus and Type 2 diabetes mellitus.

Introduction

Diabetes is a group of metabolic diseases, characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany with chronic hyperglycemia. Long-term complications of diabetes include retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations, and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease (Diabetes Care, 2008). Several pathogenic processes are involved in the development of diabetes.
These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action.

Diabetes mellitus causes lipid metabolism disorder and especially increases lipid and lipoprotein peroxidation (Giugliano et al. 1995). Lipoprotein oxidation such as LDL-C oxidation progress atherosclerosis and risk of cardiovascular disease which can increase morbidity in patients with type 2 diabetes mellitus (Nuttall et al., 1995, Mike et al., 2004). Hyperglycemia is an independent risk factor for cardiovascular diseases in diabetic patients. Since hyperglycemia causes an increased autoxidation of glucose and non-enzymatic protein glycation, oxidative stress may be increased in diabetic patients (Baynes, 1991). Increased levels of the products of oxidative damage to lipids and proteins have been detected in the serum of diabetic patients and their presence correlates with the development of complications (Gutteridge, 1995). Currently, lipid peroxidation is considered as the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead to cell death. Lipid peroxidation is a complex process known to occur in animals. It involves the formation and propagation of lipid radicals, the uptake of oxygen, a rearrangement of the double bonds in unsaturated lipids and the eventual destruction of membrane lipids, with the production of a variety of breakdown products, including alcohols, ketones, alkanes, aldehydes and ethers. Any increase in lipid profile levels or lipid peroxidation products in patients with type 2 diabetes mellitus may have adverse effects.

The prevalence of diabetes worldwide was estimated to be 2.8% in 2000 and will be 4.4% in 2030 (Diabetes Care, 2004). In developing countries, urbanization is found to be associated with diabetes besides altering diet, obesity, decreased physical activity, and other factors such as stress. By 2030, it is estimated that the number of people with diabetes >64 years of age will be > 82 million in developing countries and >48 million in developed ones (Diabetes Care, 2004). The prevalence of diabetes was assumed to be similar in urban and rural areas of developed countries (King et al., 1998).

The form of diabetes, which accounts for 90–95% of those with diabetes, previously referred to as non-insulin-dependent diabetes, Type 2 diabetes, or adult onset diabetes, encompasses individuals who have insulin resistance and usually have a relative (rather than absolute) insulin deficiency (Diabetes Care, 2014). There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of β-cells do not occur. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis occurs spontaneously in this type of diabetes, when seen, it usually arises in association with the stress of another illness such as infection. These patients are at increased risk of developing macrovascular and microvascular complications. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups and is often associated with a strong genetic predisposition, more so than is the autoimmune form of Type 1 diabetes. However, the genetics of this form of diabetes are complex and not fully defined.

**Lipid Peroxide and Lipid Profile in Diabetes Mellitus**

Lipid Peroxidation is a chain reaction initiated by the hydrogen abstraction or the addition of an oxygen
radical, resulting in the oxidative damage of polyunsaturated fatty acids (PUFA). Since polyunsaturated fatty acids are more sensitive than saturated ones, it is obvious that the activated methylene (RH) bridge represents a critical target site. The presence of a double bond adjacent to a methylene group makes the methylene C-H bond weaker and therefore the hydrogen is more susceptible to abstraction. This leaves an unpaired electron on the carbon, forming a carbon-centered radical, which is stabilized by a molecular rearrangement of the double bonds to form a conjugated diene which then combines with oxygen to form a peroxyl radical. The peroxyl radical is itself capable of abstracting a hydrogen atom from another polyunsaturated fatty acid and so of starting a chain reaction (Halliwell and Gutteridge, 1984) (Fig.-1).

In a sequence of their appearance, alkyl, peroxy and alkoxy radicals are involved. Lipid hydroperoxide (ROOH) is the first, comparatively stable, a product of the lipid Peroxidation reaction (Fig.-2).

Iron complexes (Fe²⁺, Fe³⁺) react with lipid peroxides (ROOH) to give alkoxyl and peroxy radicals, which take part in the propagation of the chain reaction and these complex metal ion-catalyzed breakdowns of lipid hydroperoxides include the cytotoxic aldehydes and hydrocarbon gases such as ethane. The free radical chain reaction propagates until two free radicals conjugate each other to terminate the chain. The reaction can also terminate in the presence of a chain-breaking antioxidant such as vitamin E (α-tocopherol).

Lipid Peroxidation causes a decrease in membrane fluidity and in the barrier functions of the membranes. The many products of lipid Peroxidation such as hydroperoxides or their aldehyde derivatives inhibit protein synthesis, blood macrophage actions and alter chemotactic signals and enzyme activity (Fridovich and Porter, 1981).

A significant increase in the lipid peroxide (MDA) and lipid profile levels were found in T2DM cases except HDL cholesterol, which is decreased, as compared to controls (Suryawanshi et al., 2006). The level of lipid peroxide increased as per the increase in concentration of blood glucose. The increase lipid peroxidation in the diabetic conditions may be
explained, as the superoxide dismutase enzyme which is antioxidant becomes inactive due the formation of superoxide radical within the cell. Lipid peroxidation leads to the damage of the tissues and organs which results into complications in diabetic patients. High levels of total cholesterol appear due to increased cholesterol synthesis. The triglyceride levels changes according to the glycemic control. The increase may be due to overproduction of VLDL-TG (Suryawanshi et al., 2006).

Serum lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test. The test includes four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides.

Material and Methods

The study was taken to find out the correlation, if any between lipid peroxidation and lipid profile levels to severity of Type 2 diabetes mellitus.

For this study, 50 clinically confirmed cases of Type II diabetes by senior endocrinologist and 50 healthy controls were taken. Degree of lipid Peroxidation was measured in terms of malondialdehyde (MDA) along with lipid profile and blood glucose in diabetes mellitus. To study the possible relationship between lipid peroxidation and metabolic control, was measured and an the plasma concentration of malondialdehyde (MDA), end product of the oxidation of polyunsaturated fatty acids, in healthy and poorly controlled Type 2 diabetic patients.

Inclusion criteria

Patients confirmed with diabetes mellitus and were diagnosed using WHO Classification (2006). Table 1 summarizes the 2006 WHO recommendations for the diagnostic criteria for diabetes and intermediate hyperglycemia.

Exclusion criteria

Normal controls who came for a normal routine checkup in OPD of SMHS Hospital and whose blood sugar level is normal.

Sample collection

A 5ml fasting blood sample was taken from both cases and controls in the Department of Biochemistry Govt Medical College, Srinagar, for sugar estimation and for lipid peroxide and lipid profile estimation. Serum was separated by centrifugation at 3000 rpm for 10 minutes. Plasma was separated within 30 min. of sample collection by centrifugation at 3000 rpm for 1 - 2 min (for sugar and lipid peroxide estimation).

Procedure for Lipid Peroxide estimation

Malondialdehyde (MDA) is used as a marker of lipid peroxidation using colorimetric reaction, which uses 1-methyl-2-phenylindole as chromogen. Condensation of one molecule of malondialdehyde with two molecule of 1-methyl-2-phenylindole under acidic condition results in the formation of a chromophore with an absorbance maximum at 586 nm. To determine specifically lipid peroxide in plasma, they are precipitated along with plasma proteins to remove water-soluble MPI reactive substances. The level of lipid peroxide is expressed in term of malondialdehyde. Since the malondialdehyde is unstable, tetramethoxy-propane which is converted quantitatively to MDA in the reaction procedure is used as standard and proper protocol was followed (Esterbauer et al., 1990).

Lipid Profile estimation

Plasma was taken and lipid profile levels were estimated by the enzymatic kit method (Abbot) using an analyzer (Architect C4000).

Blood Glucose estimation

Plasma was taken and lipid profile levels were estimated by Hexokinase kit method (Abbot) using an analyzer (Architect C4000).
Among 50 diabetic cases and 50 normal controls, lipid profile levels and lipid peroxidation were estimated. It has been observed that plasma lipid peroxide was found to be higher in diabetic patients as compared to normal (controls).

Table 2 shows, among 50 cases, 20 are males, which correspond to 40% and 30 females which corresponds to 60% and among 50 normal controls, 25 (50%) are females and remaining are males. This shows that females are at higher risk than males with diabetes.

Table 3 shows the relationship of lipid peroxidation, and various diabetic groups. Lipid peroxidation of diabetic males are compared with normal males and p <0.05, statistically significant when compared for female cases and control p value was again found to be statistically significant.

Results
Among 50 diabetic cases and 50 normal controls, lipid profile levels and lipid peroxidation were estimated. It has been observed that plasma lipid peroxide was found to be higher in diabetic patients as compared to normal (controls).

Table 2 shows, among 50 cases, 20 are males, which correspond to 40% and 30 females which corresponds to 60% and among 50 normal controls, 25 (50%) are females and remaining are males. This shows that females are at higher risk than males with diabetes.

Table 3 shows the relationship of lipid peroxidation, and various diabetic groups. Lipid peroxidation of diabetic males are compared with normal males and p <0.05, statistically significant when compared for female cases and control p value was again found to be statistically significant.

Table 4 shows the mean value of total cholesterol, serum triglyceride, LDL-cholesterol and lipid peroxide levels in diabetic group is increased compared to control group (p< 0.05). Mean value of serum HDL-cholesterol is decreased in diabetic group when compared to control and the decreased is statistically significant (p< 0.01).
Discussion

The formation of free radicals during metabolism are scavenged effectively. Oxidative stress occurs when there is an imbalance between production of free radicals and superoxide dismutases responsible for anti-oxidant activity. The toxic substances produced by an activated phagocytes during metabolic reactions causes' maximal damage to the membrane because they are active in the lipid phase. The effect of these toxic radicals is due to an increase in the formation of superoxide radicals within cells, which causes deactivation of superoxide dismutase enzyme in hyperglycemic condition, i.e. during diabetes and elevated levels of lipid peroxide in diabetes mellitus may be due to the alteration of the function of erythrocytes membrane. This affects the tissue and causes damage and secondary disorders in diabetes mellitus (Taniguchi and Naoyuki, 1992) as secondary disorders causes more complications in patients rather than diabetes itself. A significant increase in plasma malondialdehyde concentrations was found in poorly controlled diabetics when compared to healthy control normoglycaemic subjects. The increase in lipid peroxidation in diabetes mellitus is due to excess formation of the free radicals.

In the present study all groups of diabetes mellitus shows statistically significant increase in serum lipid peroxide levels as compared to normals. In the diabetes mellitus abnormal increased levels of lipid, lipoprotein and lipid peroxides in plasma may be due to the abnormal lipid metabolism (Suckling et al., 1993). Increased lipid peroxide may be due to the increased glycation of protein in diabetes mellitus, which might act as a source of free radicals. Besides the deficiency of the antioxidant activity of superoxide dismutase and glutathione peroxidase has been related to a higher concentration of peroxide and due to the lack of the antioxidant system. Mitochondria and microsomal membrane contain large amount of polyunsaturated fatty acid in their phospholipid membrane and these are more sensitive for attack due to unsaturation by free radicals resulting in high lipid peroxidation. Therefore, these may be the causes of the high rate of peroxidation and free radical in diabetes mellitus.

Plasma MDA/Cholesterol and MDA/triglyceride ratios were both higher in poorly controlled diabetics than healthy controlled subjects. In diabetic patients a positive correlation was found between plasma MDA levels and mean daily blood glucose, plasma and plasma triglycerides while no significant correlation was shown between plasma malondialdehyde and total cholesterol. This results confirms the increase of lipid peroxidation during Type 2 diabetes. The correlation with the degree of metabolic imbalance suggests a possible role for lipid peroxidation in the occurrence of glucose-induced macromolecular changes.

In the present study, it has been observed that serum cholesterol level is increased in diabetic cases when compared with the controls. So this may be attributed to less physical activity or inhibition of cholesterol catabolism. The increase in triglyceride levels may be due to insulin deficiency which results faulty glucose utilization, causes hyperglycemia and mobilization of fatty acids from adipose tissue. In diabetes the fatty acid from adipose tissue are mobilized for energy purpose and excess fatty acid are accumulated in the liver, which are converted to triglyceride as the body could not sense high glucose levels.

High level of cholesterol, triglyceride, LDL-cholesterol and low HDL-cholesterol may be due to the obesity, increase in calorie intake, decrease in physical activity in diabetes mellitus patients (Das et al., 1997; Yogi et al., 1999).

The estimation of lipid peroxide along with other lipid profile in the diabetes mellitus is very useful as it may serve as a useful monitor to judge the prognosis of
the patient and will help the patient to improve and reduce the morbidity rate. Besides, this is a cheap technique for better patient care.

References


**Corresponding Author:** Sabhiya Majid, Department of Biochemistry, Government Medical College Srinagar, India, Email : Sabumajid@yahoo.com, © 2015, IJALS. All Rights Reserved.