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Original Research Article

Estimate the Level of Lipid Peroxide and Lipid Profile in Diabetes Mellitus Patients at Patna Medical College and Hospital, Patna, Bihar

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Abstract:

Background: Diabetes mellitus is a condition like a "iceberg." An estimated 150 million people globally are expected to have diabetes. Twenty percent of the world's diabetic population is thought to currently dwell in South-East Asia. The purpose of the study was to determine the relationship between lipoprotein levels and lipid peroxidation and the severity and complications of diabetes mellitus.

Materials and Methods: This study was conducted at Department of Clinical Pathology and Biochemistry, Patna Medical College, Patna, Bihar from July 2022 to June 2023. In diabetes mellitus, the degree of lipid peroxidation was assessed using malondialdehyde (MDA), blood glucose, and the lipid profile. Diabetes mellitus (DM) is broadly classified into type 1 DM or insulin dependent diabetes mellitus (IDDM) and type 2 DM or non-insulin dependent diabetes mellitus (NIDDM).

Results: There were 52 non-diabetic subjects who served as controls and a total of 112 known cases of diabetes were examined. These cases were divided into three groups: controlled, poorly controlled, and uncontrolled, based on the concentration of fasting blood glucose level. All groups showed a significant rise in lipid peroxide (MDA) and lipid profile as compared to controls, with the exception of HDL cholesterol, which was shown to be decreased. Lipid peroxidation was significantly higher in the NIDDM group compared to the IDDM group, and it was higher in the DM patients with complications. Another observation was that the amount of lipid peroxide rose in proportion to the rise in blood glucose levels. The increased lipid peroxidation associated with hyperglycemia may be explained by the antioxidant enzyme superoxide dismutase becoming dormant as a result of superoxide radical production inside the cell. In case of diabetes, maximum lipid peroxidation causes damage to the tissues and organs, which can lead to macro vascular and micro vascular complications. Increased cholesterol synthesis is the cause of high total cholesterol levels. The glycemic control affects the levels of triglycerides. The excess VLDL-TG produced could be the cause of the rise.

Conclusion: It is concluded that maintaining adequate metabolic control over hyperglycemia can help to prevent changes in lipid metabolism and peroxidation, which may improve prognosis and delay the onset of vascular and secondary complications in diabetes mellitus.

Keywords: Malondialdehyde, Lipid Peroxide, Diabetes Mellitus.

Introduction

Arelaeus, who lived in Asia Minor and most likely wrote his account of cappadosis in the first century AD, described the illness as "a melting down of flesh into the urine." Insulin shortage led to the discovery by Van Mering and Minikowaski in 1889 that pancreactomy is the cause of the metabolic disease known as diabetes mellitus. It is defined by either insensitivity to insulin (IDDM or type II) or by the absence of insulin (NIDDM-Type 1). It is a complicated illness that affects how fat and carbohydrates are metabolized [1]. Numerous areas of mammalian lipid metabolism are impacted by insulin. It increases the production of fatty acids in the gut and in adipose tissue of the liver. It has also been shown that insulin increases the production of cholesterol. In white adipose, lipoprotein lipase activity is likewise elevated. From this vantage point, evaluating different lipid fractions and lipid peroxide in patients with diabetes mellitus may assist predict the course of the disease and reduce the likelihood of complications or other illnesses. [2] The cell membrane undergoes significant alteration when lipid peroxidation brought on by free radicals occurs [3]. Atherosclerosis, oxidative DNA damage, aging, carcinogenesis, sickle cell disease,

diabetes mellitus, and other degenerative disorders have all been linked to the pathophysiology of lipid membrane peroxidation [4]. Lipid peroxide levels in the blood thus offer valuable information regarding the prognosis of diabetes, a condition in which subsequent illnesses are frequently deadly [5]. In order to determine whether complications or secondary illnesses may be avoided, the study evaluated the association between lipid peroxide, lipids, lipoprotein fraction, and the severity of diabetes.

Material and Methods

The cubital vein was used to obtain the blood sample during the fasting and postprandial periods, as well as the 2 ml in floride bulb used for sugar estimation and the 5 ml in plain bulb used for lipid peroxide and lipid profile measurement. Centrifugation was used to separate the serum for ten minutes at 3000 rpm. Within thirty minutes of sample collection, plasma was separated by centrifugation at 3000 rpm for one to two minutes (for lipid peroxide and sugar assessment).

Test for plasma's lipid peroxide level (Esterbauer and Steinberg et al.). Malondialdehyde (MDA) was measured using the colorimetric procedure, which employs 1-methyl-2-phenylindole as a chromogen, as a measure of lipid peroxidation. A chromophore with a maximum absorbance of 586 nm is formed when one molecule of malondialdehyde condenses with two molecules of 1-methyl-2-phenylindole in an acidic environment. Lipid peroxide in plasma is particularly determined by precipitating them along with plasma proteins to eliminate water-soluble MPI reactive compounds. Malondialdehyde is used to express the amount of lipid peroxide. Tetramethoxypropane, which is quantitatively transformed to MDA during the reaction process, is employed as a reference because malondialdehyde is unstable.

Procedure

1. 7.6 nM solution of 1-methyl-2-phenylindole was prepared immediately prior to use in 33 % methanol in acetonitrile.
2. 650 micro litre aliquot of 1-methyl-2-phenylindole was placed in each test tube to which 200 micro litre of plasma was added.
3. The tube were mixed well and 150 micro litre of 10 M HCL was added.
4. After mixing once more, the tubes were sealed and incubated for 60 min. at 450C.
5. After incubation, the tubes were chilled on an ice bath and spun at 10,000 rpm for 5 min to remove the debris.
6. The absorbance at 586 nm was measured and the substracted from the blank value obtained by replacing plasma with water.
7. A calibration graph was prepared using 2 micro mol/ L, 4 micro mol/L, 6 micro mol/L and 8 micro mol/L 1,1,3,3-tetramethoxypropane in 20 nM Tris HCL, buffer pH 7.4.

Blood Sugar- Enzymatic, GOD-POD, endpoint colorimetric, single reagent chemistry. (Trinder P and Teitz N. W. by autospan kit method).

Total Cholesterol- Enzymatic endpoint Kit method, Randox Laboratory Ltd.

Serum Triglycerides- Enzymatic kit method - Transasia -Bio-medicals Ltd. (MeGowan et al., Fossati et al. and wako et al.).

HDL cholesterol- CHOD-POD kit method (Boehringer Mannheim Ltd.).

LDL and VLDL cholesterol- Burstein M. et al. and Lape-Virella et al.

Result

The study was carried out in 112 known diabetic cases and 52 healthy non-diabetic control subjects in the department of Clinical Pathology and Biochemistry, Patna Medical College, Patna, Bihar from July 2022 to June 2023.

The age group of 40–49 years old has the highest incidence of diabetes mellitus (57.14%), while the age group of 60 years and above has the lowest prevalence (24.11%).

When compared to the control group, the mean plasma lipid peroxide value is higher in cases of diabetes, and this increase is statistically significant ($p<0.01$).

In comparison to the control group, the mean values of total cholesterol, serum triglycerides, LDL-cholesterol, and VLDL-cholesterol are higher in the diabetic group ($p<0.01$).

When comparing the diabetic group to the control group, the mean value of serum HDL cholesterol is lower, and this difference is statistically significant ($p<0.01$).

Table 1: Age and sex distribution

Age	Sex		Total	Percentage
	Male	Female		
30-39	15	06	21	18.75%
40-49	38	26	64	57.14%
60 and above	14	13	27	24.11%
Total	67	45	112	100.00%

Table 2: Lipid peroxide level in plasma of various diabetes mellitus groups

Group	Plasma Lipid Peroxide (mmol/l)		
	Mean	S.D.	P value
Control (52)	3.40	±70.26	
IDDM (38)	4.56	±0.62	P<0.01
NIDDM (74)	4.92	±0.78	P<0.01
DM with complications	5.31	±0.49	P<0.01

Table 3: Relationship of lipid peroxidation, according to blood glucose level

Group of Diabetes according to blood glucose level	No. of cases	Lipid Peroxide	P value
Group A : Fasting blood sugar below 130 mg/dl (90-130 mg/dl)	32	4.12±0.38	P<0.01
Group B : Fasting blood sugar below 180 mg/dl (131-180 mg/dl)	46	4.62±0.56	P<0.01
Group C : Fasting blood sugar above 180 mg/dl (above 180 mg/dl)	34	4.98±0.72	P<0.01
Group D : Control	52	3.74±0.26	P<0.01

Table 4: Mean value of lipid peroxide and lipid fractions in controls and diabetic cases

Lipid peroxide and lipid fractions	Control (n=52)	Diabetics (n=112)
Lipid peroxide (mmol)	3.74±0.26	4.93±0.63
Total cholesterol (mg/dl)	188.65±24.61	282.00±46.23
Triglyceride (mg/dl)	110.46±36.12	26.26±11.72
HDL-cholesterol (mg/dl)	4.93±0.63	232.12±46.23
LDL-cholesterol (mg/dl)	174.36±86.67	44.86±16.46
VLDL-cholesterol (mg/dl)	152.85±48.93	38.30±18.96

Table 5: Ratio between lipid peroxide and lipid fractions in control and diabetics

Parameters Ratio	Control	Diabetics
LP : Total cholesterol	50.44	35.89
LP : Triglyceride	14.41	29.53
LP : HDL- Cholesterol	07.01	57.31
LP : LDL – cholesterol	35.36	09.06
LP : VLDL-cholesterol	31.00	07.76

In comparison to control, the ratio of lipid peroxide to total cholesterol is greater in diabetes patients. In comparison to the control group, the ratio of lipid peroxide to HDL cholesterol is lower in diabetes cases; nevertheless, the ratio of lipid peroxide to LDL and VLDL cholesterol is higher in diabetic cases. With the exception of HDL-cholesterol, there is a substantial association in this study group between the concentration of lipid peroxide and lipid fractions.

Discussion

Since activated phagocytes are active in the lipid phase, the hazardous substance they create during a response can maximally damage the membrane. The increased production of superoxide radicals within cells, which results in the inactivation of the superoxide dismutase enzyme in hyperglycemic conditions, is what causes the harmful effects of increasing hazardous radicals. This has an impact on diabetes mellitus's secondary disease and tissue damage. [10]

Free radicals are often efficiently scavenged during metabolism. When there is an imbalance between scavenging and production, oxidative stress results. Excessive production of free radicals is the cause of elevated lipid peroxidation in diabetes mellitus. Other factors that contribute to oxidative stress include ascorbic acid, reduced glutathione, autooxidation, glycosylated protein, and diminished superoxide dismutase enzyme. Serum lipid peroxide levels increased statistically significantly in all diabetes mellitus groups in the current investigation. A few of the most likely reasons why diabetes mellitus patients have higher levels of lipid peroxidation. The aberrant lipid metabolism in diabetes mellitus may be the cause of the abnormally high amounts of lipid, lipoprotein, and lipid peroxides in plasma [11].

Lipid peroxidation increased most in the group of people with diabetes mellitus who also had complications. The altered function of the erythrocyte membrane may be the cause of elevated lipid peroxide levels in diabetes mellitus. This prevents the superoxide dismutase enzyme from functioning, which builds up superoxide radicals and results in the highest level of lipid peroxidation and tissue damage in diabetics [12].

Increased protein glycation in diabetes mellitus may be the cause of the rise in lipid peroxide. It's possible that the glycated proteins themselves produce free radicals. Lipid peroxide and glucose concentration exhibit a strong correlation, which

may also contribute to elevated lipid peroxidation in diabetes mellitus. Higher peroxide concentrations have been linked to deficiencies in glutathione peroxidase and superoxide dismutase antioxidant capacity. The generation and scavenging of free radicals may be out of balance as a result of the absence of an antioxidant system. A comparatively substantial proportion of polyunsaturated fatty acid is present in the phospholipid of mitochondria and microsomal membranes. Fatty acids having 2, 4, 5, and 6 double bonds are among them. Because they have three or more double bonds, they are probably more vulnerable to free radical damage, which can lead to high levels of lipid peroxidation. Therefore, a high rate of peroxidation could be the reason why lipid peroxides and free radicals are more concentrated in people with diabetes mellitus. Lipoprotein metabolism may be impacted by apolipoprotein peroxidation. Although apo-A is thought to have antioxidant properties, these properties are lost as a result of peroxidation.

Lipid peroxide levels were higher in diabetic subjects who had vascular complications. It's possible that the elevated activity of free radical production is the cause of this rise in lipid peroxide. In the metabolism of arachidonic acid, free radicals combine to generate a hazardous endoperoxidase. The cyclooxygenase, prostaglandin, and thromboxane production are all stimulated by the lipid peroxide that is produced. Increased platelet aggregation as a result will result in vascular problems [13]. Comparing all diabetic groups to the controls, we have discovered that there is an increase in serum cholesterol. A lower level of physical activity or suppression of the breakdown of cholesterol are two potential causes of the elevated serum cholesterol levels in diabetes.

According to certain theories, a lack of insulin may be the cause of the rise in triglycerides because it leads to improper glucose use, hyperglycemia, and the release of fat from adipose tissue. Diabetes causes hyperglycemia because the body does not use blood glucose. Adipose tissue's stored fat is released for energy needs, and extra fat builds up in the liver where it is transformed into triglycerides. [14] According to the current study, insulin stimulates the production of LDL receptors; hence, a chronic insulin deficit may be linked to a reduction in LDL receptor levels. This raises LDL particle counts, which in turn raises LDL-cholesterol levels in those with diabetes mellitus. In cases of diabetes mellitus, obesity, increased calorie intake, and lack of physical exercise may be the cause of high levels of cholesterol, triglycerides, LDL cholesterol, and low HDL cholesterol. [15,16]

Conclusion

Lipid peroxide estimation and other lipid profile measurements are highly helpful in diabetes mellitus because they can be used as a good prognostic indicator. Finding risk factors early in the course of the illness will benefit the patient and lower the rate of morbidity.

References

1. Altamer, E., Vendemisle, G. and Chicco, D. Increased lipid peroxidation in Type-II poorly control Diabetic cases. Diabete. Etab. 2011; 18 (4): 264-671.
2. Jain, A.P. and Gupta, D.P. Study of blood lipids in Diabetics without any manifest vascular complications. J. Dia. Asso. Ind. 2020; XX: 29-34.
3. Agrawal, S., Banerjee, S. and Chatterjee, S.N. Effects of oxygen on ferrous sulphate induced lipid peroxidation in liposomal membrane. Ind. J. Biochem. and Biophysics. 2015; 21: 331-334.
4. Chattergee, S.N., Agrawal, S. and Amitkumar. Membrane lipid peroxidation and its pathological consequence. Ind. J. of Biochem and Biophysics. 2018; 25: 31.
5. Tappel, A.L. Lipid peroxidation damage to cell components. Clinical pathology. Federation proceeding. 2013; 32 (8): 1870-1874.
6. Yuzo, Sato, Migishi, Hotta and Nabuo, Sakamoto. Lipid peroxide level in plasma of Diabetic cases. Biochemical Medicine. 2019; 21: 104-107.
7. Nishigaki, Ikua, Masako, Hagihara and Hiroshi Tsunekawo. Lipid peroxide level and serum lipoprotein fractions in Diabetics cases. Biochemical Medicine. 2011; 25: 373-378.
8. Chandilia, H.B., Talwalkar, B.S. and Rajha, B.S. Lipid profiles in Diabetes mellitus. J. Dia. Assoc. India. 2018; 21: 155.
9. Stalenhoef, A.F.H., Demacker, P.N.M. and Lutterman, J.A. High Density lipoprotein and maturity onset Diabetes. Prog. Lipid Res. 2018; 32: 1-24.
10. Taniguchi, Naoyuki. Clinical significance of superoxide dismutase, Change in aging, Diabetes, Ischemia and Cancer. Advance in clini. chem, 2012; 29: 1-47.
11. Suckling, Keith E. and Brian, Jackson. Animal Models of Human lipid Metabolism. Prog lipid. Res. 2013; 32: 124.
12. Freitas, J.P., Filipe, P.M. and Radrigo, F.G. Lipid peroxidation in Type-II normolipidemic diabeticcases Diabetes. Res. Practy. 2017; 36 (2): 71-75.
13. Velazquez, E., Winocour, P.H., Kestenen, P. et al. Relation of lipid peroxide tomacrovascular diseases in type-II Diabetes. Diabetic Medicine. 2011; 8: 752-758.
14. Shih, K.C., Kwak, C.F. and Hwa, C.M. Acipimox attenuates hypertriglyceredemia in

Dislipidemic Non-insulin dependent diabetes mellites cases without perturbation of insulin sensitivity and glycemic control. Diabetic. Res. clin. pract. 2017; 36 (2): 113-119.
15. Das, Siddartha, Samal, Khitish Chandram and Tripathy, Bibhuti Bhashnt. Factors influencing plasma lipids and lipoprotein cholesterol in Indian NIDDM. J. Dia. Assoc. Ind. 2012;32 (2).
16. Yogi, K. et al. Lipid peroxide and human diseases. Chemistry and Physics of Lipid. 2019; 45: 337-351.