International Journal of Biochemistry Research & Review
13(1): 1-10, 2016, Article no.IJBCRR.27764
ISSN: 2231-086X, NLM ID: 101654445

SCIENCEDOMAIN *international*
www.sciencedomain.org

Assessment of Oxidative Stress and Lipid Status in Patients of Type 2 Diabetes Mellitus with and without Complications

Reenu Sharma[1], Maulik Nayak[1*] and Rita M. Shah[2]

[1]Department of Biochemistry, GMERS Medical College, Sola, Ahmedabad, Gujarat, India.
[2]Department of Biochemistry, SBKS MI and RC, Waghodiya, Gujarat, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MN designed the study, wrote the protocol and supervised the work. Author RS carried out all laboratories work and performed the statistical analysis and managed the analyses of the study. Author RMS wrote the first draft of the manuscript and managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2016/27764
Editor(s):
(1) Yi-Ren Hong, College of Medicine, Kaohsiung Medical University, Taiwan.
Reviewers:
(1) Fernando José Cebola Lidon, New University of Lisbon, Portugal.
(2) Hatice Pasaoglu, Gazi University, Turkey.
(3) Bharat Raj Singh, Institute of Engineering and Technology, School of Management Sciences, Technical Campus, Lucknow, India.
(4) Abdul Samad Aziz, Maharashtra University of Health Sciencies, India.
(5) Leonor Thomson, Universidad de la República, Uruguay.
Complete Peer review History: http://www.sciencedomain.org/review-history/15715

Original Research Article

Received 18th June 2016
Accepted 27th July 2016
Published 9th August 2016

ABSTRACT

Oxidative stress plays an important role in different disease processes. Some studies conducted on diabetic patients also support it. But very few studies have been conducted in the Indian subcontinent so far. Lipid peroxidation refers to the oxidative degradation of lipids. In this process free radicals take electrons from the lipids (generally in cell membranes), resulting in cell damage. Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. The end products of lipid peroxidation are reactive aldehydes such as malondialdehyde (MDA) as natural bi-products. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. This study was designed to assess the levels of oxidative stress in patients suffering from diabetes mellitus (DM) and to compare them with controls.

**Corresponding author: maulik20@yahoo.co.in*

Also, the study attempted to evaluate correlation between oxidative stress marker MDA and lipids as well as lipoproteins in type 2 DM subjects both with and without complications so as to analyse the role of lipid peroxidation in causing secondary pathophysiologic changes in multiple organ systems. The present study was conducted in Department of Clinical Biochemistry S.B.K.S. Medical Institute. & Research Centre, Waghodiya, Gujarat. Sixty diabetic patients were divided into two groups. Group A comprised of 30 (thirty) diabetic patients without complications and Group B comprised of 30 (thirty) diabetic patients with complications. Sixty normal healthy persons were selected for the study to serve as controls. The parameters assessed in diabetic subjects as well as healthy controls were: Serum MDA level which is a product of lipid peroxidation and Serum lipids as well as lipoproteins (Total Cholesterol, Triglycerides, High density lipoprotein cholesterol, Low density lipoprotein cholesterol). Mean values of MDA in type 2 diabetic subjects with complications were significantly higher ($P<0.001$) than values observed in type 2 diabetic subjects without complications. Values obtained for the lipids and lipoproteins in type 2 diabetic subjects with complications were significantly high ($P\leq 0.001$) compared to type 2 diabetic subjects without complications. The present study concludes that there is a significant elevation as well as correlation between oxidative stress marker MDA and various lipid parameters in type 2 diabetic subjects with complications compared to diabetic subjects without complications. This indicates increased lipid peroxidation in DM subjects with complications which may play a significant role in the development of DM associated vascular complications.

Keywords: Lipid profile; diabetes mellitus; oxidative stress; MDA.

1. INTRODUCTION

Diabetes Mellitus (DM) comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetic, environmental factors and life-style choices. Depending on the etiology of the DM, factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose utilization and increased glucose production. The metabolic deregulation associated with DM causes secondary pathophysiologic changes in multiple organ systems that impose a tremendous burden on the individual with diabetes and on the health care system. [1] DM has emerged as a major health care problem in India. According to the Diabetes Atlas published by the International Diabetes Federation (IDF), there were an estimated 40 million persons with DM in India in 2007 and this number is predicted to rise to almost 70 million people by 2025 by which time every fifth diabetic subject in the world would be an Indian [2].

Type 2 DM is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. This form of DM, accounts for approximately 90-95% of those with DM [3].

In type 2 DM increased hepatic glucose production occurs early in the course of DM, though likely after the onset of insulin secretory abnormalities in adipose tissue and obesity. Free fatty acid (FFA) flux from adipocytes is increased, leading to increased lipid [VLDL and triglyceride (TG)] synthesis in hepatocytes. This is responsible for the dyslipidemia found in type 2 DM [elevated TG, reduced high-density lipoprotein (HDL), and increased small dense low density lipoprotein particles (LDL)] [1].

According to Stanislaw et al. [4] good glycemic control (defined as near normoglycemia) as well as effective treatment of high blood pressure and dyslipidemia delay development and progression of microangiopathy [4].

DM is typically associated with increased generation of free radicals and/or impaired antioxidant defence mechanism, representing a central contribution for reactive oxygen species (ROS) in the onset, progression, and pathological consequences of DM. Increased free radical generation leads to a condition of oxidative stress. Oxidative stress leads to generation of Malondialdehyde (MDA) which is formed by both lipid oxidation and as a by-product of prostaglandin and thromboxane synthesis. Oxidation of complex lipids in vivo is largely caused by oxygen derived free radicals [5]. The major targets of these damaging species are the long chain polyunsaturated fatty acids (PUFAs) of cellular phospholipids, which are particularly prone to attack because of the arrangement of double and single bonds. The

resultant lipid peroxide frequently decomposes to a radical [6]. Which reacts with most biological molecules, including proteins and lipids. Further decomposition of these lipid peroxides produces toxic aldehydes, in particular MDA (mainly from arachidonic acid) [7].

According to DeZwart,; Mahboob et al. [8,9] Increased free radical production is said to mediate tissue injury in a wide range of diseases and DM is no exception. Free radicals are formed disproportionately in DM by glucose degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and cause severe damage in DM patients. Oxidative stress is increased in DM owing to an increase in the production of oxygen free radicals, such as superoxide, hydrogen peroxide and hydroxide radicals and deficiency of antioxidant defense mechanisms. Increased nonenzymatic and auto-oxidative glycosylation is one of the possible mechanisms that contribute to the formation of free radicals and free radical – induced lipid peroxidation in DM [8,9].

The purpose of the present study was to evaluate the role of oxidative stress and abnormal lipid levels in pathogenesis of various complications in type 2 DM.

2. METHODOLOGY

Study Subjects enrolled in this study were as follows:

A) 60 Healthy controls (HC) – selected from volunteers such as doctors, resident doctors, paramedical staff and healthy relatives / attendants of patients.
B) 60 Type 2 DM patients (30 Type 2 DM without complications – Group A & 30 Type 2 DM with complications - Group B) attending the outpatient clinics or admitted in wards of Department of Medicine Dhiraj General Hospital, S.B.K.S. MI.& R.C. Waghodiya. The duration of DM in diabetic subjects ranged from 5 to 16 years.

All the subjects were randomly selected for the study. We excluded the subjects who were habitual smokers, alcoholics, hypertensive and having active inflammatory diseases, nutritional deficiencies, estrogen therapy, malignancy and active immunological diseases. The DM subjects with complications were on oral hypoglycemic drugs.

Samples were obtained from the outpatient and inpatient department of clinical biochemistry, Dhiraj General Hospital, S.B.K.S. Medical Institute & Research Centre, Waghodiya, Gujarat. Written informed consent was collected from each patient and control subject after full explanation of the study. The study was approved by ethical committee of the institute according to the Declaration of Helsinki. Age, sex, and blood pressure were matched within the study groups. There was no significant statistical difference in age, sex distribution, BMI, and blood pressure in Type 2 DM patients and healthy control individuals. Fasting (FPG) and postprandial (PPG) plasma glucose (blood samples were drawn after 2 hrs of 75 gm postload glucose) and HbA1c% were elevated significantly among DM subjects compared to healthy control subjects ($P < 0.001$) (Table 1 & 2).

2.1 Sample Collection and Processing

The study subjects were advised to be in 12-hour strict fasting state, after which venous blood samples were drawn to obtain plasma and serum for MDA and other assays. Blood was allowed to clot for 30 minutes at room temperature and then centrifuged at 3000 rotations per minute (rpm) for

Table 1. Clinical characteristics of Healthy controls (HC) and Diabetic subjects (DM)

	HC (n=60)	DM (n=60)	P Value
Age (In years)	52.3±10.8	53.8±8.6	0.41 (NS)
Sex (M/F)	32/28	29/31	0.39 (NS)
BMI (kg/m2)	23.1±4.0	23.8±3.7	0.06 (NS)
Duration of Diabetes	----	10.9±2.7	----
Systolic BP (mm Hg)	127.8±8.4	128.2±7.9	0.20 (NS)
Diastolic BP (mm Hg)	81.4±6.95	82.2±6.5	0.14 (NS)

Age, BMI, BP-blood pressure were expressed as the mean ± SD.
NS: Non significant

Table 2. Fasting, Post Prandial Plasma Glucose and HbA_{1c} values of study subjects

	Control (n=60)	DM without complications (n=30)	DM with complications (n=30)	P value
FPG (mg/dl)	82.2±13.4	136.0±29.3	185.0±47.6	<0.001
PPG (mg/dl)	104.0±10.5	201.0±55.8	273.0±63.1	<0.001
HbA_{1c} (%)	------	9.57±0.9	11.1±1.2	<0.001

FPG- fasting plasma glucose, PPG -postprandial plasma glucose. HbA1c scores and serum levels of biochemical parameters were expressed as the mean ± SD
Statistically significant, ∗ P < 0.001

10 minutes to obtain clear unhemolysed serum (haemolysed serum samples were excluded from analysis). Aliquots of serum samples were prepared by transferring them into separate plain vials. These were labeled properly and stored at -20°C until assayed for estimation of lipids. For MDA and plasma glucose (FPG and PPG) estimation fresh samples were tested to avoid erroneous results.

2.2 Measurement of MDA

Lipid peroxidation in serum was measured by MDA estimation as described previously.[10] Lipoproteins in serum were precipitated by adding 20% trichloroacetic acid and 8.1% sodium dodecyl sulphate. Thereafter, 0.8% aqueous solution of thiobarbituric acid was added to this precipitate, mixed well, and finally heated at 95°C for 1 hour for coupling of lipid peroxide with thiobarbituric acid reagent. The resulting chromogen was extracted from the precipitate by adding n-butanol and pyridine mixture (15:1). The organic mixture was separated by centrifugation and the intensity of the organic layer was measured spectrophotometrically (Halo DB-20, Dynamica, Mayrwies, Salzburg, Austria) by using 530nm filter against water blank. The concentration of MDA in serum was determined from linear standard curve established by 1 to 8 nm of 1,1,3,3-tetramethoxypropane [10].

2.3 Measurement of Fasting and Post Prandial Plasma Glucose (FPG & PPG)

FPG and PPG were measured by Glucose oxidase-peroxidase method, [11] using the kits provided by DiaSys Diagnostic Systems GmbH.

2.4 Measurement of Serum Lipids and Lipoproteins

The lipid and lipoprotein parameters were measured by standard methods by using commercially available kits (DiaSys Diagnostic Systems GmbH).Serum total cholesterol was estimated by Cholesterol Oxidase-Peroxidase method, [12] triglycerides by Glycerol Phosphokinase-Peroxidase method, [13] high density lipoprotein-cholesterol Phosphotungstic Acid, [14] end point method, low density lipoprotein cholesterol by Direct Immunoseparation method [15] (very low density lipoprotein cholesterol were calculated by Friedwald's equation) [16].

The analyses of various parameters was performed on ERBA Mannheim's Erba XL 300 an open system clinical chemistry analyzer with throughput of 300 tests per hour.

2.5 Statistical Analysis

Differences in the parameters between the groups were analyzed by means of the student's t test. Variables were presented as mean ± standard deviation (S.D). Correlations between variables were tested using the Pearson rho (r: correlation coefficient) correlation test. Chi-square (χ^2) analysis was used for comparison of groups. Data were compared in the groups using SPSS for Windows (version 16; SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered as a statistically significance level.

3. RESULTS AND DISCUSSION

In the present study type 2 diabetic subjects with complications (6.90 nmol/ml) and without complications (5.81 nmol/ml) showed significantly higher mean values of MDA (P< 0.001; P= 0.003) compared to values of MDA observed in healthy controls (4.06 nmol/ml) (Tables 3 and 4).

MDA is a late-stage lipid oxidation byproduct that can be formed nonenzymatically or as a byproduct of cyclo-oxygenase activity. MDA is a volatile molecule that reacts, via schiff base formation, with free amine groups of protein, lipid, and DNA. It is estimated that up to 80% of

MDA is protein bound. In addition, accumulation of MDA affects membrane organization by increasing phosphatidylserine externalization. Accumulation of MDA and MDA adducts is correlated with many disease states, such as diabetes mellitus [17].

Oxidative stress, a state of lost balance between the oxidative and anti-oxidative systems of the cells and tissues, results in the over production of oxidative free radicals and reactive oxygen species (ROS). Excessive ROS generated could attack the cellular proteins, lipids and nucleic acids leading to cellular dysfunction including loss of energy metabolism, altered cell signaling and cell cycle control, genetic mutations, altered cellular transport mechanisms and overall decreased biological activity, immune activation and inflammation. These changes lead to initiation of pathogenic milieu and development of pathologies like diabetes [17].

It has been reported that oxidative stress is enhanced in response to hyperglycemia in vascular tissues of patients with DM, leading to the peroxidation of cellular membrane lipids as well as the increased oxidative modification of amino acids and DNA [18]. Ozdemir et al. [19] observed a significant increase in MDA in patients with type 2 DM compared with control group. Their study suggested that permanent structural membrane alterations occur in DM, due to increased production of ROS and decreased antioxidants in the circulation [19].

Moreover, significantly higher values of MDA (P = 0.002) were observed in type 2 diabetic subjects with complications compared to type 2 diabetic subjects without complications (Table 3 and 4).Enhanced oxidative stress under hyperglycemic conditions causes an increase in peroxide lipids in the cell membrane, which induces the intracellular expression of specific genes. To date, it has been understood that the activity of two transcriptional factors, NF-_B and AP-1 (activator protein-1), is regulated by intracellular redox states. When activated, these transcriptional factors bind to the specific binding sites in the regions upstream of various genes such as VCAM-1, ICAM-1, as well as cytokines and growth factors including MCP-1 and PDGF and then regulate the expression of those genes. Vascular disorders progress through the expression of these proteins which are involved in cell-cell interactions in the vascular wall [20].

There is much evidence that oxidative stress is involved in the etiology of several diabetic complications. Type 2 DM is associated with insulin resistance which results in failure of insulin stimulated glucose uptake by tissues. This causes glucose concentrations in blood to remain high. Consequently, glucose uptake by insulin independent tissues increases. Increased glucose flux enhances oxidant production [21,22].

The present study findings showed significantly high mean values of total cholesterol (TC), triglycerides (TG), high density lipoprotein-C (HDL-C), very low density lipoprotein-C (VLDL-C) and low density lipoprotein-C (LDL-C) respectively (P = 0.001, P <0.0001, P = 0.02, P <0.0001, P = 0.004) in type 2 diabetic subjects without complications (mean values in mg/dl: TC: 199.0; TG: 160.0; HDL-C: 49.1, VLDL-C: 31.9 and LDL-C: 117.0) compared to healthy controls (mean values in mg/dl: TC: 175.0; TG: 95.5; HDL-C: 52.2; VLDL-C: 19.1 and LDL-C: 105.0). Also, significantly high values (P < 0.0001) for

Table 3. Mean Values of FPG, MDA and Lipids in Study Subjects

S. No	Parameters	Groups studied (values as Mean ± SD)		
		Healthy controls	Group A type 2 DM without complications	Group B type 2 DM with complications
1	FPG (mg/dl)	82.2±13.4	136.0±29.3	185.0±47.6
2	MDA (nmol/ml)	4.06±1.51	5.81±1.65	6.90±1.61
3	Total C (mg/dl)	175.0±24.0	199.0±22.5	235.0±36.6
4	Triglyceride (mg/dl)	95.5±20.7	160.0±55.3	218.0±110.0
5	High density lipoprotein-C (mg/dl)	52.2±7.1	49.1±8.06	42.4±8.68
6	Very low density lipoprotein–C(mg/dl)	19.1±4.1	31.9±11.0	43.7±21.9
7	Low density lipoprotein–C(mg/dl)	105.0±24.1	117.0±27.4	148.0±32.7

Table 4. Comparison of mean values of MDA & various Lipid levels amongst the study subjects

S. No	Groups compared		Parameters					
			MDA	Total C	TG	HDL-C	VLDL-C	LDL-C
1	Healthy Controls	T	3.01	5.96	10.3	2.36	10.3	2.90
	vs	*P*	0.003	0.001	< 0.0001	0.02	< 0.0001	0.004
	Type 2 DM subjects without complications		S	S	HS	S	HS	S
2	Healthy Controls	T	6.88	12.1	10.9	7.35	10.9	7.50
	vs	*P*	< 0.001	< 0.0001	<0 .0001	<0.0001	< 0.0001	< 0.0001
	Type 2 DM subjects with complications		HS	HS	HS	HS	HS	HS
3	Type 2 DM subjects without complications	T	3.14	5.96	3.38	3.68	3.38	4.32
	vs	*P*	0.002	0.001	0.001	0.0004	< 0.0001	< 0.0001
	Type 2 DM subjects with complications		HS	S	S	S	HS	HS

HS: Highly Significant S: Significant

The accepted level of significance for all statistical analyses used in the study was P= 0.05

various lipid parameters were observed in type 2 diabetic subjects with complications (mean values in mg/dl: TC: 235.0; TG: 218.0; HDL-C: 42.4; VLDL-C: 43.7 and LDL-C: 148.0) compared to values obtained in healthy controls (Tables 3 and 4).

DM is a condition where hyperlipidemia is very common. Moreover, a positive correlation was observed between TC and MDA (r=0.36; P=0.009), TG and MDA (r = 0.31; P = 0.02) and also between LDL-C and MDA (r=0.27; P=0.05) in type 2 diabetic subjects with complications (Table 5). However, the type 2 diabetic subjects without complications did not show a significant positive correlation between lipids and MDA (Table 5). These observations indicate the coexistence of atherogenic risk factors and oxidative stress in DM subjects with complications. Chronic oxidative stress in diabetic subjects may be related to the metabolism of excess substrates available such as glucose and fatty acids present in the hyperglycemic state. DM is a condition where hyperlipidemia is very common. Moreover, lipid peroxidation increases with hyperlipidemia [23]. Peroxidation of apolipoproteins may affects the lipoprotein metabolism. It is suggested that apo-A has an antioxidant effect, but due to the peroxidation the antioxidant property of apo-A is lost [24].

Januszweski et al. [25] proposed a variation on the advanced glycation end products hypothesis i.e. the hyperglycemia exacerbates the chemical modification of proteins by lipids and that lipids and advanced glycation end products, rather than carbohydrates and advanced glycation end products, may be the immediate and major source of chemical modifications leading to tissue damage, pro-inflammatory process and chronic complications in diabetes [25].

Thus, while severe hyperlipidemia may be sufficient to induce lipo-oxidative damage, hyperlipidemia compared with hyperglycemia and possibly an increase in oxidative stress in diabetes appears to exacerbate the chemical modifications of proteins in diabetes.

Gambhir et al. [26] also found a significant positive correlation (r = 0.712) between MDA levels and TG in diabetics with complications. According to Gambhir et al. [26] hypertriglyceridemia may lead to increased production of lipid derived free radicals. The resultant oxidative stress may be implicated as a causative factor for endothelial dysfunction, which may be a primary event in the pathogenesis of atherosclerotic vascular disease.

Values obtained for the lipids that is TC, TG, HDL-C, VLDL-C and LDL-C in type 2 diabetic subjects with complications were significantly high (P <0.001) (Table 4) compared to type 2 diabetic subjects without complications.

Table 5. Correlation of serum MDA values with serum lipids in various groups of subjects

Study groups	TC			TG			HDL-C			VLDL-C			LDL-C		
	R	T	P	R	T	P	R	t	P	R	T	P	R	T	P
HC	+0.28	2.9	0.002	+0.10	0.96	0.16	-0.11	1.08	0.14	+0.05	0.48	0.31	+0.008	0.08	0.46
Group A	+0.12	0.85	0.19	+0.31	2.24	0.01	-0.15	1.08	0.14	+0.31	2.24	0.01	+0.03	0.24	0.40
Group B	+0.36	2.72	0.004	+0.35	2.66	0.005	+0.02	0.17	0.43	+0.35	2.66	0.005	+0.27	2.01	0.02

- *r = correlation coefficient HC: Healthy Controls*

TC: Total Cholesterol TG: Triglyceride HDL-C: High density lipoprotein-Cholesterol LDL-C: Low density lipoprotein-Cholesterol

Table 6. Comparative analysis of subjects with Lipid levels as per NCEP ATP III criteria

Lipid levels	Diabetic without complications vs diabetic with complications		
Serum cholesterol (mg/dl)	X^2	P	Cramer's V
i) 161-199 and 200-239	4.36	0.04 S	0.26
ii) 161-199 and 240-279	12.17	0.0005 HS	0.53
iii) 161-199 and ≥ 280	15.71	< 0.0001 HS	0.63
Serum triglycerides (mg/dl)			
i) < 150 and 150-199	0.05	0.82	0.05
ii) < 150 and 200-499	5.96	0.01 S	0.30
HDL-C (mg/dl)			
<40	7.68	0.005 S	0.30
< 40 and ≥ 60	-	-	-
LDL-C (mg/dl)			
i) < 100 and 100-129	0.83	0.36	0.16
ii) < 100 and 130-159	5.74	0.01 S	0.40
iii) < 100 and 160-189	11.15	0.0008 HS	0.61
iv) < 100 and ≥ 190	4.25	0 .03 S	0.58

- *The accepted level of significance for all statistical analyses used in the study was P= 0.05.*
- *HS : Highly Significant*
- *S: significant*

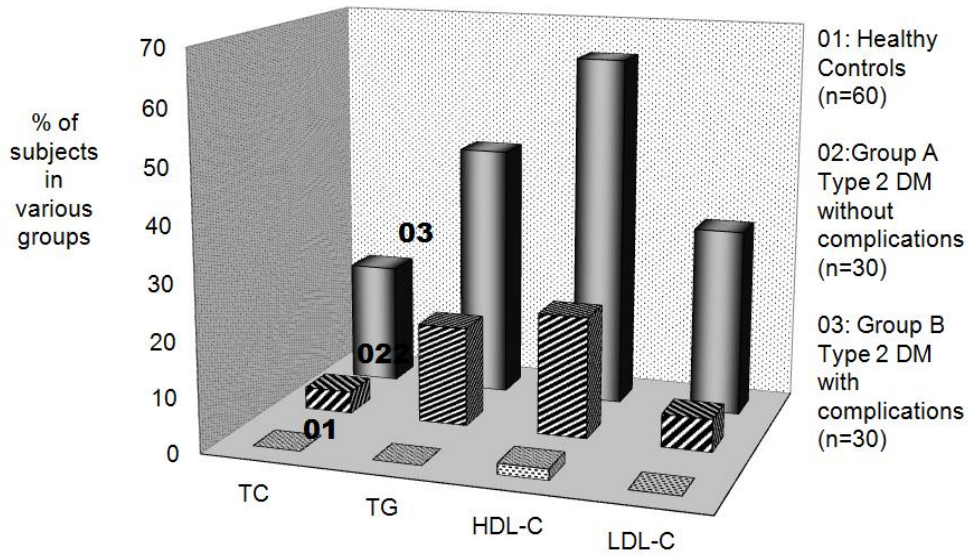

Fig. 1. Stratification of subjects (% distribution) in high risk ranges of TC: 240-279 mg/dl, TG: 200-499 mg/dl, HDL-C :< 40 mg/dl and LDL-C: 160-189 mg/dl

TC: Total Cholesterol TG: Triglyceride HDL-C: High density lipoprotein-Cholesterol LDL-C: Low density lipoprotein-Cholesterol

According to the study by Soliman [27] hyperlipidemia is reported as one of the causative factors for increased lipid peroxidation in DM. Kesavulu et al. [27] observed that the levels of LDL-C and TG were increased in diabetics with microvascular complications compared to those without these complications. Even levels of TBARS were much higher in diabetics with microvascular complications. The hyperglycemia in association with hyperlipidemia observed in diabetic patients could be the causative factor for the increased production of oxygen free radicals and lipid peroxides [28]. The TBARS levels positively correlated with HbA_{1C} and total cholesterol in the study by Kesavulu et al. [28]. Thus, lipid peroxides may play a role in the pathogenesis of microvascular complications of DM.

Chi-square analysis (χ^2) was performed (employing 2 X 2 contingency table) to compare the percentage distribution of study subjects in various risk ranges of lipids. Significant values were obtained for various lipids in diabetic subjects with complications within high risk range (as per NCEP ATP III Guidelines) compared to diabetic subjects without complications (Table 6).

The results for lipids observed in the present study are in accordance to observations by Chapman [29]. He stated that type 2 DM is characterized by atherogenic dyslipidemic profile with mild to marked elevation of triglyceride–rich lipoprotein (VLDL and VLDL remnants) concentrations, an increase in small dense LDL and apoliproprotein B (apo B) and low levels of HDL. According to Chapman [29] dyslipidemia is also characterized by a spectrum of qualitative lipid abnormalities reflecting perturbations in the structure, metabolism and biological activities of both atherogenic lipoproteins containing apo B [VLDL, intermediate density lipoprotein (IDL) and LDL] and anti-atherogenic HDL containing apo A-I and / or apo A-II.

In the present study percentage distribution of subjects within various risk ranges of lipids (as per NCEP III guidelines) was evaluated. It was observed that maximum number of subjects in borderline and high risk ranges of lipids were among type 2 diabetics with complications (Fig. 1 above).

5. CONCLUSION

In recent years, much attention has been focused on the role of oxidative stress, and it has been reported that oxidative stress may constitute the key and common event in the pathogenesis of secondary diabetic complications These findings point towards the need for early diagnosis and management of Type 2 DM patients in order to prevent the

development of oxidative stress associated diabetic complications. It may also be inferred that even in the early stages diabetic patients are exposed to oxidative stress due to hyperglycemia and oxidative stress is known to be the unifying factor in the development of diabetes complications. Hence supplementation of antioxidants may also be considered in the management of newly diagnosed Type 2 DM.

In conclusion, the estimation of lipid peroxide MDA along with lipid profile in diabetes mellitus would serve as a useful monitor to judge the prognosis of the patient. Prevention of lipid peroxidation may help to delay the development of diabetic complications. The detection of the risk factor in the early stage of the disease helps to improve and reduce the mortality rate. It gives reason to look for dependence between the oxidative stress degree, evolution of the disease and its chronic complications. This dependence could be used as prognostic marker of course evaluation of diabetes. Not the least is the possibility of reducing oxidative stress by means of different antioxidants as a supplement.

ETHICAL APPROVAL

All experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENT

The authors gratefully acknowledge authorities of Central Laboratory of S.B.K.S Medical College, Waghodiya, for providing facilities for carrying out this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dennis L. Kasper, Eugene Brunwald, Anthony S. Fauci, Stephen N Hauser, Dan L Longo, J. Larry Jemeson. HARRISON's principles of internal medicine, 16th Edition, 2152-2180.
2. Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance in India Diabetes Atlas. Gan D Ed. International Diabetes Federation, Belgium. 2006;15-103.
3. American Diabetes Association. Summary of revisions for the 2008 clinical practice recommendations. Diabetes Care. 2008;31 (Suppl. 1):S3-S4.
4. Stanislaw P, Pawel V, Agnieszka S, Aleksandra P, Joanna D, Pawel K, Patryija M, Dorota Z, Wierus Z, Wysocka B. Selected inflammatory makers as risk factors for diabetic micro-angiopathy. Diabetologia. 2006;3(Suppl.1):151-157.
5. Kume S, Takeya M, Mori T. Immunohistochemical and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. Am J Pathol. 1995; 147:654-657.
6. Spiteller G. Linoleic acid peroxidation the dominant lipid peroxidation process in low-density lipoprotein, and its relationship to chronic diseases. Chem Phys Lipids. 1998; 95:105-162.
7. Esterbauer H. Biochemical, structural and functional properties of oxidized low-density lipoprotein. Chem Res Toxicol. 1990;3:77-92.
8. DeZawart LL, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage applications in experimental animals and humans. Free Radic Biol Med. 1999;26:202-226.
9. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med. 2005;46:322-324.
10. Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chem Acta. 1978;90:37-43.
11. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem. 1969;6:24.
12. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974;20(4):470-475.
13. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clinical Chemistry. 1973; 19(5):476-478.
14. Assmann G, Schriewer H, Schmitz G, Hagele EO. Quantification of high density lipoprotein cholesterol by precipitation with

phosphohingstic acid/mgCl_2. Clinical Chemistry. 1983;29(12):2026-2030.

15. Lipids and Lipoprotelns 232 Clinical chemistry. Immunoseparation Method for Measuring Low-Density Lipoprotein Cholesterol Directly from Serum Evaluated Judith R. McNamara,' Thomas G. Cole,2 John H. Contois,' Constance A. Ferguson, 2 Jose M. Ordovas,' and Ernst J. Schaefer"3. 1995;41(2);232-240.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. Clinical Chemistry. 1972;18(6):499-502.
17. Rani V, Deep G, Singh RK, Palle K, Yadav UC. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. Life Sci. 2016;148:183-93.
18. Dymkowska D, Drabarek B, Podszywałow-Bartnicka P, Szczepanowska J, Zabłocki K. Hyperglycaemia modifies energy metabolism and reactive oxygen species formationin endothelial cells *in vitro*. Archives of Biochemistry and Biophysics, 2014;542:7–13.
19. Ozdemir G, Ozden M, Hale Maral, Kuskay S, Cetinalp P, Tarkun I. Malondialdehyde, glutathione, glutathione peroxidase and homocysteine levels in type 2 diabetic patients with without microalbuminuria. Ann Clin Biochem 2005;42:99-104.
20. Atsunori Kashiwagi. Complications of diabetes mellitus and oxidative stress. JMAJ. 2001;44(12):521–528,
21. Kowluru RA, Kennedy A. Therapeutic potential of antioxidants and diabetic retinopathy. Expert Opin Investig Drugs. 2001;10:1665-1676.
22. Chang TI, Horal M, Jain S, Wang F, Patel R, Loeken MR. Oxidant regulation of gene expression and neural tube development: insights gained from diabetic pregnancy on molecular causes of neural tube defects. Diabetologia. 2003;46(4):538-545.
23. Moriel P, Plavnik FL, Zanella MT, Bertolami MC, Abdalla DS. Lipid peroxidation and antioxidants in hyperlipidemia and hypertension. Biol Res. 2000;33:105–12.
24. Arora R, Vig AP, Arora S. Lipid peroxidation: A possible marker for diabetes. J Diabetes Metab. 2013;S11: 007.
25. Januszewski AS, Alderson NL, Metz TO, Thorpe SR, Baynes JW. Role of lipids in chemical modification of proteins and development of complications in diabetes. Biochemical Society Transactions. 2003; 31(Part 6):1413-1416.
26. Gambhir JK, Saxena R, Prabhu KM, Madhu SV, Gambhir DS. Postprandial hypertriglyceridiemia and oxidative stress in type 2 diabetic patients with macroangiopathy. Indian Heart J. 2004;56: 384.
27. Soliman GZA. Blood lipid peroxidation (superoxide dismutase, malondialdehyde, gutathione levels in Egyptian type 2 diabetic patients). Singapore Med J. 2008;49(2):129-136.
28. Kesavulu MM, Giri R, Rao BK, Apparao CH. Lipid peroxidation and antioxidant enzyme levels in type 2 diabetics with microvascular complications. Diabetes Metab. 2000;26:387-392.
29. Chapman MJ. Metabolic syndrome and type 2 diabetes: Lipid and physiological consequences: Dyslipidaemia 2007; 4(Suppl. 3):S5-S8.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15715