



ANTIOXIDANT STATUS IN NON-INSULIN DEPENDENT DIABETES MELLITUS

CARLOS NOEL MENEZES¹ AND CHITRA Y DHUME^{*2}

¹*Department of Biochemistry, Goa Medical College, Bambolim –Goa, India*

^{2*}*Department of Biochemistry, Goa Medical College, Bambolim –Goa, India*

ABSTRACT

Diabetes Mellitus is the most common of the endocrine disorders. Diabetes patients have significant defects of Anti-oxidant protection, which may enhance their susceptibility to oxidative damage and development of diabetic complications. The present study comprised of estimation of the biochemical parameters and two special investigations i.e. Serum Malondialdehyde and Blood Glutathione levels in sixty cases [20 normal healthy individuals and 40 N.I.D.D.M. patients] attending the outpatient department of Goa Medical College. Serum Malondialdehyde and Blood GSH levels were significantly increased along with Serum Uric acid, Serum total Cholesterol, Triglycerides, LDL and VLDL-Cholesterol as compared to apparently healthy individuals. HDL-Cholesterol levels were significantly decreased.

KEYWORDS: Diabetes Mellitus, Malondialdehyde and Blood Glutathione



CHITRA Y DHUME

Department of Biochemistry, Goa Medical College, Bambolim –Goa, India

INTRODUCTION

Diabetes mellitus is a metabolic syndrome, which is associated with a series of hormone dependent metabolic abnormalities, long term complications and microvascular lesions. There is hyperglycemia which is a primary disorder of carbohydrate metabolism with multiple etiological factors that generally involve absolute or relative insulin deficiency or resistance or both, thus leading to glucose intolerance, impairment in renal function and hepatic insufficiency¹. Since the etiology of Diabetes mellitus continues to evade the researchers and the microcellular metabolism which is altered due to lack of insulin is still not clearly understood, diabetes mellitus continues to be a dilemma till date and invites research on several fronts. Diabetes mellitus is considered as an 'Iceberg' disease, which has affected at least 30 million people throughout the world. Its prevalence in most adult populations is 2-5%; in some populations the rate is considerably higher. Epidemiological studies have established a prevalence of disease in adults to be 2.4% in rural and 4.0-11.6% in urban dwellers². Diabetes mellitus also alters the lipid profile and the protein metabolism. Type II N.I.D.D.M. is generally associated with increased serum uric acid, serum cholesterol and decreased H.D.L.-cholesterol. Fasting samples also show an increase in the lipid profile and an increase in the lipoproteins. Diabetic patients have significant defects of antioxidant protection, which may enhance their susceptibility to oxidative damage and development of diabetic complications. Regarding the status of Antioxidants, Oxygen free radicals are implicated in many types of pathological diseases including Diabetes mellitus and aging³. Oxidative stress has been proposed to play an important role in Diabetes mellitus, right from its genesis to the development of micro vascular complications. Changes in the protein profile are those associated with lack of insulin and liver damage. There is usually a decrease in the serum proteins, serum albumin and serum globulin and an altered albumin/globulin ratio. The serum transaminases are found to be normal or increased, especially in cases of N.I.D.D.M.

Our present study makes an effort to study Blood Glutathione, Lipid Peroxidation marker like Malondialdehyde, basic biochemical lipid profile, serum uric acid and fasting blood sugar levels in N.I.D.D.M. Patients and apparently healthy individuals.

MATERIALS AND METHODS

The present study comprised of estimation of the biochemical parameters and two special investigations i.e. Serum Malondialdehyde and Blood Glutathione levels in sixty cases [20 normal healthy individuals and 40 N.I.D.D.M. patients] attending the outpatient department of Goa Medical College. Appropriate permission from the institutional ethics committee was taken prior to the commencement of the study. Informed consent was obtained from all participants. The period of study was between July 2010 to July 2012.

Study Group

This consisted of 40 patients of N.I.D.D.M. seeking medical aid in the outpatient department of Goa Medical College. All the 40 patients were above 40 years of age and belonged to either sex. These N.I.D.D.M. patients were taking oral hypoglycemic drugs regularly. Their diagnosis was based on history, thorough physical examination and blood glucose levels. Special care was taken to exclude from the study group, patients having diseases like megaloblastic anemia, liver disease, acute renal failure, hypothyroidism, hyperthyroidism, tuberculosis, lymphomas and iron deficiency anemia.

Control Group

This group comprised of twenty healthy persons above 40 years of age of either sex. A thorough history was taken and clinical examination carried out in each case to rule out any diseased state. In both the groups, a detailed history was obtained and a thorough clinical examination was carried out. Blood glucose was estimated by Glucose Oxidase Method⁴. Total Cholesterol was estimated by

Enzymatic Method⁵. HDL- Cholesterol was estimated using accelerator selective detergent Method⁶. Serum Triglycerides were estimated by Glycerol Phosphate Oxidase Method⁷. LDL-Cholesterol was calculated by using Friedewald and Fredrickson's formula. Blood Urea was estimated by Urease Method⁸. Serum Creatinine was estimated by

kinetic alkaline picrate method⁹. Serum Uric acid was estimated by Uricase Method¹⁰. Serum Albumin was estimated by BCG method¹¹. Serum Malondialdehyde was estimated by Ohkawa, Ohishi and Yagi Method¹². Blood Glutathione was estimated by Beutler's Method¹³.

RESULTS

Parameter	Controls		NIDDM patients		S.E.	T Value	p Value	
	Mean	SD \pm	Mean	SD \pm				
FBSL (mg %)	85.1	11.4	177.15	36.54	8.38	10.98	0.001	
Cholesterol (mg %)	185.45	20.82	248.05	44.21	10.45	5.99	0.001	
H.D.L (mg %)	40.05	3.74	36.175	6.34	1.53	2.53	0.001	
L.D.L (mg %)	124.7	18.73	182.82	44.94	10.51	5.52	0.001	
V.L.D.L (mg %)	19.2	6.18	30.22	5.29	1.53	7.29	0.001	
TG (mg %)	97.6	27.69	152.72	26.65	7.39	7.45	0.001	
Blood Urea (mg %)	26.65	4.51	29.52	2.53	0.9068	3.16	0.01	
Se. Creatinine (mg %)	0.795	0.2136	0.807	0.1801	0.0519	0.23	0.10	
Se. Proteins (g %)	6.28	0.3873	5.50	1.0781	0.2478	3.25	0.01	
Se. Bilirubin (mg %)	0.465	0.024	0.375	0.033	0.0523	0.21	0.10	
Se.Na (meq/l)	134.25	5.62	143.90	6.48	0.2478	3.25	0.10	
Se.K (meq/l)	3.99	0.6903	3.75	0.842	0.3478	2.25	0.10	
Se.Cl (meq/l)	93.75	4.62	97.85	3.82	9.52	4.62	0.10	
Se. Uric Acid (mg %)	4.18	0.6928	6.10	1.5895	0.3704	5.18	0.001	
Se. MDA (nmol/ml)	0.90	0.0241	1.29	0.0316	0.065	6.24	0.001	
Blood GSH (mg %)	61.82	4.4018	77.53	10.8825	2.53	6.20	0.001	

Table No.1
Values of different parameters in Controls and NIDDM patients.

Groups	Males	Percentage %	Females	Percentage %
Controls (20)	16	80	4	20
NIDDM (40)	27	67.5	13	32.5

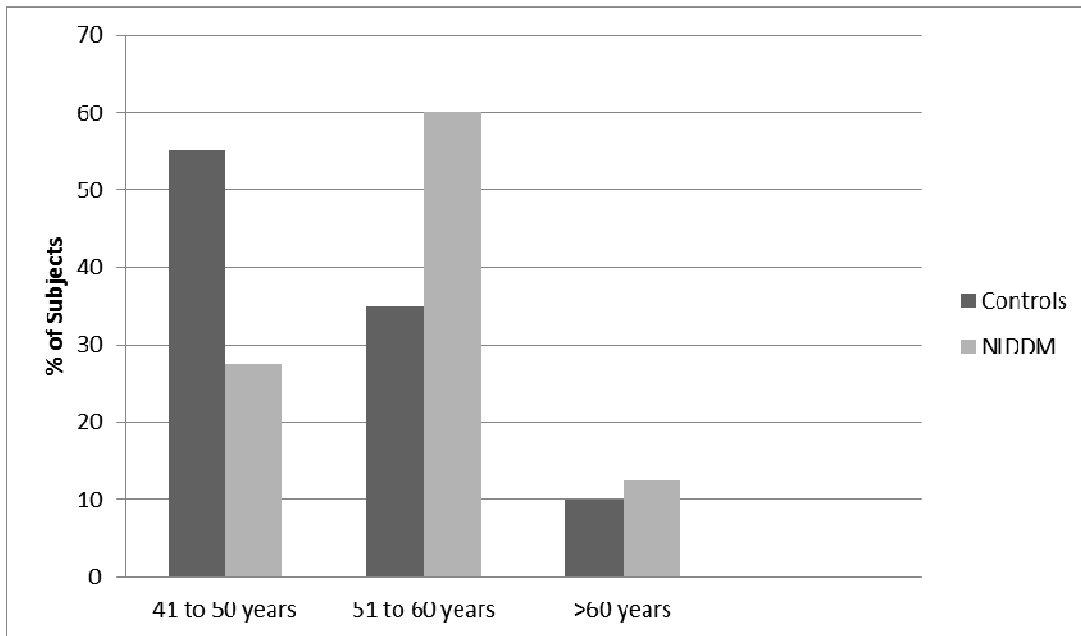
Table No.2
Showing Sex Distribution of Study

An overall male predominance was observed in the case material with 67.5% incidence in the study group and 80% incidence in the Control group.

Duration of illness (in years)	Number of patients	Percentage %
1 to 5	19	47.5
6 to 10	15	37.5
11 to 15	6	15

Table No.3
Showing Distribution of NIDDM cases according to their duration of illness

Graph no. 1
Showing the Age incidence of the patients in the control and study groups



NIDDM Patients	Number of patients with complications	%
40	26	65%

Nature of Complications	Number of Patients
Neuropathy alone	12
Neuropathy + Retinopathy	2
Neuropathy + Ulcer (foot)	2
Neuropathy + Skin infection	1
Neuropathy + UTI	1
Neuropathy + Retinopathy + Ulcer (foot)	1
Skin infection alone	4
Urinary tract infection alone	2
TOTAL:	26

Table No.4
Showing complications in patients with NIDDM and the break-up of the complications

Out of the 26 NIDDM patients who had complications, an overwhelming majority (20 patients) had neuropathy.

DISCUSSION

Diabetes mellitus is better understood today in terms of multi organ manifestations and variable complications by this basis of

oxidative stress or lipid peroxide damage. It is a heterogeneous primary disorder of carbohydrate metabolism, leading to

hyperglycemia. Diabetes produces acute complications like neuropathy, retinopathy, nephropathy, cardiovascular complications like ischemic heart disease and other cardiovascular involvement, thus leading to premature death in such cases. The mortality rate in diabetes is considerably high all over the world. Elevated blood sugar levels are mainly used to detect diabetes. Biochemical research has made tremendous efforts to find better and easier ways to diagnose the disease in the early stages of its better treatment, further follow-up and management. Remarkable improvement in the expectancy of life and health standards is attributed- to- various- medical- and biochemical- researches conducted so far. It is towards this end that the present study is intended to contribute its little mite. The analysis of the 'study group revealed that the maximum number of cases of N.I.D.D.M. were between 51 to 60 years [55%] followed by 41 to 50 years [35%] (Table 3). The duration of the illness was 1 to 5 years in most of the cases [47.5%] followed by 6 to 10 years [37.5%] (Table 6). Changes in the various biochemical parameters in N.I.D.D.M. have been dealt with in this study. Increased serum cholesterol levels were seen in 55% of N.I.D.D.M. cases as compared to no case in the control group. On the other hand, there was a 35% rise in the serum triglycerides in the N.I.D.D.M. group as compared to a 2.5% rise in the control group. In the lipid metabolism, it was seen that the frequency of hypercholesterolemia was much higher than that in the total population, whereas hypertriglyceridemia was not that frequent. Three types of Hyperlipoproteinemias can occur in diabetes mellitus. Classical hyperlipemia consists of massive elevation of serum triglyceride levels that occur in patients who have suffered from insulin deficiency or in patients of insulin resistance for many weeks or months. Eruptive Xanthomas, lipemia retinalis and hepatomegaly can occur. The second type of hypertriglyceridemia in diabetics is associated with ketoacidosis. The third type of hypertriglyceridemia is mild to moderate elevation that persists even when patients appear to be adequately treated for their diabetes. The raised value of beta lipoproteins is due to the atherosclerotic changes that take

place in the intimal wall. High levels of triglycerides are also observed due to free fatty acid mobilization. There is no change seen in the serum electrolytes in this study. Usually there is no change seen, unless the disease is uncontrolled. The change is usually seen as a rise in serum potassium. A mildly significant rise in the blood urea is seen while there is no statistically significant increase in serum creatinine. There is a significant decrease in the serum total proteins. In N.I.D.D.M., it is established that the hyperglycemia is the cause of AGE products and also the generation of free radicals. Since lipids are largely involved, M.D.A. can be considered as a simple, reliable assessment index of the oxidative stress. Our results confirm the hypothesis that N.I.D.D.M. is characterized by enhanced lipid peroxides and the resulting spill over in circulation. Our control values are 0.90 ± 0.02 nmol./ml. and the N.I.D.D.M. patients had values of 1.29 ± 0.03 nmol./ml.

Other authors have reported control values of a) 2.86 ± 0.92 nmol./ml.

b) 3.41 ± 0.75 nmol/ml¹⁴

N.I.D.D.M. Values reported here:

a) 5.86 ± 0.66 nmol/lml, ¹⁵

b) 62.7 ± 10.1 microgram/dl¹⁶. This goes to prove that N.I.D.D.M. subjects are constantly dealing with free radical stress.

OXIDATIVE PROTECTION SCREEN

An objective evaluation of antioxidant protective capacity and free radical damage assessment is very important in the practice of clinical medicine and research. The defense is best expressed as TOP index as per details in the review. Free radicals are continuously produced during metabolism. In N.I.D.D.M. subjects, they are in large measure and more so manifest as early atherosclerotic changes and damage and heart disease. Tocopherols inhibit lipid peroxidation by scavenging the lipid peroxy radicals. Vitamin E requires vitamin C for regeneration alongside coenzyme Q10. The ideal would be to assess the TOP index i.e. patients plasma is challenged by free radical generator as detailed in the review of literature. However, blood glutathione [G.S.H.] was an easily accessible material and is a reliable facet of anti-oxidative defense availability. Lipid peroxides attack and destroy cell membranes. Anti-oxidants are produced within the cells- as per

ability of cell, requirement, age, inheritance, nutrition and stress. It is researched and proven that those with higher than usual levels of anti-oxidants enjoy better health. Our controls had GSH values of 61.82 ± 4.40 mg%. Other authors have values of a] $58.55 - 62.7$ mg%¹⁷ b] $42 - 50$ mg %¹⁸ and, c] $30-51.40$ mg%¹⁹. Our N.I.D.D.M. cases had GSH values of $77.53 + 10.88$ mg%. Other reported values of GSH in N.I.D.D.M. are a] $46.42 - 49.61$ mg%²⁰. b] $38.72-41.62$ mg%²¹ c] $41.23-43.01$ mg%²². This apparent difference in our N.I.D.D.M. GSH values can be accounted for. Anti-oxidants in diets- vitamin A, C, E were adequate as per diet history. Also, certain minerals empower these anti-oxidants eg. Selenium, copper, zinc and manganese and also L-cysteine rich in fish oriented diet taken by the Goans at large. Also large helpings of salads and general aversion to fried foods helps the system in decreasing generation of free radicals. Also as these patients were controlled, the GSH was at higher levels indicating a well-balanced anti-oxidative screen or defence parameter. These case studies confirm that generation of lipid peroxides and the attendant GSH fluxes are related to diabetic control and also to perhaps other antioxidants in the diet like vitamin A, E and C etc. Initial levels prior to the detection of diabetes mellitus and the history would perhaps tell a different story. Once the diabetics are controlled, the complications which have occurred are still manifest. But if the defenses to oxidative stress have met the challenge, the MDA & GSH levels are a reflection of the current metabolic status. The renal parameters vary within normal limits. The lipid profile was on expected lines. The proteins and A/G ratio showed a mildly significant statistical decrease. The Goan population ingests a fish predominant diet and salads are an integral part of meals. Due to the high literacy status and health awareness, they are aware of the role of vitamins and anti-oxidants and take sprouted cereals as added supplements. Longevity is well seen in Goa and therefore quality of life is of social and medical importance. Early detection and diagnosis of

a disease like N.I.D.D.M. would be a step forward in preventive medicine. To achieve this end, early evaluation of free radicals and the oxidative stress and anti-oxidant match molecules in large population groups in longitudinal studies could establish a regimen to curb the N.I.D.D.M. disease or surely at least to prevent the complications of N.I.D.D.M.

CONCLUSION

The age distribution of the patients and the controls ranged from 40 to 60 years. The levels did not vary with the age of the individuals in both the control and N.I.D.D.M. groups. 65% of patients had some complications like neuropathy, coronary heart disease, ketosis, retinopathy etc. The complications were not correlated with the biochemical values. 55% patients were seen to be having hypercholesteremia and 45% patients were having raised Serum Triglycerides in this study. Duration of illness and severity of the disease was found to have no effect Serum MDA and Blood GSH. Regarding serum lipoproteins, there was a *decrease* in HDL Cholesterol, an increase in LDL-cholesterol. VLDL-cholesterol was increased which was statistically significant. Blood urea showed a mildly significant increase while the increase in Serum Creatinine was statistically insignificant. No significant increase was seen in the Serum electrolytes- sodium, potassium and in chloride. There was a decrease in the total Serum Proteins which was mildly significant. There is no statistical significance in the comparative Albumin/Globulin ratios. There was a highly significant rise in the Serum Uric acid in this study. There was no significant change in the Serum Bilirubin. There is a highly statistically significant increase in the Serum Malondiadehyde. The GSH levels also register a highly statistically significant increase.

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