

Study of Ischemia Modified Albumin in Type 2 Diabetes as a Marker of Severity

Dr Sowjanya U.V.P.U.¹, Dr C. Sridevi², Dr. D.M.M. Rajkumari³,
Dr. A. Kasibabu⁴

^{1,2,3,4} (Department of Biochemistry, Andhra medical college, Visakhapatnam, AP., India)

Abstract: Hyperglycaemia, hyperlipidaemia and oxidative stress have been implicated in the development of chronic complications related to Diabetes mellitus (DM). Ischemia and hypoxia are observed in diabetic patients, in addition to these which are reflected in increased levels of oxidative damage they may modify albumin and hinder its binding capacity to Cobalt

Objective: To evaluate plasma levels of IMA (Ischemia modified albumin) in patients with type 2 diabetes mellitus and its complications and to determine their relationship with Glycated haemoglobin and MDA (malondialdehyde) which are proven markers for development of complications.

Materials and methods: 40 T2 DM patients without complications (Group1) and 40 DM patients with complications like neuropathy, and retinopathy (Group 2) were enrolled in the study along with 40 age-sex matched controls. They all were clinically evaluated and blood samples were analysed for plasma IMA, and MDA along with the routine biochemical parameters like FPG, HbA1c, total Cholesterol, TAG & HDL and their association was analysed with the disease process.

Observations: Plasma IMA was significantly higher in the patients compared to controls which was more marked in the Group 2 cases (51.53 ± 4.05 , 75.50 ± 10.93 , and 98.94 ± 0.93 U/ml, in Controls, Group 1 and Group 2 respectively). HbA1c (6.38 ± 0.24 , 7.92 ± 0.68 , $5.24 \pm 0.38\%$ in group 1, 2, controls) and MDA (5.17 ± 0.57 , 6.49 ± 0.42 , 1.48 ± 0.18 nmol/ml in group 1, 2, controls) were also found to be considerably raised in the cases compared to controls. Plasma IMA and MDA revealed a positive association with each other ($r=0.844$ in group 1 and $r=0.814$ in group 2 patients). Correlation analysis between IMA and HbA1c also revealed a significant positive relationship ($r=0.903$ in group 1 and $r=+0.822$ in group 2 patients) suggesting that increased oxidative stress results in ischemia and widespread endothelial damage with increased generation of IMA in chronic diseases like Diabetes mellitus.

Conclusion: Plasma IMA can be used as an auxiliary marker for severity in Type 2 Diabetes Mellitus.

Keywords: Diabetes Mellitus, HbA1c, IMA; MDA

I. Introduction

Diabetes mellitus is the major healthcare problem worldwide. Diabetes mellitus is a metabolic disorder of multiple aetiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both¹. It is not a single disease entity but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia.

Diabetes mellitus tends to run in families. It is associated with dyslipidemia, atherosclerosis and predispose to certain specific microvascular abnormalities including retinopathy, nephropathy and neuropathy. It increases the risk of macrovascular abnormalities like stroke, myocardial infarction and peripheral vascular diseases. It also decreases the resistance to infection, especially if diabetes is poorly controlled.

Diabetes shows "ice berg" phenomenon. The global prevalence of diabetes is expected to increase from 4% in 1995 to 5.4% by the year 2025.² The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030.³ Currently the countries with the largest number of diabetic patients are India, China and United States² In India alone, diabetes is expected to increase from 40.6 million in 2006 to 79.4 million by 2030.⁴

In Indian diabetics most of the long standing micro and macro vascular complications are more common compared to other ethnic groups.⁴ Long term vascular complications represent the main cause of morbidity and mortality in type 2 diabetic patients.

Diabetes mellitus is not only a metabolic disorder but also a vascular disease. Metabolic abnormalities which include hyperglycemia increase oxidative stress and activate the Renin Angiotensin system. Type 2 diabetes Mellitus is associated with increased incidence, severity, and delayed recovery from ischemic and hypoxic events that participate in disturbances of endothelial function and extracellular matrix remodelling and lead to the development of angiopathies.^{5,6} Oxidative stress has been implicated in the development of chronic complications related to diabetes mellitus.⁷ Some previous studies have reported that compared to healthy

controls, patients with type 2 diabetes (T2DM) and chronic microvascular complications have higher serum levels of IMA (Ischemia Modified Albumin).^{8,9}

Structural modifications of the albumin molecule can occur as a result of increased generation of reactive oxygen species (ROS). It has been suggested that a sensitive biomarker of cardiac ischemia, ischemia-modified albumin (IMA), is also an indicator of oxidative stress.¹⁰ It is suggested that the pathophysiological events of ischemia, including hypoxia, acidosis, and ROS generation, result in conformational changes of albumin. These changes alter the ability of the molecule to bind free metal ions such as cobalt, which became the basis for IMA determination by the albumin cobalt-binding test (ACB test)^{11,12} The definitive and precise mechanism for IMA production in vivo is still unknown. The N-terminus is most frequently mentioned as the binding site for free metal ions. However, an in vitro study under aerobic conditions showed that Co (II) binds preferentially to the metal binding sites A and B, not to the N-terminus of human albumin.¹³

Recently, more and more studies are dealing with the role and diagnostic significance of IMA in different disorders of non-cardiac origin. Higher IMA levels were observed in physiological and pathological pregnancy, brain ischemia, infection, liver disease, trauma, some neoplasms, pulmonary embolism, peripheral vascular disease, and systemic sclerosis and skeletal muscle ischemia.^{14,15}

IMA levels were also higher in diabetic patients, especially those with complications, and were related to the levels of HbA1c. IMA levels are connected with diabetic complications and may be helpful in assessing the disease's development and predicting the severity of its course and perhaps even the effectiveness of therapy.

II. Materials And Methods:

80 patients with types 2 diabetes mellitus treated at the Endocrinology department, King George Hospital, Andhra medical college, Visakhapatnam, India and 40 healthy adults (control group) were studied. The subjects included in the study were age and sex matched.

Inclusion criteria

- Confirmed cases of type 2 diabetes mellitus
group 1- without complications
group 2- with complications (retinopathy, neuropathy, MI, etc.,)

Exclusion criteria

- Any acute illness
- Inflammatory conditions
- Other conditions which interfere with results like liver, renal, neural diseases

Methods

IMA was measured by Cobalt albumin binding method¹⁶. The IMA estimation involved adding 0.1% cobalt chloride (CoCl₂.6H₂O) to serum, and incubated for adequate cobalt-albumin binding. 0.15% Dithiothreitol (DTT) was added as a colorizing agent and the reaction was quenched 2 min later by 0.9% NaCl. Using a spectrophotometer at 470 nm, colour development with DTT was compared to a serum-cobalt blank without DTT and IMA values were expressed in U/ml extrapolated from the standard graph. One IMA unit is defined as "gm of free cobalt" in the reaction mixture per ml of serum sample. HbA1C was measured by Ion exchange resin method, fasting blood glucose by GOD POD method, lipid profile by routine enzymatic methods and MDA by Thiobarbituric acid reaction (Mahalouz et al)¹⁸.

III. Results And Observations:

A comparative study with 80 type 2 diabetic patients and 40 controls was undertaken. The cases were clinically evaluated and divided into two groups. Group 1 consisted of 40 DM patients without complication and 40 DM patients with complications were in Group 2. All patients were diagnosed on the basis of clinical picture as well as biochemical investigations.

The metabolic derangement was very evident in the study group with significantly higher FBS and dyslipidaemia being observed in the cases compared to controls. This difference was even more marked in Group 2 patients comprising of DM cases with complications in comparison to Group 1 patients of DM without any complications. (Table1)

Special parameters like IMA, MDA and HbA1c were significantly higher in the cases compared to controls (p<0.001) (Table 2). The difference in the above mentioned parameters was also statistically significant between Group 1 and Group 2 cases, (p<0.001) the rise being more marked in the Group 2 patients. Thus the role played

by ischemia, oxidative stress is apparent in the disease process of Diabetes mellitus and its associated complications.

Table 1: Routine biochemical parameters in the study population (*p<0.001)

Group	FBS mg% Mean ± SD	Chol mg% Mean ± SD	TG mg% Mean ± SD	LDL mg% Mean ± SD	HDL mg% Mean ± SD
Group 1	154.5 ± 22.5*	170.5 ± 18.06*	189.9 ± 24.09*	95.96 ± 18.06*	36.55 ± 2.6
Group 2	203.65 ± 16.51*	186.8 ± 33.8*	191.4 ± 54.3*	117.8 ± 35.07*	30.7 ± 2.6*
controls	98.65 ± 10.32	144.9 ± 10.9	132.72 ± 21.65	80.18 ± 10.77	38.25 ± 4.6

Table 2: The level of IMA, MDA, and HbA1C in the study population, (p<0.001).

Group	IMA U/ml Mean ± SD	MDA nmol/ml Mean ± SD	HbA1c % Mean ± SD
Group 1	75.50 ± 10.93	5.17 ± 0.57	6.38 ± 0.24
Group 2	98.94 ± 0.93	6.49 ± 0.42	7.92 ± 0.68
Controls	51.53 ± 4.05	1.48 ± 0.18	5.24 ± 0.38

Plasma IMA was positively correlated with serum MDA, a marker of oxidative stress and free radical generation through Pearson's Correlation analysis. Plasma IMA was positively correlated with HbA1c, a marker of glycemic control and indicator of development of complications. (Table 3) Thus the positive association of raised plasma IMA with MDA and HbA1c may be an effect of increased oxidative stress and free radical generation leading to widespread inflammation of vascular endothelium. The resultant tissue hypoxia might have contributed to the increased modification of albumin attributing towards raised IMA level.

Table 3 shows Karl Pearson's coefficient (r) of correlation of IMA with MDA, HbA1C, and Duration of the disease in diabetic patients (group 1 & 2) and controls

Correlation with IMA	Group 1	Group 2	Controls
MDA	+0.844	+0.814	+0.712
HbA1C	+0.903	+0.822	+0.772
Duration	+0.665	+0.928	-

IV. Discussion:

Persistent hyperglycaemia and lipid derangements are a quite essential feature of long standing Diabetes mellitus. Various causes like non-enzymatic glycation, polyol pathway, protein oxidation leads to free radical generation contributing to oxidative stress. Increased level of **MDA**, an established marker of oxidative stress, is seen in Diabetic patients indicating excessive oxidative stress in this chronic disorder. This study registered a high value of **IMA** in Diabetes cases compared to controls, more marked in Group 2 patients pointing towards increased oxidative stress associated with the progression of the disease process and is in agreement with many other studies on the role of oxidative stress and free radicals in the pathogenesis of complication in long standing Diabetes mellitus. Increased oxidative stress is associated with hypoxia and chronic inflammation.

Various studies have highlighted Ischemia modified albumin as a marker in acute myocardial infarction whose levels rise in the blood even before Troponins with a sensitivity of 82%.

Chronic hypoxia associated with oxidative stress leads to a conformational change in the N-terminal of the albumin. This change leads to an inability of the molecule to bind covalently to the transition metal ions like Cobalt, Copper, and Nickel etc.¹² It has been proposed by various authors that IMA increases wherever the albumin passes through ischemic tissues. Hence, though approved as a marker in cardiac ischemia, now it is being studied in context of other diseases where there is a prevalence of increased oxidative stress and ischemia, like Diabetes mellitus, liver diseases, obese post-menopausal women, complicated pregnancies and cancers. The precise mechanisms for production of Ischemia modified albumin during ischemia are not known, but have been localized to modifications of the N-Asp-Ala-His-Lys sequence of human albumin and are proposed to be related to production of free radicals during ischemia and/or reperfusion, reduced oxygen tension, acidosis, and cellular alterations such as disruption of sodium and calcium pump function.

Hyperglycaemia and added derangements in carbohydrates, fat and protein metabolism increase oxidative stress and activate the renin-angiotensin system. Type 2 diabetes mellitus (T2DM) is concomitant with augmented incidence, severity, and delayed recovery from the ischemic and hypoxic events that contribute to the instabilities of endothelial function and extracellular matrix remodelling with consequential development of micro and macro angiopathy.

The present study found that IMA levels **increased** progressively with duration of the disease. The lowest value observed in healthy controls was significantly different from those in diabetics with complications. But these were also different between diabetic patients with and without complications.

Analysing the relationships between IMA and parameters applied in routine diagnosis of diabetes mellitus, significant correlation with HbA1c, MDA was found. The mean \pm SD values of IMA in group 1 & 2 and controls were found to be 75.50 ± 10.93 , 98.94 ± 0.93 , 51.53 ± 4.05 U/ml respectively. The mean values of IMA were higher in cases (group 1&2) when compared to controls and the increase was statistically highly significant ($p < 0.001$). The mean values of IMA were also higher in patients with complications when compared to patients without complications and the increase was found to be statistically significant ($p = < 0.001$).

A positive correlation was found between IMA and HbA1c, ($r = 0.904$, 0.822 , 0.772 in group 1, 2, & controls respectively). A positive correlation was also found between IMA and MDA, ($r = 0.844$, 0.814 , 0.712 in group 1, 2, controls respectively) and also between IMA and Duration of the disease ($r = 0.665$, 0.928 in group 1 & 2).

V. Conclusion:

Thus it was apparent that **IMA** levels increased in the presence of increased oxidative stress as indicated by the level of MDA which is an established marker of oxidative damage in diabetic patients, more in patients with complications.

The cost effectiveness of the test procedure may allow serial estimations of **IMA** at regular intervals in every Diabetic case along with other routine parameters like HbA1c, FBS, serum Creatinine and Microalbuminuria.

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