Original Research Article

Evaluation of The Association Between Ischemia Modified Albumin (IMA), Glycemic And Lipid Status In Diabetic Nephropathy

Abstract

Introduction: Hyperglycemia induced oxidative stress in type 2 diabetes mellitus modify various biomolecules to cause diabetic nephropathy (DN). IMA (Ischemia-modified albumin) is one such oxidative stress marker already examined in various clinical events but have not yet been evaluated in different stages of DN.

Aim: To estimate and assess the relationship of IMA with glycemic status and lipid parameters in all stages of DN.

Study Design: Cross-sectional study

Place and Duration of Study: Study was conducted at Department of Biochemistry, Kasturba Medical College Hospitals, Mangaluru conducted between 2014 and 2015.

Materials & Methods: There were 60 type 2 diabetic cases and 30 healthy controls. Diabetic cases were further categorized into three equal groups on the basis of UACR (urine albumin-creatinine ratio), DN stage I having UACR less than 30 mg/g, DN stage II having UACR 30 to 300 mg/g, and DN stage III having UACR ≥ 300 mg/g of creatinine. Using enzyme-linked immunosorbent assay serum IMA level was estimated whereas automated analyzers was used for serum creatinine, HbA1c, urine albumin and urine creatinine analysis.

Results: Lowest level of IMA (109 ng/mL) measured in DN stage I, which was significantly different from those in DN stage II (154 ng/mL) and DN stage III (178 ng/mL). The significant positive correlation between IMA and fasting blood glucose, glycated hemoglobin were present in stage II and stage III DN. In this study significant positive correlation of serum IMA to serum total cholesterol, low density lipoprotein cholesterol and negative correlation with high density lipoprotein were revealed in all stage of DN.

Conclusion: Current study postulates that early evaluation of serum IMA in diabetic patients with deranged lipid profile will provide an index of nephropathy development. This will help in prognosis and controlling complication in diabetes mellitus.

Key words: *Diabetic nephropathy; dyslipidemia; ischemia-modified albumin; oxidative stress; urine albumin creatinine ratio*

ABBREVIATIONS

IMA- Ischemia Modified Albumin, T2DM- type 2 diabetes mellitus, DN- diabetic nephropathy, UACR- urine albumin-creatinine ratio, ROS- reactive oxygen species, FPG- fasting plasma glucose, A1C- glycated hemoglobin, 2hPG- 2-h plasma glucose, TC-Total Cholesterol, TG-Triglyceride, LDL- Low Density

Lipoprotein, HDL- High Density Lipoprotein, SBP-Systolic Blood Pressure, DBP-Diastolic Blood Pressure, GOD-glucose oxidase, CHOD-PAP- cholesteroloxidase-peroxidase aminophenazone, ANOVA- analysis of variance

1. INTRODUCTION

Recently WHO epidemiological data reports that India has the highest number of type 2 diabetes mellitus (T2DM) patient in the world. Still, the cause of long term complications in T2DM is not entirely understood, and controversies exist about why they occur in some patients and not in others [1]. Besides genetic predisposition, hypertension, hyperglycemia, and dyslipidemia had an essential role in pathogenesis and progression of vascular complication in T2DM leading to diabetic nephropathy (DN) [2-4]. Till now urine albumin-creatinine ratio (UACR; mg/g of creatinine) in spot or random urine sample is considered best marker for screening and diagnosing DN [5]. Based on UACR, DN is categorized into DN stage I or normoalbuminuria if UACR < 30 mg/g creatinine; DN stage II or microalbuminuria if UACR between 30 - 300 mg/g creatinine and if UACR ≥ 300 mg/g creatinine as macroalbuminuria or DN stage III [6,7]. As per National Kidney Foundation and American Diabetes Association guidelines multiple specimens of albuminuria is required to enhance precision for DN in diabetics [8]. Whereas, Third National Health and Nutrition Examination Survey and United Kingdom Prospective Diabetic Study reported absence of albuminuria in one-third of adults with T2DM and chronic renal insufficiency indicating microalbuminuria alone is no longer optimal to identify DN. So, better marker is required to diagnose DN at an early stage [9-11].

Aetiopathogenesis of DN is connected with the generation of free radicals or reactive oxygen species (ROS) which causes hypoxia, ischemia and vascular inflammatory changes [12]. Oxidative damage and sub-endothelial inflammation due to hyperglycemia, hyperlipidemia and high blood pressure lead to conformational changes of different biomolecules also in N-terminal of albumin causing formation of IMA [13-15]. Blood IMA levels increases due to hyperglycemia, hyperlipidemia, and inflammation resulting in reduce metal binding capacity of albumin to bind cobalt [16]. Rise of IMA is identified as biomarker of ischemic events in many diseases like cardiac ischemia, pulmonary embolism, cirrhosis, cerebrovascular attack, kidney diseases, etc. [17,18]. Recently Ahmad et al. reported high IMA levels in DN patients showing ischemic and hypoxic events in them. Their finding reinforced the utility of estimation of IMA as an auxiliary marker in diagnosing early vascular injury in DN [19]. A significant positive correlation was reported in few diabetic studies between serum IMA and UACR, fasting plasma glucose (FPG), and glycated hemoglobin (A1C) [19-22].

Endothelial damage due to hyperlipidemia by the reactive oxygen species causes overproduction of endothelial activation cells and accumulation of leukocytes in the walls of arteries to cause atherosclerosis. Oxidative stress in dyslipidemia also transiently modify the N-terminal metal binding capacity of albumin causing the formation of IMA [23]. A positive correlation between raise serum IMA and dyslipidemia in acute cerebrovascular disorders, renal disease, coronary heart disease, and metabolic syndrome were reported [24-28]. Although there are many research done on oxidative stress molecules (IMA) and lipid profiles in T2DM, their evaluation in different stages of renal complication in diabetes has not been reported so far. This study was planned with an objective to assess the association of lipid parameters, glycemic status with IMA in different stages of DN.

2. MATERIALS AND METHODS

2.1 Study Population

This observational study comprising of 90 subjects from the Kasturba Medical College Hospitals, Mangaluru conducted between 2014 and 2015. The participants in this study were between 30 to 65 years of age. As per American Diabetic Association criteria for the diagnosis of diabetes glycated hemoglobin (A1C) ≥ 6.5% or fasting plasma glucose (FPG) ≥126 mg/dL or 2-h plasma glucose (2hPG) value in the 75-g oral glucose tolerance test ≥ 200 mg/dL pre-diagnosed 60 T2DM cases and for comparison 30 healthy age-gender matched controls were enrolled in this study [29]. All 60 diabetic cases were further categorized into three equal groups on the basis of UACR (urine albumin-creatinine

ratio), DN stage I or normoalbuminuria in Group I if UACR < 30 mg/g creatinine; DN stage II or microalbuminuria in Group II having UACR between 30 - 300 mg/g creatinine and in Group III if UACR ≥ 300 mg/g creatinine as macroalbuminuria or DN stage III. 30 healthy control were included in Group IV.

Institutional ethical clearance was obtained to start the study and Informed consent was obtained from all the enrolled participants. All measures were carried out in compliant with the Helsinki declaration. To nullify the effect of analytical matrix on IMA measurement only those participants having normal serum albumin level (3 to 5.5 g/dL), no history of liver dysfunction, myocardial infarction or stroke, infection, malignancy, pregnancy and patients on steroid or hormonal therapy in last three months were included in this study.

2.2 Measurements

From the patient's medical records, clinical history and routine biochemistry tests such as FPG and 2hPG (GOD-Peroxidase method), A1C (in Bio-Rad Turbo II Variant auto analyzer by Ion-exchange High-Performance Liquid Chromatography method total cholesterol (Enzymatic Colorimetric CHOD – PAP), LDL-Cholesterol, triglycerides (Enzymatic Colorimetric GPO – PAP), HDL-Cholesterol (Enzymatic Direct), serum and urine albumin (Turbidimetric method), serum and urine creatinine (Jaffe's method) were analyzed in Clinical Biochemistry Laboratory on automated clinical chemistry analyzers (Hitachi Modular P-800) using Roche commercial kits were recorded. Values of UACR for each participant were calculated manually using calculator.

Leftover serum for each enrolled subjects from the clinical biochemistry lab was collected and refrigerated at −20 °C in Eppendorf tube for further estimation of IMA. It was estimated using solid-phase enzyme-linked immunosorbent assay (ELISA) based on double-sandwich principle with kits from Shanghai Yehua Biological Technology Co., Ltd, on ELx 800 by BioTek® Instruments, Inc. The sample was added to the precoated monoclonal antibody wells. Immune complex with streptavidin–horseradish peroxidase was formed after incubating labeled antibodies with biotin. Unbound enzymes were washed, and the color was produced after substrate was added into it. The colored solution was then estimated by using colorimeter at 450 nm. This gives the concentration of IMA as intensity of color produced was positive proportional to the concentration of analyte [30]. Assay range of IMA kit was 2–600 ng/mL having sensitivity of 1.08 ng/ mL with intra-and inter-test CV being, 10%, and 12% respectively.

2.3 Statistical Analysis

The data collected was entered and analyzed by using IBM software SPSS (Statistical Package for Social Sciences Chicago, IL, USA) version 20.0 for windows. Continuous or parametric data were expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) with Tukey's as the Post-Hoc test was used for comparison of means between all four groups [31]. Pearson's correlation coefficient analysis was done to find out the association between IMA, glycemic and lipid parameters. The *p*-value less than 0.05 was considered statistically significant.

3. RESULTS

In this study, most of the subjects were between 30 – 65 years of age with equivalent gender distribution in diabetic groups. Table 1 shows the general characteristics among groups and represented as mean ± standard deviation. Compare to group I duration of diabetes and blood pressure were higher in group II and III.

Table 1. Comparison of general characteristics among the groups

	Group I (N=20)	**Group II (N=20)**	**Group III (N=20)**	**Group IV (N=30)**
Age(Years)	51.1±7.1 (30-58)	52.9±4.1 (46-60)	54.8±9.9 (32-65)	50±7.2[a,b,c] (30-64)
Male / Female	12 / 8	12 / 8	9 / 11	16 / 14

SBP(mm/Hg)	145±8[c,d]	152±8[d]	159±20[a,d]	122±6[a,b,c]
DBP(mm/Hg)	89±5[d]	89±6[d]	92±8[d]	80±2[a, b, c]
Diabetes duration (Years)	4.5 ± 0.90	9.5 ± 1.6	14.5 ± 2.0	

*N-total participants, SD-standard deviation. [p <0.001 is significant indicated a Vs Group I, b is Vs Group II, c is Vs Group III, d is Vs Group IV, * denotes p<0.05 p-Values by ANOVA followed by Post Hoc Tukey's test.]*

3.1 Glycemic status

The significant difference was present in FPG, 2hrPG, and A1C of diabetic patients when compared with control groups. In diabetes subjects, 2hrPG and A1C in group III were found highest and significant difference compared to group I and II (Table 2).

Table 2. Comparison of Biochemical parameters among the groups

	Group I (N=20)	Group II (N=20)	Group III (N=20)	Group IV (N=30)
FPG (mg/dl)	164 ± 62[c*,d]	180 ± 34[d]	193 ± 16[a*,d]	80 ± 8[a,b, c]
2hrPG (mg/dl)	249 ± 43[c, d]	274 ± 33[c*, d]	320 ± 79[a,b*, d]	129 ± 10.7[a,b, c]
A1C (%)	7.5 ± 0.8[b* ,c, d]	8.9 ± 0.7[a*, c, d]	10.7 ± 2.4[a, b, d]	4.8 ± 0.5[a ,b, c]
Serum Creatinine (mg/dl)	1.1 ± 0.3[b, c]	1.5 ± 0.2[a, d]	1.7 ± 0.37[a, d]	0.97 ± 0.2[b, c]
UACR (mg/g)	18.8 ± 7[b, c]	126 ± 62.3[a,c,d]	535 ± 144[a,b, d]	16.9 ± 6[b, c]
TC (mg/dl)	247 ± 65[b*,c,d*]	334 ± 90[a*,d]	372 ± 128[a,d]	165 ± 34[a*,b,c]
LDL (mg/dl)	139 ± 41[d*]	154 ± 50[d]	173 ± 69[d]	101 ± 18[a*,b,c]
TG (mg/dl)	139 ± 50[c]	167 ± 44[c,d]	226 ± 53[a,b,d]	111 ± 28[b,c]
HDL (mg/dl)	35 ± 13[b*,c,d*]	23 ± 8[a,*d]	19 ± 7[a,d]	43 ± 9[a*,b,c]
IMA (ng/ml)	109 ± 50[b*,c, d]	154 ± 43[a*, d]	178 ± 68[a, d]	45.6 ± 24[a,b, c]

*[p <0.001 is significant indicated a Vs Group I, b is Vs Group II, c is Vs Group III, d is Vs Group IV, * denotes p<0.05 p-Values by ANOVA followed by Post Hoc Tukey's test.]*

3.2 Lipid Status

Mean levels of TC, LDL and TG were found highest in group III than in group II and lowest in group I whereas reverse order was found with HDL (Table 2). A significant mean difference between TC and HDL were found in group I compared to group II and III whereas TG value in group III was significantly greater related to group I and II. All lipid parameters were significantly high in group III related to group I, but no significant difference was present among group II and III.

3.3 Ischemia Modified Albumin

Compared to the control group statistical difference in mean levels of IMA were found in all three groups. IMA level in group III was highest and significant difference present with group I. However, a significant difference was also found present when mean IMA level of group I compared with group II and III (Table 2 and Fig. 1).

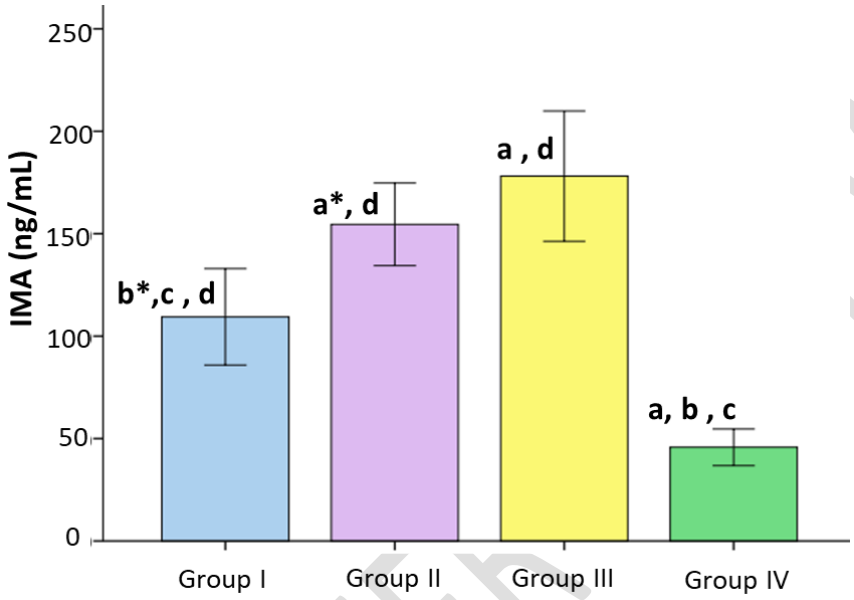

Fig. 1. Error bar showing comparison of IMA in different groups

*p <0.001 is significant indicated a Vs Group I, b is Vs Group II, c is Vs Group III, d is Vs Group IV, * denotes p<0.05 p-Values by ANOVA followed by Post Hoc Tukey's test.*

3.4 Correlation of IMA with Glycemic status

Table 3 shows Pearson's correlation between IMA, FPG, 2hrPG, and A1C. The significant positive correlation of IMA was present in group II and group III with FPG and A1C. IMA had no association with 2hrPG in any group whereas it had positive association with UACR in all the three groups indicating its influence in pathogenesis of nephropathy.

3.5 Correlation of IMA with Lipid status

Association of IMA with lipid parameters were shown in Table 3. TC and LDL were positively associated with an increase in IMA level in all three groups of diabetes. HDL had negative correlation with IMA in group I, group II and group III. No correlation between IMA and TG were found in any groups. When IMA was compared with blood pressure, SBP was significantly associated compared to DBP in all diabetes groups.

Table 3. Correlation between serum IMA with routine biochemical parameters in each groups

	Group I (N=20)		Group II (N=20)		Group III (N=20)		Group IV (N=30)	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*

FPG (mg/dl)	0.02	0.9	**0.48***	**0.03**	**0.51***	**0.02**	0.05	0.7
2hrPG (mg/dl)	0.2	0.37	0.02	0.94	0.02	0.9	0.08	0.67
A1C (%)	0.05	0.83	**0.47***	**0.03**	**0.51***	**0.02**	0.08	0.66
Serum Creatinine (mg/dl)	0.38	0.09	0.41	0.06	**0.44***	**0.05**	0.01	0.98
UACR (mg/g)	**0.45***	**0.04**	**0.46***	**0.04**	**0.5***	**0.02**	0.35	0.05
TC (mg/dl)	**0.56***	**0.01**	**0.45***	**0.04**	**0.87***	**0.001**	0.05	0.77
LDL (mg/dl)	**0.49***	**0.03**	0.15	0.52	**0.59***	**0.01**	0.08	0.65
TG (mg/dl)	0.17	0.46	0.09	0.6	0.18	0.4	0.05	0.76
HDL (mg/dl)	**-0.49***	**0.02**	**-0.61***	**0.01**	**-0.64***	**0.01**	0.31	0.09
SBP (mmHg)	**0.47***	**0.03**	**0.53***	**0.01**	**0.6***	**0.01**	**0.47***	**0.01**
DBP (mmHg)	0.38	0.09	**0.49***	**0.02**	**0.58***	**0.01**	0.09	0.6

*[p <0.001 is significant indicated a Vs Group I, b is Vs Group II, c is Vs Group III, d is Vs Group IV, * denotes p<0.05 p-Values by ANOVA followed by Post Hoc Tukey's test.]*

4. DISCUSSION

In T2DM, there is a complex interrelationship between modifiable and nonmodifiable risk factors leading to micro and macrovascular complications. Besides hypertension, important modifiable factors in pathophysiology and progression of vascular complication in diabetes leading to DN include hyperglycemia, dyslipidemia, albuminuria, anemia, lifestyle, etc. [2,32,33]. In present study, compared to healthy controls higher mean IMA levels were present in all T2DM cases. This demonstrates either the establishment of ischemic events in uncontrolled diabetics or production of IMA as a biomarker of oxidative stress due to hypertension, hyperglycemia, and dyslipidemia [34]. In support to other studies, among T2DM, lowest values were detected in DN stage I (normoalbuminuria), which was significantly different from those in the DN stage II and stage III groups, but there were no significant differences noted between DN stage II and stage III groups [19,35]. As expected, diabetes cases were having high FPG, 2hrPG and A1C compared to control group. Continuous exposure of hyperglycemia in diabetes causes many biochemical sequelae like glucose autoxidation, nonenzymatic glycation, increase of advanced glycation end products, activation of polyol, protein kinase C pathway, etc. inducing oxidative stress [15,16]. Lin et al. explained the association of FPG and A1C in causing DN may be due to high collagen formation, cell growth and cytokines release [36]. More reactive oxygen species (ROS) formation occurs because of oxidative stress in diabetes due to imbalance between and enzymatic and nonenzymatic antioxidants [37]. Also, ROS is now well established responsible factor for biochemical modification of proteins, lipids, carbohydrates, DNA, etc. damaging glomerulus membrane and endothelium lining in DN [21].

IMA is one such oxidative stress biomolecule due to modification at N-terminal of albumin produced by ROS induced ischemia in DN. Results of the study suggest that hyperglycemia-induced stress in DN

provokes albumin alteration in the early stage of the disease. As the disease progress more and more ischemia or oxidative damage happens due to cumulative effect of hypertension, hyperglycemia and altered lipid profiles leading to increased IMA levels in stage III than stage II and I [19]. Serum IMA and UACR were positively correlated in this study associate albuminuria with the disease progress in DN [35]. Bilgi et al. reported no positive correlation regarding evaluation of relationship between urine IMA and albuminuria in DN [38]. Whereas the diagnostic efficacy of serum IMA levels as early indicator of DN was reported in our previous study compared to malondialdehyde and advanced oxidative protein products [19].

Positive association of IMA with a glycemic index (FPG and A1C) authenticates that hyperglycemia provokes hypoxia, oxidative stress, and ischemia leading vascular complications in type 2 diabetes [13,22,39]. The present study attempted to find out the relationship of IMA with lipid status in DN. A positive correlation of serum IMA level was found in DN with TC and LDL in all the stage. This finding shows role of hypercholesterolemia in generation of IMA levels in DN. Increased blood viscosity due to lipid changes causing decreased blood flow in DN. These changes increase instability of plaques, helping in formation of blood clots provoking atherosclerosis, thereby aggravating ischemia and resulting in increased IMA levels [24]. Supporting our results Refaat et al. study also reported positive correlation of serum IMA with glycosylated hemoglobin, TC and LDL in T2DM with dyslipidemia [40].

T2DM with good glycemic control have normal LDL status resulting no rise of IMA levels. On the other side, in poor glycemic control glucose nonenzymatically bound to lysine residues in a variety of protein residues. Glycation of apolipoprotein B decreases LDL receptors activity resulting its altered metabolism. Triglyceride enrich HDL, increased cholesterol to protein ratio and depletion of apolipoprotein A1 in T2DM might increases catabolic rate of HDL in diabetes than in normal [2]. In support to study results significantly low HDL cholesterol level and negative correlation of HDL cholesterol with IMA in diabetes [23,40]. So, mechanism of albuminuria in DN may be prolonged hyperglycemia which increases glycation and oxidation of lipoproteins that enhances their binding to glycosaminoglycan of glomerular basement membrane. Deposition of glycated lipid molecules in mesangial cells passes chemotactic signal for its proliferation and to macrophages. Receptor mediated monocyte or macrophage causes formation of mesangial or glomerular foam cells. Other mechanism for albuminuria includes mesangial expansion due to accumulation of oxidized lipid in hyperglycemia [2]. In limitation, confounding effect of hypertension, drugs used to lower BP, T2DM, DN, lipids were not mentioned. This could have influence on generation of IMA levels and DN pathophysiology. So, taking all these criteria into consideration further study is required to account the utility of this novel marker IMA as an early marker to diagnose microalbuminuria in DN cohort.

5. CONCLUSION

Hyperglycemia-induced glycation of lipoproteins causes increase values of IMA levels in the blood as well as in the extracellular matrix of glomerulus basement membrane, bring out albuminuria in diabetes. Significant increase of IMA in diabetic nephropathy without albuminuria and its strong association with glycated hemoglobin, fasting plasma glucose, total cholesterol, and HDL cholesterol reinforces the utility of estimating IMA in early diabetes. As per knowledge this is going to be a first study to report correlation of hyperglycemia and dyslipidemia with serum IMA. So, we hypothesize monitoring serum IMA in early diabetes may help clinician in detection and monitoring progress of nephropathy. However, further studies are required to fully assess the potential clinical use of IMA in a larger population.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication as per international or university standard.

ETHICAL APPROVAL

All authors hereby declared that 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.

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