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"Assessment and correlation of IMA with Oxidative Stress, Glycemia Status in Diabetic Nephropathy Patients"

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Abstract:

Background: Increased incidence, severity, and a delayed recovery from stress-induced ischemia contributing to endothelial dysfunction are linked to diabetes problems. This can result in angiopathy, particularly diabetic nephropathy (DN). This study aimed at assessment and correlation of IMA with Oxidative Stress, Glycemia Status in Diabetic Nephropathy Patients. **Subjects and Methods:** subjects were selected from the Medicine & Nephrology OPD of INDEX Medical College and Hospital for the study and remaining 100 were age and sex matched healthy controls. **Results:** IMA shows strongly positive correlation with blood pressure, fasting blood sugar (FBS), PPBG, HbA1c, cholesterol, LDL, triglycerides, serum creatinine and UACR ($P<0.05$). There is negative correlation between IMA and HDL, GSH. **Conclusion:** IMA may function as a sensitive marker of diabetic nephropathy and an indicator of glycemic control.

Keywords: Diabetic nephropathy, ischemia-modified albumin, type 2 diabetes mellitus, malondialdehyde & vascular injury.

Introduction:

One of the most prevalent endocrine diseases, diabetes mellitus (DM) is characterized by persistent hyperglycemia, which leads to a number of microvascular problems, including diabetic nephropathy [1]. Reduced glomerular filtration rate and albumin excretion in urine are two characteristics of diabetic kidney disease [2]. The

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following risk factors contribute to endothelial dysfunction and damage, which in turn causes atherosclerosis: high blood pressure, insulin hormone receptors resistance, and vascular inflammation [3]. Numerous indicators, including high sensitivity C-reactive protein (Hs-CRP), microalbuminuria, and hyperhomocysteinemia causes endothelial dysfunction; the degree of these indicators is a strong predictor of macrovascular disease.[4]. Renal failure is a potential result of diabetic nephropathy (DN), a significant microvascular complication of type2 diabetes. Human Serum albumin's N-terminal amino acids are altered during, ischemia of human tissues brought on by hypoxia and oxidative stress, resulting in the formation of IMA, a marker of endothelial cell injury.[5] Within minutes of artery occlusion, free radical formation, a weakened antioxidant defense system, and the ischemia that goes along with diabetes cause alterations in the tertiary structure of human serum albumin, which is why it's called ischemia-modified albumin (IMA). Increased production of reactive oxygen species (ROS) may lead to structural alterationsin the albumin molecule. These days, myocardial ischemia is recognized as a sign of oxidative stress and can be detected using IMA, a sensitive biomarker.[6] Studies on the function and diagnostic value of IMA in non- cardiac illnesses have recently been reported. After measuring it using a manual spectrophotometry method, Piwowar et al. revealed the relationship of IMA with diabetes for the first time, revealing a 75% elevated value of IMA in diabetes compared with control.[7] Additionally, peripheral vascular disease, cerebral ischemia, infection, liver illness, trauma, certain neoplasms, diabetic ketoacidosis, pulmonary embolism, and both complex and straightforward pregnancies were found to have elevated IMA levels.[8,9] Increased activity offree radicals resulting from malignant endothelial cell exposure to glucose, which oxidizes lipid molecules, is linked to complications in diabetes.[10] Polyunsaturated fatty acids peroxidize to form malondialdehyde (MDA), one of the extremely reactive three carbon dialdehyde byproducts. Arachidonic acid metabolism for prostaglandin synthesis also produces MDA.[11] It has been determined that this stress signal reflects the problems associated with diabetes.[12] Although associative research have been conducted on IMA and MDA with DN, there is minimal practical application in terms of reference ranges and diagnostic criteria that is not consistent across different groups.Our goal was to evaluate and correlate IMA in patients with diabeticnephropathy with oxidative

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stress and glucose status.

Subjects and Methods:

The present study was conducted in the Department of Biochemistry, INDEX Medical College and Hospital in collaboration with Department of Medicine & Nephrology during the period from July 2021 to September 2023.
200 subjects were selected from the medicine & Nephrology OPD of INDEX Medical College and Hospital for the study and remaining 100 were age and sex matched healthy controls. Participants were divided into three groups:

Group A: (n=100) Type 2 Diabetes Mellitus with Diabetic Nephropathy.

Group B: (n=100) Type 2 Diabetes Mellitus without Diabetic Nephropathy.

Group C: (n=100) control group (healthy individuals).

Following the Institutional Review Board's approval and each participant's informed written permission. Participants ranged in age from 35 to 70 years old and were of both genders. Patients with liver dysfunction, those who experienced ischemic events within the previous three months, such as acute myocardial infarction or cerebrovascular stroke, those who had an infection within the previous six weeks, those who were cancer patients, and those on steroid drugs were all excluded from the study. The primary focus of the research participant evaluations was microvascular problems, specifically nephropathy. Following a history-taking session, each participant underwent a clinical examination, including a body mass index (BMI) and blood pressure (B.P.) assessment. Early morning fasting 4ml blood sample and spot midstream urine sample was collected for analysis. Ischemia modified albumin (IMA). HBA1c, random blood glucose, serum creatinine, urinary Micro albumin, creatinine and urinary albumin/creatinine ratio were estimated. The levels of serum IMA were measured using a Albumin Cobalt (II) binding assay method. The Serum is left for 10 to 20 minutes to coagulate at room temperature, after centrifugation of the sample for 20 minutes at a speed of 2500 rpm, the supernatant was removed then centrifugation was repeated in case of precipitation, then samples were stored at - 20°C. The IBM

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software SPSS (Statistical Package for Social Sciences Chicago, IL, USA) version 20.0 for Windows was used to enter and evaluate the data that had been gathered. The standard deviation (SD) of continuous or parametric data was reported as mean. To compare the means of the Three groups, one-way analysis of variance (ANOVA) using Tukey's as the Post-Hoc test was employed [13]. To determine the relationship between IMA, glycemic, and lipid markers, Pearson's correlation coefficient analysis was performed. A statistically significant p-value was defined as one that was less than 0.05.

Observation and Results:

The present study included 300 subjects, out of which 100 were type 2 diabetic with DN (Group A), 100 were type 2 diabetic without DN (Group B) and 100 were healthy controls (Group C). Table-1 shows the Anthro The mean age of the type 2 diabetic with DN subjects was having higher 56.8±6.4 years, type 2 diabetic without DN subjects has 52.76±7.1 years and in 41.0±6.73 years for healthy controls. The mean BMI of type 2 diabetic with DN subjects was higher 28.43±2.34, type 2 diabetic without DN subjects 25.54±2.59 as compared to 23.93±1.23 healthy controls [Table 1]. Similarly, compared to group A duration of diabetes and blood pressure were higher in group B and group C. The demographic and laboratory data of our studied groups are shown in Table 1.

Variables	Gtoup-A (Mean±S.D.)	Gtoup-B (Mean±S.D.)	Group-C (Mean±S.D.)	P-value
Age in years	56.8±6.4	52.76±7.1	41.0±6.73	0.001
BMI kg/m2	28.43±2.34	25.54±2.59	23.93±1.23	046
SBP in mm of Hg	129.78±12.63	127.5±7.99	121.2±10.94	0.001
DBP in mm of Hg	81.91±8.25	79.52±4.11	78.44±6.63	0.01

Table-1: Shows the general demographic characteristics in between Controls & Type 2 DM without DN

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The significant difference was present in FBs, PPBS, and HbA1C of diabetic patients when compared with control groups. In diabetes subjects, FBS, PPBS and HbA1C in group A were found highest and significant difference compared to group B and C (Table 2). Mean levels of TC, LDL and TG werefound highest in group A than in group B and lowest in group C whereasreverse order was found with HDL (Table 2).

Variables	Group A (Mean±S.D.)	Group B (Mean±S.D.)	Group C (Mean±S.D.)	P-value
FBS (mg/dl)	152.77±20.97	138.72±16.74	82.46±7.05	0.01
HbA1c (%)	8.61±1.15	7.44±1.32	4.07±0.7	0.01
T. cholesterol (mg/dl)	222.07±35.0	215.76±30.92	138.5.0±11.71	0.01
Triglycerides (mg/dl)	184.52±31.7	159.01±54.32	113.6±15.2	0.01
HDL-c (mg/dl)	35.73±5.06	38.54±8.97	51.48±5.24	0.42
LDL-c (mg/dl)	149.43±38.12	145.19±38.05	64.29±12.12	
Sr. creatinine (mg/dl)	3.69±0.39	3.92±0.58	0.82±0.13	0.01
MDA (micro mole/litre)	2.97±0.83	2.56±1.25	1.66±0.35	0.01
UAC ratio (mg/g)	230.32±29.43	18.95±3.3	8.22±2.37	0.01
Ischemia-modified albumin (IMA) (U/ml)	138.31±18.9	83.16±7.62	45.15±1.9	0.01

Table-2: Comparison of biochemical parameters in between controls, type 2 DM without DN & type 2 DM with DN.

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A significant mean difference between TC and HDL were found in group A compared to group B and C whereas TG value in group A was significantly greater related to group B and C. All lipid parameters were significantly high in group A related to group B & C. Similarly, Mean levels of serum creatinine, urinary creatinine ratio, Ischemia-modified albumin and Malondialdehyde werefound highest in group A than in group B and lowest in group C.

Variables	**Type 2 DM without DN**		**Type 2 DM with DN**	
	(r)	**P**	**(r)**	**P**
Age in years	0.04	0.692	0.30	0.02
Body mass index	0.03	0.767	0.14	0.16
Systolic blood pressure	0.12	0.23	0.28	0.004
Diastolic blood pressure	0.09	0.37	0.24	0.016
Fasting blood sugar	0.32	0.001	0.36	0.002
Post prandial blood sugar	0.34	0.001	0.42	0.001
Glycated hemoglobin	0.21	0.03	0.52	0.001
Urinary albumin creatinine ratio	0.13	0.197	0.56	0.001
Total cholesterol	0.19	0.05	0.37	0.001
Triglycerides	0.27	0.006	0.59	0.001
High density lipoprotein cholesterol	-0.11	0.275	-0.16	0.11
Low density lipoprotein cholesterol	0.24	0.016	0.55	0.001
Malondialdehyde	0.22	0.027	0.64	0.001
Reduced glutathione	-0.03	0.767	-0.14	0.073

Table-3: Correlation between ischemia modified albumin with biochemical parameters

studied in both type 2 DM without DN & type 2 DM with DN.

Ischemia-modified albumin (IMA) shows significantly positive correlation with FBS, PPBS, HbA1c, TC, TG, LDL and MDA with type 2 DM without DN. Similarly, IMA shows strongly positively correlated with blood pressure, fastingblood sugar (FBS), PPBG, HbA1c, cholesterol, LDL, triglycerides, serum creatinine and UACR ($P<0.05$). The insignificant negative correlation between IMA and HDL, GSH.

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Discussion:

Micro and macrovascular problems in type 2 diabetes are caused by a complicated interplay between controllable and nonmodifiable risk factors. In addition to hypertension, other significant modifiable factors in the pathogenesis and evolution of vascular complications in diabetes that result in diabetic nephropathy include anemia, albuminuria, dyslipidemia, hyperglycemia, lifestyle choices, etc. [14]. In the current investigation, Group A and Group B both had greater mean IMA levels than the healthy controls. This shows that uncontrolled diabetes either causes ischemic events or produces IMA as a biomarker of oxidative stress brought on by hypertension, hyperglycemia, and dyslipidemia [15]. As predicted, the cases' groups' FPS, PPBS, and HbA1C were higher than those of the control group. Many biochemical consequences of persistent raised blood glucose in diabetes includes nonenzymatic glycation of proteins, lipids, nucleic acid molecules gives rise to advanced glycation end products (AGEs), polyol activation, activation of the protein kinase C pathway(PKC), and oxidative stress [16]. According to Lin et al., increased collagen synthesis, cellproliferation, and cytokines release may be the cause of the connection between FPG and HbA1C in DN [17]. Due to an imbalance between enzymatic and nonenzymatic antioxidants, diabetes causes oxidative stress and increased production of reactive oxygen species (ROS) [18]. Furthermore, it is now widely known that ROS is the cause of the biochemical alterations of protein, lipids, carbohydrates, DNA, and other substances that harm the glomerulus membrane and endothelial lining in DN [19]. One such oxidative stress biomolecule is IMA, which is created when ROS-induced ischemia in DN modifies the albumin N-terminal. The study's findings imply that in the early stages of the disease, albumin change is triggered by stress caused by hyperglycemia in diabetic kidney disease. This study found a positive correlation between serum IMA and UACR, which links albuminuria to the advancement of DN illness [16]. Regarding the assessment of the link between urine IMA and albuminuria in DN, Bilgi et al., found no positive correlation [20]. In contrast, our earlier study compared the diagnostic efficiency of serum IMA levels as an early indication of DN to that of malondialdehyde and advanced

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oxidative protein products [11]. It is confirmed by the positive correlation between IMA and glycemic index (FPS and HbA1C) that hyperglycemia causes hypoxia, oxidative stress, and ischemia, which in turn causes vascular problems in type 2 diabetes [21]. The goal of the current study was to determine how IMA and lipid status related to type 2 diabetes with and without DN. When type 2 DM without DN is present, ischemia-modified albumin (IMA) strongly positively correlates with FBS, PPBS, HbA1c, TC, TG, LDL, and MDA. Similarly, there is a strong positive correlation ($P<0.05$) between IMA and serum creatinine, fasting blood sugar (FBS), PPBG, HbA1c, cholesterol, LDL, and triglycerides, as well as blood pressure. The IMA shows negative correlation with MDA & GSH. This result demonstrates how hypercholesterolemia contributes to the production of IMA levels in DN. Reduced blood flow in DN because of increased blood viscosity brought on by lipid alterations. These alterations make plaques more unstable, which aids in blood clot formations that causes atherosclerosis, hence exacerbating ischemia and raising IMA levels. Refaat et al.'s investigation, which corroborated our findings, also found a positive association between serum IMA & TC, LDL, glycosylated hemoglobin, and T2DM with dyslipidemia [22]. Good glycemic management of type 2 diabetes results in normal LDL status and no increase in IMA levels. Conversely, glucose is nonenzymatically attached to lysine residues in a range of protein residues in cases of poor glycemic control. Increased cholesterol to protein ratios, apolipoprotein A1 depletion, and triglyceride enrichment of HDL may cause diabetes-related elevated HDL catabolic rate compared to normal. In line with the study's findings, there is a notably low HDL cholesterol level and a negative connection between HDL and IMA in diabetes [22]. Thus, sustained hyperglycemia may be the mechanism causing albuminuria in diabetic kidney disease (DN), as this promotes the lipoprotein oxidation and glycation & improves lipoprotein binding to the glycosaminoglycan of the glomerular basement membrane. Glycated lipid molecules deposited in mesangial cells send a chemotactic signal to macrophages, enabling them to proliferate. Mesangial or glomerular foam cell production is mediated by receptors on monocytes or macrophages. Mesangial enlargement brought on by an accumulation of oxidized lipid in hyperglycemia is another mechanism for albuminuria.

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Conclusion:

In conclusion, the urine microalbuminuria, random blood sugar, HbA1c, urine albumin creatinine ratio, and length of diabetes were all positively correlated with the significantly higher levels of ischemia-modified albumin. This was especially true for type 2 diabetes with diabetic neuropathy. In the same way, Ischemia-modified albumin (IMA) with type2 DM without Diabetic Nephropathy alsohas a strong positive correlation with FBS, PPBS, HbA1c, TC, TG, LDL, & MDA. IMA may therefore be applied as a Type 2 diabetes risk marker, either inthe presence or absence of diabetic nephropathy. Consequently, we suggest that IMA be used as a sensitive test of diabetic nephropathy and an indicator of glycemic control.

References:

1. American Diabetes Association(2011) Diagnosis and classification of diabetes mellitus.Diabetes.Care ;34(1): S62-9.
2. Andy KH Lim (2014) Diabetic nephropathy – complications and treatment. Int J Nephrol Reno Vasc Dis, 7: 361–381.
3. Mogensen. C.E, Poulsen. P(1994). Epidemiology of microalbuminuria in diabetes and in the background populationCurr. Opin. Nephrol. Hypertens, (3).248–256 .
4. Perticone.F, Ceravolo.R, Pujia.A, G. et al (2001).Prognostic Significance of Endothelial Dysfunction in Hypertensive Patients.Circulation ,(104) 191–196 .
5. Dekker MS, Mosterd A, Van t ,Hof AW, Hoes AW(2010). Novel biochemical markers in suspected acute coronary syndrome: systematic review and critical appraisal. Heart, (96):1001–10.
6. Ma SG, Jin Y, Hu W, Bai F, Xu W, Yu WN. Evaluation of ischemia-modified albumin and C reactive protein in type 2 diabetics with and without ketosis. Biomark Insights. 2012;7:19–26.
7. Piwowar A, Knapik-Kordecka M, Warwas M. Ischemia-modified albumin level in type 2 diabetes mellitus – preliminary report. Dis Markers. 2008;24(6):311–7.
8. Apple FS, Wu AH, Mair J, et al. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem. 2005;51:810–

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24.

9. Van Rijn BB, Franx A, Sikkema JM, Van Rijn HJ, Bruinse HW, Voorbij HA. Ischemia modified albumin in normal pregnancy and preeclampsia. Hypertens Pregnancy. 2008;27:159–67.
10. Palanduz S, Ademoglu E, Gokkuşu C, Tamer S. Plasma antioxidants and type 2 DM. Res Commun Mol Pathol Pharmacol. 2001;109:309–18.
11. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic Biol Med. 1990;9:515–40.
12. Kaefer M, De Carvalho JA, Piva SJ, et al. Plasma malondialdehyde levels and risk factors for the development of chronic complications in type 2 diabetic patients on insulin therapy. Clin Lab. 2012;58:973–8.
13. Kim HY. Analysis of Variance (ANOVA) comparing means of more than two groups.Restor Dent Endod. 2014;39(1): 74-7.
14. Adinortey MB, Gyan BE, Adjimani J, Nyarko P, Sarpong C, Tsikata FY, et al. Dyslipidaemia associated with type 2 diabetics with micro and macrovascular complications among Ghanaians. Indian J Clin Biochem. 2011;26(3):261-8.
15. Gaze DC. Ischemia modified albumin: A novel biomarker for the detection of cardiac ischemia. Drug Metab. Pharmacokinet. 2009;24(4):333–41.
16. Patil P, Rao AV, Shetty S. Association of ischemia modified albumin with glycaemic status in type 2 diabetes mellitus. Int J Recent Sci Res. 2017;8(1):15374-8.
17. Lin CC, Chen CC, Chen FN, Li CI, Liu CS, Lin WY, et al. Risks of diabetic nephropathy with variation in hemoglobin A1c and fasting plasma glucose. The American Journal of Medicine. 2013;126 (11):1017.e1-10.
18. Marrocco I, Altieri F, Peluso I. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. Oxid Med Cell Longev. 2017;6501046.
19. Piwowar A, Knapik-Kordecka M, Warwas M. Ischemia-modified albumin level in type 2 diabetes mellitus – preliminary report. Dis Markers. 2008;24(6):311–7.

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE12, 2023

20. Bilgi M, Keser A, Katlandur H, Sahin E, Kalkan AO, Yildiz M, et al. Evaluation of the relationship between microalbuminuria and urine ischemia-modified albumin levels in patients with diabetic nephropathy. J Clin Lab Anal. 2017;31(3).
21. Sadik L, Yogoub Z, Sayed N, El Nour A, Abide El Hameed M, Satee B. The levelof Ischemic Modified Albumin (IMA) as risk marker for Cardio Vascular Disease (CVD) among some diabetic patients (type II) in Khartoum State-Sudan. Sudan Journal of Medical Sciences. 2017;12(4): 231-9.
22. Refaat S, Ghaffar NAE, Khalil A. The Relationship between ischemia modified albumin and lipids in type 2 Egyptian diabetic patients. Advan. Biol. Res. 2014;8 (1):18-22.