Original Article

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Biochemistry Section

Ischaemia-modified Albumin Estimation and Usefulness of Different Albumin Adjustment as a Marker to Predict Kidney Damage in Diabetes Mellitus

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ABSTRACT

Introduction: Urine Albumin Creatinine Ratio (UACR) has been the standard for detecting albuminuria in diabetes mellitus, but the presence of "non albuminuric renal impairment" has encouraged the search for novel markers. Recently, increased serum Ischaemia Modified Albumin (IMA) levels have been implicated in cases of Diabetic Nephropathy (DN). However, its estimation by conventional albumin cobalt assay is confounded by serum albumin levels.

Aim: To estimate IMA and albumin-adjusted IMA levels to assess their utility in diagnosing early renal damage in diabetes mellitus.

Materials and Methods: This hospital-based cross-sectional study was conducted from March 2014 to January 2015 at Kasturba Medical College, Mangaluru, Karnataka, India. The study included 30 healthy individuals and 60 patients with diabetes mellitus, which were further divided equally into three groups based on UACR. The groups were as follows: normoalbuminuria group (UACR <30 mg/g of creatinine), microalbuminuria group, and macroalbuminuria group (UACR 30-300 and >300 mg/g of creatinine, respectively). Serum IMA levels were estimated using Enzyme-linked Immunosorbent Assay (ELISA), while Glycated Haemoglobin (HbA1c), serum creatinine, urine albumin, and urine creatinine were measured using auto-analysers. Albumin-adjusted IMA values were measured, and correlation assays between IMA and serum albumin concentration were analysed in each group using one-way Analysis of Variance (ANOVA) and Pearson's correlation.

Results: In the present study, out of the 60 diabetes mellitus patients, 27 were females and 33 were males, with a mean age of 53±17 years. Among the 30 controls, 14 were females and 16 were males, with a mean age of 51±17.5 years. Serum albumin had a significant negative correlation with IMA in the microalbuminuria ($r=-0.4$, $p<0.01$) and macroalbuminuria ($r=-0.58$, $p<0.001$) groups. A significant mean difference was found in serum IMA, albumin-adjusted IMA, IMA index, and IMA ratio between the normoalbuminuria group and the other groups. Receiver operating characteristic analysis was used to predict the early stage of nephropathy (albuminuria). High sensitivity and specificity were measured for IMA (97.5% and 78%, respectively) and IMA ratio (97.5% and 76%, respectively) at cut-off percentages of 99 and 24.5, respectively.

Conclusion: The IMA ratio has greater diagnostic importance compared to other adjusted IMA values in discriminating diabetic patients with micro- and normoalbuminuria.

Keywords: Albuminuria, Fasting glucose, Nephropathy, Serum creatinine, Urine albumin

INTRODUCTION

The DN is a worldwide prominent cause of End-Stage Renal Disease (ESRD) that leads to diabetes-related morbidity and mortality [1]. The Chennai Urban Rural Epidemiology Study (CURES) reported a high prevalence of microalbuminuria (27%) than overt nephropathy (macroalbuminuria), which is 2.2% in an Asian urban Indian cohort [2]. In diabetic patients, overt DN progresses insidiously into ESRD once it is diagnosed and is intervened sparsely due to limited available resources. Therefore, it is better to diagnose and treat DN at an early stage to prevent its progression [3].

Microalbuminuria has been recommended as a non invasive marker to diagnose renal disease at an early stage [3]. However, the third National Health and Nutrition Examination Survey (NHANES) has pointed out the absence of albuminuria in one-third of adults with Type 2 Diabetes Mellitus (T2DM) and chronic renal insufficiency, indicating that microalbuminuria alone is no longer optimal to identify DN. Therefore, a better marker is required to diagnose DN at an early stage [4].

Human serum albumin is a well-recognised acute-phase protein that has anti-inflammatory and antioxidant abilities due to its high concentration and ligand-binding or buffering property. In T2DM, complications arise due to increased glycation or free radical injury of circulating proteins, which induce structural modification of serum albumin. Ultimately, these changes lead to reduced antioxidant and anti-inflammatory actions of albumin, causing the development of stress-induced complications like DN. Structural modification in the N-terminal (amino terminal) of serum albumin occurs within minutes due to hypoxia, ischaemia, Reactive Oxygen Species (ROS), and acidosis, especially at the aspartyl-alanyl-histidyl-lysine series, resulting in an interim change to the metal primary binding site, such as cobalt, nickel, and copper [5,6]. Previous research has reported about this modified serum albumin as IMA for the early diagnosis of myocardial ischaemia, which has later been studied in different diseases like stroke, pulmonary embolism, diabetic ketosis, systemic sclerosis, neuroblastoma, skeletal muscle ischaemia, etc. [4].

Serum IMA was estimated by applying the indirect colourimetric Albumin Cobalt Binding assay (ACB) principle. This method states that in physiological or non ischaemic conditions, there are minimal free metal ions/cobalt ions present because they mostly bind to serum albumin [7]. Based on this principle, a negative correlation was anticipated between serum IMA and albumin levels because it represents falsely high IMA in those with very low serum albumin levels [8]. To overcome the confounding effect of albumin on estimated IMA levels, different albumin adjustments for serum IMA estimation were suggested [9-11].

To the best of authors knowledge, there is no previous study in a diabetes cohort that was designed to estimate IMA by ELISA with a more sensitive and specific method and evaluate IMA results considering serum albumin within the reference range. The present study was planned with the aim of determining whether albumin adjustment is required in DN patients with normal serum albumin to interpret their serum IMA levels.

Hence, the present study was conducted to estimate IMA and albumin-adjusted IMA's to assess their utility in diagnosing early renal damage in diabetes mellitus.

MATERIALS AND METHODS

This hospital-based cross-sectional study was conducted at Kasturba Medical College, Mangaluru, Karnataka, India, from March 2014 to January 2015. The study was carried out after obtaining approval from the Institutional Ethical Committee (IEC no: KMC MLR 11-14/239), and informed consent was obtained from all enrolled participants. All measures were conducted in compliance with the Helsinki Declaration [12].

Inclusion criteria: A total of 60 type 2 diabetic patients, based on the American Diabetes Association (ADA) guidelines, with or without DN according to UACR [13], were included in the study. For comparison, 30 age and gender-matched healthy controls were randomly selected.

Exclusion criteria: All diabetic participants with a history of ischaemic artery disease like cardiovascular disease, cerebrovascular disease, pulmonary embolism, arterial occlusion or deep vein thrombosis, acute febrile illness, asymptomatic infection, malignant or chronic inflammatory diseases were excluded. Diabetic patients with additional nephropathy, foot ulcers, pregnancy, and those on insulin therapy were also excluded from the study. Participants with a history of drug use that might influence lipid metabolism, blood coagulation system, or liver or renal function tests, such as anticoagulants, hormone replacement therapy, oral contraceptives, steroids, and antilipidemic drugs, were also excluded. To nullify the effect of the analytical matrix on IMA measurement, only participants with serum albumin levels <3 g/dL and >5.5 g/dL were excluded from this study.

Sample size calculation: The total sample size for the study was calculated to be 90 using the formula $N=\frac{2(Z_{\alpha}^{2}.Z_{\beta}^{2}).\alpha^{2}}{\delta^{2}}$ at a 95% confidence interval

and 90% power of the study. Out of the 90 subjects, 60 were diabetes patients and 30 healthy controls were included. All 60 diabetic participants were further grouped into three groups of 20 each based on UACR. The normoalbuminuria group had UACR <30 mg/g of creatinine, the microalbuminuria group had UACR 30-300 mg/g of creatinine, and the macroalbuminuria group had UACR >300 mg/g of creatinine, respectively [14,15].

Study Procedure

Data on age, sex, medical history, medication, duration of diabetes, presence of nephropathy, and relevant routine biochemistry tests such as Fasting Blood Glucose (FBG) and 2-hour Postprandial Blood Glucose (PPBG) were collected. HbA1c levels were measured using the ion-exchange high-performance liquid chromatography method. Serum and urine albumin levels were measured using the bromocresol green method, and serum and urine creatinine levels were measured using the Jaffe's method. Commercial kits and automated clinical chemistry analysers were used for these measurements.

Sample collection and IMA estimation by ELISA: Leftover serum samples from eligible participants were collected from the clinical biochemistry lab and stored at -20°C. IMA levels were estimated using a solid-phase Enzyme-linked Immunosorbent Assay (ELISA) based on the double-sandwich principle. Kits from Shanghai Yehua Biological Technology Co. Ltd. were used, and the ELISA was performed using the ELx 800 instrument by BioTek Instruments, Inc. The sample was added to the wells coated with a purified human monoclonal antibody for IMA. After incubation with a conjugated antibody specific for IMA and streptavidin-horseradish peroxidase, the unbound enzymes were washed, and the colour was produced by adding tetramethylbenzidine substrate. The reaction was terminated by adding sulfuric acid, and the colour intensity was measured at 450 nm using a spectrophotometer. The concentration of IMA was determined based on the intensity of the colour produced was positive proportional to the concentration of analyte [16]. Assay range of IMA was 2-600 ng/mL having sensitivity of 1 .08 ng/mL with intra and intertest assay coefficient of variance (CV%) being, 1 0% and 1 2%, respectively.

Analysis of serum albumin on IMA measurement: Different analytical methods were used to assess the effect of serum albumin on the estimated IMA levels. The first method, proposed by Lippi G et al., (2007), calculated the albumin-adjusted IMA index (Adj. IMA) as the individual serum albumin concentration divided by the median albumin concentration of the population, multiplied by the IMA value [9]. The second method, derived by Lee YW et al., was modified into the albumin-adjusted IMA index (IMA index) by multiplying the serum albumin concentration (g/dL) by 23, adding the IMA value (U/mL), and subtracting 100 [10]. In the present study, authors used the formulae provided to calculate the IMA index in ng/mL. The third formula, used by van Rijn BB et al., (2008) and Inci A et al., was the IMA Ratio (IMAR), calculated as IMA divided by serum albumin [11,17].

STATISTICAL ANALYSIS

The data were analysed using IBM Statistical Package for Social Sciences (SPSS, Chicago, IL, USA) version 20.0. Normally distributed variables were expressed as mean and Standard Deviation (SD), while categorical variables were expressed as percentages. Statistical analysis comparing baseline variables between two groups was performed using an unpaired or independent sample t-test. One-way ANOVA with Tukey's post-hoc test was used to compare means among all four groups. Box plots were used to represent the median values in different groups. The correlation between variables was tested using Pearson's correlation coefficient, and the results were graphically plotted using Microsoft excel 2013 software. The optimal cut-offs for IMA, Adj. IMA, IMA index, and IMA ratio were determined experimentally using Receiver Operator Characteristic (ROC) analysis. The sensitivity and specificity of these variables were evaluated by calculating the areas under the curves in the ROC curve. A test variable was considered statistically significant at the 0.05 confidence level.

RESULTS

The general baseline characteristics of the 90 participants were studied. The diabetic group consisted of 60 individuals with a mean age of 53±17 years, while the control group consisted of 30 individuals with a mean age of 51±17.5 years. The mean serum albumin levels between the two groups were within the normal range and showed no significant difference. However, the levels of Fasting Blood Glucose (FBG), 2-hour postprandial blood glucose (2hPPBG), HbA1c, Urine Albumin-Creatinine Ratio (UACR), serum Ischaemia modified Albumin (IMA), adjusted IMA (Adj. IMA), IMA index, and IMA ratio were significantly higher (p-value <0.001) in the diabetic group compared to the control group [Table/Fig-1].

Variables	Cases (N=60) Mean±SD	Control (N=30) Mean±SD	p-value
Age (years)	53±17 (36-70)	51±17.5 (34-69)	0.058
Sex (F/M)	27/33	14/16	0.05
FBG (mg/dL)	179.5±43.0	80.1±7.9	<0.001
2hPPBG (mg/dL)	281.3±61.9	129.3±10.8	<0.001

HbA1C (%)	9.1±2	4.8±0.48	<0.001
UACR (mg/g)	226.8±241	16.9±6.1	<0.001
Serum creatinine (mg/dL)	1.45 ±0.4	0.98±0.24	<0.001
Albumin (g/dL)	3.8±0.6	4±0.6	0.085
IMA (ng/mL)	147±61	46±24	<0.001
Adj. IMA	139±44	45±20	<0.001
IMA index	135±51	38±21	<0.001
IMA ratio	39±20	11±6	<0.001

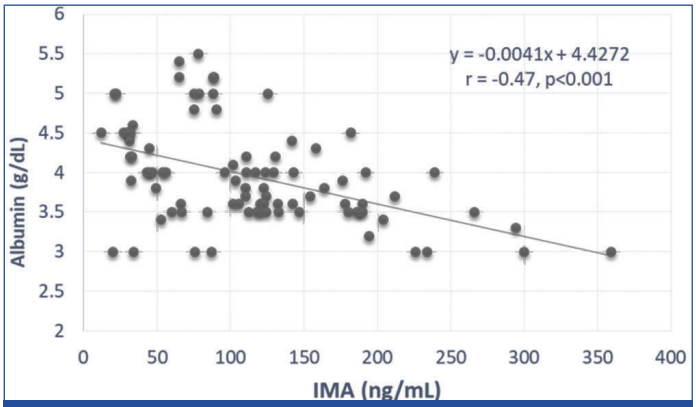
[Table/Fig-1]: Baseline biochemical characteristics of the enrolled participants (N=90) grouped based on ADA guidelines [12].
N: Total number of subjects; SD: Standard deviation; F/M: Female/male; FBG: Fasting blood glucose; 2hPPBG: 2 hour postparandial blood glucose; A1C: Glycated haemoglobin; UACR: Urine albumin creatinine ratio; IMA: Ischaemia modified albumin; Adj. p<0.05: Statistical significance

Based on UACR, the 60 diabetic cases were equally segregated and compared with the control group. The microalbuminuria group and the control group had similar serum albumin levels, but the macroalbuminuria group had significantly lower mean levels of serum albumin (3.6±0.4 g/dL) compared to the normoalbuminuria group (4.2±0.8 g/dL) and the control group (4.1±0.6 g/dL). The normoalbuminuria group had significantly lower levels of serum IMA, Adj. IMA, IMA index, and IMA ratio compared to the other groups. The macroalbuminuria group had the highest mean levels of serum IMA, Adj. IMA, IMA index, and IMA ratio, which were statistically significant compared to the normoalbuminuria and control groups [Table/Fig-2].

Variables	Normoalbuminuria (n=20)	Microalbuminuria (n=20)	Macroalbuminuria (n=20)	Control (n=30)
Albumin (g/dL)	4.2±0.8^{c}	3.8±0.3	3.6±0.4^{a,d}	4.1±0.6
IMA (ng/mL)	109±50^{b*,c,d}	155±43^{a*,d}	178±68^{a,d}	46±24^{a,b,c}
Adj. IMA	107±14^{b,c,d}	149±39^{a,d}	162±51^{a,d}	45±21^{a,b,c}
IMA index	99.4±14^{b*,c,d}	142±41^{a*,d}	162±63^{a,d}	38±21^{a,b,c}
IMA ratio	26±11^{b*,c,d*}	41±14^{a*,d}	51±24^{a,d}	11±6^{a*,b,c}

[Table/Fig-2]: Mean comparison of parameters between groups based on different stages of nephropathy (N=90).
Results stated as mean±standard deviation. p<0.001 is signified a for Normoalbuminuria group, b or Microalbuminuria, c for Macroalbuminuria, d for control group, *p<0.05
N: Total number of subjects; IMA: Ischaemia modified albumin; Adj. IMA: Albumin adjusted IMA index

When comparing the overall serum albumin levels with serum IMA in all participants, a significant negative correlation (r=-0.47, N=90, p<0.001) was found [Table/Fig-3]. Serum albumin also had a significant negative correlation with IMA in the microalbuminuria (r=-0.4, p<0.01) and macroalbuminuria (r=-0.58, N=20, p<0.001) groups. The IMA ratio showed a significantly strong negative correlation in all groups of this study [Table/Fig-4].

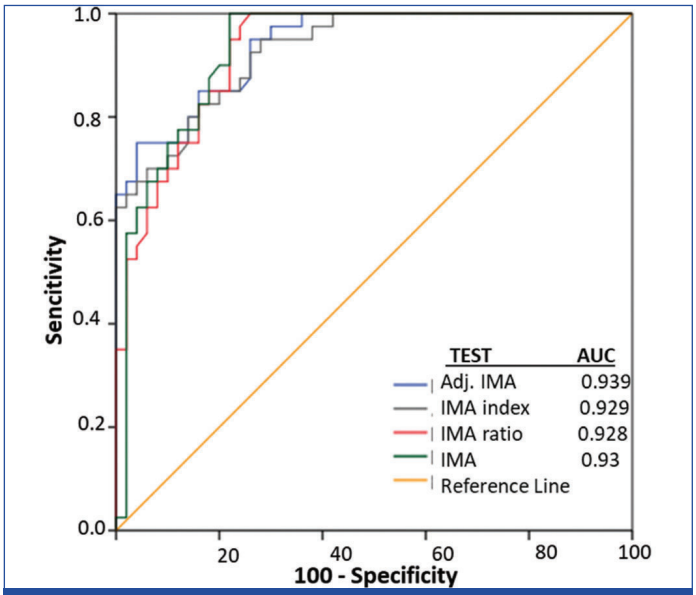


[Table/Fig-3]: Scatter graph displaying negative correlation between serum albumin and IMA in total study subjects (N=90).

Receiver Operating Characteristic (ROC) analysis was used to determine the optimal cut-offs for serum IMA, Adj. IMA, IMA index, and IMA ratio to predict early stage nephropathy (albuminuria). The areas under the ROC curves were 0.93, 0.939, 0.929, and 0.928, respectively [Table/Fig-5].

Variables		Normoalbuminuria (n=20)	Microalbuminuria (n=20)	Macroalbuminuria (n=20)	Control (n=30)
IMA	r	-0.68*	-0.4	-0.58*	-0.13
	P	0.001	0.08	0.05	0.5
Adj. IMA	r	-0.24	-0.03	-0.34	-0.15
	p	0.3	0.9	0.15	0.4
IMA index	r	-0.25	-0.24	-0.48*	-0.45*
	p	0.2	0.3	0.03	0.01
IMA ratio	r	-0.92*	-0.65*	-0.7*	-0.6*
	P	0.001	0.05	0.001	0.001

[Table/Fig-4]: Correlation between serum albumin and IMA, Adj. IMA, IMA index and IMA ratio between different stages of nephropathy (N=90).
N: total number of subjects; IMA: Ischaemia modified albumin; Adj. IMA: Albumin adjusted IMA index {Lippi G et al., (2007)}, IMA index: Albumin-adjusted IMA index {Lee YW et al., (2007)}, IMA ratio {van Rijn BB et al., (2008) and Inci A et al., (2016)}



[Table/Fig-5]: Receiver operating characteristics (ROC) curves for peak serum IMA, Adj. IMA, IMA index and IMA ratio to distinguish albuminuria (N=40) from normoalbuminuria (N=50).
N: Total number of subjects; IMA: Ischaemia modified albumin; Adj. IMA: Albumin adjusted IMA index

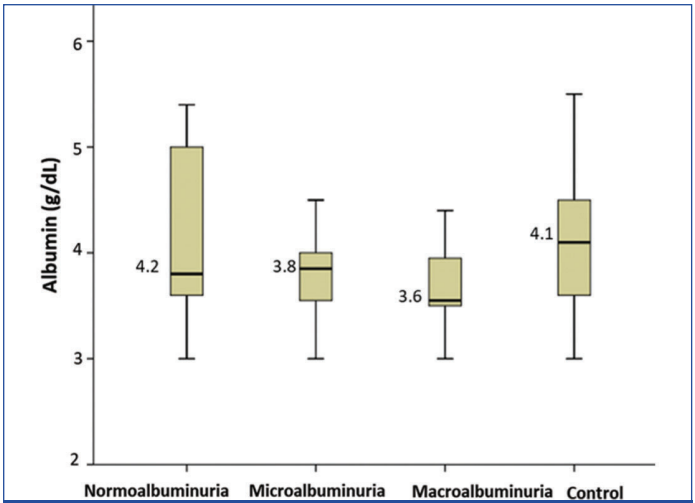
The ideal analytical cut-offs for serum IMA, Adj. IMA, IMA index, and IMA ratio, which had the highest sensitivity and specificity in evaluating albuminuria from normoalbuminuria, were determined to be 99 ng/mL (97.5% and 78%, respectively), 102 (95% and 74%, respectively), 94 (92.5% and 74%, respectively), and 24.5 (97.5% and 76%, respectively) [Table/Fig-6]. Box plots showing the median serum albumin levels in each group were within the normal range [Table/Fig-7].

Parameters	Cut-off	AUC	Sensitivity (%)	Specificity (%)
Adj. IMA	102	0.939	95	74
IMA index	94	0.929	92.5	74
IMA ratio	24.5	0.928	97.5	76
IMA	99	0.93	97.5	78

[Table/Fig-6]: Comparison of predictive ability of serum IMA, Adj. IMA, IMA index and IMA ratio to diagnose albuminuria (N=40) from normoalbuminuria (N=50).
N: Total number of subjects; IMA: Ischaemia modified albumin; Adj. IMA: Albumin adjusted IMA index {Lippi G et al., (2007)}, IMA index: Albumin-adjusted IMA index

DISCUSSION

Early and accurate diagnosis of nephropathy is crucial at an early stage to reduce mortality and morbidity in diabetes due to renal failure. Serum and urine protein are considered to be good indicators of glomerular hyperfiltration, an early stage of kidney damage [3]. Serum Ischaemia modified Albumin (IMA) is a modified



[Table/Fig-7]: Box plot representing serum albumin (median) in different stages of nephropathy.

albumin molecule that has been considered an effective biomarker for diagnosing early Diabetic Nephropathy (DN) [4]. In the present study, significantly higher levels of IMA were found in individuals with diabetes mellitus compared to the control group, indicating that hyperglycemia-induced oxidative stress leads to structural modifications of albumin [18-21]. However, the authors found an insignificant decrease in albumin levels in the diabetic group, and they recommended that the interpretation of IMA results should be done with caution.

Consistent with other studies, an inverse correlation between IMA and serum albumin levels was observed, even within the normal albumin concentration range. At this narrow range of albumin levels (3 to 5.5 g/dL), a weaker negative association was found between IMA and albumin compared to previous studies [7]. Previous reports have indicated that for every 1 g/dL decrease in serum albumin, there is a 2.6% increase in IMA levels. However, many studies have stated that the influence of albumin on IMA is within the normal range [22].

Considering the limitations of the analytical measurement of IMA, which is based on the Albumin Cobalt-Binding (ACB) principle, all confounding factors that can cause an increase in albumin (such as haemoconcentration, iatrogenic factors, high protein diet, severe dehydration, etc.) or a decrease in albumin (such as burns, malabsorption syndrome, chronic infection, kidney disease, etc.) should be thoroughly evaluated [9]. To overcome the influence of albumin on IMA levels, different researchers have proposed their own methods to eliminate this effect. Some studies have recommended using the median albumin levels of the population to adjust for the individual serum albumin effect on IMA results [23]. Other researchers have applied formulae-based derived equations, such as the one proposed by Lee YW et al., to minimise the impact of albumin on IMA levels [10]. A third method to decrease the influence of albumin was utilised by van Rijn BB et al., and Inci A et al., who proposed using the ratio of individual serum IMA and albumin to adjust for the effect of albumin on IMA levels [11,17].

In the present study, the levels of IMA were assessed in each stage of nephropathy. In the macroalbuminuria group, or overt Diabetic Nephropathy (DN) patients, significantly lower albumin levels were found compared to the early DN and control groups. To address the confounding effect of low albumin on IMA results in this study, all three available albumin adjustment formulas were utilised and correlated with serum albumin in each stage of DN to determine their usefulness. As suggested by Lippi G et al., the median albumin value of 3.9 g/dL was calculated for all participants (N=90) in the present study and was used to minimise the influence of the low median albumin level in the macroalbuminuria group [9]. The mean differences between each group for all three adjusted IMA values were found to be similar to the unadjusted IMA level, indicating the importance of using normal albumin as an inclusion criterion for patients. This result supports previous studies done in pregnancy (Ozdemir S et al.,) [22], coronary angioplasty (Demir H et al.,) [8], and acute myocardial ischaemia (Kumar et al.,) [22] that used the Lippi G et al., formulas and showed similar differences even after adjusting IMA [9].

Although negative correlations were reported between serum albumin and unadjusted IMA, as well as all three adjusted IMA values, in all stages of nephropathy, a strong negative association was found only in early DN. The non significant relationship of the adjusted IMA (Lippi G et al.,) with albumin in any group and the presence of only a significant negative correlation of the IMA index (Lee YW et al.,) in overt DN and the control group indicate the independence of IMA from albumin in the present study [9,10]. This discrepancy could be due to the differences in the method of IMA analysis and the formulas used by Lee YW et al., who established their formulas based on IMA analysis using the Cobas Integra 800 (Roche Diagnostics, Germany) and the ACB commercial kit (Inverness Medical Innovations, USA). They proposed the calculation of the IMA index as follows: serum albumin concentration (g/dL)×23+IMA (U/mL)-100 [10]. In contrast, Lippi G et al., estimated IMA using the ACB colourimetry method and suggested formulas using the median albumin level of the enrolled population as (individual serum albumin concentration/median albumin concentration of the population)×IMA. Lee YW et al., expressed the IMA results in U/mL according to the kit manual, while Lippi G et al., reported them in ABSU (absorbance units) [9]. Both of these formulas were based on the ACB principle for estimating IMA but reported with different units. Another reason for the independence of IMA from albumin may be the inclusion of a population with albumin concentrations within the reference range, which limits the bias effect on IMA estimation.

On the other hand, the IMA ratio had a significant negative correlation in all groups, indicating the dependency of albumin in IMA estimation as well as the requirement for a sensitive and direct method for IMA analysis. In the present study, a direct and quantitatively specific ELISA method was used to estimate albumin structural modification. In contrast, the ACB assay used by other researchers, as given by Kumar KA et al., is an indirect method with certain limitations. The main drawback of this method is that it is not confirmed whether the exogenous cobalt chloride ($CoCl_2$) added in a fixed concentration of 1.5 equivalents per albumin molecule will bind to the N-terminal of albumin or attach to either cobalt binding site A or B, as albumin has other metal binding sites as well. Another limitation is the denaturation of albumin at the disulfide (S-S) bond caused by the thiol-based 1,4-dithiotheritol colourising agent used in the Oran I and Oran B, ACB assay. This may lead to the displacement of cobalt from its binding site, resulting in a false positive result [24,25].

In the diagnosis of DN, authors mostly use the UACR as a screening tool for detecting albuminuria in diabetic patients. IMA and adjusted IMA values were tested for diagnosing early DN (microalbuminuria) from normoalbuminuria using ROC analysis to evaluate the discriminatory power of each test. Based on these criteria, appropriate cut-offs for IMA, adjusted IMA, IMA index, and IMA ratio were reported in the present study, but differences in sensitivity and specificity between these tests were also found. Due to the high sensitivity and specificity of IMA (97.5% and 78%, respectively) and the IMA ratio (97.5% and 76%, respectively), these two tests can be used to differentiate microalbuminuria from normoalbuminuria. This indicates that serum IMA alone can be utilised with high specificity to diagnose early nephropathy at a cut-off point of >99 ng/mL, supporting the results of the previous study [4]. However, the high correlation of serum albumin with the IMA ratio in all stages of DN compared to the unadjusted IMA reveals its usefulness and diagnostic precision. Thus, the IMA ratio can be used

as a convenient method to adjust for the effect of albumin, and its cut-off level can be used to diagnose microalbuminuria.

Limitation(s)

The present study was somewhat limited by its cross-sectional design. It is challenging to establish a cause-and-effect relationship due to the somewhat small number of individuals in the study.

CONCLUSION(S)

The advantage of simultaneously measuring both IMA and IMA albumin ratio in the same individual samples, along with other routine biochemical parameters, makes them useful auxiliary markers in estimating DN. The IMA ratio demonstrates greater diagnostic performance in discriminating between patients with micro- and normoalbuminuria compared to IMA alone, and appears to be almost perfect for diagnosing these individuals. The development and commercialisation of an improved kit for measuring the IMA marker using highly sensitive immunoassay techniques is essential to ensure high precision and accuracy in its measurement. This would allow for easy incorporation of IMA into clinical research and day-to-day laboratory practice.

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