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Comparison of diagnostic efficacy of IMA and albumin adjusted IMA index in diabetic and non-diabetic acute stroke patients

Jayaraj G Gudi[1]*, Shivaleela M Biradar[2], Anil Malleshappa[3], Karkal Ravishankar Naik[4]

[1]Assistant Professor of Biochemistry, Sapthagiri Institute of Medical Sciences and Research Centre, Bengaluru, [2]Assistant Professor of Biochemistry, BLDE (Deemed to be University) Shri B M Patil Medical College, Vijayapura, [3]Professor of Biochemistry, KAHER's Jawaharlal Nehru Medical College, Belagavi [4]Professor of Neurology, KAHER's Jawaharlal Nehru Medical College, Belagavi.

***Corresponding author: Jayaraj G Gudi,** Assistant Professor of Biochemistry, Sapthagiri Institute of Medical Sciences and Research Centre, Bengaluru,
E-mail: jayarajgudi12@gmail.com

Abstract

Background: Acute stroke is one of the important causes of death and disability around the world. Type 2 Diabetes Mellitus (T2DM) increases the risk of acute stroke. This study was undertaken to evaluate the efficacy of a novel biomarker Ischemia Modified Albumin (IMA) in diagnosing the cases of stroke in diabetic and non diabetic patients along with albumin adjusted Ischemia Modified Albumin index, a modification to IMA values as suggested by few authors.

Materials and method: This study included 30 patients of diabetic stroke, 30 non-diabetic stroke patients along with 30 healthy volunteers serving as controls. IMA was estimated using Albumin cobalt binding test. The receiver operating characteristic (ROC) curve was plotted for IMA and albumin adjusted IMA index separately in 'diabetic stroke' and 'non-diabetic stroke' patients group and measures of diagnostic efficacy were calculated.

Results: Area under the curve (AUC) of serum IMA was 0.968 in 'diabetic stroke' group as compared to 0.917 in 'non-diabetic stroke' group. The AUC of albumin adjusted IMA index was 0.800 in 'diabetic stroke' group as compared to 0.811 in 'non-diabetic stroke' group. All the measures of diagnostic efficacy of albumin adjusted IMA index were much lower as compared with that of serum IMA.

Conclusion: Albumin adjusted IMA index is not a better marker for diagnosis of stroke in both diabetic and non-diabetic patients as compared to serum IMA.

Keywords: Ischemia Modified Albumin, Diagnostic Efficacy, albumin adjusted IMA index, Acute Ischemic Stroke, Diabetes Mellitus, Biomarker

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Introduction

Acute ischemic stroke leading to neuronal death due to cerebral ischemia has become a major cause of death and disability. Stroke adds substantially to the burden of disease both in developed and developing countries. According to a study India has the burden of acute stroke higher than developed countries.[1]

Early diagnosis and proper medical treatment can substantially reduce the complications of acute stroke. Currently the confirmatory diagnosis of acute stroke and its classification requires evidences of injury in radiological investigations such as computed tomography (C.T.) and magnetic resonance imaging (M.R.I.) which is almost always necessary.[2]

Though they provide critical information, their high cost and unavailability is a major limitation to use them. Many a times radiological signs areabsent in the early stages of stroke.[3] Hence there is a need of new markers of ischemia which are easy to measure, available at any place. Thus identification and characterization of biomarkers which can be measured soon after the ischemic stroke and having rapid and easy measurement method along with cost effectiveness would be advantageous for early diagnosis. Considering this possibility, many scientists have assessed numerous biomarkers for stroke diagnosis but none have been approved clinically. In 2005, Food and Drug Administration of US approved the use of Ischemia modified albumin (IMA) for the early diagnosis of myocardial ischemia.[4] A steep rise in studies on ischemia modified albumin was noted soon after that which assessed relation of IMA with various ischemic conditions including myocardial ischemia.

Serum albumin becomes ischemia modified albumin after losing its binding capacity to divalent metal ions such as copper, cobalt etc. Quantification of this is possible by the estimation of its reduced metal binding capacity. Studies evaluating IMA levels in acute stroke are lacking. Few studies have simply estimated IMA levels and reported increased IMA levels in acute ischemic stroke. But the data is insufficient. As per our knowledge, this is the first study evaluating the diagnostic efficacy of IMA in acute stroke as compared to the gold standard CT / MRI till now.

When the serum IMA levels are being used to diagnose the acute stroke, it becomes more important to study the factors affecting serum IMA levels apart from ischemic process itself. Type 2 Diabetes Mellitus is one such factor which appears to influence serum IMA levels[5]. The raised serum IMA levels have been shown in few studies on diabetic people even in absence of apparentischemia[6]. There are few studies in the literature indicating the possibility of serum albumin concentration affecting serum IMA levels too.Gidenne et al. showed a negative relationship between IMA levels as measured by albumin cobalt binding assay and serum albumin concentrations[7].We showed variable effect of serum albumin concentration on IMA levels in our previous study[8]. Hence, in order to nullify the effect of serum albumin

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concentration, Lippi et al proposed calculation of albumin adjusted IMA index by the following formula[9].

$$\text{Albumin adjusted IMA index} = \frac{\text{Individual serum albumin concentration}}{\text{Median albumin concentration of population}} \times \text{IMA}$$

Thus, albumin adjusted IMA index appears to be devoid of the effect of varying serum albumin concentration on IMA and hence can become a better marker of ischemia than IMA itself.
Hence, this study compares the diagnostic efficacy of IMA and albumin adjusted IMA index for the diagnosis of acute stroke in diabetic and non-diabetic patients.

Material and method

The present study includes 30 diabetic and 30 non-diabetic patients with confirmed diagnosis of acute stroke through neuro-imaging modality admitted in intensive care unit of a tertiary care hospital. Control group included 30 healthy volunteers attending the blood bank of the hospital.

The 'Diabetic Stroke group' included acute stroke patients with confirmed type 2 diabetes mellitus who were on anti-diabetic treatment with oral hypoglycemic drugs and / or insulin. 'Non-diabetic stroke group' included acute stroke patients without the history of diabetes mellitus. Apparently healthy subjects without known ischemic diseases like myocardial infarction, peripheral vascular diseases were included in 'control' group. Acute stroke patients coming to the hospital within 12 hours after the onset of symptoms were only recruited to the study. Any patient with history suggestive of recent ischemic events like acute coronary syndrome, myocardial infarction, pulmonary embolism, peripheral vascular disease were excluded from the study. Patients with type 1 diabetes mellitus were also excluded from the present study.

Method of data collection

After clearance from institutional ethics committee for research on human participants study participants were recruited into the study. Written informed consent was obtained prior to their participation in the study from all the participants. Patients with symptoms of acute stroke presenting within 12 hours of onset were only recruited in the study. Clinically relevant information as per the proforma was obtained. 5 ml of venous blood was drawn from the patients after the clinical diagnosis of acute stroke.Later, based on the evidences in CT/MRI of brain, acute stroke diagnosis was confirmed. Only those patients with definitive radiological evidences of acute stroke were included in the case group. Based on the history of diabetes mellitus, they were further divided into two groups viz. diabetic stroke and non-diabetic stroke groups.

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Venous blood was allowed to clot for 15 minutes and soon after that serum was separated by centrifugation. Separated serum was stored at -80°C until further biochemical analyses. Ischemia modified albumin was estimated using the method of Albumin Cobalt Binding test which was described by Bar Or et al[10].Initially 200 microlitres of serum was taken in a test tube. To that, 50 microlitres of cobalt chloride was added. After incubation for 10 minutes, Dithiothreitol was added. Further 1 ml of sodium chloride was added to stop the reaction after 2 min incubation. The absorbance of the reaction mixture was read at 470 nm using a spectrophotometer and values were recorded in absorbance units (ABSU).

Statistical analyses

Statistical Package for Social Sciences (SPSS) software, version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.) was used to perform the required statistical analyses. The quantitative data was expressed as mean ± SD. Categorical data was expressed as frequency. The generated data was compared using two-way ANOVA with post hoc Bonferroni test at the 5% level of significance. Receiver operating characteristic (ROC) curves were plotted for serum IMA and albumin adjusted IMA in both the groups separately. Area under the curve (AUC) was calculated using the software. Suitable cut off levels for serum IMA and albumin adjusted IMA were chosen manually and measures of diagnostic accuracy were calculated.

Results

The present study included 30 diabetic stroke patients, 30 non-diabetic stroke patients and 30 apparently healthy controls. Male subjects were more in all the three groups.

Table 1: Baseline characteristics of the three groups

Parameters		**Diabetic stroke (n = 30)**	**Non-diabetic Stroke (n = 30)**	**Control (n = 30)**	**p value**
Gender	Male	23	27	25	-
	Female	07	03	05	-
Age (Mean years ± SD)		61.7 ± 10.03	60.2 ± 15.4	34.6 ± 8.44	a < 0.001 b < 0.001 c = 0.657
Systolic BP (mmHg) (Mean ± SD)		150 ± 24	146 ± 22	125 ± 10	a < 0.01 b < 0.01 c = 0.521
Diastolic BP (mmHg) (Mean ± SD)		94 ± 12	96 ± 10	82 ± 8	a < 0.01 b < 0.01 c = 0.71
Type of	Ischemic	25	22	NA	-

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stroke	Hemorrhagic	05	08		
***a** = Diabetic stroke vs Control, **b** = Non-diabetic stroke vs Control, **c** = Diabetic stroke vs Non-diabetic stroke (NA*- Not applicable)					

There was a statistically significant difference observed when all the three groups were compared with respect to mean age. Statistically significant difference was seen in both mean systolic & diastolic BP between controls and other two groups ($p< 0.01$). But the difference between 'diabetic stroke' group and 'non-diabetic stroke' group was not statistically significant ($p= 0.521$ & $p= 0.71$) [Table 1].

Table 2: Mean and SD values of Ischemia Modified Albumin (IMA) in the three groups and results of ANOVA

Groups	N	IMA levels (Mean ± SD) ABSU	95% Confidence Interval for Mean		ANOVA test p- value
			Lower Bound	Upper Bound	
Diabetic stroke	30	0.23 ± 0.03	0.22	0.24	<0.001
Non-diabetic stroke	30	0.21 ± 0.03	0.20	0.22	
Controls	30	0.16 ± 0.03	0.15	0.17	

ANOVA test was employed to compare the serum IMA levels of three groups. Patients of stroke with (0.23 ABSU) and without diabetes mellitus (0.21 ABSU), both had higher mean IMA levels as compared to controls (0. 16 ABSU). The difference was statistically significant ($p < 0.001$). [Table 2]

Table 3: Mean and SD values of Albumin adjusted Ischemia Modified Albumin (IMA) index in the three groups and results of ANOVA

Groups	N	Albumin adjusted IMA index (Mean ± SD) ABSU	95% Confidence Interval for Mean		ANOVA test p- value
			Lower Bound	Upper Bound	
Diabetic stroke	30	0.19 ± 0.03	0.18	0.21	<0.001
Non-diabetic stroke	30	0.19 ± 0.03	0.18	0.21	
Controls	30	0.15 ± 0.03	0.14	0.16	

Albumin adjusted IMA levels were calculated in three groups and also subjected to ANOVA test. Patients of stroke with (0.19 ABSU) and without diabetes mellitus (0.19 ABSU), both had higher mean levels of albumin adjusted IMA as compared to controls (0.15 ABSU).

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The difference was statistically significant ($p < 0.001$). [Table 3] But, no difference was seen between diabetic and non-diabetic stroke groups.

CT/MRI were considered as 'reference test' and used to diagnose acute stroke in both diabetic and non-diabetic patients. Receiver Operating Characteristic (ROC) curve was plotted using SPSS software for both IMA and albumin adjusted IMA index.

Table 4: Comparison of Diagnostic efficacy of IMA and Albumin adjusted Ischemia Modified Albumin (IMA) index in diabetic stroke patients group

Measures of Diagnostic Accuracy	**Ischemia Modified Albumin At a Cut off: 0.196 ABSU**	**Albumin adjusted Ischemia Modified Albumin (IMA) index At a cut off: 0.173 ABSU**
Sensitivity	90%	73%
Specificity	87%	70%
Positive Predictive value (PPV)	87%	69%
Negative Predictive value (NPV)	90%	74%

In 'diabetic stroke' patients, the area under the ROC curve (AUC) for IMA was 0.968 (95% CI: 0.933- 1.000) showing IMA as an excellent diagnostic test ($p< 0.001$) [Graph 1]. On analyzing the ROC curve, a cut-off of 0.196 ABSU of IMA was chosen and diagnostic accuracy measures were calculated. At this cut- off, sensitivity was 90%, specificity was 87%, Positive predictive value was 87% and Negative predictive value was 90% [Table 4].

Table 5: Comparison of Diagnostic efficacy of IMA and Albumin adjusted Ischemia Modified Albumin (IMA) index in non-diabetic stroke patients group

Measures of Diagnostic Accuracy	**Ischemia Modified Albumin At a Cut off: 0.192 ABSU**	**Albumin adjusted Ischemia Modified Albumin (IMA) index At a cut off: 0.192 ABSU**
Sensitivity	87%	47%
Specificity	83%	53%
Positive Predictive value (PPV)	84%	75%
Negative Predictive value (NPV)	86%	62%

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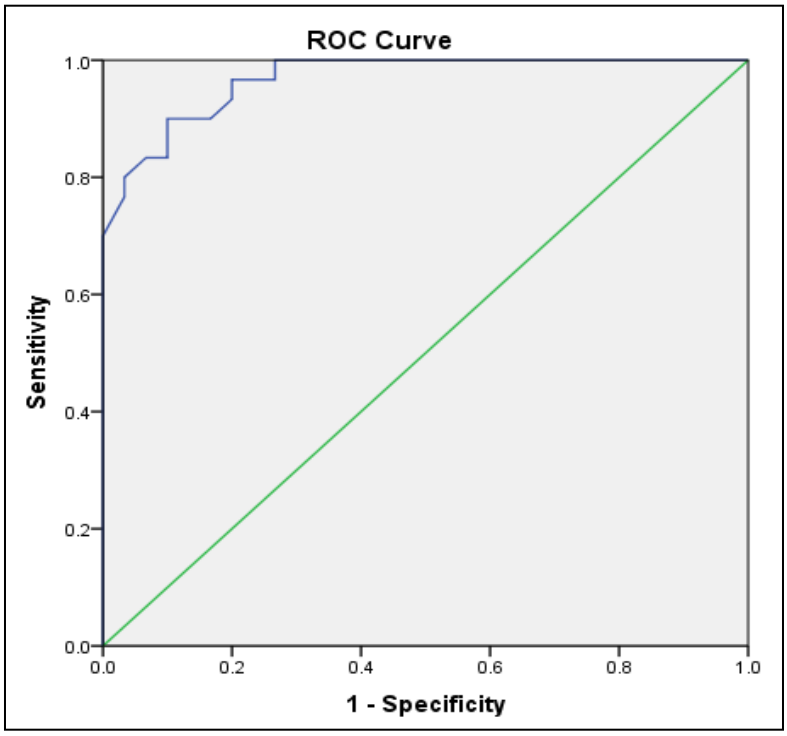
In 'non-diabetic stroke' group, AUC for IMA was 0.917 (95% CI: 0.847- 0.987) which showed IMA as excellent diagnostic test in this group too (p< 0.001) [Graph 3]. On analyzing the ROC curve, a cut-off of 0.192 ABSU of IMA was chosen and diagnostic accuracy measures were calculated. At this cut- off, sensitivity was 87%, specificity was 83%, Positive predictive value was 84% and Negative predictive value was 86% [Table 5].

Considering albumin adjusted IMA index as an index test, ROC curve was plotted in both diabetic stroke and non-diabetic stroke groups. AUC of albumin adjusted IMA index in diabetic stroke group was 0.800 (95% CI: 0.688- 0.912) (p<0.001) [Graph 2]. AUC in non-diabetic stroke group was 0.811 (95% CI: 0.705- 0.917) (p<0.001)[Graph 4]. At a cut off of 0.173 ABSU of albumin adjusted IMA in diabetic group, sensitivity was 73%, specificity was 70%, positive predictive value was 69% and negative predictive value was 74% [Table 4]. At a cut off of 0.192 ABSU of albumin adjusted IMA in non-diabetic group, sensitivity was 47%, specificity was 53%, positive predictive value was 75% and negative predictive value was 62% [Table 5].

Graph 1: Receiver Operating Characteristic Curve for IMA in diabetic stroke patients group



Graph 2: Receiver Operating Characteristic Curve for albumin adjusted IMA in diabetic stroke patients group

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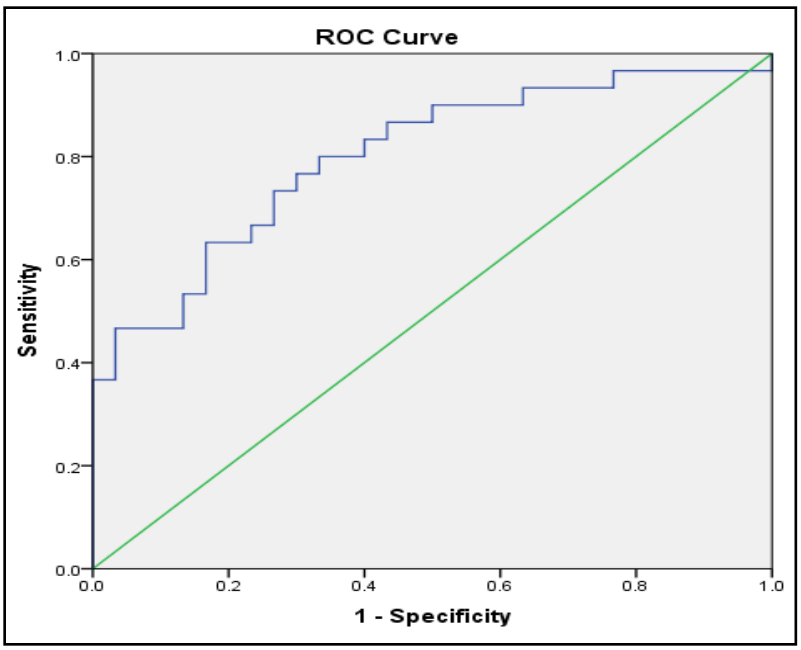
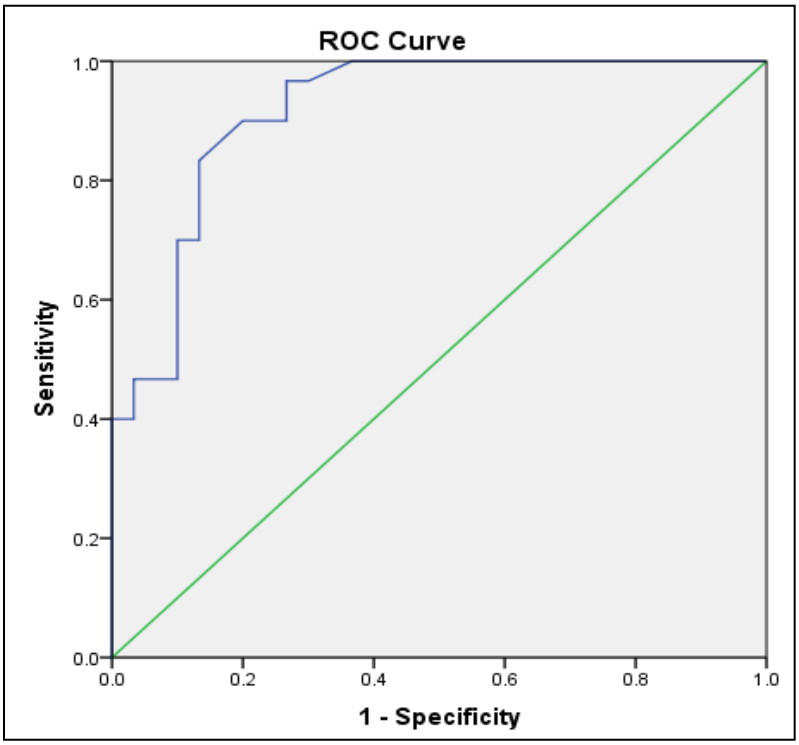


Graph 3: Receiver Operating Characteristic Curve for IMA in non-diabetic stroke patients group



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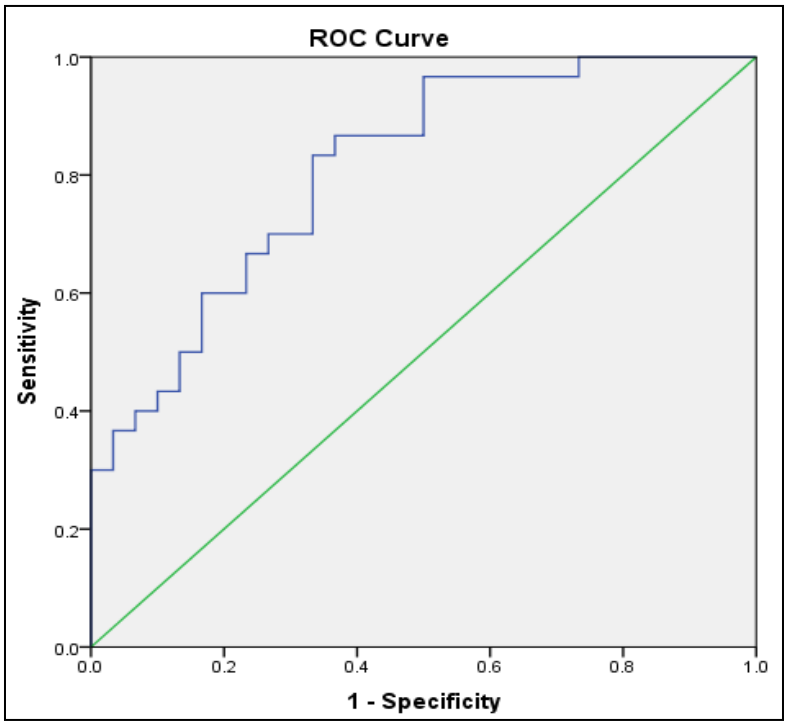
Graph 4: Receiver Operating Characteristic Curve for albumin adjusted IMA index in non-diabetic stroke patients group



Discussion

There has been increase in the studies on IMA soon after the FDA approval of role of IMA in the acute coronary syndrome patients. Plenty of studies reported favorable results for the use of IMA in cases of myocardial injury. Serum IMA levels were shown to be increased in various ischemic conditions and few situations of absence of apparent ischemia such as Diabetes Mellitus suggesting possibility of mechanism of formation of IMA which is common between ischemic and non-ischemic conditions. Some of the possible mechanisms may be hypoxia, acidosis, free radical mediated injury etc. Oxidative stress associated with endothelial inflammation leading to ischemia appears to be the underlying etiology in conditions such as Diabetes Mellitus.[11] Further studies are warranted for the explanation of exact mechanism of formation of IMA.

Acute stroke is one of the ischemic condition where in IMA appears to be elevated. Many studies have reported the same in various sub types of acute stroke. A study by Gunduz et al reported higher serum IMA levels in infarction, hemorrhage patients than control group. They concluded that IMA can serve as a diagnostic marker in acute stroke[12].

Oxidative stress was proposed as the major mechanism of formation of IMA due to the recent studies which showed increased IMA levels in various deceases for which oxidative stress

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was the reason[13, 14]. Diabetes Mellitus is one such condition where there is enormous evidence for the presence of oxidative stress. Hence it is logical to expect higher IMA levels in diabetic patients than non-diabetics even in the absence of acute ischemic events. Piwowar et al reported 75% higher IMA levels in diabetics as compared to non-diabetics. Hence they concluded that hyper glycemia and associated oxidative stress could be the probable reason for increased IMA levels[15]. There is a paucity of studies evaluating IMA levels in diabetes in association with any ischemic events.

Plenty of studies are available having assessed the diagnostic efficacy of IMA in acute coronary syndrome. But, there is no much evidence available in the literature measuring diagnostic efficacy in acute stroke patients. No study till date has assessed measures of diagnostic efficacy of IMA in acute stroke patients who are grouped based on the presence or absence of DM. G. Lippi et al. study proposed a further modification of serum IMA levels in order to minimize the effects of different serum albumin levels in different patients[9]. The same study suggested calculation of albumin adjusted IMA index to be better indicator than IMA level itself. We have reported the differing effect of serum albumin levels in diabetic and non-diabetic stroke groups.This study did not include age matched controls ($p<0.001$). But there was no significant difference between two patient groups with respect to age. A study conducted by Govender et al has shown that the effect of age and sex on IMA levels appears to be minimal or nil.[16]

The AUC for serum IMA in both the patient groups is similar to a study done by Ahn and colleagues who reported AUC of 0.928 (95% CI: 0.857–0.999) in acute stroke group which increased to 0.990 (95% CI: 0.970–1.000) after adjusting IMA values with the albumin values.[17] But in our study the measures of diagnostic efficacy viz, sensitivity, specificity, positive predictive value and negative predictive value decreased drastically after the calculation of albumin adjusted IMA index.A study conducted by Dalsaniaet. al. also showed excellent diagnostic value of IMA in stroke patients with AUC of 0.951 (95% CI: 0.889-0.984). This study predicted that IMA has a sensitivity of 94% and specificity 90%.[18]

Conclusion

Our study clearly demonstrated through the measures diagnostic efficacy that, serum IMA level can be an excellent diagnostic aid in both diabetic and non-diabetic stroke. Further, we have shown that the diagnostic efficacy of IMA increases in presence of diabetes mellitus compared to non-diabetics. However, albumin adjusted IMA index was found to have lesser sensitivity and specificity and may not be useful to diagnose the stroke in both diabetic and non-diabetic groups.

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The future studies should focus on identifying clinically useful cut-off levels for serum IMA in diabetics and non-diabetics both. Standardization of estimation methods is also necessary in order to generalize and compare the IMA results.

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