

## Effect of diabetes mellitus on the diagnostic efficacy of ischemia modified albumin in acute stroke

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### Abstract

**Introduction:** Acute stroke is a major cause of disability and death all over the world. Ischemia modified albumin (IMA) carries a potential use in the early diagnosis of acute stroke. Stroke is frequently associated with Diabetes mellitus (DM) which is known to affect the serum IMA level. This study compares the diagnostic efficacy of IMA in stroke patients with and without DM.

**Materials and Methods:** The present study included 60 acute stroke patients with and without DM (30 in each group) and 30 healthy controls. Albumin cobalt binding test was used to estimate IMA. The receiver operating characteristic (ROC) curve was plotted for 'Stroke with DM' and 'Stroke without DM' separately and measures of diagnostic efficacy were calculated.

**Results:** Area under the curve (AUC) of serum IMA was 0.968 in 'Stroke with DM' group as compared to 0.917 in 'Stroke without DM' group. Sensitivity, specificity, positive predictive value and negative predictive value were 90%, 87%, 87% and 90% respectively in 'Stroke with DM' group and 87%, 83%, 84% and 86% respectively in 'Stroke without DM' group.

**Conclusion:** IMA is an excellent marker for the diagnosis of acute stroke in both presence and absence of DM. The presence of DM increases the diagnostic efficacy of IMA in acute stroke patients.

**Keywords:** Ischemia modified albumin, Diagnostic efficacy, Albumin cobalt binding test, Acute ischemic stroke, Diabetes mellitus, Biomarker.

### Introduction

Acute stroke is a serious condition in which occurs cerebral neuronal death as a result of cerebral ischemia leading to death or permanent disability. Stroke adds substantially to the health burden both in developed and developing countries. According to a study the burden of acute stroke in India is higher than developed countries.<sup>1</sup>

The after effects of acute stroke can be reduced greatly by early identification of stroke and proper medical management. At present the acute stroke is being identified by detailed history and clinical signs and symptoms. Radiological investigations including computed tomography (C.T.) and magnetic resonance imaging (M.R.I.) provide evidences to confirm the stroke diagnosis. The clinical manifestations of stroke are highly variable because of the complexity of the brain and its vasculature. Thus neuroimaging is almost always necessary for the diagnosis and classification of the stroke.<sup>2</sup> But these facilities are not devoid of limitations. They are costly and not available everywhere. More importantly radiological evidences are often absent in the early stages of stroke.<sup>3</sup> So we need markers which can be easily estimated at bedside or in laboratory which could help the clinician support the diagnosis of acute stroke. Thus identifying a biochemical marker which appears early after the ischemic event, cost effective and having rapid and easy measurement method is of immense help in rapid diagnosis.

Several researchers have evaluated a number of biomarkers for the diagnosis of stroke but none of them are approved for the clinical use. Soon after the Food and Drug Administration of US approved the use of Ischemia

modified albumin (IMA) for the early diagnosis of myocardial ischemia in 2005,<sup>4</sup> there was a sudden increase in the studies correlating serum IMA levels with various ischemic conditions including myocardial ischemia. Normal serum albumin loses its divalent metal binding capacity due to the effect of ischemia leading to Ischemia modified albumin. It can be quantified by assessing its reduced metal binding capacity. Though the ischemia modified albumin has been studied in various ischemic contexts, the studies correlating IMA levels with 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. No study has evaluated the diagnostic efficacy of IMA in acute stroke as compared to the gold standard CT / MRI till now.

While interpreting the serum IMA levels to diagnose acute stroke, it is imperative to understand various factors affecting its serum concentration. Diabetes Mellitus (DM) is one such factor which has been reported to influence the serum IMA concentration.<sup>5</sup> Few studies have concluded that DM increases the IMA level even in absence of ischemia.<sup>6</sup> Hence, we decided to study the effect of DM on the diagnostic efficacy of IMA by comparing acute ischemic stroke patients with and without DM.

### Materials and Methods

**Study Population:** This cross sectional study included the patients of acute stroke diagnosed with definitive neuroimaging evidences either with or without DM, attending the casualty or admitted in intensive care unit of a university teaching hospital. Apparently healthy volunteers

attending the blood bank of the hospital were taken as controls.

**Sample Size:** This study included 90 subjects of either sex, aged more than 18 years, of whom there were 30 patients of acute stroke with diabetes mellitus, 30 patients of acute stroke without diabetes mellitus and 30 normal healthy controls. The sample size was calculated considering the mean and standard deviations of previous similar studies.

**Inclusion Criteria:** Patients of acute stroke with confirmed type 2 diabetes mellitus who were on treatment for hyperglycaemia with oral hypoglycaemic drugs and / or insulin were included in 'Stroke with DM group'. Acute stroke patients without the history of diabetes mellitus were included in 'Stroke without DM' group. Healthy subjects without known ischemic diseases like myocardial infarction, peripheral vascular diseases were included in 'control' group.

**Exclusion Criteria:** Acute stroke patients presenting after 12 hours of onset of symptoms of stroke were excluded from the study. Any patient with history suggestive of recent ischemic events like acute coronary syndrome, myocardial infarction, pulmonary embolism, peripheral vascular disease were not included. Patients with type 1 diabetes mellitus were also excluded from the present study.

**Method of Collection of Data:** Institutional ethical committee clearance was obtained before starting the study. Written informed consent was obtained from all the subjects involved in the study. Initially patients presenting with the symptoms of acute stroke within 12 hours of onset of symptoms were included in the study. Clinical data with other relevant information were obtained as per the proforma. Blood sample was drawn from all the patients soon after the clinical diagnosis. Patients with confirmed diagnosis of acute stroke by brain CT/MRI were only included in the case group. Detailed history including diabetes history was taken and included them in different groups accordingly.

Serum was separated immediately from venous blood and used for the estimation of ischemia modified albumin and other required biochemical parameters. Albumin Cobalt Binding test as described by Bar Or et al. was used to estimate serum IMA.<sup>7</sup> Fifty microliters of cobalt chloride were added to 200 microliters of serum and was incubated for ten minutes. Later dithiothreitol was added and incubated for 2 min. Then, 1 ml of sodium chloride was used to stop the reaction. The absorbance of the reaction mixture was read at 470 nm using a spectrophotometer and values were recorded in absorbance units (ABSU).

### Statistical Analyses

All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) software, version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). The quantitative data was expressed as mean  $\pm$  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 in both the groups separately. Area under the curve (AUC) was calculated using the software. Suitable cut off level for serum IMA was chosen manually and measures of diagnostic accuracy were calculated.

### Results

This study included 30 subjects in each of the groups. Number of male subjects was higher than females in all the groups. Statistically significant difference was observed when the two patient groups were compared with the control group with respect to mean age whereas no significant difference was found between two patient groups. Statistically significant difference was seen in both mean systolic & diastolic BP between controls and other two groups ( $p < 0.01$ ). But the difference between 'Stroke with DM' group and 'Stroke without DM' group was not significant ( $p = 0.521$  &  $p = 0.71$ ) [Table 1].

'Stroke with DM' (0.23 ABSU) and 'Stroke without DM' (0.21 ABSU) groups had higher mean serum IMA levels when compared with control group (0.16 ABSU). ANOVA test showed statistically significant difference in mean IMA levels between the three groups ( $p < 0.001$ ) [Table 2].

Considering radiological investigations (CT/MRI) as the 'reference standard test' and IMA as the 'index test' in 30 patients of 'Stroke with DM' group and healthy 'controls' the diagnostic accuracy parameters were analyzed. Receiver Operating Characteristic (ROC) curve was plotted using SPSS software.

The area under the ROC curve (AUC) was 0.968 (95% CI: 0.933-1.000) which shows IMA is an excellent diagnostic test in 'stroke with DM' group ( $p < 0.001$ ) [Graph 1]. A suitable cut-off of 0.196 ABSU of IMA was chosen based on the analysis of ROC curve 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 3].

Area under the ROC curve (AUC) was 0.917 (95% CI: 0.847- .987) which shows IMA is an excellent diagnostic test in stroke without DM group as well ( $p < 0.001$ ) [Graph 2]. A suitable cut-off of 0.192 ABSU of IMA was chosen based on the ROC curve analysis 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 3].

**Table 1: Baseline characteristics of the three groups**

Parameters		Stroke with DM (n = 30)	Stroke without DM (n = 30)	Control (n = 30)	P value
<b>Gender</b>	Male	23	27	25	-
	Female	07	03	05	-
<b>Age</b> (Mean years ± SD)		61.7 ± 10.03	60.2 ± 15.4	34.6 ± 8.44	a < 0.001 b < 0.001 c = 0.657
<b>Systolic BP</b> (mmHg) (Mean ± SD)		150 ± 24	146 ± 22	125 ± 10	a < 0.01 b < 0.01 c = 0.521
<b>Diastolic BP</b> (mmHg) (Mean ± SD)		94 ± 12	96 ± 10	82 ± 8	a < 0.01 b < 0.01 c = 0.71

*a = Stroke with DM vs Control, b = Stroke without DM vs Control, c = Stroke with DM vs Stroke without DM*

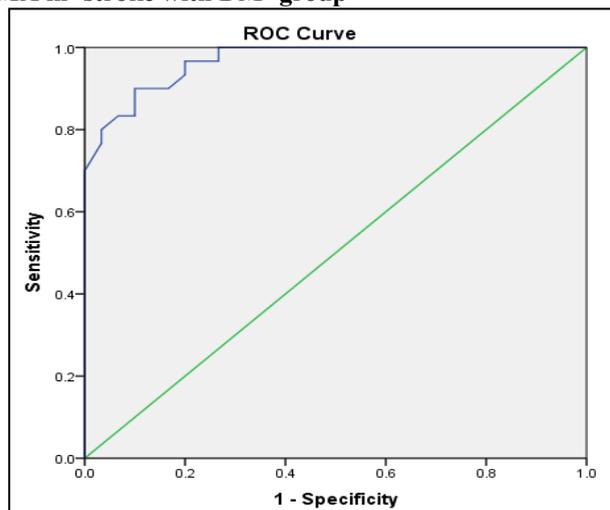
**Table 2: Mean and SD values of Ischemia Modified Albumin (IMA) in the three groups and results of ANOVA**

Groups	N	IMA levels (ABSU) Mean ± SD	95% Confidence Interval for Mean		ANOVA test
			Lower Bound	Upper Bound	
Controls	30	0.16 ± 0.03	0.15	0.17	F-value 52.50, p-value <0.001
Stroke with DM	30	0.23 ± 0.03	0.22	0.24	
Stroke without DM	30	0.21 ± 0.03	0.20	0.22	

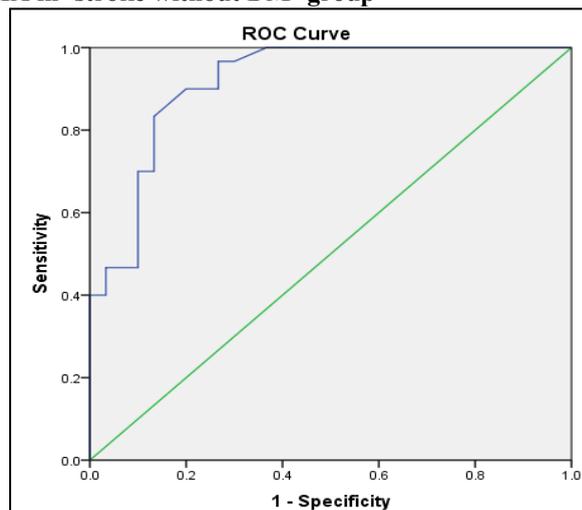
**Table 3: Diagnostic efficacy of IMA in ‘stroke with DM’ group and ‘stroke without DM’ group**

Measures of Diagnostic Accuracy	Stroke with DM group At a Cut off: 0.196 ABSU	Stroke without DM group At a cut off: 0.192 ABSU
Sensitivity	90%	87%
Specificity	87%	83%
Positive Predictive value (PPV)	87%	84%
Negative Predictive value (NPV)	90%	86%

**Graph 1: Receiver operating characteristic curve for IMA in ‘stroke with DM’ group**



**Graph 2: Receiver operating characteristic curve for IMA in ‘stroke without DM’ group**



## Discussion

Following the promising results in various studies on the acute coronary syndrome patients and FDA approval of IMA for the diagnosis of myocardial injury there has been an upsurge in the studies involving IMA. Though many studies have reported increased serum IMA levels in various ischemic conditions indicating the ischemia as the trigger for the formation of IMA, there are few studies reporting high IMA values in the absence of apparent ischemia like diabetes mellitus suggesting possibility of other mechanisms of formation of IMA. Hypoxia, acidosis, superoxide radical injury, exposure to free iron and copper, etc. are some of the possible mechanisms.<sup>8</sup> Although the exact mechanism of formation of IMA is yet to be explained, oxidative stress associated with the sub-endothelial inflammation and the resultant chronic ischemia appears to be the underlying etiology in certain non ischemic conditions like diabetes mellitus.<sup>9</sup>

Acute stroke is one of the many ischemic conditions wherein IMA appears to be elevated. Significance of IMA in different aspects in acute stroke patients is seldom studied. Most of the studies reported have studied the role of IMA in the diagnosis of acute stroke and its subtypes while some others studied its prognostic significance. Gunduz et al. estimated serum IMA level in patients with brain infarction, brain haemorrhage and sub-arachnoid haemorrhage.<sup>10</sup> Serum IMA level in all the three patients groups were higher than control group. They concluded that IMA can serve as a diagnostic marker in cerebrovascular accidents.

For patients of acute ischemic stroke presenting within the window period, revascularization with intravenous thrombolysis is the choice of treatment.<sup>11</sup> But few patients of acute ischemic stroke may not show signs of ischemia when the imaging is done very early. Evaluation of the IMA in this subset of imaging negative patients may help the clinician in decision-making in the emergency setting.

Recently IMA levels have been shown to be increased in many diseases where oxidative stress is proposed as the main mechanism supporting the hypothesis that IMA generation depends strongly on the high oxidative stress state.<sup>12</sup> Since the most common cause of mortality in diabetic patients is macro-vascular complications such as myocardial infarction and stroke, it is important to identify the effect of diabetes mellitus on the markers shown to be useful to predict ischemia.

A few studies have been reported in the literature which evaluated the effect of DM on IMA levels though not in association with stroke. A study done by Piwowar et al revealed 75% higher IMA levels in diabetics than non diabetics. Hence they concluded that hyperglycemia and associated oxidative stress could be the reason for the higher IMA levels in diabetic patients.<sup>13</sup>

Though there are a number of studies which have evaluated the diagnostic efficacy of IMA in acute coronary syndrome patients, studies analyzing the diagnostic efficacy measures in acute stroke patients are limited. Moreover no study has been reported in the literature evaluating the

diagnostic efficacy of IMA in stroke patients who are grouped based on the presence of DM.

In the present study the controls were not age matched with the stroke patients which is evident by the significant difference between the mean age of patients in both 'stroke with DM' and 'stroke without DM' groups and controls. But 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.<sup>14</sup>

In the present study, Area under the ROC curve (AUC) for IMA in 'Stroke with DM' and 'Stroke without DM' groups showed that estimation of IMA is an excellent diagnostic test with AUC being 0.968 and 0.917 respectively. Ahn and colleagues also 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. This study reported sensitivity of 87.5% & specificity of 89.3%.<sup>15</sup> A study conducted by Dalsania et 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%.<sup>16</sup>

## Conclusion

The present study has clearly demonstrated that IMA is an excellent biomarker for the diagnosis of acute stroke in patients both with and without DM. All the parameters of diagnostic efficacy have shown that IMA could predict presence of stroke in diabetic people more efficiently than people without diabetes mellitus. Thus it can be concluded that presence of diabetes mellitus in acute stroke patients increases the diagnostic efficacy of serum ischemia modified albumin. Along with the recent acute ischemia during the stroke, ongoing chronic ischemia and oxidative stress might have been responsible for further increase in IMA levels in diabetics than non-diabetics.

Further studies are needed to confirm and generalize the above findings. Future studies should focus on identifying clinically appropriate cut-off for serum IMA levels both in presence and absence of diabetes mellitus. It is also important to standardize and validate appropriate method for IMA estimation for better comparison of results all around the world.

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## Conflict of Interest: None.

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