

## ISCHEMIA MODIFIED ALBUMIN AMONG TYPE 2 DIABETIC PATIENTS VISITING A TERTIARY CARE CENTER OF KATHMANDU

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Received: 10 Jun, 2020

Accepted: 4 Aug, 2020

Published: 27 Sept, 2020

**Key words:** Diabetes mellitus; Hyperglycemia; Ischemia modified albumin; Oxidative stress.

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### Citation

Thapa S, Rayamajhi RJ, Gautam S, Dahal S. Ischemia modified albumin among type 2 diabetic patients visiting a tertiary care center of Kathmandu. Journal of Chitwan Medical College. 2020;10(33):74-8.



Peer Reviewed

### ABSTRACT

**Background:** Chronic hyperglycemia in diabetes may increase oxidative stress causing ischemia and long-term complications of diabetes. It may also alter albumin, increasing the concentration of serum ischemia modified albumin. The present study was conducted to estimate serum ischemia modified albumin level and to assess its relationship with parameters of glycemic control in diabetic patients.

**Methods:** In this study, 130 type 2 diabetic patients were enrolled and blood samples were analyzed for ischemia modified albumin, glycated hemoglobin, fasting and postprandial blood glucose. Parameters of glycemic control were estimated using routine standard methods and serum ischemia modified albumin was measured manually by spectrophotometric cobalt-albumin binding assay. Participants with glycated hemoglobin level less than 7% were labeled as group 1 and participants with glycated hemoglobin value more than or equal to 7% were labeled as group 2.

**Results:** Group 2 participants had significantly higher mean serum ischemia modified albumin as compared to group 1 ( $p < 0.001$ ). There was significant positive correlation between ischemia modified albumin and parameters of glycemic control; glycated hemoglobin ( $r = 0.300$ ,  $p = 0.001$ ), fasting blood glucose ( $r = 0.239$ ,  $p = 0.006$ ), postprandial blood glucose ( $r = 0.318$ ,  $p < 0.001$ ). However the relationship of ischemia modified albumin with age, body mass index and duration of diabetes were statistically insignificant.

**Conclusions:** The present study shows increase in serum ischemia modified albumin with increase in all three glycemic parameters. This finding suggests that ischemia modified albumin can be used as a marker of hyperglycemia induced oxidative stress in diabetes.

### INTRODUCTION

Diabetes mellitus is a metabolic disease with increasing prevalence and considered as the seventh leading cause of direct or indirect death.<sup>1</sup> At present, glycated hemoglobin (HbA1c), fasting blood glucose (FBG) and postprandial blood glucose (PPBG) levels are measured to monitor glycemic control in diabetic patients.<sup>2</sup> With rise in glycemic parameters in diabetic patients, oxidative stress may also increase, due to increase production of free radicals. This will cause chronic ischemia leading to macro and micro vascular complications.<sup>3,4</sup>

Chronic ischemia modifies the N-terminus of albumin thus altering the binding capacity of albumin with different metals. Such albumin molecule is known as ischemia modified albumin (IMA).<sup>5</sup> Studies suggest that serum IMA concentration increases in myocardial injury and may increase in diabetes as well due to hyperglycemia induced ischemia.<sup>6-9</sup>

In the context of Nepal, prevalence of diabetes has been found to be 8.4%, and with increase in life expectancy, diabetes associated complications are also being more prevalent.<sup>10</sup> Studies have been conducted in Nepal for the use of IMA as a biomarker for acute coronary syndrome.<sup>11</sup> The present study was

conducted to measure IMA in type 2 diabetic patients and assess the relationship of IMA with HbA1c, fasting blood glucose and post prandial blood glucose. This will help to use IMA as an alternative marker of hyperglycemia induced oxidative stress, which is cost-effective and further, may help in monitoring the development of complications as a result of hyperglycemia.

### METHODS

This was a cross-sectional study conducted at Kathmandu Medical College Teaching Hospital (KMCTH) from June 2019-February 2020 after obtaining ethical approval from Institutional Review Committee of KMCTH (Ref.: 20052019114). Clinical biochemistry laboratory was chosen as a study site to directly meet the patients for interviewing demographic profile and to run the tests for glucose and HbA1c and department of Biochemistry was chosen for measurement of serum IMA in UV spectrophotometer. Non-probability convenience sampling technique was used and diagnosed type 2 diabetic patients visiting KMCTH for regular glucose monitoring were included in the study. The informed and written consent was taken from the interested participants.

The sample size was calculated using formula  $n=(1.96)^2 \cdot (\sigma)^2 / e^2$ , where,  $n$  is the number of samples required,  $\sigma$  (standard deviation of serum IMA among diabetic patients)= 0.215 at 95% confidence interval<sup>12</sup>  $e$  (error rate) was taken as 4%.

The sample size was estimated to be 110.94 and it was approximated to 130.

The study excluded conditions, which might increase oxidative stress level and serum IMA like history of acute myocardial infarction or stroke, malignancy, acute or chronic infections and those under corticosteroid medications. The participants were interviewed for demographic profiles like age, sex, and duration of diabetic history and medications history. Height and weight were measured and BMI was calculated using the formula  $\text{weight}/(\text{height})^2$ .

Sample collection: Blood samples were routinely drawn by venipuncture technique and collected in two tubes, serum separator tube for glucose and IMA determination and EDTA containing tube for analyzing glycated hemoglobin. Fasting blood sample was collected for measurement of fasting blood glucose, HbA1c and IMA and two hours post meal sample was collected for postprandial blood glucose measurement. The sample in serum separator tube was centrifuged to separate serum. Fasting blood glucose and postprandial blood glucose were measured immediately and then serum was stored at -20° Celsius refrigerator for measurement of IMA but not for more than four weeks. Routine standard colorimetric enzymatic method was used to measure serum glucose with glucose reagents from Human Diagnostics and Boronate Affinity Quenching Technology was used to measure HbA1c using HumaMeter A1c kit by Human Diagnostics.<sup>13, 14</sup> Ischemia modified albumin (IMA) was measured based on the method described by Bar-Or et al using UV/VIS spectrophotometer by Cecil.<sup>15</sup> For the measurement of IMA, 200 microliters of patient serum was added to 50 microliters of cobalt chloride solution of 1 grams/liter concentration. The mixture was vigorously mixed and incubated for 10 minutes. Then 50 microliters of Dithiothreitol solution of 1.5 grams/liter concentrations was mixed and incubated for 2 minutes. The sodium chloride solution of 1.0 ml of a 9.0 grams/liter concentration was added and mixed. The blank was prepared similarly with the exclusion of DTT and the absorbance of the assay mixture was read against blank at 470 nm in UV spectrophotometer. The values were expressed as absorbance unit (ABSU).

Diabetic participants were divided into two groups based on HbA1c level. Group 1 included diabetic participants with HbA1c level less than 7% and considered as patients with good glycemic control and those having 7% or more HbA1c levels were labeled as group 2 and considered as patients with poor glycemic control.<sup>16</sup>

Statistical analysis was done using Statistical Packages for Social Sciences version 16. Descriptive statistics was repre-

sented as Mean  $\pm$  Standard deviation (S.D) with 95% confidence intervals for continuous data (age, BMI, FBG, PPBG, HbA1c and IMA) and categorical data (gender and HbA1c group) was depicted as frequency number. Pearson correlation was used to assess relation between IMA and age, BMI, FBG, PPBG and HbA1c. Spearman correlation test was used to assess relation between IMA and duration of diabetes. Statistical significance was assumed at  $p < 0.05$ .

## RESULTS

Group 1 with participants having good glycemic control had 69 and group 2 with poor glycemic control had 61 diabetic participants. Characteristics and clinical values (Mean  $\pm$  S.D.) of group 1 and group 2 participants are given in Table 1. The mean age of group 1 participants was slightly higher than of group 2. BMI, fasting blood glucose and postprandial blood glucose were slightly higher in group 2 participants as compared to group 1 participants.

**Table 1: Baseline characteristics of clinicodemographic and biochemical parameters of group 1 and group 2**

Characteristics	Group 1 (HbA1c <7%)	Group 2 (HbA1c $\geq$ 7%)
Number	69	61
Gender (Female/Male)	39/30	32/29
Age (years)	58.28 $\pm$ 11.10	57.02 $\pm$ 10.35
Duration of diabetes (years)	4.27 $\pm$ 3.80	8.56 $\pm$ 5.44
BMI (kg/m <sup>2</sup> )	23.53 $\pm$ 2.05	25.44 $\pm$ 1.70
FBG (mg/dl)	100.85 $\pm$ 19.43	141.57 $\pm$ 39.03
PPBG (mg/dl)	137.81 $\pm$ 40.22	208.21 $\pm$ 62.66
HbA1c (%)	6.15 $\pm$ 0.529	8.04 $\pm$ 1.10

Group 1 participants had significantly lower mean serum IMA levels than group 2 (0.16 $\pm$ 0.07 versus 0.21 $\pm$ 0.08;  $p < 0.001$ ) (Table 2). There was no significant difference observed for mean serum IMA levels between male and female (0.18 $\pm$ 0.08 versus 0.18 $\pm$ 0.07;  $p = 0.97$ ).

**Table 2: Comparison of mean serum IMA between group 1 and 2**

Groups	Serum IMA Mean $\pm$ S.D	Mean difference	p-value
Group 1 (HbA1c <7%)	0.16 $\pm$ 0.07	-0.055	$p < 0.001$
Group 2 (HbA1c $\geq$ 7%)	0.21 $\pm$ 0.08		

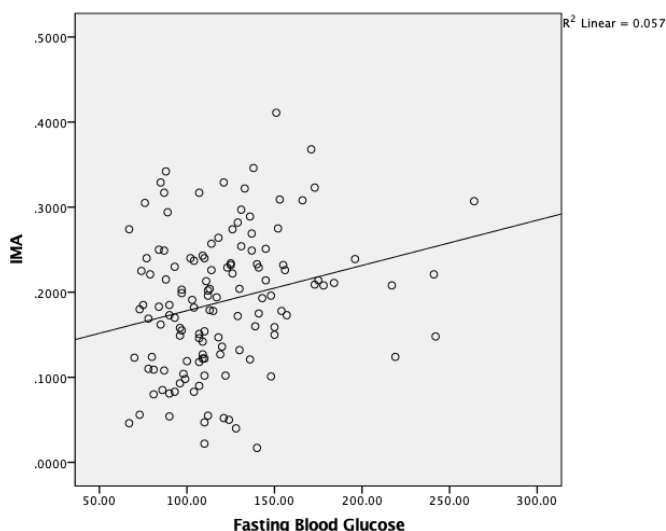
Serum IMA had weak but significant positive correlation with HbA1c ( $r = 0.300$ ,  $p = 0.001$ ), FBG ( $r = 0.239$ ,  $p = 0.006$ ), and PPBG ( $r = 0.318$ ,  $p < 0.001$ ) (Table 3). However, the relationship of age, BMI and duration of diabetes with IMA was not statistically significant. Correlation of IMA with HbA1c, FBG and PPBG are

shown in Figure 1, 2 and 3 respectively.

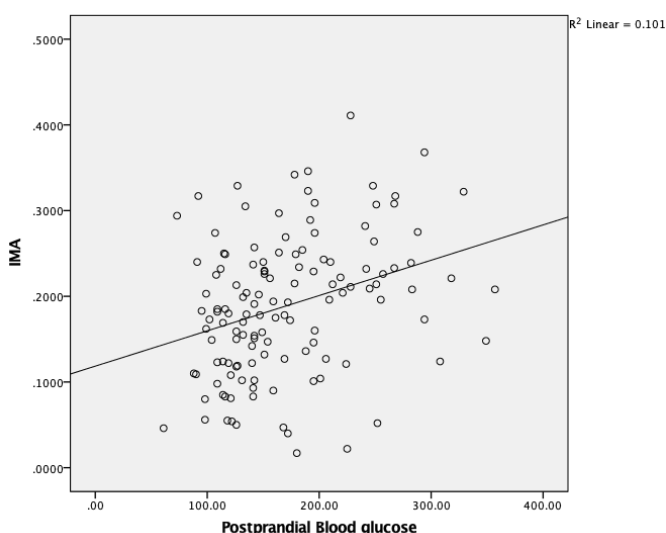
**Table 3: Correlation of IMA with different clinical parameters**

Parameters	Correlation	p-value
Age (years)	-0.072 <sup>a</sup>	0.417
Duration of diabetes (years)	0.116 <sup>b</sup>	0.190
BMI (kg/m <sup>2</sup> )	0.164 <sup>a</sup>	0.06
FBG (mg/dl)	0.239 <sup>a</sup>	0.006 <sup>**</sup>
PPBG (mg/dl)	0.318 <sup>a</sup>	<0.001
HbA1c (%)	0.300 <sup>a</sup>	0.001 <sup>**</sup>

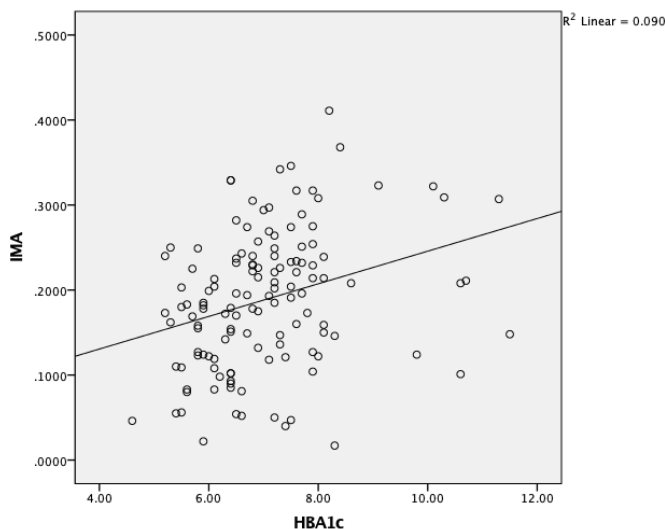
a= Pearson correlation, b=Spearman correlation, \*\* statistically significant



**Figure 1: Scatter plot graph showing positive correlations between serum IMA and fasting blood glucose with p=0.006, r=0.239, which is statistically significant**



**Figure 2: Scatter plot graph showing positive correlations between serum IMA and postprandial blood glucose with p<0.001 r=0.318, which is statistically significant**



**Figure 3: Scatter plot graph showing positive correlations between serum IMA and HbA1c with p=0.001, r=0.300, which is statistically significant**

### DISCUSSION

The present study was conducted to analyze IMA levels in serum and assess its relationship with different parameters of glycemic control among diabetic patients. Diabetic participants with poor glycemic control had significantly higher serum IMA than diabetic participants with good glycemic control. There was weak but significantly positive relationship between IMA and HbA1c, fasting blood glucose and postprandial blood glucose. IMA has emerged as a novel biomarker for differentiating ischemic and non-ischemic patients as chronic ischemia damages albumin to raise the concentration of IMA in blood.<sup>17</sup> Hyperglycemia can induce chronic ischemia by increasing production of free radicals. Free radicals may be generated by different mechanisms such as from auto-oxidation of glucose, glycation of proteins and antioxidative enzymes, which limit their capacity to detoxify oxygen radicals and stimulate cytochrome P450-like activity by excessive nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) produced by glucose metabolism.<sup>18</sup> These free radicals damage the specific binding sites of albumin for transition metals like cobalt, copper, nickel. N-Aspartate-Alanine-Histidine-Lysine with histidine being the most essential forms the specific binding site, which is also the most susceptible region for degradation of albumin by free radicals. Hence hyperglycemia produced chronic ischemia can raise the concentration of IMA in blood.

The current study correlated IMA with HbA1c, fasting blood glucose and postprandial blood glucose and observed weak but significantly positive correlation between IMA and these glycemic parameters. This finding shows that hyperglycemia plays role in increasing oxidative stress thus modifying albumin to increase the serum IMA levels in diabetes. Similar results were observed by Piwowar et al. which showed significantly higher plasma IMA among diabetic patients with poor glycemic control when compared with those who had good glycemic control.<sup>8</sup> The findings of this study were also in accordance to a study by Kaefer M et al. which found weak but significant

correlation between IMA and fasting glucose in type 2 diabetic patients.<sup>7</sup>

HbA1c has been closely associated with diabetic complications; more the HbA1c level, higher is the risk of complications. With increasing prevalence of diabetes mellitus, risk of complications associated with it is also increasing. Chronic hyperglycemia induced oxidative stress in diabetic patients causes glucose auto-oxidation, glucose flux through polyol pathway and gradual formation of advanced glycated end products. These changes may oxidize proteins, lipids and DNA and thus damages the tissues ultimately causing sub endothelial inflammation and chronic ischemia. Long-term complications of diabetes include macro vascular complications such as myocardial infarction, stroke, periphery artery disease and micro vascular complications such as retinopathy, neuropathy and nephropathy.<sup>3,4</sup> Shao Gang et al. studied the relation of peripheral artery in relation with HbA1c and IMA in diabetic patients and found that HbA1c was closely associated with peripheral artery disease. The increase in IMA was related with the severity of PAD. This concludes that IMA can act as a biomarker of PAD.<sup>19</sup> Reddy et al. in their study found higher levels of IMA in diabetic patients with diabetic retinopathy compared to non-diabetic participants suggesting the role of oxidative stress in the development of diabetic retinopathy.<sup>20</sup>

In the current study, the mean age of diabetic participants was slightly higher among group 1 participants as compared to group 2, whereas, BMI and duration of diabetes were slightly higher among group 2 participants than group 1 but there was no significant correlation between these profiles and IMA. There was no any significant difference in mean IMA between male and female in diabetes. Shao-gang MA et al. and Piwowar et al. in their different studies also did not find any statistically significant relationship of IMA with age, gender and duration of the disease. On the contrary, Arslan et al. observed positive correlation of serum IMA with obesity and metabolic syndrome.<sup>21</sup> Chawla et al. also reported no significant correlation between the duration of diabetes mellitus and serum IMA

that was similar to this study.<sup>9</sup> However Swojanya UVPU et al. found significant positive correlation between IMA and duration of the disease.<sup>22</sup>

The present study has some limitations. This study recruited diabetic patients by non probability convenience sampling from a single tertiary care center so the findings clearly lacks generalization to all diabetic populations. The current study did not measure the presence of diabetic complications so we could not correlate IMA with the presence of diabetic complications. This study strongly recommends the necessity to conduct further study from different tertiary center including nondiabetic, diabetic without and with complications groups from different tertiary centers to assess if IMA correlates with the diabetic complications.

## CONCLUSION

Conditions like diabetes increase oxidative stress level, which is a known risk factor for the development of different diabetic complications. The present study suggests that IMA can be used as an economic marker of hyperglycemia associated rise in oxidative stress in diabetes which may further help to evaluate and monitor the development of complications as an outcome of hyperglycemia. This study further recommends a large-scale multicenter study including non-diabetic and diabetic patients, with and without complications to observe whether IMA correlates with the diabetic complications or not.

## ACKNOWLEDGEMENT

We would like to acknowledge all the staffs of Clinical Biochemistry Laboratory of KMCTH for their assistance with data collection and sample processing for this study.

**CONFLICT OF INTEREST:** None

**FINANCIAL DISCLOSURE:** None

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