ORIGINAL ARTICLE ASIAN JOURNAL OF MEDICAL SCIENCES

The study of association of serum ischemia-modified albumin and prediabetes in women: A case–control study

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Submission: 25-06-2022 Revision: 23-08-2022 Publication: 01-10-2022

ABSTRACT

Background: Prediabetes, diabetes, and their associated complications adversely affect women's health worldwide. Women in the reproductive age group are more prone for developing diabetes and its associated complications such as gestational diabetes and infertility. Hence, it is the need of the hour to diagnose women at risk of pre-diabetes. Oxidative stress, ischemia, and hypoxia have been suggested as important factors in developing prediabetes and its sequels. **Aims and Objectives:** The present study aimed to study the association of serum ischemia-modified albumin (IMA), namely, a marker of oxidative stress, ischemia, and hypoxia with prediabetes in the women of reproductive age group. **Materials and Methods:** The present study was carried out in 50 females in the reproductive age group of 19–45 years. The study population was further sub-divided into two groups: Group I consisted of 25 prediabetic patients and Group II consisted of 25 healthy controls. Serum samples of the subjects were analyzed for serum IMA, blood sugar profile, and other routine biochemistry tests. **Results**: The present study showed that mean serum IMA was raised in prediabetic group as compared to healthy controls. The serum IMA also positively correlated with blood sugar profile. **Conclusion:** Increased serum IMA could be used as a biomarker for predicting prediabetes in females of reproductive age group. This, in turn, would be helpful in preventing the burden of various complications known to occur in the natural course of prediabetes and diabetes.

Key words: Hypoxia; Ischemia-modified albumin; Ischemia; Oxidative stress; Prediabetes

Access this article online

Website: http://nepjol.info/index.php/AJMS

DOI: 10.3126/ajms.v13i10.46117

E-ISSN: 2091-0576

P-ISSN: 2467-9100

INTRODUCTION

Prediabetes is defined as an intermediate state between euglycemia and hyperglycemia. It is an asymptomatic condition which increases the risk of type 2 diabetes mellitus and its associated complications. The prevalence of prediabetes ranges from 3% to 36% worldwide depending on the criteria used for diagnosing prediabetes.[1] India has been considered as the "World Capital of Diabetes" and the prevalence of diabetes in India is around 14%.[2] Prediabetes is a reversible condition and it can be well managed by lifestyle modification and medications if needed. Its early detection can help prevent the development of diabetes and its associated complications.[3] Being an asymptomatic condition, prediabetes may remain undetected for a long time, but later on may present as frank diabetes with or without complications.[4] Hence, there is a need for an early biomarker to diagnose prediabetes so that the endemic and pandemic of diabetes can be curbed to some extent.[5] At present, women in India are facing numerous health issues due to negligence of health which ultimately adversely affects their physical and mental wellbeing.[6] Diabetes and its associated complications are one of them. Women in reproductive age group are more prone for developing diabetes and its associated complications such as Gestational Diabetes, Polycystic ovary syndrome, infertility, sleep apnea, and heart diseases.[7]

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Ischemia, hypoxia, and oxidative stress have been considered as important factors in the development of prediabetes and diabetes.[8] Ischemia-modified albumin (IMA) being a novel marker of ischemia, hypoxia, and oxidative stress,[9] which can, therefore, also prove to be an early biomarker for prediabetes.

Aims and objectives

The present study aimed to evaluate the association of serum IMA and prediabetes in the women of reproductive age group.

MATERIALS AND METHODS

Type of study

The present study was a hospital-based observational type cross-sectional study.

Place of study

The present study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine, AIIMS Nagpur from April 2020 to March 2021. The study was conducted after the approval of the Institutional Ethical Committee (letter no. IEC/Pharmac/2020/159 dated – 17/09/2020) and in accordance with the Declaration of Helsinki.

Sampling and sample size

Randomized selection of participants was done by lottery method until the desired sample size was achieved.

The sample size was calculated using "openEpi" software. The mean of IMA was predicted as 1 U/ml and SD=0.26 U/ml in the prediabetes arm and the mean of IMA as 0.74 U/ml and SD=0.31 U/ml in the healthy subject arm.[10] The power of study was taken as 80%, confidence interval=95%, and chance of error=5%. The total sample size after calculation was 25 in each arm. Hence, the study population consisted of 50 female subjects in the reproductive age group (19–45 years). It was further subdivided into two groups:

Prediabetics group

Twenty-five diagnosed prediabetes females. The study subjects were recruited from among those attending the outpatient department of Medicine in AIIMS, Nagpur during the study period.

Normoglycemic healthy individuals group

Twenty-five females had fasting blood sugar less than 110 mg/dl and no signs or symptoms of an ongoing acute or chronic inflammatory condition or ischemic heart disease. They were classified as normoglycemic healthy individuals. They were recruited from among the healthy volunteers who came for routine medical checkup, medical fitness, etc. in the same outpatient department.

Inclusion criteria of prediabetics (cases)

Prediabetes individuals diagnosed as per World Health Organization (WHO) criteria,[11] that is, with a fasting blood sugar of 110–125 mg/dl and/or an impaired glucose tolerance test of 140–200 mg/dl were recruited for the study.

Exclusion criteria of pre-diabetics (cases)

Women with any history of acute or chronic illness, ischemic heart disease or diabetes mellitus, tuberculosis, any autoimmune disorder, connective tissue disorders, lung, renal, or hepatic disease or with a history of drug intake which may affect blood glucose levels such as steroids and antihypertensive drugs were excluded from the study.

Method of data collection

After obtaining a bilingual written informed consent, demographic data of the study subjects were collected. Detailed history was taken and thorough clinical examination was carried out.

Sample collection and analysis

Blood samples of the study population were collected in the Central Clinical Biochemistry Laboratory of AIIMS Nagpur. Serum was separated from whole blood and processed for routine bio-chemical investigations such as fasting blood glucose (FBG), postprandial blood glucose (PPBG), HbA1c, liver function test (LFT), kidney function test (KFT), and lipid profile. The residual samples were stored at −20°C for the analysis of IMA. Serum IMA in both the groups was measured on a spectrophotometer (M/s Motras Scientific Instruments Pvt Ltd., Modal – UV Plus, New Delhi, India). Comparison of serum IMA levels was done in prediabetes versus healthy normoglycemic individuals.

Principle of estimation of serum IMA (colorimetric assay detecting free unbound cobalt left behind)

The amino terminal of human serum albumin is modified in ischemic events. This reduces the affinity of albumin molecule to bind to transition metals like cobalt which results in an increase of unbound cobalt. The free unbound cobalt, then, reacts with dithiothreitol (DTT) to form a colored complex. The intensity of colored complex is directly proportional to the concentration of unbound cobalt which, in turn, is proportional to the level of serum IMA that can be measured spectrophotometrically.

Reagent preparation, storage, and stability

Reagents

Three reagents were used to measure IMA in serum – (1) 0.1% cobalt chloride, (2) DTT (1.5 mg/ml of H_2O), and (3) 0.9% NaCl. These reagents were stored at 2–8°C. The assay

method involved adding 200 µl of serum to 50 µl of 0.1% cobalt chloride. After mixing the solution gently, a waiting time of 10 min was given for adequate cobalt albumin binding. 50 µl of colorizing agent DTT (1.5 mg/ml H_2O) was, then, added. The reaction was quenched 2 min later by adding 1 mL of 0.9% NaCl. The color development was compared to a serum blank without DTT using a spectrophotometer at 470 nm and estimated in absorbance units (ABSU).[12]

Statistical analysis

The data were analyzed by appropriate statistical methods using the Statistical Package for the Social Sciences (SPSS) – 21st version, Chicago, Illinois, United States and GraphPad Prism, San Diego, CA, United States. Data were expressed as mean±SD. Students t-test was used to compare the serum IMA levels in prediabetes versus healthy individuals. Correlation analysis was done for analyzing the relationship between serum IMA and the biochemical parameters of blood LFT, KFT, sugar profile, and lipid profile. Receiver operating characteristic (ROC) curve analysis was used to establish the cut off of serum IMA to discriminate between prediabetes and healthy individuals. P<0.05 was considered as significant.

RESULTS

The mean age of study population was 22.8 years in cases and 22 years in control group, which shows that the mean age of distribution was nearly equal in both the study groups. The present study was thus age matched with control group. The demographic and biochemical characteristics of study population are shown in Table 1.

As shown in Table 1 and Figure 1, the mean serum IMA was significantly higher in cases as compared to controls. Table 2 and Figure 2 show correlation analysis of serum IMA with the biochemical parameters. Serum IMA positively correlated with FBG, PPBG, HbA1c, serum ALP, uric acid, triglycerides, LDL, VLDL, and negatively correlated with serum HDL with statistical significance (P<0.05).

Area under ROC curve for serum IMA was 0.978, P<0.001, 95% confidence interval=0.933 to 1.0, as shown in Figure 3. ROC curve of serum IMA predicts that at a cutoff 0.61 ABSU, the IMA has 96% sensitivity and 100% specificity for discriminating prediabetes individuals from healthy controls.

DISCUSSION

The present study conducted in the females of reproductive age group demonstrated that the mean serum IMA was raised in prediabetic cases as compared to healthy controls. The mean serum IMA in cases was 0.68 ABSU and in control group was 0.53 ABSU. There was a significant rise in serum IMA levels in prediabetes as compared to the control group (P<0.001). The serum IMA also positively correlated with fasting plasma glucose, postprandial plasma glucose, and HbA1c. The present study is the only study, in which females of reproductive age group were assessed to determine serum IMA levels in prediabetics as per our best knowledge and available resources.

The WHO has defined "Prediabetes" as an intermediate hyperglycemic state. The cutoff of blood sugar for diagnosing prediabetes is Fasting Blood Sugar of 110-125 mg/dl and/or an impaired glucose tolerance test of 140–200 mg/dl as per the WHO criteria. The American Diabetes Association has included HbA1c between 5.7% and 6.4% to diagnose prediabetes.[11] Prediabetes is a condition of impaired fasting glucose and/or impaired glucose tolerance. Prediabetes individuals are at risk of developing diabetes mellitus, cardiovascular disease, atherosclerosis, dyslipidemia, etc.

Serum IMA has been considered as a novel biomarker of hypoxia, ischemia, and oxidative stress. Oxidative free radicals generated during oxidative stress alter the

Table 1: Demographic and routine biochemical parameters of study population

Parameters	Cases (n=25) (mean±SD)	Controls (n=25) (mean±SD)	P value
Age (in years)	22.8±2	22±1.8	0.783
Height (in meters)	1.63±0.374	1.62±0.332	0.691
Weight (in Kg/m^2)	64±12	67±12	0.343
BMI (m^2/Kg)	27±6	26±5	0.960
SBP (mm of Hg)	155±9	122±9	<0.001*
Urea (mg/dl)	30±7.3	25.8±5	0.207
Creatinine (mg/dl)	0.9±0.3	0.8±0.4	0.128
Uric acid (mg/dl)	5.9±2.2	4.4±1	0.001*
Bilirubin (mg/dl)	0.84±0.4	0.72±0.5	0.367
ALT (IU/L)	57±21	54±17	0.542
AST (IU/L)	45±10	39±18	0.193
ALP (IU/L)	91±26	75±32	0.067
Total protein (g/dl)	7.8±0.86	7.6±0.9	0.527
Albumin (g/dl)	4±0.5	4.4±0.5	0.060
Total cholesterol (mg/dl)	189±31	186±26	0.726
Triglyceride (mg/dl)	155±19	128±39	0.004*
HDL (mg/dl)	38±8	45±11	0.011*
LDL (mg/dl)	156±35	105±20	0.001*
VLDL (mg/dl)	37±15	24±7	0.001*
FBG (mg/dl)	119±8	94±6	<0.001*
PPBG (mg/dl)	161±12	125±11	<0.001*
HbA1c (%)	6.1±0.2	5.1±0.4	<0.001*
Serum IMA (ABSU)	0.68±0.04	0.53±0.04	<0.001*

*P≤0.05 is considered statistically significant. BMI: Body mass index, SBP: Systolic blood pressure, ALT: Alanine transaminase, AST: Aspartate transaminase, LDL: Low density lipoprotein, HDL: High density lipoprotein, VLDL: Very low density lipoprotein, FBG: Fasting blood glucose, PPBG: Postprandial blood glucose, IMA: Ischemia-modified albumin

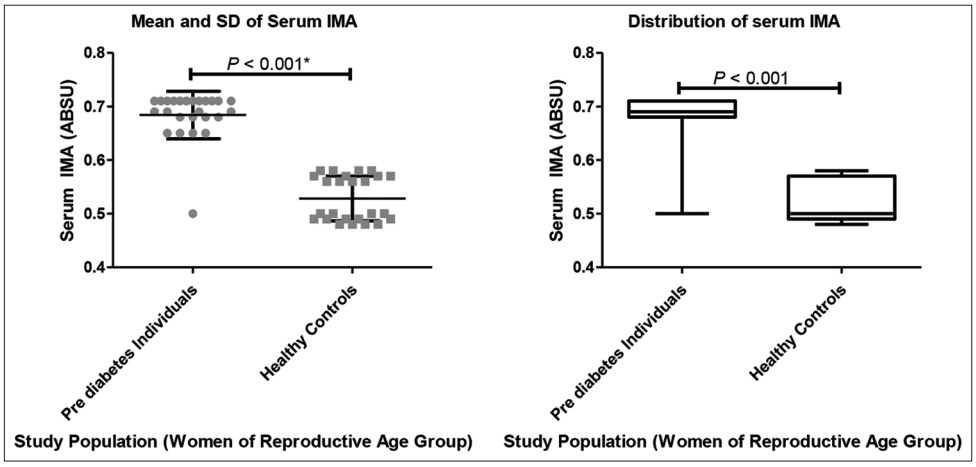

Figure 1: Distribution of serum ischemia-modified albumin in the study population

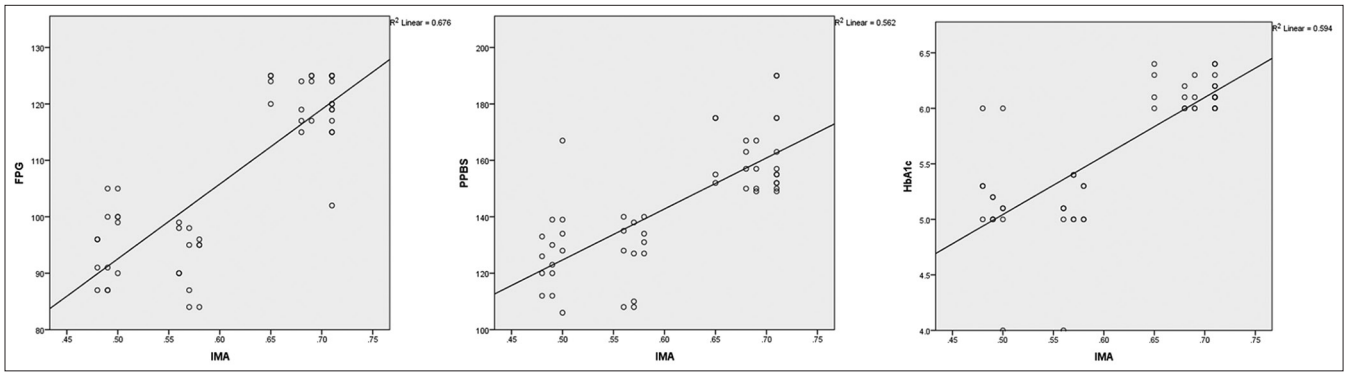

Figure 2: Correlation analysis of serum ischemia-modified albumin (IMA) with blood glucose profile of study population. Serum IMA is positively correlated with fasting blood glucose, postprandial blood glucose, and HbA1c

Table 2: Correlation analysis of serum IMA with study variables

Parameters	Serum IMA	
	r value	P value
Urea	0.343*	0.015
Creatinine	0.018	0.900
Total bilirubin	0.161	0.263
Direct bilirubin	0.84	0.561
ALT	0.089	0.539
AST	0.189	0.188
ALP	0.361*	0.010
Total protein	−0.060	0.677
Albumin	−0.204	0.155
Uric acid	0.409*	0.003
FBG	0.822*	<0.001
PPBG	0.750*	<0.001
HbA1c	0.771*	<0.001
Total cholesterol	0.065	0.654
Triglyceride	0.318*	0.025
HDL	−0.376*	0.007
LDL	0.580*	<0.001
VLDL	0.392*	0.005

*P≤0.05 is considered statistically significant. r: Pearson's correlation coefficient. FBG: Fasting blood glucose, PPBG: Postprandial blood glucose, ALT: Alanine transaminase, AST: Aspartate transaminase, ALP: Alkaline phosphatase, HDL: High density lipoprotein, LDL: High density lipoprotein, VLDL: Very low density lipoprotein, IMA: Ischemia-modified albumin

N – terminus of albumin which, now, becomes "IMA." Serum IMA has been studied in myocardial infarction, acute coronary syndrome, sepsis, pulmonary embolism, deep vein thrombosis, cerebrovascular disease, carbon mono oxide poisoning, end stage renal disease, chronic hepatitis C, diabetes mellitus, hypertension, and much more.[13] Serum IMA has also been considered as novel biomarker of endothelial damage in diabetic patients.[14] The present research group has also studied serum IMA as an early biomarker in epilepsy.[15]

Several mechanisms other than hypoxia, ischemia, and oxidative stress can increase serum IMA such as acidosis, exposure to free iron, and copper.[16] These factors are proposed to play an important role in the modification of albumin to produce serum IMA. Thus, these factors could be involved in the whole spectrum of the conditions ranging from prediabetes, diabetes to its complications.

The findings of the present study are consistent with similar other studies. However, these studies were conducted in both male and female populations. A study carried out by Piwowar Agnieszka et al., showed that mean serum IMA

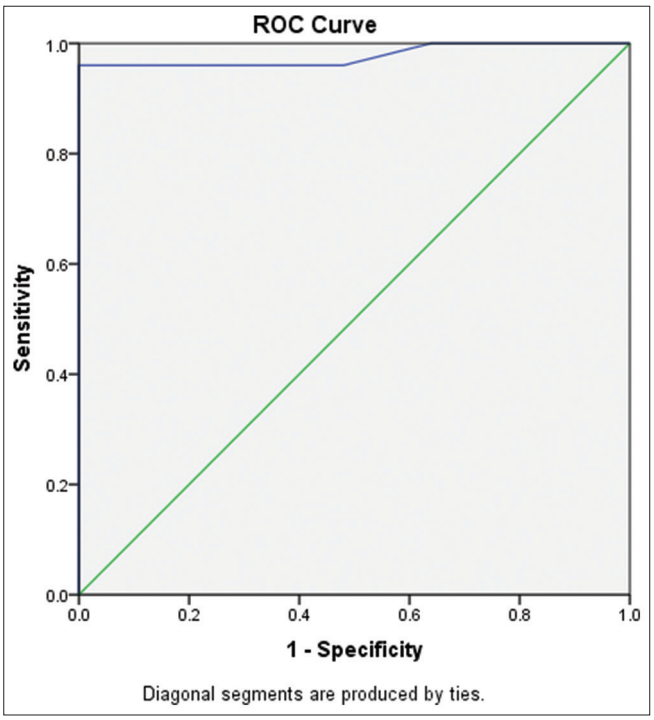

Figure 3: Receiver operating characteristic curve analysis of serum ischemia-modified albumin (IMA) in the study population. This curve shows that serum IMA covers 0.97 area of total area and has good discriminatory ability for prediabetes from healthy control

was significantly higher in diabetes as compared to healthy control.[17] Another study conducted by El-Eshmawy et al., found out that mean serum IMA was significantly higher in prediabetes as compared to healthy controls.[18] This group had recruited 100 prediabetes and 50 control of both sexes. However, a study conducted by Pande et al., found out that the mean serum IMA was lower in prediabetic individuals as compared to healthy control.[10] The finding of this study is contradictory to the results of the present study and other supportive studies.[17,18]

The present study could provide a new insight in understanding the risk factors involved in the development of prediabetes, diabetes, and its associated complications. This, in turn, will aid in the overall management of apparently healthy females at risk of developing diabetes by early diagnosis and decrease in the occurrence of various complications associated with it.

The strength of the present study is a well-characterized study population and standardized techniques to measure various study parameters. The present study has few limitations such as a small study sample size selected from the local population. Furthermore, the study being a hospital-based case–control study cannot be used to predict cause and effect relationship. Serum IMA being a non-specific marker for other ischemic conditions as well, therefore could not be used alone to study in prediabetes individuals. Large prospective, interventional studies need to be undertaken to confirm the cause and effect relationship and to assess the protective role of antioxidants in humans in preventing the development and progression of prediabetes and its sequels.

Limitations of the study

Small sample size, single centric and cross-sectional study are the few limitations of the present study. Follow up and multi centric studies, with large sample size are needed to establish the clinical utility of IMA in the diagnosis of prediabetes.

CONCLUSION

The present study shows that serum IMA could be used as an early biomarker to diagnose prediabetes in females of reproductive age group. With this, the burden of various complications of prediabetes can be prevented in the natural course of diabetes.

ACKNOWLEDGEMENT

Authors are thankful to ICMR STS Program 2020.

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Authors Contribution:
AA- Concept, statistical analysis and preparation of the manuscript; **RJ-** Concept, statistical analysis and interpretation of results, preparation and revision of the manuscript; **UC-** Reviewed the literature and manuscript preparation; **AJ-** Study design, statistical interpretation, finalization of the draft manuscript; **RT-** Concept and design of the study, prepared first draft of manuscript; **AS-** Concept and design of the study, prepared first draft of manuscript; **AMP-** Data collection; **BDR-** Preparation and revision of the manuscript.

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Source of Support: Nil, **Conflict of Interest:** None declared.