Original Research Article

A correlative study of serum ischemia modified albumin and total thiol in prediabetes and diabetes mellitus

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A B S T R A C T

Introduction: Ischemia is a worldwide scare and denotes decreased oxygen supply to various tissues of our body. Ischemia modified albumin, a biomarker to diagnose cardiac ischemia has a role in oxidative stress. Diabetes mellitus carbohydrate metabolic disorder is an oxidative stress condition. Total Thiol is an antioxidant, which reduces the reactive oxygen species. There have been lot of research which proved the association of diabetes with ischemia modified albumin. In this study we tried to compare the association of ischemia modified albumin with prediabetes as well as diabetes.

Materials and Methods: After obtaining the ethical approval from institutional ethics committee, 120 serum samples were collected (40 - normal healthy subjects, 40 - prediabetes, 40 - diabetes mellitus) and analyzed for ischemia modified albumin using spectrophotometric Co(II)-albumin binding assay and total Thiol was measured using spectrophotometric method. Glycated hemoglobin, lipid profile values were obtained from laboratory information system. Data were compiled and appropriate statistical tests were used wherever necessary. p value <0.05 was said to be significant.

Result: This study reported decreased levels of ischemia modified albumin and increased levels of total thiol in both prediabetes as well as diabetes mellitus as compared to normal control. Significant positive correlation between ischemia modified albumin and total thiol was obtained.

Conclusion: Oxidative stress maker IMA can be neutralized by an anti-oxidant total thiol. People consume antioxidant to prevent progression of chronic diseases. Extra supplementation of antioxidant may not be much helpful in cases where naturally antioxidants are produced due to the diseases. Thus measuring both helps in prevention of extra supplementation of anti-oxidants.

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1. Introduction

Diabetes mellitus is a carbohydrate metabolic disorder characterized by hyperglycemia due to insulin deficiency or insulin resistance. It is considered as a major threat to human health in the 21st century. Long term uncontrolled diabetes mellitus leads to changes in arterial wall like thickening of capillary basement membrane, deposition and oxidation of membrane lipids mainly LDL cholesterol and cellular proliferation resulting in vascular complications like atherosclerosis.[1,2] The pathogenesis of development of atherosclerosis by diabetes mellitus is complex involving chronic inflammatory condition, oxidative stress, hypoxia and ischemia. A large number of studies have been proposed to say that reactive oxygen species produced during ischemia modify the N-terminal of albumin moiety producing ischaemia modified albumin. Not only ischaemia the studies have also shown its role in non ischaemic conditions like liver cirrhosis and metabolic syndrome.[3,4]

Prediabetes is a state of abnormal glucose homeostasis characterized by the presence of impaired fasting glucose, impaired glucose tolerance, or both and when the glycated hemoglobin value is 5.7-6.5%. Prediabetics have high preponderance to encounter diabetes mellitus in future.[3]

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Ischemia modified albumin (IMA) has emerged as a novel biomarker for cardiac ischemia and oxidative stress. During oxidative stress there will be release of oxygen free radicals, these resulting in conformational changes in the N-terminus of albumin and thus this molecule is called as IMA.[4] There have been large number of studies describing the role of IMA as a marker for diseases related to inflammation and ischemia induces a cascade of proinflammatory reactions that lead to the generation of reactive oxygen species (ROS). The levels of IMA are higher in various inflammatory and oxidative stress-associated diseases.

Thiols are sulfhydryl group (-SH) containing organic compounds. They constitute a major portion of antioxidants in the body and they play a significant role in defense against reactive oxygen species. It occurs both extracellularly and intracellularly in free form and protein bound form and interact with highly reactive oxygen species and reduces the oxidative stress thus maintaining the homeostatis. The levels of total thiol is reduced in disorders associated with oxidative stress. A lbumin makes the major portion of the protein bound thiols. Apart from defense against free radicals, thiols share significant role in detoxification, signal transduction, apoptosis and various other functions at molecular level.[5,6] Oxygen free radicals produced during normal physiological process or pathological conditions are normally neutralized by the anti-oxidant defense mechanism. If defense mechanism is unable to neutralize the oxidants then lead to progression of pathological condition.

Thus this study was done to evaluate the circulating levels of IMA and total Thiol in prediabtes and diabetes mellitus and correlate the above parameters.

2. Materials and Methods

2.1. Ethical statement

Ethic approval was obtained from institutional ethics committee Kasturba Hospital Manipal before collecting the sample.

2.2. Department involved

The study was conducted in department of Biochemistry, Kasturba Medical College, Manipal.

2.3. Biological material used

4 ml of whole blood collected in red capped vacutainer. Serum separated was used for the estimation of ischemia modified albumin and total thiol.

2.4. Inclusion criteria

Apparently normal healthy subjects, prediabetics and diabetics were included.

2.5. Exclusion criteria

Subjects with chronic disorders like liver cirrhosis, kidney diseases were excluded.

Sample size: totally 120 samples were grouped as following.

Group 1: 40 - normal healthy subjects
Group 2: 40 – prediabtes subjects
Group 3: 40 – diabetes mellitus subjects

2.6. Method of estimation of IMA

IMA levels were determined by the colorimetric method developed by Bar-Or et al.[7] The amino terminal end (N-terminus) of the albumin molecule appears to be the primary binding site for transitional metals, such as cobalt, copper and nickel. The methodology involved the addition of 200 μL of patient's serum to 50 μL of 1 g/L cobalt chloride solution, followed by vigorous mixing, and 10-min dark incubation. Fifty μL of dithiothreitol (DTT; 1.5 g/L) was then added and mixed. After 2 min of incubation, 1.0 mL of a 9.0 g/L solution of NaCl was added. The absorbance of assay mixtures was read at 470 nm. The blank was prepared similarly with the exclusion of DTT. The values are expressed in U/ mL.

2.7. Method of estimation of total Thiol

Serum total thiol is measured by Dinitrobenzene (DTNB)-Ellman's method and the absorbance is measured at 412nm using spectrophotometer.

2.8. Method of estimation for Lipid profile

Total cholesterol, triglycerides and HDL-Cholesterol were analyzed by enzymatic, colorimetric method in auto analyzer (Cobas 6000 Roche Diagnostics India Pvt Ltd). LDL-Cholesterol values were obtained by using the Friedewald formula.

2.9. Statistical analysis

Data of above parameters were compiled and appropriate statistical tests were used wherever necessary. p value <0.05 were said to be significant.

3. Results

In the present study we investigated relationship between oxidative stress marker and anti oxidant levels. This study reported slightly decreased levels of IMA in prediabetes (p=0.78) and statistically significant decreased levels of IMA in diabetes mellitus (p=0.026) as compared to normal control (Table 1), a controversy result is obtained when compared to the previous literatures. To further support the contradictory results of IMA, an anti-oxidant total thiol showed statistically significant increase in

prediabetes (p=0.001) and diabetes mellitus (p=<0.001) group compared to normal controls (Table 1). To further prove this assumption correlation was assessed between thiol and IMA which showed positive correlation both in prediabetes (p=0.004) and diabetes mellitus (p=0.003) groups (Figures 1 and 2)
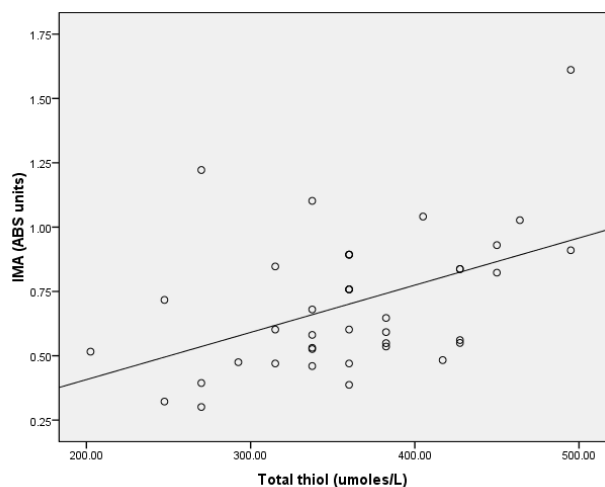

Fig. 1: Correlation of serum IMA with total thiol in prediabetes Pearson ' s correlation r= 0.454, p= 0.004
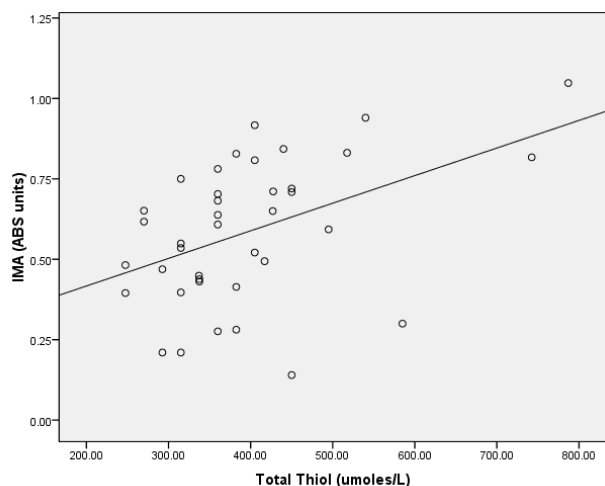

Fig. 2: Correlation of serum IMA with total thiol in diabtes mellitus Pearson's correlation r= 0.464, p= 0.003

4. Discussion

Diabetes mellitus and its micro and macro vascular complications are related to the duration and extent of glycemic state.   Diabetes mellitus as well as its complications have oxidative stress etiology.   There are growing number of studies suggesting that reactive oxygen species produced during oxidative stress condition changes the N-terminal sequence of albumin moiety, thereby producing ischemia modified albumin.[8–10]

The role of IMA in diabetes mellitus and its complications have been proved in many of the previous studies. This study mainly focused on levels of IMA in prediabetics and diabetes mellitus along with the levels of total thiol, an antioxidant. Total thiol constitute the major portion of total antioxidants among all the antioxidants of the body and play significant role in defense mechanism.[7] There are many studies which showed decreased total thiol concentration in many medical conditions including diabetes mellitus. This study is one of the kind where we observed contradictory results.

The contradictory result obtained from this study can be explained by the hypothesis that as prediabetes and diabetes mellitus are oxidative stress conditions in which levels of oxidative stress marker like IMA is usually increased which can be harmful.

To combat the increased reactive oxygen species body produces more amount of anti-oxidants. When the levels of anti-oxidants are elevated they neutralize the oxidants. Overshooting levels antioxidants might be a reason for negative inhibition on the production of oxidants. Therefore in this study we found decreased IMA and increased total thiol in prediabetes and diabetes mellitus.   Extra supplementation of antioxidants reacts not only with free radicals also with other molecules so sometimes converting other molecules to oxidants.   Therefore antioxidants can also act as pro oxidants. They can inactive the action of naturally occurring antioxidants. Thus care must be taken while prescribing the antioxidants supplements.

5. Conclusion

To conclude oxidative stress maker IMA can be neutralized by an anti-oxidant total thiol. Simultaneously monitoring the both helps in prevention of extra supplementation of anti-oxidants in these conditions.   Future studies are required to understand and focus on antioxidant supplementation and its effects on pre diabetics.

6. Limitation

The study was conducted in 3 groups of small sample size, therefore larger study is required to obtain reliable result.

The study subjects did not represent the whole population. The number of males and females were not equally distributed. In addition to the above given reason, there could be differences in sample storage temperature and handling, which may affect IMA values. It is advisable take measures to avoid pre-analytical variability since these influence test results and the entire study to a great extent. We have taken measures to reduce the pre analytical errors by monitoring the temperature.   If there were

Table 1: Mean and standard deviations of biochemical parameters among the study groups

Biochemical parameters	Group 1	Group 2	Group 3
Ischemia Modified Albumin (U/mL)	0.74 ± 0.31	0.70 ± 0.26	0.58 ± 0.21 **
Total Thiol (μmoles/L)	289.1 ± 58.12	360.65 ± 67.37 *	393.24 ± 116.78 **
Glycated hemoglobin (%)	5.4 ± 0.24	6.20 ± 0.26	8.88 ± 1.74
Total cholesterol (mg/dL)	187.7 ± 39.58	184 ± 45.53	186.25 ± 56.58
Triglycerides (mg/dL)	140.89 ± 86.86	175.32 ± 84.36	183 ± 98.48
LDL- Cholesterol (mg/dL)	112.12 ± 43.51	104.93 ± 42.4	104.07 ± 56.26
HDL- Cholesterol (mg/dL)	44.32 ± 12.35	42.19 ± 10.67	42.2 ± 10.63

Student t-test

*- statistically significant difference between group 1 and group 2

Table 2: Showing the p value for biochemical parameters between the groups

Biochemical parameters	Group 1 and Group 2	Group 1 and Group 3
Ischemia Modified Albumin (U/mL)	0.74 NS	0.026
Total Thiol (μmoles/L)	0.001	<0.001
Glycated hemoglobin (%)	<0.001	<0.001
Total cholesterol (mg/dL)	0.39 NS	0.86 NS
Triglycerides (mg/dL)	0.13 NS	0.03
LDL- Cholesterol (mg/dL)	0.47 NS	0.41 NS
HDL- Cholesterol (mg/dL)	0.28 NS	0.41 NS

NS- not significant

any temperature fluctuations then samples were stored in different equipment. Differences in patient's serum albumin levels could also have affected IMA results, adjusting IMA values with albumin values might help to overcome this issue.

7. Source of funding

None.

8. Conflict of interest

None.

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