Original article

Ischemia modified albumin (IMA) and albumin adjusted-IMA (AAIMA) as biomarkers for diabetic retinopathy

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Abstract

Introduction: Oxidative stress has important role in the pathophysiology of diabetic retinopathy (DR). Ischemia modified albumin (IMA) has been recently considered as a marker of oxidative damage in diabetes. However, there is scarcity of published information about both IMA and albumin adjusted-IMA (AAIMA) in DR patients. **Objectives:** To evaluate the serum levels of IMA and AAIMA in patients with DR and in healthy controls. **Material and methods:** This was a cross sectional study. Serum was obtained to measure lipids, albumin and IMA from the the patients with DR and non-diabetic subjects. The IMA level was measured by a colorimetric albumin cobalt binding (ACB) assay and the values were presented as absorbance units (ABSU). The IMA levels were adjusted for albumin interference and the AAIMA by using a formula [Individual serum albumin/median albumin concentration of the population X IMA]. **Results:** This study was done on 18 DR and 20 non-diabetic patients. The mean Serum IMA values in DR group and controls were 0.50±0.17 and 0.32±0.17, respectively (P=0.002). The mean serum AAIMA values in DR group and controls were 0.48±0.20 and 0.32±0.17, respectively (P=0.01). The albumin and HDL-Cholesterol levels were significantly lower in DR patients compared to controls (p=0.004 and p=0.01, respectively). **Conclusions:** The level of IMA and AAIMA were higher in cases of DR compared to that of non-diabetic subjects. The levels of albumin and HDL-Cholesterol were lower in DR patients compared to controls.

Keywords: Albumin, diabetes, diabetic retinopathy, ischemia modified albumin, oxidative stress

Introduction

The world's diabetes epidemic is expected to increase from 382 million in 2013 to 592 million in 2035 (Forouhi et al 2014). Diabetes causes complications like cardiovascular problems, nephropathy, neuropathy, and retinopathy (DR). The factors associated with development and progess of DR are high glucose, dyslipidemia, oxidative stress, chronic inflammatory condition, endothelial dysfunction, hypoxia and ischemia (Turk et al, 2011; Kowluru and Chan, 2007). High glucose levels is crucial to generate free radicals and oxidative stress resulting in damage to lipids and proteins (Kaefer et al, 2010; Manohar et al, 2013).

Human serum albumin (HSA) is the most abundant plasma protein in humans with 585 amino acids (Roche et al, 2008). Oxidation

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of HSA under pathological conditions such as diabetes is associated with significant structural and functional modifications of the molecule of albumin that markedly affect its biological activity (Anraku et al, 2001). Ischemia modified albumin (IMA) is a novel biochemical marker and it increases in diseases associated with ischemia and oxidative stress. The IMA following exposure to free radicals can be measured by alterations of the binding capacity of HSA to exogenous cobalt (Bar-or et al, 2000). This albumin cobalt-binding assay (ACB) for IMA estimation is affected by serum albumin level (Koc et al, 2011).

The relationship between IMA and diabetes has already been reported (Kaefer et al, 2010; Ukinc et al, 2009). However, to the best of our knowledge Turk et al have presented the IMA serum levels increased in patients with DR (Turk et al, 2011). The albumin concentration in DR patients and its relationship to IMA formation is not studied. Therefore we investigated serum levels of IMA and AAIMA in DR patients and healthy controls.

Material and methods

Study population

This study was conducted between November 2013 and April 2014. The study was approved by the Institutional Ethics Committee (No. BPSGMC/RC045IEC/13). After obtaining informed consent, participants were recruited into two groups: type 2 diabetic patients with retinopathy and healthy controls. Participants were recruited from the out-patient department of ophthalmology. Diabetes was defined as patients with fasting blood sugar (FBS) >126 mg/dl. Diabetic retinopathy has been defined according to the previously established criteria (Wu et al, 2013; Wilkinson et al, 2003). Diabetic retinopathy group included 10 patients with mild nonproliferative, 6 patients with moderate nonproliferative and one subject each with severe nonproliferative and hypertensive diabetic retinopathy. Exclusion criteria for the study included malignant disease, infectious disease, liver disease, kidney disease, smokers, alcoholics, obesity and chronic or acute illnesses.

Laboratory analysis

Blood samples were collected from all patients and control participants after an overnight fast by venous puncture technique into Vacutainer (BD Diagnostics, Plymouth, UK) tubes with sodium fluoride plus EDTA or no anticoagulants. Specimens were centrifuged at 2500×g for 15 min. Plasma was used to measure the levels of fasting blood sugar (FBS), while the serum was used to assess the levels of total cholesterol, HDL cholesterol, triglycerides and albumin by using standard methods on Roche/Hitachi Modular P-800 automated analyzer. LDL cholesterol was calculated using Friedwald formula, as described previously (Friedwald et al, 1972). The glycated hemoglobin (HbA1c) level was measured by immunoturbidimetry method using tetradecyltrimethylammonium bromide (TTAB method) using commercial kits from Roche diagnostics, Germany on Roche/Hitachi Modular P-800 analyzer.

Serum IMA was measured by colorimetric assay based on biochemical properties of albumin to bind exogenous cobalt, as previously described by Bar-or et al (2000). This assay quantitatively measures unbound cobalt remaining after cobalt–albumin binding has occurred. Dithiothreitol (DTT) in the assay reacts with unbound cobalt to form a colored product. The results were expressed as absorbance units (ABSU). Because of the dependence of IMA values on albumin concentrations, IMA results were adjusted for albumin interference (Individual serum albumin/median albumin concentration of the population X IMA), as reported by Koc et al (2011).

Statistical analysis

Data were analyzed using Statistical Package for Social Studies (SPSS 11.5) (SPSS Inc,

Chicago, IL, USA). The male-female gender differences were tested using Chi square test. Continuous variables were first studied for normality distribution using the Shapiro-Wilk test, a more appropriate test for small sample sizes. For variables exhibiting normal distribution, between group differences were evaluated by using unpaired t-test. The Mann–Whitney U test was used to evaluate between group differences for variables with non-normal distribution. Results obtained were presented as mean and standard deviation (SD) with 95% confidence intervals of mean. Correlations between the variables were assessed by Spearman correlation analysis. Statistical significance was assumed at $P<0.05$.

The sample size was calculated using G*Power version 3.1. At an α-error of 0.05, to achieve an actual power of 0.82, a sample size of 16 in each group is required to test the difference between two independent means (two groups).

Table 1: Study variables among control and diabetic retinopathy groups

Variables	Control group (n=20; m/f=12/8)				Patient group (n=18; m/f=10/8)				P value
	Mean±SD	SEM	95%CI	25th percentile	Mean±SD	SEM	95%CI	25th percentile	
Age	51.60±5.69	1.27	48.93-54.26	46.75	56.94±10.57	2.49	51.68-62.0	52.25	NSa
BMI	22.80±2.99	0.66	21.40-24.20	20.05	24.16±3.22	0.76	22.55-25.76	22.44	NSb
Disease duration (years)	-	-	-	-	9.61±3.39	0.80	7.92-11.30	7.0	0.00
FBS (mg/dL)	98.50±24.86	5.55	86.86-110.13	87.0	166.61±67.0	15.79	133.29-199.93	117.75	0.00b
HbA1C (%)	6.35±0.99	0.22	5.88-6.81	5.80	10.26±3.21	0.75	8.66-11.86	7.37	0.00b
Cholesterol (mg/dL)	165.80±35.60	7.96	149.13-182.46	141.75	153.50±47.15	11.11	130.05-176.94	125.25	NSb
Triglycerides (mg/dL)	115.10±61.19	13.68	86.46-143.73	80.25	139.72±52.80	12.44	113.46-165.98	108.0	NSb
HDL-cholesterol (mg/dL)	43.30±6.68	1.49	40.16-46.43	38.50	34.66±12.97	3.05	28.12-41.12	27.0	0.013a
LDL-cholesterol (mg/dL)	99.48±35.06	7.84	83.06-115.89	85.10	90.88±37.22	8.77	72.37-109.40	60.50	NSb
Albumin (g/dL)	4.39±0.27	0.61	4.26-4.51	4.12	3.82±0.76	0.18	3.44-4.20	3.37	0.004a
IMA (ABSU)*	0.32±0.17	0.03	0.24-0.40	0.18	0.50±0.17	0.04	0.42-0.59	0.38	0.002a
AAIMA#	0.32±0.17	0.03	0.24-0.40	0.17	0.498±0.20	0.04	0.38-0.58	0.27	0.011a

FBS: fasting blood sugar, HbA1C: glycated hemoglobin, IMA: ischemia modified albumin, AAIMA: albumin adjusted-IMA, *Absorbance units, #(Individual serum albumin/median albumin concentration of the population X IMA). aShowed normal distribution-tested using parametric t-test. bShowed non-normal distribution-tested using non-parametric Mann-Whitney U test. NS: not significant.

Results

The clinical and biochemical characteristics of the cases and comparison group are described in Table 1. We found no significant difference between two groups for age, gender and BMI. Age, serum levels of HDL, albumin, IMA and AAIMA showed normal distribution and results were therefore presented as mean±SD. The DR group had significantly higher levels of fasting glucose, glycated hemoglobin, IMA and AAIMA compared to normal individuals.

The serum IMA in DR patients and healthy group were 0.50±0.17 ABSU and 0.32±0.17 ABSU (P=0.002), respectively.

When corrected for serum albumin levels, the AAIMA values of patients and control groups were 0.48±0.20 ABSU and 0.32±0.17 ABSU (P=0.01), respectively. In contrast, the levels of HDL-cholesterol and albumin were significantly lower in patients than in controls.

The serum values of albumin in patients and control groups were 3.82±0.76 g/dL and 4.39±0.27 g/dL (P=0.004), respectively. The HDL values of patients and control groups were 34.66±12.97 mg/dL and 43.30±6.68 mg/dL (P=0.01), respectively. There were no significant differences in total cholesterol, triglycerides and LDL-cholesterol between groups. There were no significant bivariate correlations between variables in the patient group.

Discussion and conclusion

The results of the present study indicate that IMA levels increase in patients with diabetic retinopathy as compared to controls. Our finding is in agreement with a recent report (Turk et al, 2011). However, this is the first report demonstrating both IMA and AAIMA in patients with diabetic retinopathy. It is important to study IMA result adjusted for albumin levels, because of dependence of IMA values on serum albumin concentrations (Koc et al, 2011). We (Reddy et al, 2014a; Reddy et al, 2014b; Reddy et al, 2014c) and others (Koc et al, 20110) have previously reported the interference from albumin and need for adjusting IMA values for serum albumin concentrations. Although IMA is well documented in different types of diabetes and diabetic nephropathies (Piwowar et al, 2009; Ma et al, 2012a; Ma et al, 2012b), there have been less studies of IMA in diabetic retinopathy.

Our finding of an increase in IMA values in diabetic retinopathy indicates structural/functional/chemical modification of HSA, resulting in an increased IMA formation. Increased IMA formation is regarded as a marker of ischemia and oxidative stress. The normal healthy HSA has high ability to bind with cobalt. In contrast, disease conditions results in decreased ligand binding ability of HSA to cobalt, consequently, leading to increased IMA formation. Previous studies reported IMA as a novel indicator of oxidative stress, endothelial dysfunction and ischemia in diabetic patients (Kaefer et al, 2010; Ukinc et al, 2009). Its association with hyperglycemia and inflammation has been well demonstrated by Kaefer et al (2010).

Diabetic disease is accompanied by over production of free radicals and generation of oxidative stress, which in turn, contribute to the onset, progression and pathological complications of disease (Aronson, 2008). Hyperglycemia in diabetes, ischemia and hypoxia are important factors implicated in the development of associated complications, such as diabetic retinopathy (Kowluru et al, 2007). As reported previously, hyperglycemia promotes an increase of IMA in diabetes, by mechanisms of hypoxia, oxidative stress and inflammation (Piwowar et al, 2008). Importantly, hyperglycemia per se is greatly involved in the over production of free radicals, which can in turn, damage the normal HSA. Therefore, our finding of increased IMA values clearly suggests molecular damage resulting in structural and functional impairment of serum albumin in diabetic retinopathy, provoked oxidative stress. This is well in agreement with a recent report of Turk et al (2011), who proposed IMA as a novel marker for early detection of microvascular hypoxia, preventing potential retinal complications. Although, increase in IMA and hyperglycemia is evident in our study, there were no significant correlations of IMA with other study variables, probably due to small subject sample in the patient group.

We also found significant decrease in serum albumin and HDL-Cholesterol levels in the patient group compared to controls. This finding is suggestive of impaired physiological antioxidant status. The significant antioxidant, anti-inflammatory and antiatherogenic effects of HDL have been reported to be protective against oxidative stress and cardiac ischemia (Tomas et al, 2004). HDL is known to modulate endothelial function and low HDL levels are predictive of cardiovascular events (O'Connell et al, 2001). A recent report in diabetes showed increased oxidative damage and impaired anti-inflammatory and antioxidant activities of HDL (Morgantini et al, 2011). Apart from regulating osmotic pressure, albumin has several important physiological functions. It acts as a transport protein for metals, fatty acids, cholesterol, bile pigments, and drugs. Most importantly, it is a predominant antioxidant contributing to more than 70% of serum antioxidant properties (Manohar et al, 2013; Bourdon et al, 2001). Yukl et al (2003) reported a decrease in albumin synthesis and low levels of serum albumin diabetic patients compared to control group. Furthermore, increased level of oxidatively modified HSA (observed as IMA formation in this study) may impair HSA antioxidant function in disease conditions (Kawakami et al, 2006). Therefore, the decrease in albumin levels in our study clearly indicates insufficient antioxidant properties, resulting in increased free radical damage and high IMA concentrations.

However, measurement of IMA by albumin cobalt binding assay applies the ability of normal albumin binding to cobalt. In case of modified albumin, the capacity of albumin binding to cobalt decreases, leaving more unbound/ free cobalt to react with DTT developing colour that indicates IMA value in absorbance units. Therefore, it is very much possible that alterations in albumin concentrations may affect IMA values. It has also been reported that a 1% change in albumin concentration can produce a 2% opposite change in IMA values (Zapico-Muñiz et al, 2004). Given the previous evidence of decreased serum albumin levels in diabetic disease (Yukl et al, 2003), and the interference of albumin level with the IMA estimation (Koc et al, 2011; Zapico-Muñiz et al, 2004), it is important to provide IMA values corrected for albumin interference. In the present study, IMA results were corrected for albumin interference and found that, even after correcting for albumin changes, the AAIMA values were significantly higher in the patient group. This suggests that, increased IMA formation in our study is not due to decrease in albumin concentrations, as such no correlation between IMA and albumin was found. This further indicates lower albumin level as an additional risk in DR owing to its important physiological antioxidant roles. It is evident from these observations that there was an impaired oxidative/antioxidant status in favor of oxidative stress in DR patients.

Our study limitations include; small sample size and lack of diabetic group without retinopathy as one of the comparison group. However, our present study results clearly demonstrate increased oxidative stress in DR group compared to non-diabetic controls. As biochemical changes suggestive of oxidative stress are known to contribute to the development of DR, measurement of IMA could be recommended as a simple, novel and inexpensive measure of oxidative stress. However, our findings warrant a strong recommendation for further cross sectional studies involving non-diabetic, diabetic with and without retinopathy groups with large sample size.

Conclusion

We have shown an increase of IMA in the patient group. When the IMA results were corrected for interference from albumin changes, the AAIMA exhibited significant differences between patient and control groups.

We also have shown a decrease of albumin and HDL-cholesterol in the patient group. These biochemical changes suggestive of oxidative stress are known to contribute to the development of DR. As IMA measurement is simple and novel method for oxidative stress damage, we suggest that IMA values should be evaluated with caution on evaluation of serum albumin concentrations in patients with diabetic retinopathy. As the processes by which the oxidative stress contributes to diabetic complications are still unclear, further comprehensive studies with large sample size are required to understand the mechanisms of increased IMA and decreased albumin in the development of diabetic retinopathy.

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