Ischemia modified albumin, an early and novel predictor of complications of hypertension in south asian region

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ABSTRACT

Background - Hypertension is a common occurrence in our society. Microalbuminuria is already evaluated in hypertension as a marker of cardiovascular risk and target organ damage. Ischemia modified albumin is a novel marker of oxidative stress. Materials and methods - 84 hypertensive patients (36 without microalbuminuria and 48 with microalbuminuria) were selected for the study. 29 age and sex matched control subjects were also selected. Ischemia modified albumin was assayed in all the study subjects by albumin cobalt binding assay in semiautoanalyser. Urinary albumin creatinine ratio was also measured in all study subjects. Statistical analysis was done in graphpad software & statistical calculator software. Results: ANOVA showed significant increase of ischemia modified albumin (p<0.005) and microalbuminuria (p<0.0001) between three groups. Furthermore student t test showed the significant increase of ischemia modified albumin in hypertensives without microalbuminuria (p<0.05) than controls. Conclusion - Ischemia modified albumin assay is a simple, rapid and cost effective test, can predict the microalbuminuria and can aware the future complications of hypertension in sufficient early stage.

Keywords: Conjunctival autograft, Primary pterygium, Recurrent pterygium

Introduction

Hypertension is a common public health problem currently and affects all the socioeconomic classes. Essential hypertension, often asymptomatic and silent killer, till at diagnosis it can present with ischemic heart disease(IHD), stroke, myocardial infarction(MI), and other vascular disorders[1]. By the year 2025, 29.2% of the population are expected to have hypertension. Approximately 54% of all strokes and 47% of IHD were contributed to high blood pressure [2]. Hypertension was reported as 4th contributor of premature death in developed countries and 7th in developing countries [3].

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In Indian Scenario, it affects 5.99% and 6.99% in males and females in urban population[1]. Ischemia modified albumin (IMA) is a recent biomarker used for the diagnosis of acute coronary syndrome [4]. IMA was first described as a marker of myocardial ischemia, but lately it is being intensively investigated in other disorders, including diabetes, and in other conditions associated with oxidative stress [5,6]. IMA was also studied in hypoxic conditions like preeclampsia [7] or birth asphyxia[8]. Using the advanced techniques it has been observed that N-terminus of albumin is particularly susceptible for degeneration by ischemia cord ima [1] Roy et al has suggested that reactive oxygen specis (ROS) can modify the N terminus of albumin which can result in decreased affinity to cobalt. The resulting molecule, IMA and its reduced binding to cobalt can be utilized by the albumin cobalt binding assay (ACB) [9] IMA has been studied in hypertension. Kumar A et al found that the IMA has increased significantly in hypertensives than normotensives. They also indicated the prognostic value IMA in hypertensives [10]. Non diabetic
Microalbuminuria, was already considered as a variable under evaluation of essential hypertension [11]. Microalbuminuria has also considered as an integrated marker of cardiovascular risk [11,12]. Microalbuminuria has been also studied in essential hypertension and target organ damage [13,14]. Urine albumin levels also predict cardiovascular morbidity and mortality[15]. Urine albumin concentrations measured in 'spot' morning collections are frequently standardised for concurrent creatinine excretion to derive albumin creatinine ratios (ACRs). This procedure corrects for unknown urine volumes but needs differentiation of males from females in whom creatininuria is lower because of reduced muscle mass, a fact not always taken into account [16,17]. In addition, creatinine excretion in the urine depends not only on gender but also on age and race [18, 19]. Systemic BP may exert some modulating influence on albuminuria even in normalalbuminuric hypertensive patients[20]. An interesting and emerging issue is high normal albuminuria (somewhere between 8-15 μg/min or 15-30 mg/24 h) as a condition of increased cardiovascular risk [11]. From the above facts we observed that IMA and microalbuminuria in the form of albumin creatinine ratio have proven their own efficiencies as a prognostic marker of hypertension. A very few studies have conducted to observe the association between gradual increase of oxidative stress and development of complications of hypertension. Considering all these facts, we tried to assess a reliable and early marker, which can guide us to predict the severity and complications of hypertension.

Materials and methods

Study design
Cross sectional hospital based study

Study site
The study was carried out at the Department of Biochemistry, Calcutta National Medical College and Hospital (CNMCH)

Duration of study
The duration of study was six months (01.10.2013-31.03.2014)

Selection of cases and controls
A total of 84 hypertensive patients (36 with normal UACR and 48 of increased ACR (49 males and 35 females), aged 40 to 60 years) were selected. Informed consent was taken from them. Cases were selected from clinically newly diagnosed hypertensive patients attending the medicine outdoor patient department of Calcutta National Medical College. 29 (19 male and 10 female) age and sex matched healthy control subjects without any family history of hypertension were also selected for the study, with consent.

Inclusion criteria: Subjects between the age group of 40-60 years were selected. Samples from cases were collected before institution of any antihypertensive treatment. The criteria for diagnosis of hypertension were systolic pressure of >140 mm of Hg and diastolic pressure of >90 mm of Hg.

Exclusion criteria
Hypertensive patients who were already on antihypertensive treatment were excluded from the study. Study subjects were examined systematically to exclude any disease (Secondary hypertension) or factors known to cause or associated with hypertension. Subjects on drugs like steroids, oral contraceptive pills, and thyroxin were excluded from the study. Patients with diabetes mellitus, renal insufficiency, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

Ethical Clearance
Before commencement of the work, Ethical Clearance was obtained from the Institutional Ethics Committee, according to the Helsinki Declaration. Written informed consent was taken from cases and control subjects.

Methods for analysis of test parameters Blood pressure measurement
Blood Pressure of the study subjects were measured in sitting posture. The instrument used was mercury sphygmomanometer (cuff size 12.5x40 cms). Systolic and diastolic blood pressures were taken according to the criteria. Two readings were recorded from them with a difference of 5 minutes as per World Health Organization guidelines[21]. If high blood pressure (≥140/90 mmHg) was noted, a third reading was taken after 30 min. The lowest of the three readings was taken as blood pressure. Thus the patients were diagnosed as hypertensive.

Sample collection: Blood
5 ml of venous blood is collected and serum was separated. Estimation of IMA was done immediately.

Urine: First morning samples of urine from the same patients were taken.

ACB Assay for IMA
Principle
ACB assay for determination of the level of IMA in serum is done by addition of a known amount of cobalt
(II) to a serum specimen and measurement of the unbound cobalt (II) from the absorbance of the colored complex between dithiothreitol (DTT) and free cobalt by spectrophotometer which is indicative of the level of IMA [22]. Intensity of the colored complex varies inversely with the ACB.

**Assay protocol**

A volume of 200 µL of serum was mixed with 50 µL of 1 g/l cobalt chloride (CoCl2) solution. Vigorous mixing was done followed by incubation for 10 min. Then 50 µL of 1.5 g/l solution of DTT was added and mixed following which an incubation for 2 min. Finally, 1 ml of 9 g/l of NaCl was added, and absorbance was read at 470 nm in a spectrophotometer [23]. The blank was prepared similarly with the exclusion of DTT. Standard curve was prepared using different concentrations of CoCl2 and values of IMA were expressed in units/ml.

Urine concentrations of creatinine were determined using the method described by Jaffe [24]. The concentration of albumin in urine was determined according colorimetric method described by Shosinsky [25]. The results vary linearly with range of albumin concentration up to 6 g/l. The intra-assay and inter-assay CV was 3.2% and 6.5%, respectively. On this basis, we calculated the urinary albumin/creatinine ratio and divided individuals into groups with normal albuminuria (UACR <30 mg/g), and microalbuminuria (UACR 30-300 mg/g).

**Statistical analysis**

Done by using graphpad software & statistical calculator software

**RESULTS**

ANOVA showed there was a significant difference in mean serum IMA levels between 3 groups (Table 1). As revealed by student t test, difference among group 1 (Controls) and group 2 (Hypertensives with normal ACR) was significant, but among group 2 and group 3 (Hypertensives with increased ACR) was not significant (Table 2).

**Table 1: ANNOVA shows IMA & UACR between three groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Normotensive (n=29)</th>
<th>Hypertensive with normal UACR (n=36)</th>
<th>Hypertensive with increased UACR (n=48)</th>
<th>F Value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA(Units/ml)</td>
<td>95.93±30.43</td>
<td>115.25±18.15</td>
<td>125.27±29.48</td>
<td>5.571</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>UACR(mg/g)</td>
<td>22.19± 1.99</td>
<td>20.50±7.25</td>
<td>218.36± 50.23</td>
<td>489.494</td>
<td>&lt;0.000</td>
</tr>
</tbody>
</table>

**Table 2 : Student’s t-test shows significant difference of IMA between 2 groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA level of control &amp; hypertensive groups with normal UACR</td>
<td>0.0023</td>
</tr>
<tr>
<td>IMA level of hypertensive groups with normal UACR &amp; increased UACR</td>
<td>0.0758</td>
</tr>
</tbody>
</table>

**Discussion**

Hypertension is widely established as a potential risk factor for atherosclerosis and cardiovascular disease which is the leading cause of morbidity and mortality in all developed and developing countries in the world including India[26]. Increased oxidative stress is one of the principal mechanisms by which it may exert its pathological influence[27].According to Piwowar A, IMA may be a good clinical marker with higher specificity to reveal individuals with upper normal albuminuria. Diagnostic usefulness of IMA in estimating kidney dysfunction is the ability to distinguish individuals without any kidney disturbance ( normoalbuminuria) from those with kidney disturbance (micro or upper normal albuminuria)[28]. The levels of IMA was found to increase progressively with the degree of albuminuria in patients with diabetes. A significant positive correlation coefficient was also observed between UACR and plasma IMA levels in them.[29].Ischemic hypoxia modifies albumin at N-terminus residues reducing its affinity to Cobalt [30,31]. Chief factor involved in modifying the metal binding domains of albumin molecule is the generation of reactive oxygen species due to ischemia reperfusion injury. Plasma IMA levels are reported to correlate with parameters of oxidative stress like advanced oxidation protein products and thiol groups [32]. Microalbuminuria is a predictor of cardiovascular events[33]. Hillege et al[34] stated that urinary albumin is a risk marker with, while Klausen et al [35] showed that...
only slightly raised level of albumin well in the normoalbuminuric range is related to increased cardiovascular risk. A lot of studies reviewed the association of microalbuminuria and cardiovascular risk.[36,37]. Above studies mentioned that microalbuminuria was clustered with other cardiovascular risk factors like age, diabetes, hypertension, left ventricular hypertrophy, obesity, metabolic syndrome etc.

Careful screening of those factors and posthoc selection of healthy individuals in large population still revealed the marked and overwhelming independent predictive power of microalbuminuria. It was further supported by recent Framingham publication that microalbuminuria remains a strong predictor of cardiovascular event even in normotensive, non diabetic and normal renal function individual. Microalbuminuria or proteinuria occurs as a consequence of renal damage. Physiologically the glomerular filter forms a barrier to prevent albumin to pass into kidney tubules. Furthermore, proximal tubule is equipped with an effective albumin reabsorption system that metabolizes albumin to fragments and amino acids. Damage to glomerular barrier or proximal tubule can lead to increased excretion of albumin or its fragments. Moreover an increased tubular burden of albumin reabsorption may damage the proximal tubule leading to interstitial inflammation and loss of functioning kidney tissue [38]. Subsequently urinary albumin is increasing showing transition from normo to microalbuminuria. Urine microalbumin excretion may be associated with susceptibility to subsequent organ damage [39]. Hypertension brings about increased oxidative stress, hypoxia and endothelial dysfunction leading to renal damage[29]. UAE testing improves the accuracy of cardiovascular risk assessment in patients with hypertension [40,41]. We observed that normoalbuminuric hypertensive patients are having increased oxidative stress reflected by increased IMA level (Table-1). But microalbuminuric hypertensive patients have shown further increase of IMA. Our study has suggested that gradual increase of oxidative stress causes further damage of renal filtration barrier and proximal tubular cells. Increased oxidative stress causes transition from normo to microalbuminuric stage in uncontrolled hypertensive patients. Our study indicated that IMA levels in hypertensive patients predicts microalbuminuria, so intensive effort to control oxidative stress in this level can prevent microalbuminuria. This findings are similar to the findings of Kumar A [10]. The correlation between microalbuminuria and mortality was obvious [42]. All-cause mortality was 9.4% among patients without microalbuminuria versus 18.2% among those with microalbuminuria. The risk for all-cause mortality in patients with microalbuminuria was also elevated, especially among those with concomitant hypertension[43]. Microalbuminuria independently predicted future stroke when compared to patients with normoalbuminuria[44]. The presence of microalbuminuria during the first week of hospitalization for acute myocardial infarction is a strong prognostic marker for inhospital mortality, particularly among patients with hypertension, and of long-term recurrent coronary events or mortality [45]. More recent observation was that, high normal albuminuria (somewhere between 15 and 30 mg/d) is associated with hyperfiltration which anticipated a decline in renal function [46]. IMA can be used with higher specificity to reveal individuals with upper normal albuminuria [31]. So prevention before the development of microalbuminuria in hypertension patients can keep the patient more safe. It is known that IMA levels rise within minutes after ischemia and return to baseline within 4 to 6 hr [47]. The study conducted by Sameer S et al documented that IMA is an early marker of ischemia, increases before any detectable change in cardiac troponins, and is elevated even in the absence of myocardial necrosis. This is especially useful to rule out ischemia in the emergency setting, thereby making IMA a potentially useful biomarker [48].

Conclusion
The results of our study have clearly suggested that the IMA level in hypertension indicates the status of oxidative stress. Furthermore it may be helpful in assessing oxidative stress during the development of kidney dysfunction. From the above findings, we can assume that, IMA can be incorporated as a diagnostic test parameter in hypertensives to avoid the future acute coronary complications. IMA test holds promises as it is simple, rapid and cost-effective. Furthermore it appears before microalbuminuria. IMA result may help in moving the patients into a low risk category by initial evaluation based on the clinical presentation.

References
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