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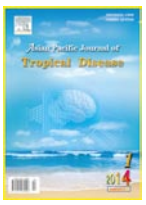

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Prognostic implications of ischemia modified albumin in known cases of 86 elderly hypertensive South Asian aged 56-64 years - a hospital based study

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PEER REVIEW

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Comments

Overall, the paper carries meaningful information and the result of the study would open up further studies based on hypertensive and IMA, and in future some links could come out with coronary complications and hypertension with the linking molecule IMA.
Details on Page S433

ABSTRACT

Objective: To study associated ischemia modified albumin in hypertensive participants and to compare the results with normotensive healthy controls.
Methods: A total of 86 hypertensive patients and 86 age-sex matched normotensive healthy volunteers were selected for this study. The study was conducted for a period of 3 years from September 2007 to August 2010. Biochemical parameters and other parameters such as smoking habits, systolic and diastolic blood pressure and family history were recorded. Lipid profile, ischemia modified albumin, malondialdehyde and conjugated diene were measured by standard methods and results were compared between patients and controls.
Results: Total cholesterol, triglycerides, low density lipoprotein cholesterol were significantly higher (P<0.001) in hypertensive subjects when compared to normotensive control. Also, significant differences were seen in high density lipoprotein cholesterol levels between both groups (P<0.001). The index of lipid per oxidation comprising both malondialdehyde and conjugated dienes were significantly higher in hypertensive compared to normotensive controls. Ischemia modified albumin levels were significantly increased among hypertensive compared to normotensive controls (P<0.001).
Conclusions: Hypertensive patients have increased oxidative stress and are accompanied with rise in ischemia modified albumin. Ischemia modified albumin could be incorporated as a diagnostic test parameter in hypertensive to avoid the future acute coronary complications.

KEYWORDS

Hypertension, Ischemia modified albumin, Malondialdehyde, Conjugated diene, South Asian

1. Introduction

Essential hypertension, or hypertension of unknown cause, accounts for invariably more than 90% of diagnosed cases of hypertension[1]. It is one of the risk factors for cardiovascular diseases[2]. In addition, it is also associated with subclinical vascular impairment as endothelial dysfunction and as an early marker of atherosclerosis[3].

Various proposed mechanisms have been laid over the past years in the causation of hypertension. Among them, it could be vasoconstrictive mechanisms, the sympathetic nervous system, the endothelin system, the vasopressin system and more recently the reactive oxygen species which is suggestive in the development of experimental or human hypertension[4]. Yet another cause is increased vascular oxidative stress which could be involved in the pathogenesis of hypertension, a major risk factor for cardiovascular disease mortality[5]. One of the most important oxidative processes is oxidation of lipids and lipoproteins which are oxidized low-density lipoprotein (Ox-LDL)[6]. Ox-LDL is a major cause of vessel wall injury and atherosclerosis[7]. Ox-LDL has a prominent role in the

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pathogenesis of atherosclerosis, and the elevation of ox-LDL levels in atherosclerotic plaques is an important event in the development of atherosclerosis[8]. It may also be involved in atherogenesis by inducing smooth muscle cell proliferation and smooth muscle foam cell generation[9]. Under oxidative stress not only LDL, but other serum lipids are exposed to oxidation[10]. High density lipoprotein (HDL) is one of the most important independent protective factors for the arteriosclerosis which underlies coronary heart disease[11].

Recently, a new parameter ischemia modified albumin (IMA) has been developed and found to be very useful for the detection of acute myocardial ischemia. IMA was first discovered in 1990, further substantiated by positive findings in 1992, where elevated levels were observed in acute myocardial infarction and unstable angina and finally in 2003, this test was approved by Food and Drug Administration for use in the United States[12]. The circulating albumin molecules, in the presence of ischemia get modified and this modification does not rely on cell death[13]. Albumin has a metal binding site at the N-terminal residues which gets modified due to oxidation by free radicals generated during ischemia by acetylation or depletion of one or more amino acids residues that looses the ability to bind cobalt[14]. Hence cobalt happen to be used as an indicator in the assay. In the test, free cobalt atoms are incubated with patient's serum. When there is increased ischemia, the modified albumin is not able to bind with cobalt, hence the amount of free cobalt is directly proportional to the extent of formation of modified albumin, thus the damage due to free radicals in ischemia process. IMA test holds promises as it is simple, rapid and cost-effective test. It detects the majority of patients (82%) with unstable angina or so called acute coronary syndrome[15]. IMA testing is promising to be a major breakthrough in cardiac testing; a negative IMA result may help in moving the patients into a low risk category by initial evaluation based on the clinical presentation. IMA study thereby provides a major cost saving. Since not much studies based on IMA in relation to hypertension have been conducted in hypertensive patients and since hypertension is associated with cardiovascular complications, so the current study was undertaken to observe the extent of IMA generation with increasing blood pressure and to observe the extent of IMA generation with respect to oxidative stress, so that IMA assay in hypertensive patients could be used as prospective measure to avoid future acute coronary complications.

2. Materials and methods

A total of 86 hypertensive patients and 86 age-sex matched normotensive healthy volunteers were selected for this study. The study was conducted for a period of 3 years from September 2007 to August 2010. The design of this study was pre-approved by the institutional ethical committee board of the Institution and informed consent was obtained from the patients and controls.

Biochemical parameters and other parameters such as smoking habits, systolic and diastolic blood pressure and family history were recorded.

2.1. Diagnostic criteria of patients

All the patients had their blood pressure measured using standard mercury manometer. At least two readings at 5 min intervals as per World Health Organization guidelines were recorded[16]. 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.

2.2. Exclusion criteria

Patients with diabetes mellitus, renal insufficiency, hepatic disease or taking lipid lowering drugs or antioxidant vitamin supplements were excluded.

Venous blood was collected after overnight fast of 12 h and ethylene diamine tetraacetic acid was added and samples were processed for lipid profiles.

Blood collection and biochemical methods used. About 10 mL of blood was collected after overnight fasting in different containers.

2.3. Serum for lipid profile, IMA, malondialdehyde and conjugated dienes measurements

Remaining blood was taken and serum was separated. Serum was used for determination of IMA, malondialdehyde and conjugated dienes.

2.3.1. Lipid profile

Total cholesterol, triglycerides, and HDL-cholesterol were analyzed enzymatically using kit obtained from (Randox Laboratories Limited, Crumlin, UK). Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol using the following formulae:

LDL-c= TC-TG/5-HDL-c (mg/dL)

2.3.2. Ischemia modified albumin (IMA) studies

2.3.2.1. Principle

The assay is based on the premise that myocardial ischemia causes modifications of the human serum albumin (HSA) that are demonstrated by the reduced exogenous cobalt (II) binding. The concentration of IMA can be determined by addition of a known amount of cobalt (II) to a serum sample

and measurement of the unbound cobalt (II) by colorimetric assay using dithiothreitol. An inverse relationship thus exists between the level of albumin bound cobalt and the intensity of the color formed.

2.3.2.2. Procedure

Preparations for the Co (II) albumin binding protocol involved the addition of 200 μL of patient serum to 50 μL of a solution of 1 g/L cobalt chloride, followed by vigorous mixing and 10 min of incubation. Dithiothreitol (50 μL of a 1.5 g/L solution) 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 the assay mixture was read at 470 nm. The blank was prepared similarly with the exclusion of dithiothreitol.

2.3.3. Thiobarbituric acid reactive substances

Malondialdehyde (MDA) levels were estimated by thiobarbituric acid reaction[17]. Using 40% tricholoroacetic acid, proteins were precipitated from 0.5 mL serum, and precipitated proteins were incubated with thiobarbituric acid reagent in a boiling water bath for 1 h. After bringing down to room temperature, the colored complex formed was measured using spectrophotometer at 532 nm. 1, 1, 2, 3-tetraethoxypropane (1 nmol/L) was used as a standard for MDA estimation. Concentrations were expressed in nmol/L.

2.3.4. Conjugated dienes (CD)

CD levels were measured by the method of Recknagel and Glende with little modification[18]. Briefly, the principle of the assay is based on the rearrangement of double bonds in polyunsaturated fatty acids leading to the formation of CD, which absorb light at 233 nm. The oxidation index of the lipid sample at 233 nm and 215 nm is computed which reflect the diene content and the extent of peroxidation. The lipid peroxidation products measured in serum were treated with antioxidant butylated hydroxytoluene twice, immediately after obtaining and before adding the test reagents to suppress artifactual changes during handling and assay procedures. The first stage of lipid peroxidation consists of the molecular rearrangement of the double bonds in polyunsaturated fatty acids residues of lipids, which leads to CD formation and conversion of CD in hydroperoxide. Serum was chosen to avoid possible influences of substances required for plasma preparation. Serum sample (150 μL) and (150 μL) of 0.9% NaCl (reagent blank contains only isotonic saline) were incubated at 37 °C for 25 min. About 0.25% butylated hydroxyl toluene (150 μL) was added and the lipids were extracted by heptane/isopropanol (1:1). Then samples were acidified by 5 mol/L HCl and extracted by cold heptane (1600 μL). After centrifugation for 5 min at 3000 r/min, the absorbance of heptane fraction were measured spectrophotometrically at absorbance maximum between 220 nm and 250 nm. The amount of hydroperoxides produced was calculated using Molar Coefficient of 2.52×10^4 m^{-1}.

All chemicals of analytical grade were obtained from Sigma Chemicals, India.

2.4. Statistical analysis

Data on lipid profile and PON1 activity was entered in Microsoft Excel for windows 2007. The mean±SD was obtained using excel software. The two-sample-*t*-test value was obtained between the patients and the control. The distribution of '*t*'- probability was calculated depending on '*n*' and significance of test was obtained. For $P<0.001$ was considered as highly significant.

3. Results

Total cholesterol, triglycerides, LDL-cholesterol were significantly higher ($P<0.001$) in hypertensive subjects when compared to normotensive control (Table 1). Also, significant differences were seen in HDL-cholesterol levels between both groups ($P<0.001$).

Table 1
Lipid profile, lipid peroxidation and IMA in hypertensive and normotensive controls.

Variables	Normotensive	Hypertensive	*P* value (95% *CI*)
Age	60.55±3.43	62.14±2.35	0.0034 (61.72-62.55)
Total cholesterol†	164.68±13.56	196.32±11.28	<0.001 (194.32-198.31)
HDL-cholesterol†	53.21±7.23	40.56±5.67	<0.001 (39.55-41.56)
Triglycerides†	109.23±10.34	137.53±13.29	<0.001 (135.18-139.87)
LDL-cholesterol†	85.69±10.34	123.87±16.32	<0.0001 (120.98-126.75)
MDA (nmol/L)	5.23±1.23	18.94±3.23	<0.0001 (18.36-19.51)
Conjugated dienes (μmol/L)	30.35±3.21	47.76±6.43	<0.001 (46.62-48.89)
IMA (IU/mL)	79.34±4.31	98.51±10.95	<0.0005 (96.57-100.44)
Systolic blood pressure††	112.23±6.47	138.45±11.53	<0.001 (136.41-140.48)
Diastolic blood pressure††	73.56±7.71	91.23±6.89	<0.001 (90.01-92.44)

All values are mean±SD, $n=86$. Values in the parenthesis indicate the number of subjects. †: mg%; ††: mm/Hg; NT: normotensive; HT: hypertensive.

The index of lipid per oxidation comprising both malondialdehyde and conjugated dienes are shown in Table 1 and Figure 2. Serum malondialdehyde and conjugated dienes were significantly ($P<0.001$) increased in hypertensive compared to normotensive controls.

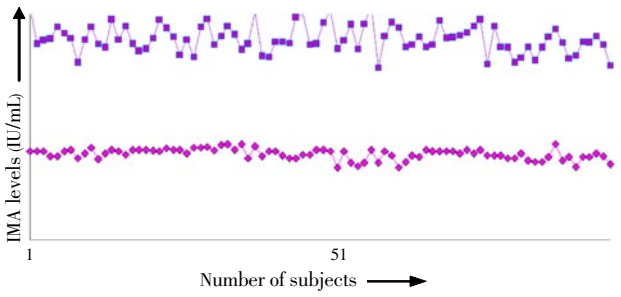

Figure 1. IMA levels in normotensive (■) *vs.* hypertensive (♦) subjects.

IMA levels were significantly increased among hypertensive compared to normotensive controls (Table 1, Figures 1-3) (P<0.001).

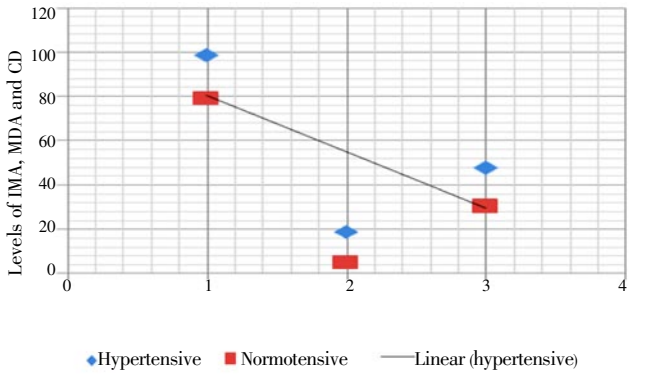

Figure 2. Comparison of IMA, MDA and CD levels in hypertensive and normotensive.

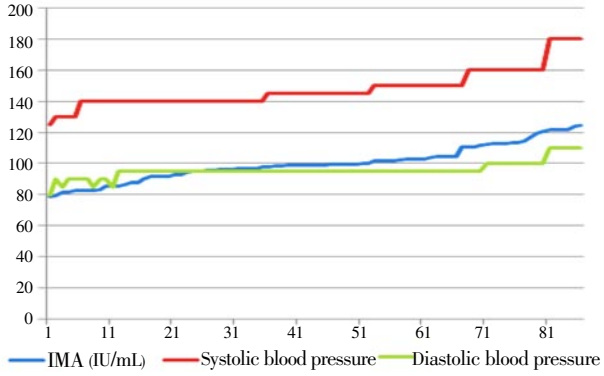

Figure 3. Relationship of IMA with changes in blood pressure in hypertensive patients.

4. 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[19]. Increased oxidative stress is one of the principal mechanisms by which it may exert its pathological influence[20]. In our earlier study, we evaluated the levels of IMA in normolipidemic acute myocardial infarct patients on admission to Intensive Coronary Care Unit. We further correlated it with the extent of lipid peroxides formed in Infarction. In this hospital based study, the relationship between oxidative stress and IMA levels were evaluated in hypertensive participants in mid-adult life as hypertension. Since it is a well established risk factor of acute myocardial infarction and the detection of IMA in early stages in hypertensive patients assumed to be beneficial as a prognostic applications of IMA test for its utility in the early detection and management of hypertensive patients. IMA is the most suitable and potential candidate for this purpose and it has been studied with enthusiasm. HSA is the most abundant multifunctional protein in the blood, consists of 585 amino acids residues and (66.5 kDa), is synthesized in the liver and has a half-life of about 19 d. Cobalt (II) binding to albumin occurs at the N-terminal residue of HSA. The mechanism of alteration of these residues is not yet understood but it appears to be reversible. In the present study, the levels of IMA were detected in hypertensive patients. It is assumed that the oxidative damage of the N-terminal residues is repaired slowly.

Ischemia occurs oxygen demand exceeds the oxygen supply and if this condition is not reversed, diseases such as hypertension and subsequently myocardial infarction precipitates. During this phase, the circulating albumin gets altered causing modification in the N-terminal residues, thus decreasing its affinity to bind to Co (II). Reperfusion of the ischemic cells not only restores the blood supply but also causes massive production of free radicals, resulting in the imbalance of oxidative- anti-oxidative process. The excess production of reactive oxygen species may initiate lipid per-oxidation in cell membrane. These processes may result in a loss of contractile function of the blood vessels and lead to hypertension. Whatever might be the cause of the decreased endogenous antioxidants, the net result is accumulation of H_2O_2, one of the most damaging products of the free radical metabolism.

The elevated levels of IMA can be of prognostic importance and in the current study we observed drastic elevation of IMA in hypertensive patients compared to normotensive healthy controls. Our study is supported by the findings of other studies reported elsewhere[21,22].

Even though the determination of IMA is promising, the results can only be referred as a prelude to what might prove to be significant change in the approach to hypertensive patients and their subsequent treatment plan. An extensive study with a higher set of patients is thus required which should compare the IMA test with other lipid per-oxidation markers such as the malondialdehyde and conjugates diene. The current study consider that IMA test as a valuable biochemical test of evaluating hypertensive patients and its risk for acute myocardial ischemia, and it can be applied in detection of hypertension patients for early prevention of acute myocardial infarction.

The mean total cholesterol level of hypertensive participants [(196.32±11.28) mg/dL] was significantly (P<0.001) higher when compared with controls [(164.68±13.56) mg/dL]. The mean high density lipoprotein-cholesterol level in the hypertensive [(40.56±5.67) mg/dL] was significantly lower (P<0.001) compared with normotensive controls [(53.21±7.23) mg/dL]. Triglyceride values observed in hypertensive participants was [(137.53±13.29) mg/dL] significantly higher than controls [(109.23±10.34) mg/dL]. The mean LDL-cholesterol levels in hypertensive participants was [(123.87±16.32) mg/dL] significantly higher than controls [(85.69±

10.34) mg/dL].

Earlier studies carried out on 40 hypertensive patients also observed a significantly higher levels of total cholesterol, triglyceride and LDL-cholesterol compared to normotensive controls[23].

Yet another study carried out in Spain in hypertensive patients with dyslipidemia treated with lipid lowering therapy also observed a significantly higher levels of triglycerides and lower levels of HDL-cholesterol in hypertensive patients compared to normotensive controls. It was reported from the study that despite of treatment with lipid lowering drugs, the lipid profile in hypertensive patients remained on the higher levels when compared with controls and this could be area of concern as it could be a potent cardiovascular risk[24]. Even the study carried out among hypertensive Nigerians reported significantly elevated levels of total cholesterol, triglyceride and LDL-cholesterol, but the levels of HDL-cholesterol were comparable with normotensive[25].

Also, studies conducted earlier among hypertensive subjects in Malaysia[26], Brazil[27] and Turkey[28,29] reported similar findings which concurs with the current study. So it can be presumed that all hypertensive subjects have higher lipid profiles compared to normotensive.

The mean serum lipoprotein (a) MDA and CD levels in hypertensive participants were higher when compared with controls. Studies conducted earlier also observed similar findings as observed in the current study[30,31].

Hypertensive patients have increased oxidative stress and are accompanied with rise in IMA. IMA could be incorporated as a diagnostic test parameter in hypertensive to avoid the future acute coronary complications.

Though the observation made in the current study can not draw a definitive conclusion, due to inadequacy in sample size which is just 86. To validate the findings of the current study, multicenter study with large sample size should be carried out to support the findings of the current observation from this study.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

IMA is an early indicator of acute coronary syndrome and it is of prognostic importance in ruling out patients with future predictions of acute myocardial infarction. The conformational structure of albumin is altered at N-terminal residues with changes in few amino acids and thus it's affinity to bind to cobalt decreases. So far the study was restricted to cardiovascular diseases patients and currently it has been studied also in diabetes mellitus. The current study was conducted in hypertensive patients with an hypothesis of its increased concentration would be altered in hypertensive patients.

Research frontiers

It is the few among the first studies done in hypertensive patients. So far no studies have been reported regarding the relation of IMA in hypertensive patients. The result of the study would be of applied importance as it would implicate the diagnostic assay of IMA in hypertensive patients.

Related reports

So far, no such reports have been published or reported on the diagnostic efficacy of IMA in hypertensive patients. So the results of the current study would initiate future study based on hypertensive patients and some concrete links could be developed on the mechanism/s of alternation in albumin structure in hypertension apart from what have been advocated in coronary heart disorders.

Innovations & breakthroughs

Due to increased oxidative stress in hypertensive patients, the circulating albumins structures get modified, which is the basic mechanism of changes in IMA in hypertension.

Applications

Hypertensive patients have increased oxidative stress and are accompanied with rise in is IMA. IMA could be incorporated as a diagnostic test parameter in hypertensive to avoid the future acute coronary complications.

Peer review

Overall, the paper carries meaningful information and the result of the study would open up further studies based on hypertensive and IMA, and in future some links could come out with coronary complications and hypertension with the linking molecule IMA.

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