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ORIGINAL ARTICLE

A Study On Lipid Profile, Oxidation Stress And Carbonic Anhydrase Activity In Patients With Essential Hypertension

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ABSTRACT

BACKGROUND OF THE STUDY: The incidence of cardiovascular diseases (CVDs) is rising and they are predicted to be the biggest causes of death by 2020 in India. Various epidemiological studies have highlighted the increasing incidence of hypertension, which is assumed to be one of the major risk factors in CVD. Even though effective treatment measures have been extended against hypertension, it still remains inadequately managed.

Increased levels of serum cholesterol, TG and VLDL have been observed in patients with hypertension. Considering the view points and the relationship between hypertension, lipid profile and carbonic anhydrase modulation in diuretic therapy and scanty literature reports in this region, the current study was focused to determine the association of lipid profile and carbonic anhydrase activity in essential hypertension patients.

AIMS AND OBJECTIVES: The aim of the present study was to determine the serum lipid profile, malondialdehyde levels and carbonic anhydrase activity in known cases of essential hypertensive patients and to compare the results with age-sex matched healthy controls in our community.

MATERIALS AND METHODS: One hundred fifty-six participants (107 males; 49 females) were enrolled for the present study, with their ages ranging from 32 to 66 years. Seventy hypertensive patients (42 men and 28 women, 32- 64 yrs of age) and 86 normotensive healthy controls (65 men and 21 women, 32-66 yrs of age) were recruited for the study. Patients with essential hypertension were included in the study. Smokers, obese subjects and patients on anti-hypertensive drugs for >3 months were excluded from the study. Also, patients on lipid lowering drugs and antioxidant vitamin supplements were also excluded. Lipid profile, malondialdehyde levels and carbonic anhydrase activity were analyzed by standard methods in both the groups of subjects. The data from the patients and controls were compared by the Student's *t*-test. The values are expressed as mean ± standard deviation (SD). Microsoft Excel for Windows 2003 was used for statistical analysis. *P*-values <0.05 were considered to indicate statistical significance.

RESULTS: The lipid profile variables were significantly higher when compared to the healthy controls. The differences were highly significant in total cholesterol and triglycerides, but were not significant in HDL-C, LDL-C and VLDL. The study also observed higher levels of malondialdehyde in hypertensive patients ($p<0.005$) as compared to the levels in the normotensive controls. The carbonic anhydrase activity

in hypertensive patients was also observed to be highly significant ($p<0.001$) as compared to that in the healthy controls.

CONCLUSION: The lipid profile is bound to be altered in essential hypertension cases, along with increased oxidative stress and is associated with an increased activity of carbonic anhydrase.

KEY WORDS: Essential hypertension, Carbonic anhydrase, Malondialdehyde, Lipid profile.

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INTRODUCTION

According to the World Health Report of 2002, cardiovascular diseases (CVDs) will be the largest cause of death and disability by 2020 in India [1]. In 2020, 2.6 million Indians are predicted to die due to coronary heart disease, which constitutes 54.1 % of all CVD deaths [2]. Nearly half of these deaths are likely to occur in young and middle aged individuals (30-69 years). The contributing factors for the growing burden of CVDs are the increasing prevalence of cardiovascular risk factors, especially hypertension, dyslipidaemia, diabetes, overweight or obesity, physical inactivity and tobacco use. It is an area where major health gains can be made through the implementation of primary care interventions and basic public health measures by targeting the diet, lifestyles and the environment.

In this area of thrust, one of the earliest studies carried out in India, figured at a 4% prevalence of hypertension (Criteria: >160/95) amongst the industrial workers of Kanpur [3].

Subsequently, the Indian Council of Medical Research (ICMR) conducted a study in 1994, involving 5537 individuals (3050 urban residents and 2487 rural residents) which demonstrated a 25% and 29% prevalence of hypertension (Criteria: ≥ 140/90 mm of Hg) among males and females respectively in urban Delhi and 13% and 10% hypertension in rural Haryana.

Further, Gupta R [4] from Jaipur, through three serial epidemiological studies (Criteria: ≥ 140/90 mm of Hg) which he carried out during 1994, 2001 and 2003, demonstrated a rising prevalence of hypertension (30%, 36%, and 51% respectively among males and 34%, 38% and 51% among females). In 2002, Hazarika et al [5] reported a 61% prevalence of hypertension among men and women aged >30 years in Assam.

Reports based on the above quoted studies suggest that the prevalence of hypertension have been on the rise among Indians. It is known that obesity is a major contributory factor for the increasing prevalence and the longevity of hypertension. Even though effective treatment measures have been extended against hypertension, it still remains inadequately managed. Essential hypertension is the most prevalent hypertension type, affecting 90–95% of the patients suffering from hypertension. Even though no direct cause has been identified, factors such as sedentary lifestyle, stress, obesity, hypokalaemia, salt sensitivity, alcohol intake, and vitamin D deficiency are

reported to increase the risk of developing essential hypertension. The risk also increases with aging, some inherited genetic mutations, and having a family history of hypertension. Insulin resistance which is a component of syndrome X, or the metabolic syndrome, is also thought to contribute to hypertension. Recent studies have implicated low birth weight as a risk factor for adult essential hypertension.

Currently, gene therapy is being practiced in the control of hypertension. Overexpression of the vasodilator genes, as well as the antisense knockdown of the vasoconstrictor genes have been successfully used in animal models of hypertension.

Hypertension is also associated with elevated reactive oxygen species and the impairment of endogenous antioxidant mechanisms. Increased levels of serum cholesterol, TG and VLDL have been observed in patients with hypertension. It has been shown that oxidized lipoprotein inactivates NO and aggravates hypertension. The association of carbonic anhydrase (CA) with hypertension has been established and the changes in its activities are obvious with altered metabolism, especially in hypertension, diabetes mellitus and hyperlipidaemia.

Thiazide diuretic therapy which is used in hypertension, can dose-dependently elevate serum total cholesterol levels, modestly increase low-density lipoprotein cholesterol (LDL-C) levels and raise triacylglycerol (TG) levels, while minimally changing high-density lipoprotein cholesterol (HDL-C) concentrations. All diuretics, including loop diuretics, cause these lipid changes. The mechanisms of diuretic-induced dyslipidaemia remain uncertain, but have been related to worsened insulin sensitivity and/or the reflex activation of the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system in response to volume depletion.

Considering the view points and the relationship between hypertension, lipid profile, carbonic anhydrase modulation in diuretic therapy and scanty literature reports in this region, the current study was focused to determine the association between lipid profile and carbonic anhydrase activity in essential hypertension patients.

AIM AND OBJECTIVE:

The aim of the present study was to determine the serum lipid profile and carbonic anhydrase activity in known cases of essential hypertensive patients and to compare the results with age-sex matched healthy controls in our community.

MATERIALS AND METHODS:

One hundred and fifty-six participants (107 males; 49 females) were enrolled for the present study, with their ages ranging from 32 to 66 years. Seventy hypertensive patients (42 men and 28 women, 32- 64 yrs of age) and 86 normotensive healthy controls (65 men and 21 women, 32-66 yr of age) were recruited for the study. The study was pre-approved by the Ethical Committee of this institution's Review Board.

Inclusion Criteria: Patients with essential hypertension.

Exclusion Criteria: Subjects who were smokers, obese and on anti-hypertensive drugs for more than three months were excluded from the study. Also, patients on lipid lowering drugs and antioxidant vitamin supplements were also excluded.

Sample Collection

Twelve hours fasting blood samples were collected from healthy volunteers and patients with insulin resistance. The patients who were selected for the study were registered in the Out Patients Department (OPD) of the College of Medicine and JNM Hospital, Kalyani. Ten ml of blood sample was collected from the participants, of which 5ml of blood was collected in a sterile test tube, allowed to clot and then carefully centrifuged at 3000 r.p.m for 10 minutes.

Clear serum were collected and kept at - 4^0C until the tests were performed. The serum samples which were obtained were used for the analysis of the biochemical parameters.

Lipid Profile TC, TG and HDL-cholesterol were analyzed enzymatically by using a kit which was obtained from Randox Laboratories Limited, Crumlin, UK. Plasma LDL-cholesterol was determined from the values of total cholesterol and HDL-cholesterol by using the following formula [6]:

$$\text{LDL-cholesterol} = \text{TC} - \frac{\text{TG}}{5} - \text{HDL-cholesterol (mg/dl)}$$

MDA Assay: MDA levels were estimated by the thiobarbituric acid (TBA) reaction [7]. Using 40% tricholoroacetic acid, proteins were precipitated from 0.5 ml serum, and the precipitated proteins were incubated with TBA reagent in a boiling water bath for one hour. After bringing the solution down to room temperature, the coloured complex which was formed, was measured by using a spectrophotometer at 532 nm. 1, 1, 2, 3-tetraethoxypropane (1 nmol/l) was used as a standard for MDA estimation. The concentrations were expressed in nmol/l.

Assay of Carbonic anhydrase activity [8]**:** The assay system consisted of 100 µl of sample (serum) containing 1.4 ml of 0.05 M Tris-SO4 buffer (pH 7.4) and 1.5 mL of 3mM p-nitrophenyl acetate. The change in absorbance at 348 nm was measured over a period of 3 min., before and after adding the sample. One unit of enzyme activity was expressed as 1 µmol of released p-nitrophenol per minute at room temperature. All chemicals for carbonic anhydrase activity assay were obtained from Merck Chemicals.

Statistical analysis: The data from the patients and controls were compared by using the Student's *t*-test. The values were expressed as mean ± standard deviation (SD). Microsoft Excel for Windows 2003 was used for statistical analysis. *P*-values <0.05 were considered to indicate statistical significance.

RESULTS:

The findings of the current study, based on the essential hypertensive patients, are summarized in [Table/Fig 1].

[Table/Fig 1]: Baseline Variables in Patients and Controls (mean ±SD)

Variable	Normotensive (n=86)	Hypertensive(n=70)
Age (yrs)	42.4 ±12.5	40.2 ± 7.8 †
Sex (M/F)	65/21	42/28 †
Systolic blood pressure (mmHg)	106.6 ±13.4	166 ±18.6‡
Diastolic blood pressure (mmHg)	76.7 ±7.4	98 ±8.1‡
Total Cholesterol (mg/dl)	146.6 ±23.6	208 ± 20.18‡
Triglycerides (mg/dl)	135.2 ±15.6	194.5 ±19.3§
HDL-c (mg/dl)	45.4 ±3.8	36.6 ±4.8 †
LDL-c (mg/dl)	74.16 ±4.5	132.5 ± 14.8 †
VLDL (mg/dl)	27.04 ±5.1	38.9 ±8.2 †
Malondialdehyde (µmol/ml)	1.83 ±0.6	5.8 ±1.8§
Carbonic anhydrase activity (µ mol/l)	4.23 ±2.5	11.8 ± 3.6 ‡

† NS; ‡ (p <0.001); § (p < 0.005)
p ≤0.05 considered significant by unpaired, two tailed, student 's *t* test

The differences in both systolic and diastolic blood pressure were highly significant (p<0.001) among essential hypertensive patients when compared to those in the normotensive healthy controls. When lipid profile variables were compared, the results were highly significant in total cholesterol and triglycerides, but were not significant in HDL-C, LDL-C and VLDL. The study also observed higher levels of malondialdehyde in the patients (p<0.005) as compared to the controls. The carbonic anhydrase activity in hypertensive patients was also observed to be highly significant (p<0.001) as compared to that in the healthy controls.

DISCUSSION:

The current study was focused to determine the changes in carbonic anhydrase activity and lipid profile, accompanied by the measurement of malondialdehyde levels in essential hypertension patients. In the current study, we observed significantly higher (p<0.001) levels of total cholesterol and triglycerides (p<0.005) with a concomitant increase in serum malondialdehyde concentration (p<0.005) and carbonic anhydrase activity (p<0.001). It is commonly noticed that hypertension is associated with metabolic abnormalities and

oxidative stress, which was observed in our study. Earlier studies which were conducted in India [9] also found elevated levels of total cholesterol and triglycerides in hypertensive patients, as also observed in our study.

[Table/Fig 2]: Analysis of Variables based on blood pressure (mean ±SD)

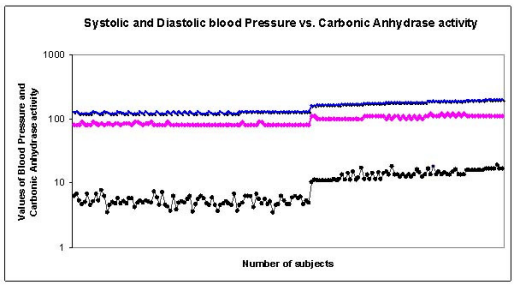
Variable	Group I (n=)	Group II (n=)	Group III (n=)
	Systolic (181.4 ± 16.5)	*Systolic (156.8 ± 12.1)*	*Systolic (133.7 ± 19.7)*
	Diastolic (109.4 ±2.5)	*Diastolic (97.1 ±2.5)*	*Diastolic (88.5 ±2.5)*
Age (yrs)	42.4 ± 12.5	40.2 ± 7.8 †	40.2 ± 7.8 †
Total Cholesterol (mg/dl)	172.6 ± 7.0	192.1 ± 32.2 ‡	201 ± 30.5 ‡
Triglycerides (mg/dl)	177.5 ± 5.8	191 ± 118.1 §	209.1 ± 96.7 §
HDL-c (mg/dl)	39.8 ± 5.2	36.3 ± 5.3 †	33.6 ± 5.3 †
LDL-c (mg/dl)	100.3 ± 0.65	117.3 ± 3.3 †	125.6 ± 5.8 †
VLDL (mg/dl)	35.5 ± 1.65	38.2 ± 23.26 †	41.82 ± 19.34 †
Malondialdehyde (µmol/ml)	4.6 ± 1.4	5.3 ± 1.2 §	6.7 ± 1.5 §
Carbonic anhydrase activity (µ mol/l)	7.9 ± 9.9	9.3 ± 3.2 ‡	10.8 ± 3.5 ‡

† NS; ‡ (p <0.001); § (p < 0.005);

Gambhir et al, 2007 also conducted [10] a study to investigate the carbonic anhydrase (CA) activity in erythrocytes from normotensive and essential hypertensive subjects, which reported decreased CA activities in the normotensive subjects as compared to the hypertensive ones, which does not conform to the findings of our study, where we observed increased carbonic anhydrase activity with increase in blood pressure. Researchers from Bangladesh conducted a prospective study which was based in the Northern region of Bangladesh, to investigate the lipid profile status in hypertensive patients as compared to healthy normotensive controls. Their study revealed similar findings of elevated serum total cholesterol, triglycerides and LDL-cholesterol in the hypertensive subjects as compared to the controls, as observed in our study [11]. Another comparative prospective study which was conducted in patients of type II diabetes mellitus (DM) with and without hypertension, revealed significantly elevated serum total cholesterol, triglycerides and LDL-cholesterol in hypertensive type II DM subjects as compared to the normotensive type II DM subjects [12].

Another study which was carried out in Bangladesh to appraise the lipid profile in hypertensive patients also observed similar findings as observed in our study [13].

Some studies strongly associate hypertension and dyslipidaemia and suggest that both may add up to increase the patients' susceptibility to the development of coronary heart disease. A study conducted on Nigerian hypertensive patients found a significantly higher lipid profile, except for HDL-Cholesterol which was strongly similar to that found in our study [14]. Another retrospective study was done, based on Indians, to estimate the prevalence of abnormal lipid profile in hypertensive patients. The medical records of patients coming for a health check-up, were screened for hypertensive subjects. The prevalence of abnormality in the lipid ratios in most (57%) of these subjects attending the regular check up, proved the association between hypertension and lipid profile [15]. Further studies were conducted in India on type 2 diabetes mellitus (DM) hypertensive males and females to evaluate the lipid profiles in them and it was observed that the mean TC, TG, VLDL-C, HDL-C and LDL-C concentrations and the TG/HDL and LDL/HDL ratios were higher in type 2 DM hypertensive patients as compared to that in the non-diabetic control subjects.



[Table/Fig 3]: Carbonic Anhydrase Activity vs. Blood Pressure

The study also observed that hypertensive type 2 diabetic females had significantly higher serum TC than the hypertensive non-diabetic males. This suggested that hypertensive type 2 diabetic females were exposed more profoundly to the risk factors including atherogenic dyslipidaemia as compared to the males [16]. Another study which was conducted in Western Nepal on confirmed cases of hypertension to observe

their lipid profiles and oxidative stress, observed elevated malondialdehyde (MDA) levels and lipid profiles as compared to the controls, which was in conformity with the findings of our study [17]. In another prospective study which was conducted in the Northern region of Bangladesh to investigate the serum lipid profile viz the level of total cholesterol (TC), the Triglycerides (TGs), HDL-cholesterol and LDL-cholesterol of the hypertensive patients as compared to the healthy controls also revealed similar findings as observed in our study [18]. Yet another study was conducted, based on the objective of evaluating oxidative status, antioxidant activities, and reactive oxygen species byproducts in whole blood and mononuclear peripherals cells and their relationship with blood pressure. Sixty-six hypertensive patients and 16 normotensive volunteers as a control group were studied. In both groups, whole blood and peripheral mononuclear cells oxidized/reduced glutathione ratio and malondialdehyde was significantly higher, and the activity of superoxide dismutase, catalase, and glutathione peroxidase was significantly lower in hypertensive patients as compared to the normal subjects. In our study, we observed the malondialdehyde levels to be significantly higher in hypertensive patients as compared to that in the healthy controls [19].

In the final analysis, it appears that essential hypertension is always associated with elevated serum TC concentrations and other lipid abnormalities. The major concern of this observation was that the subjects also had oxidative stress due to the abnormalities of lipids and their ratios. Therefore, the analysis of other risk factors which are associated with hypertension will be of immense importance in the eventual assessment of the risk status, as lipid abnormalities and oxidative stress result in the future risk of Coronary artery disease.

CONCLUSION

The lipid profile is bound to be altered in essential hypertension along with increased oxidative stress and it is associated with an increased activity of carbonic anhydrase.

WHAT THIS STUDY SAYS

The timely assessment of the lipid profile in all cases of hypertension is a must, as this would further aggravate the disorders and the risks which lead to coronary artery disease. The determination of carbonic anhydrase activities in hypertensive patients is also important, as it correlates with the degree of severity of the disorder.

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