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Original Research Article

A STUDY OF MULTI-MARKER APPROACH IN HYPERTENSIVE PATIENTS TO PREDICT THE RISK OF HEART FAILURE ATTENDING A TERTIARY CARE HOSPITAL.

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ABSTRACT

Background: HTN is a major contributor to the development of Heart Failure. A growing array of biological pathways which include deleterious mechanisms promoting HF development & progression, as well as compensatory cardio-protective pathways explain the risk of HF. Components of these pathways can be utilised as biomarkers of this condition in order to facilitate the diagnosis, prognostication & potentially direct the management.
Aim of the Study: To assess the predictive ability of selected biomarkers like N-terminal Fragment of the medullary natriuretic Peptide (NT-Pro BNP) and High Sensitive C-Reactive Protein (Hs-CRP) for the risk of Heart Failure in Hypertensive patients. The objective of this study was to measure the serum levels of biomarkers with different patho-physiological backgrounds i.e., NT-Pro BNP and Hs CRP in hypertensive patients to give additive prediction value for incident Heart Failure.
Material and Methods: A cross-sectional study was done with 100 subjects divided into 2 groups. 50 patients with Hypertension with Overt Heart failure served as (Group 1) and 50 patients with Hypertension without Heart Failure served as (Group 2) in the age group of 40-70 years. Fasting serum NT pro BNP levels, Hs-CRP levels were estimated. Data was analysed using IBM-statistical package for social sciences (SPSS) and Graphpad Prism. Unpaired-t test was performed to test the significance of difference in means of NT pro BNP levels, Hs CRP between the two groups. Pearson's correlation was done, to study the correlation between serum NT pro BNP, Hs CRP.
Results: NT-pro BNP levels are elevated in Group 1 than in Group 2, the Mean ± SD of serum NT pro BNP in group 1 was 1178.29 ± 604.4 pg/ml and in group 2 was 148.17± 74.04 pg/ml. Serum Hs-CRP levels are increased in group 1 than in group 2, the Mean of serum Hs-CRP in group 1 was 5.83 mg/L and in group 2 was 2.35 mg/L. To study the correlation among both parameters within Group-1, Pearson's correlation was done. No such association is seen among both parameters in HF hypertensive patients (p=0.05). On the basis of receiver operating characteristic (ROC) curve analysis, we found that NT-pro BNP > 328.9 pg/ml,Hs CRP >3.3 mg/L were significant predictors of overt heart failure in patients with hypertension.
Conclusion: Heart Failure occurs when Heart cannot pump blood(systolic failure) and when it is not filled(diastolic failure) adequately. HF is a complex syndrome associated with various patho-physiological and biochemical disorders. No single bio-marker can detect the features of HF. Hence the promising biomarkers like NT-pro BNP were chosen relevant to their

underlying pathophysiology along with other markers like Hs-CRP in this study for Heart Failure assessment and help in its prognosis.
Keywords: Hypertension (HTN), Heart-Failure (HF), NT-pro BNP, Hs CRP.

INTRODUCTION

Hypertension Cardiovascular disease is the main cause of death worldwide. Hypertension is a public health problem and important area of research due to its high prevalence and being major risk factor for cardiovascular diseases and other complications.[1] Diagnosis of HTN is based on guidelines for Prevention, Detection, Evaluation, and management of High BP in Adults by AMERICAN HEART ASSOCIATION (AHA).

HTN: ≥130/ >80 mm of Hg

HEART-FAILURE HF is a complex syndrome associated with various patho-physiological and biochemical disorders. No single biomarker can detect the features of HF.[2]Heart failure (HF) is caused by structural and functional defects in myocardium resulting in impairment of ventricular filling or the ejection of blood. The most common cause for HF is reduced left ventricular myocardial function; however, dysfunction of the pericardium, myocardium, endocardium, heart valves or great vessels alone or in combination is also associated with HF. Some of the major pathogenic mechanisms leading to HF are increased hemodynamic overload, ischemia-related dysfunction, ventricular remodelling, excessive neuro-humoral stimulation, abnormal myocyte calcium cycling, excessive or inadequate proliferation of the extracellular matrix, accelerated apoptosis and genetic mutations.[3]

NT-Pro BNP(N-TERMINAL PRO BRAIN NATRIURETIC PEPTIDE) Plasma concentrations of cardiac-derived natriuretic peptides have been firmly associated with cardiac function.[4,5] In particular, increased concentrations of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), as well as the N-terminal fragments of their biosynthetic precursors (N-terminal pro ANP and pro BNP), are hallmarks of congestive heart failure. Accordingly, the European Society of Cardiology has suggested that measurement of the cardiac derived peptides may be helpful in diagnosing chronic heart failure.

CRP (C-REACTIVE PROTEIN) In HF release of CRP occurs due to Cytokine Production like IL-6 caused by Hepatic cell damage.It is produced in Cardiac myocytes under hypoxic stress. In isolated left and right HF and in CHF(CONGESIVE HEART FAILURE) there is hepatic centrilobular necrosis as a result of decreased CO,hypo-perfusion,hypoxia and venous congestionleading to Increased IL-6 and thus hepatic CRP production. Increased CRP causes an increased risk of myocardial infarction and subsequently HF.

MATERIALS AND METHODS

Setting: A cross-sectional study was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad. Source of samples and data: · Department of Biochemistry, Osmania General Hospital · Department of General medicine, Osmania General Hospital. Department of Cardiology, Osmania General Hospital

The study included 100 subjects who were divided into 2 groups in the age group of 40-70 years. Group 1 with 50 Hypertensive patients with HF and Group 2 included 50 Hypertensive patients without HF.

Table No. 2: Classification of study subjects Group Subjects No. of subjects Group 1 Hypertensive patients with HF 50 Group 2 Hypertensive patients without HF 50 Informed consent was taken from all the individuals who took part in the study. Ethical clearance was obtained for the study from the ethical committee of Osmania Medical College.

Inclusion Criteria: Group 1: Patients with Hypertension for atleast 2 years with HF in the age groups of 40 – 70 years. Group 2: Patients with Hypertension for atleast 2 years without overt HF in the age groups of 40 – 70 years.

Exclusion Criteria:♣ Patients with known history of Hyper/Hypo thyroid state ♣ Pulmonary Hypertension ♣ Unstable HTN ♣ NYHA class-IV HF ♣ Pregnancy and Lactation ♣ Obstructive / Restrictive Pulmonary disease ♣ Cancer ♣ Anemia ♣ Alcohol abuse ♣ Drugs abuse

Specimen Collection: 4 ml of fasting venous blood was collected under aseptic precautions into serum vacutainers. Haemolysed and lipemic samples were not included.Blood was allowed to clot and serum was separated after centrifugation at 3000 rpm for 5 minutes.The serum was stored in Eppendorf tubes at -20ºC. 27 PARAMETERS ESTIMATED: 1) Serum NT-pro BNP2) Serum Hs-CRP

SERUM NT-pro BNP Method: Sandwich Enzyme linked Immuno sorbent assay(ELISA).

Serum Hs-CRP Method: Measurement of antigen-antibody reaction by end point method, Latex turbidimetry.

RESULTS

The present study was undertaken in the Department of Biochemistry, Osmania Medical College and Osmania General Hospital, Hyderabad. A total of 100 patients were recruited for the study which included 50 Hypertensive patients with Heart Failure as Group-1 and 50 Hypertensive patients without Heart Failure as Group-2. Mean age in group 1 and group 2 were matched and p= 0.11 which is statistically not significant.

The data was analysed using version 9.3.0.GraphPad Prism software.ROC curve analysis was done using SPSS software version 28.0.1.0 and MEDCALC version 20.019. The results were expressed as mean ± SD of various parameters in different groups. [Table 1]

In order to assess the significance of the differences observed in the mean values of different parameters observed in different groups studied, the data is subjected to unpaired t - test. The significance of difference of mean values of different groups and within the groups is represented by p values and p value <0.05 is considered as significant. [Table 3]

Table 1: Mean and SD of Age and Blood Pressure in two groups

	Group-1(n=50)	Group – 2(n=50)	P(NON-HF vs HF)
AGE	50.98±7.75	48.62±6.66	0.11
SBP	127.4±7.23	131.3±9.68	0.02
DBP	76.8±6.83	83.8±6.51	0.0001

Table 2: Study parameters in group 1 and group 2

Parameter	Group 1(n=50)			Group 2(n=50)		
	Mean	± SD	SEM	Mean	±SD	SEM
Serum NT-pro BNP	1178.29	604.4	85.5	148.17	74.04	10.5
Serum Hs-CRP	5.83	1.62	0.22	2.35	0.84	0.12

Table 3: Pearson's Correlation Coefficient for The Parameters

Parameter	NT pro BNP	Hs- CRP
NT pro BNP	1	0.05
Hs- CRP	0.05	1

Correlation is significant at the 0.05 level(2-tailed)

Table 4: Unpaired t- test between Group 1 and Group 2

Parameter	t valve	P valve	Degree of freedom
Serum NT pro -BNP	11.96	< 0.0001	98
Serum Hs-CRP	13.45	< 0.0001	98

Table 5: ROC curve analysis between two groups

BIOMARKER	AUC(%)	SENSITIVITY	SPECIFICITY	Cut off value	p value
NT pro BNP	100	100	100	328.9	<0.0001
Hs -CRP	99	100	92	3.3	<0.0001

Table 6: Independent risk factors for HF in progressing Logistic Regression

PARAMETER	ODDS RATIO
NT pro BNP	1.182
Hs-CRP	674.9

DISCUSSION

In the present study, the study subjects have been divided into 2 groups based on HF. Group 1 includes Hypertensive patients with Heart failure and Group 2 includes Hypertensive patients without Heart failure. Serum N-terminal Fragment of the medullary natriuretic peptide (NT-Pro BNP), Serum High sensitive C-Reactive Protein (HsCRP), systolic blood pressure (SBP), diastolic blood pressure (DBP) levels were assessed in group 1 and group 2. There was no statistically significant difference in age groups among group 1 and group 2 (P-value=0.11) using the unpaired t-test. To assess the significance of the difference observed in the values of differences in the 2 groups, the unpaired t-test was performed. The significance of the difference between the means was expressed as a P-value and a P-value of less than 0.05 was considered statistically significant.

The mean ± SD of Serum N-terminal fragment of the medullary natriuretic peptide (NTPro BNP) in Group 1 was 1178.29 ± 604.4 pg/ml and the mean ± SD in Group 2 was 59 148.17 ± 74.04 pg/ml of Serum NT- Pro BNP. To assess the significance of the differences observed in the mean values of serum NT-Pro BNP in the two groups, the data were subjected to the unpaired t-test. A P-value of <0.0001 was obtained which is statistically significant.

The mean ± SD of Serum High sensitive C-Reactive Protein (Hs-CRP) in Group 1 was 5.83 ± 1.62 mg/L and the mean ± SD of Serum Hs-CRP in Group 2 was 2.35 ± 0.84 mg/L. To assess the significance of the differences observed in the mean values of serum Hs-CRP in the two groups, the data were subjected to the unpaired t-test. A Pvalue of <0.0001 was obtained which is statistically significant.

The mean ± SD of Systolic Blood Pressure (SBP) in Group 1 was 127.4 ± 7.23 mmHg and the mean ± SD of SBP in Group 2 was 131.3 ± 9.68 mmHg. To

assess the significance of the differences observed in the mean values of SBP in the two groups, the data were subjected to the unpaired t-test. A P-value of 0.02 was obtained which is statistically significant.[6] The mean ± SD of Diastolic Blood Pressure (DBP) in Group 1 was 76.8 ± 6.83 mmHg and the mean ± SD of DBP in Group 2 was 83.8 ± 6.51 mmHg. To assess the significance of the differences observed in the mean values of DBP in the two groups, the data were subjected to the unpaired t-test. A P-value of 0.0001 was obtained which is statistically significant.

To study the correlation between serum NT-Pro BNP and Hs-CRP among group-1 i.e., hypertensive patients with Heart Failure, Pearson's correlation was done

The B-type natriuretic peptides BNP and NT-pro BNP provide a cheap and accessible diagnostic test for heart failure (HF) and left ventricular dysfunction.[7] Clinical guidelines advocate their use in the diagnostic work-up in case of HF suspicion to limit the number of potential cases requiring echocardiography by ruling out the condition where thenatriuretic peptide level is low, although recommended rule-out cut-off points vary between studies and guidelines.[8,9] According to present ESC guidelines, the optimal exclusion cut-off point for NT-pro BNP in patients presenting with acute onset or worsening of symptoms is 300 pg/mL. For patients presenting in a non-acute way, the optimum exclusion cut-off point is 125 pg/mL for NT-proBNP. The sensitivity and specificity of NT-proBNP for the diagnosis of HF are lower in non-acute patients. There are no significant differences in plasma concentration of NT-proBNP between patients with heart failure of various origins. However, to diagnose heart failure, knowledge of the non-cardiac factors that influence NT-proBNP is crucial. Anemia, which is common in heart and renal failure, is one of the independent factors affecting natriuretic peptides. In chronic kidney disease, anaemia is mainly caused by reduced erythropoietin production.[10] For these reasons, NT-proBNP was concluded to be of diagnostic value in patients with heart failure and proper renal functions. Previous studies have identified a variety of non-cardiac factors influencing natriuretic peptide levels, including age, sex, BMI, renal function, hepatic damage and diastolic pressure. Heart failure itself is associated with adverse structural remodeling, which is caused by alterations in volume or pressure load, local ischemia, fibrosis and myocyte death due to apoptosis or necrosis.

Of the various inflammatory biomarkers identified, C-reactive protein (CRP) is the most extensively studied and validated, namely in cardiovascular disease. CRP is a highly sensitive but nonspecific product of inflammation, whose synthesis is triggered by a noxious stimulus like infection, trauma, tissue necrosis as well as by the inflammatory cytokines related to the noxious stimuli. It is an acute-phase protein mainly synthesized in hepatocytes, but it can also be synthesized in some extrahepatic tissues like vascular smooth muscle, atherosclerotic plaques and intracardial tissues.[11]CRP and the currently measured high-sensitivity CRP (HsCRP) are the most sensitive and less specific inflammatory markers and are considered the paradigm of inflammatory markers and inflammatory activation. CRP measurement has been consistently suggested in successive guidelines as a useful tool for cardiovascular risk stratification. CRP can itself contribute to cardiovascular disease burden through a multitude of proatherogenic, proinflammatory and prothrombotic effects.[12] HsCRP has an important role specifically in the heart failure setting. The concentration of HsCRP is a strong predictor of incident heart failure both in the general population as well as in high-risk populations of patients with coronary disease.[13]70 Higher CRP has been reported to predict ominous outcomes in both chronic,[14] and acute heart failure.[15]CRP has been correlated with the severity and prognosis of HF, as well as with the response of HF patients to treatment. Independently of HF aetiology (ischemic heart disease idiopathic dilated cardiomyopathy, valvular heart disease), a higher HsCRP concentration is related to a more severe disease course, decreased left ventricle ejection fraction, worse quality of life and treatment effect, higher New York Heart Association HF class, more activated neurohormones (brain natriuretic peptides, noradrenaline, aldosterone), higher rate of rehospitalization, in case of acute HF – hospitalization to intensive care units, higher mortality in hospital and long-term mortality. HsCRP concentration in healthy adults correlates with arterial blood pressure and independently of gender predicts the risk of AH development in men and women: HsCRP > 3.0 mg/L is related to a 3 times higher risk of AH development, compared to those with HsCRP < 1.0 mg/L. In case when AH is already present, HsCRP is associated with vascular stiffness, development of atherosclerosis, target organ damage, cardiovascular event risk and correlates with systolic, diastolic and mean blood pressure. Inflammation mediates myocardial fibrosis and predisposes the development of diastolic dysfunction, which in turn causes HF. Such a hypothesis was supported by a study involving young Afro-Americans (107 patients: mean age, 48 ± 10 years), having no heart or kidney diseases and consuming no alcohol. More than half (52%) of the patients had diastolic dysfunction, which was independently associated with hsCRP.

Limitations of Study

1. The present study is carried out in limited sample size; it should have been carried out on a larger group of population. 2. The study subjects are within 40-70 years of age, so a wider age group should have been included in the study. 3. The present study was conducted

as a prospective, consecutive recruitment of patients with hypertension.

CONCLUSION

In summary, this study analysis suggests that inflammation(CRP),adverse structural remodeling (NT-pro BNP) and endothelial dysfunction are implicated in the pathogenesis and development of Heart Failure from Hypertension in individuals. NT-pro BNP has been proved the best among the parameters as the predictive test with a high sensitivity and specificity i.e. it acts as the Gold standard biomarker in Heart Failure.(AUC=1).Hs-CRP stands next as useful biomarker in predicting risk of HF. In evaluation of Heart Failure, or for screening purpose, these biomarkers can be estimated on a routine basis as they are also cost effective and readily available commercially. Hypertensive patients could be monitored for these indexes once a year or earlier when HF symptoms occur to detect potential risk of developing heart failure, renal complications of hypertension and the risk of myocardial hypertrophy in order to determine the indications for accurate staging complications of hypertension and modification and intensification of pharmacotherapy. This study data support the premise, that abnormalities in multiple pathways antedate the onset of overt Heart Failure. Nonetheless, the predictive value of existing biomarkers for assessing future Heart Failure risk from Hypertension is modest. Identification of additional biomarkers would be necessary before "Multi-Marker" strategies for predicting Heart Failure could be considered useful.

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