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Association of apoptotic marker cytokeratin18 with blood pressure in non-alcoholic fatty liver disease patients

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Abstract

Objective: Non Alcoholic fatty liver disease (NAFLD) associated with hypertension (HTN) is an emerging health issue globally. It is associated with increased levels of apoptotic marker CK18. Main objective of this study was to explore association of cytokeratin18 (CK18) with hypertension (HTN) in NAFLD patients.

Methodology: Descriptive cross sectional study was conducted in Mayo hospital Lahore. Hundred NAFLD subjects were enrolled from OPD of radiology department after approval from ethical review committee. Anthropometric measurements were taken and blood pressure (BP) was measured by mercury sphygmomanometer. Blood samples were drawn from each patient for CK18 levels with ELISA. Data was analyzed by SPSS 20. Continuous variables were presented as mean± SD. Association between CK18 and HTN were analyzed by regression analysis and results were presented as β coefficient. P value<0.05 was taken significant.

Results: Mean age of studied subjects was 43.8±5.34 with height (m), weight (kg) and BMI 1.59±0.063 m, 78.2±11.17 kg, 30.5±4.07kg/m^2 respectively. Systolic and diastolic blood pressures were 106±12.8, 72± 12.8mmHg. CK 18

was not significantly associated with systolic (P value 0.55) and diastolic BP (P value 0.37) most probably due to small size of study.

Conclusion: Most of the NAFLD patients were hypertensive and have raised CK18 levels than normotensive subjects. So, raised levels of CK18 in NAFLD subjects might be helpful in early screening of HTN. However, significant association was not observed probably due to small sample size.

Keywords: CVS, CK18, DBP, HTN, MS, NAFLD, SBP (write full forms for all these abbreviations)

Introduction

Nonalcoholic fatty liver disease (NAFLD) is emerging health issue worldwide affecting both obese and non-obese individuals.[1] It is an evolving epidemic in western as well as Asian population. Its prevalence is continuously increasing and affecting 15%-20% of the Asian population.[1]

It involves multisystem regulatory pathways including hepatic and extra hepatic tissues such as cardiovascular system (CVS) and renal system.[1] Abnormalities in these systems are the leading cause of many metabolic disorders like dyslipidemia, insulin resistance, diabetes mellitus type2 (T2DM) and hypertension (HTN). Dyslipidemia associated with NAFLD is due to excess production of atherogenic lipoprotein and it is characterized by increased levels of serum triglycerides, decreased levels of HDL cholesterol and excess release of pro-inflammatory mediators. These markers are the contributing factors for atherosclerosis, hypertension (HTN) and cardiovascular diseases (CVD). HTN and CVD have poor prognosis and said to be the one of the major causes of morbidity and mortality in NAFLD patients.[2, 3] Cytokeratin (CK18) is a well-known marker for apoptosis and inflammation. It is not normally found in vascular smooth muscle but is highly expressed during the formation of atherosclerotic plaques and found to be raised in hypertensive NAFLD patients.

[4] Evidences are available showing correlation of (CK18) with BP and has reported its pivotal role in pathophysiology of these disorders .[4,5]

Association between liver enzymes like serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and Gamma-glutamyl transferase (GGT) and hypertension is well established. Many previous researchers have documented positive association of these enzymes with HTN in NAFLD.[3] Very few epidemiological studies focused on the relationship of CK18 with HTN and reported varying results. [6] Some have reported positive correlation between CK18 and systolic blood pressure (SBP),[7] rebutted to this, some did not find any significant association between CK18 and BP.[8] Due to these inconsistent results, association between these parameters is still to be hypothesized and required new researches to focus their relationship.

Current study w**as** aimed to explore the association of CK18 with Blood pressure in NAFLD patients as no documentations concerning relationship between these parameters are available in Pakistan to the best of authors' knowledge.

Methodology

Current descriptive cross sectional study comprises of 100 patients was conducted at King Edward Medical University (K.E.M.U), working in collaboration with Mayo hospital, Lady Willingdon Hospital, Lady Aitchison Hospital, Kot Khawaja Saeed Hospital, Shahdara Hospital, and Govt Syed Mitha teaching Hospital Lahore affiliated to it. Sample size of 100 cases was estimated by using prevalence of B.P, confidence interval 95%, 80% power of test and α 5%.

After approval from ethical review committee, 100 NAFLD patients from radiology OPD of Mayo hospital by convenient sampling technique was enrolled following inclusion criteria based on Nondiabetics with age ranging 40-60 years with fatty liver subjects (male, female) recruited in the study.

Subjects with Fatty liver were diagnosed on the basis of high echogenicity textured liver on ultrasound. However subjects with hepatitis B and C and known diabetics were excluded from the study. Informed written consent was taken from each patient and confidentiality was assured. All the relevant demographic information and history were taken on a pre-designed Performa. Physical examination was done and blood pressure was measured with mercury sphygmomanometer by auscultatory method. Triplicate readings were taken in the sitting position, after relaxing the patient for 5 minutes and average were entered in the data.[9] Blood pressure cut off points for hypertensive patients were taken according to the new guidelines 2017 for hypertension provided by American Heart Association and the American College of Cardiology.[10]

According to the new criteria, normal BP and elevated blood pressure are < 120/80 mm Hg and 121to 129/ >80 mm Hg, respectively. Blood pressure 130-139 / 80-89 mm Hg and ≥140/ ≥90 mm Hg were taken as stage I and II hypertension. [10]

After taking informed consent, 5 ml of blood samples were taken and serum was separated in a centrifuge machine for 30 minutes to determine the level of CK18M30 in both groups by sandwich ELISA (Elabscience, Catalog No. E-EL-H2073 in Pathology lab of KEMU. Microtiter plate Elisa plate was pre-coated with CK18M30 antibody. Standards or samples were added to ELISA microwells and the target antigen binds to capture the antibody. This is followed by addition and incubation of Avidin-Horseradish Peroxidase and biotinylated antibody specific for CK18M30. Substrate solution was added after washing. After adding stop solution optical density at 450nm was noted by ELISA reader plate.

Statistical analysis was done using SPPS 21.0. Descriptive variables are presented as frequencies, while mean and standard deviation for continuous variables. Categorical variables are presented as percentages. Association was analyzed by regression analysis.

Results

This study included 100 NAFLD patients of mean ±SD age of 43.8 ± 5.34. Descriptive statistics of study is presented in table 1. Out of total NAFLD population, 24 were normotensive and 76 subjects were hypertensive. Figure 1 is showing the comparison of blood pressure among study groups. Mean ± SD SBP and DBP of normotensive subjects were 106±12.8 and 72±12.8 mmHg, respectively. SBP and DBP of hypertensive subject were 131±14.1 and 94±10.2 mmHg, which was significantly different from normotensive subjects (P value 0.001*)

Figure 2 is indicating that CK18 is higher in hypertensive than normotensive subjects (18.3±2.8 v/s 10.5± 5.6) and difference was statistically significant with P value 0.016*, However Regression analysis did not show significant association of CK18 with systolic (P value 0.55) and diastolic blood pressure, (p value 0.37) (Table 2).

Discussion

NAFLD is becoming the major health burden in Western, American as well as in Asian population. It is also considered as hepatic manifestation of metabolic syndrome (MS).[11]

Patients with NAFLD have probability for developing co-morbidities including hypertension[12], atherosclerotic cardiovascular disease (ASCVD), type 2 diabetes mellitus and chronic renal diseases. [13] Associated factors like obesity, atherogenic dyslipidemia, and greater carotid intima-media thickness followed by atherosclerosis, a prothrombotic and a proinflammatory state are greater contributor in developing deteriorating consequences. [12] All these proinflammatory conditions result in inflammation and elevation of inflammatory marker like CK18.[14] The Framingham et al documented that, high BP due to decline in HDL level is the most common element of NAFLD.[15] On

the other hand, hypertension is attributed to an inflammatory disease by various researchers.[12]

Interest of the current study is to enlighten the significant association between CK18 and BP for early detection, and effective management of hypertension, which is key determinant of cardiovascular risk and leading cause of morbidity, disability and mortality in NAFLD worldwide.[3] Strong evidences showed that the rise in 20mmHg systolic blood pressure (SBP) and 10 mm Hg diastolic blood pressure (DBP) are contributing in doubling the risk of mortality from stroke and cardio vascular diseases.[10]

In current study, individuals with 131±14.1and 94±10.2mmHg SBP and DBP are considered as hypertensive as per new guide lines by American Heart Association[10]. Speliotes etal have reported that NAFLD patients showed higher SBP, which is supporting our results.[15] Qian LY etal study is also in agreement with our results as they also reported higher limits of blood pressure in NAFLD patients and they further considered blood pressure as the independent risk factor of NAFLD.[12] Various previous studies are also in favor of our results documenting higher BP in NAFLD patients .[16,17]

One of the previous evidences revealed that NAFLD with raised levels of emerging inflammatory biomarker CK18 is an independent risk factor for hypertension.[7] In present study, we analyzed the association of blood pressure with serum CK18 by regression analysis . It was found to be higher in hypertensive than normotensive NAFLD patients (P value =0.016*) but no significant association of CK18 was found with SBP (P value 0.55) and DSB (P value 0.37) as it requires larger number of sample individuals, while small sample size is the limitation of this study. However, present study is in line with Mattey DL who did not find any significant association between CK18 and BP in European population. [8]

Concern of this study was to highlight the association between CK18 and BP, though significant association was not observed. However elevated CK18 in

hypertensive may emulate damage of cells having CK18 in cardiac vasculature and atherosclerotic plaques. It might be a useful surrogate marker for the identification of patients at risk of developing hypertension and subsequent IHD and targeted management to prevent life threatening morbidities and disabilities. Effective screening strategies including new biomarkers like CK18 should be recommended for early detection of co-morbidities of NAFLD to prevent harmful consequences and promoting healthy life style.

Conclusion

Most of the NAFLD patients were hypertensive associated with raised inflammatory biomarker CK18.

Limitation

Small sample size is the limitation of study because significant association requires sample size on a broader scale.

This is the cross sectional study so we could not establish a casual association between BP and elevated CK18levels in NAFLD patients.

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Conflict of interest: None.

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Table 1: Descriptive of studied subject (n= 100)

Variables	Mean	Standard Deviation(SD)
Age (years)	43.8	5.34
Height (m)	1.59	0.063
Weight (kg)	78.2	11.17
Body mass index (BMI) kg/m^2	30.5	4.07
Systolic Blood Pressure (SBP) mmHg	125.09	17.5
Diastolic blood pressure (DBP) mmHg	88.62	14.21

Table 2: Association between CK18 and Blood Pressure

Blood pressure	B	S.E	r	P value	95% C.I
Systolic blood pressure (SBP)	0.045	0.075	0.06	0.55	0.014-0.195
Diastolic blood pressure (DBP)	0.05	0.06	0.09	0.37	0.066-0.176

Dependent variables: SBP and DBP, Independent variables: CK18= Cytokeratin 18
β = beta coefficient resulting from regression, SE= standard error, r= Pearson correlation value,
C.I = Confidence Interval. Statistically significant value at P ≤ 0.05

Figure 1: Comparison of Blood Pressure

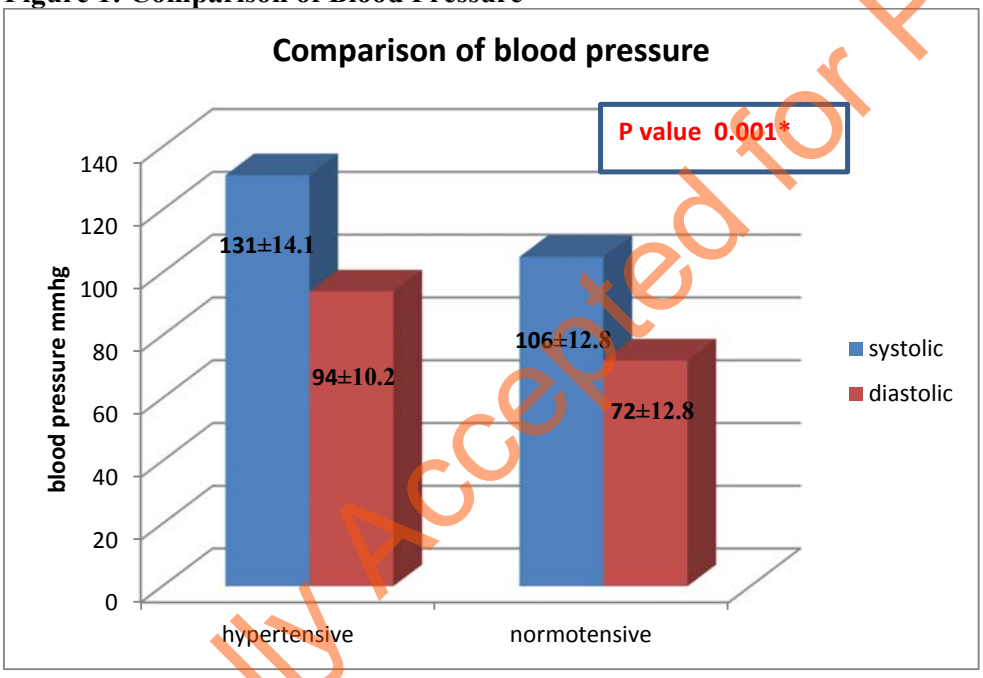

Figure 2: Comparison of CK18 between Hypertensives and Normotensives in NAFLD Patients