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Short Communication

Serum Branched Chain Amino Acids May Determine the Severity of Coronary Artery Disease as Evidenced by Regression Modelling

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AM collected the data. TAS, NA and AM wrote the manuscript. TAS and SI analysed the data. NA did Elisa testing. FM presented the concept and superved the study.

Key words
Branched chain amino acids, BCAA, Coronary artery disease, CAD, Diabetes mellitus

ABSTRACT

Branched chain amino acids (BCAA) are essential amino acids, their role in metabolic disorders has only recently been established. However, their association with coronary artery disease (CAD) is still ambiguous. The current study sought to determine the relationship of BCAAs with severity of CAD adjusting for diabetic status. The objectives of this study were to determine the factors affecting serum BCAA levels in CAD patients adjusting for age, body mass index (BMI), gender and Gensini score, and to determine the risk factors for the severity of CAD. In our cohort. 119 patients who underwent angiography were recruited from the Department of Cardiology, King Edward Medical University/ Mayo Hospital, Lahore from January to July 2019. Their gender and BMI were recorded. They were diagnosed as diabetic on the basis of HBA1c levels. Serum BCAA levels were measured by using commercially available colorimetric kit. Multiple regression modeling was applied using serum BCAA levels as the dependent variable and age, gender, BMI, Gensini score and diabetic status as independent variables. Another multiple regression model with Gensini score as the dependent variable was also created. Overall three factors affected BCAA levels; age (Beta=-0.32, p <0 .001), male gender (Beta=-0.32, p=<0.001) and diabetic status (Beta=0.20, p=0.03). Significant factors affecting severity of CAD as indicated by Gensini score were BMI (p=0.016, Beta=-0.22) and age (p=0.019, Beta=0.23). To conclude, serum BCAA levels I CAD patients are affected by age, male gender and diabetic status. Secondly, the severity of CAD is mainly affected by BMI and age. High BCAA levels are not linked with the severity of CAD either in diabetics or in non-diabetics.

Amino acids are the building blocks of proteins. Nine out of twenty amino acids are essential as they cannot be synthesized by the human body. Among them, three are branched-chain amino acids (BCAAs) which include valine (Val), leucine (Leu) and isoleucine (Ile). The relationship between BCAAs, atherosclerosis and coronary artery disease (CAD) has been an active area of research. Many studies have shown that high serum BCAA levels are associated with insulin resistance and diabetes mellitus (Newgard *et al*., 2009; Wang *et al*., 2011; Ruiz-Canela *et al*., 2018; Yoon, 2016; Zhao *et al*., 2016). Serum BCAA levels are also associated with the risk of CAD independent of diabetes and other risk factors for CAD (Ruiz-Canela *et al*., 2018; Yang *et al*., 2015, 2016) Elevated levels of BCAAs are also positively associated with the risk factors for CAD including carotid intima media thickness (cIMT), body mass index (BMI), blood pressure, fasting blood glucose, triglycerides, obesity, apoB, apoCII, apo CIII and negatively associated with protective factors for CAD namely HDL-C and apoAI (Yang *et al*., 2014). We aimed to further elucidate the relationship between BCAAs, CAD and diabetic status by the regression modelling.

Materials and methods

This cross-sectional comparative study was carried out in the Department of Cardiology, King Edward Medical University/ Mayo Hospital, Lahore from January to July 2019 after receiving the institutional review board approval. A hundred and nineteen subjects were recruited by non-probability convenient sampling after taking informed consent. The sample size was calculated by applying 95% confidence level, 90% power of test with

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expected mean value of BCAA in diabetics as 183.6±35.5 and non-diabetics as 162.3±35.5 using sample size formula for comparison of means (Shah *et al*., 2010).

Subjects, both men and women, undergoing angiography due to clinical suspicion of CAD were included. Diabetics and non-diabetics were segregated according to blood HBA1c levels. We excluded patients receiving lipid lowering agents in the preceding 6 months since the serum lipids are associated with BCAA levels (Yang *et al*., 2016) in addition to being an independent risk factor for CAD.

Demographic data was recorded. Drug history was documented for the last 6 months. Height in meters and weight in kilograms were recorded by a standard height weight machine. Five ml blood was drawn from the patients by venipuncture from the brachial vein with the help of a disposable syringe using sterile technique. Two milliliters (ml) blood was poured into CBC vial containing EDTA for HBA1c and three ml blood was poured into serum vial for BCAA levels measurement. Serum was extracted by centrifugation and stored at -20°C. HbA1c was measured with a commercially available kit (SPHERA, catalog no 7546) using the protocol suggested by the manufacturer in the immunology laboratory of University of Health Sciences, Lahore. BCAA analysis was performed by spectrophotometry using commercially available kit (Abcam, ab83374) as per the instructions given in the kit.

CAD severity was assessed by Gensini scoring system. All data was analyzed using SPSS-23. Factors affecting serum BCAA levels in all patients of CAD were assessed by using multiple linear regression modeling. All continuous variables which were not normally distributed were log transformed. BCAA levels were used as the dependent variable whereas age, BMI, Gensini score, gender and diabetic status were entered as the independent variables in the regression model. Reliability of the model was assessed by ANOVA and relevance was measured by R^2*(see footnote) change. As a further sensitivity analysis, the relationship was further explored in diabetics and non-diabetics separately.

In addition, risk factors for severity of CAD were evaluated by multiple linear regressions. Dependent variable was Gensini score and independent variables included age, BCAA levels, BMI, gender and diabetic status. Reliability of the model was assessed by ANOVA and relevance was measured by R^2 change. As a further sensitivity analysis regression was repeated in diabetics and non-diabetics separately. P-value ≤0.05 was taken as significant.

Results

Patient characteristics are summarized in Table I. The regression model was used to assess the effect of age, BMI, gender, Gensini score and diabetic status on serum BCAA levels was statistically significant (ANOVA $p<0.001$) and adjusted R^2 revealed that our proposed risk factors accounted for 0.18 of changes in serum BCAA levels. Overall three factors affected serum BCAA levels significantly as shown in Table II. These included age, gender and diabetic status. The age of patients (Beta= -0.32, $p<0.001$), male gender ($p<0.001$, Beta=-0.32) and diabetic status ($p=0.03$, Beta=-0.20) were significantly associated with serum BCAA levels. The severity of CAD disease as indicated by Gensini score ($p=0.06$, Beta=-0.04) and BMI ($p=0.41$, beta=-0.07) showed no significant association with serum BCAA levels.

Table I. Characteristics of patients recruited in this study.

Patient characteristics	Non-diabetics (n=64)	Diabetics (n=55)
Gender		
Men	59	41
Women	5	14
Gensini score (Median ±IQR)	33.00±38.80	49.50±41.00*
Age (years) (Mean±SD)	47.41±10.16	51.93±6.13**
HbA1C (%)	5.58±0.35	8.96±1.69***
BMI (kg/m^2)	23.65±4.00	24.48±4.78

IQR, Inter-quartile range; SD, Standard deviation; HbA1C, Percentage of glycated Hemoglobin; BMI, Body mass index. Student's t-test of significance: * $p<0.05$; ** $p<0.01$; *** $p<0.001$

When diabetics were considered separately, male gender came out to be the only significant risk factor for raised serum BCAA levels ($p=0.01$, Beta -0.37). In case of non-diabetics, age ($p=0.001$, Beta= -0.40) and gender ($p=0.04$, beta=-0.24) were significant factors affecting serum BCAA levels as shown in Table II. Moreover, we found that age was positively associated with Gensini score ($p=0.019$, Beta 0.23) whereas BMI was negative associated ($p=0.016$, Beta -0.22). However, serum BCAA levels, gender and diabetic status did not significantly affect the CAD score (Table II).

The current study shows that higher serum BCAA levels are associated with younger age ($p<0.001$), male gender ($p<0.001$) and diabetic status ($p=0.03$) in CAD patients. Our results are similar to the previous studies. Alfaqih *et al*., reported that serum BCAA levels were significantly associated with diabetes mellitus ($p<0.001$) (Alfaqih *et al*., 2018). Similarly, a study by (Nakamura *et al*., 2014) showed that levels of valine, leucine and isoleucine were raised in patients with higher fasting

insulin levels and HbA1C as compared to controls (Nakamura *et al*., 2014). This is consistent with our results as we found that BCAA levels were significantly higher in diabetic patients even after adjusting for age, BMI, gender and severity of CAD. However, our study is the first of its kind to elucidate these relationships in a subset of CAD patients.

Table II. Factors affecting serum BCAA levels in all, diabetic and non-diabetic patients of CAD and severity of CAD.

Model	UC		SC	t	Sig.
	B	Std. error	Beta		
All patients of CAD[a]					
Constant	6.342	0.776		8.175	0.00
Natural log of age (years)	-0.508	0.140	-0.318	-3.621	0.000
Natural log of BMI (kg/m^2)	-0.136	0.164	-0.073	-0.831	0.408
Gender of patient	-0.281	0.078	-0.318	-3.589	0.000
Calculated Gensini score	0.000	0.001	-0.043	-0.483	0.630
Diabetic status	0.131	0.059	0.201	-2.228	0.028
Diabetic patients of CAD[b]					
Constant	6.742	1.759		3.832	0.000
Log age	-0.638	0.366	-0.232	-1.746	0.087
Gender of patient	-0.288	0.104	-0.367	-2.772	0.008
Log BMI	-.0142	0.247	-0.078	-0.573	0.569
Log Gensini	-0.004	0.053	-0.009	-0.069	0.945
Non-Diabetic patients of CAD[c]					
Constant	5.848	0.889		6.578	0.00
Log age	-0.518	0.152	-0.404	-3.42	0.001
Gender of patient	-0.278	0.134	-0.243	-2.076	.042
Log BMI	-0.079	0.237	-0.041	-.0331	0.74
Log Gensini	0.02	0.04	0.07	0.57	0.57
Risk factors for severity of CAD[d]					
(Constant)	3.17	3.14		1.01	.31
Log BCAA	0.11	0.30	0.04	0.37	0.71
Log age	1.12	0.47	0.23	2.38	**0.02**
Gender of patient	0.17	0.27	0.06	0.65	0.52
Log BMI	-1.27	0.52	-0.22	-2.44	**0.02**
Diabetic status	0.36	0.19	0.18	-1.90	**0.06**

UC, unstandardized coefficients; SC, standardized coefficients.

[a] For all patients, a. dependent variable: Log BCAA; ANOVA p<0.001; adjusted R2=18. *[b] For diabetic patients, a. dependent variable: Log BCAA; ANOVA P=0.04, adjusted R2=11.7; *[c] for non-diabetic patients, a. dependent variable: Log BCAA; ANOVA P=0.002; adjusted R2=0.25. [d]risk factors for severity of CAD

The causal relationship between diabetes mellitus and serum BCAA remains vague. In a recent study by Mahendran *et al*. (2017) the investigators used a genetic risk score of the known variants associated with circulating BCAA levels or insulin resistance as instrumental variables to examine the causal effect of fasting plasma BCAA levels on insulin resistance and vice versa (Mahendran *et al*., 2017). They concluded that it was in fact increased insulin resistance which led to raised levels of BCAA. However, more studies are required to clarify this relationship further.

Discussion

Several studies have previously reported the association of BCAA with CAD (Yang *et al*., 2015; Shah *et al*., 2010). To the best of our knowledge, our study reported the association of serum BCAA levels with the severity of CAD as given by Gensini score for the first time. In our cohort, this relationship did not reach a statistical significance (p=0.71). Furthermore, there was no relationship between BCAA levels and BMI in our cohort. Previous research by (Yang *et al*., 2015) has shown a direct positive correlation of BMI with BCAA levels (Yang *et al*., 2015). However, this finding in a cohort of Chinese patients with more than 50% coronary artery stenosis and included healthy controls as well. The differences in inclusion criteria may account for the dissimilarity in the results of the previous and the current study.

In our study, serum BCAA levels were also related to male gender (beta=+0.32, p<0.001) and increasing age (beta=0.32, <=0.001). Our results are congruent with the published literature. Pitkanen *et al*. (2003) have reported that men have higher levels of essential amino acids as compared to women. This is hypothesized to be related to differences in the muscle mass and protein metabolism between the genders (Tipton, 2001). The same study also reports decreased BCAA levels with increasing age which is also consistent with our findings (Tipton, 2001). These findings have recently been validated with several new studies (Calvani *et al*., 2018; Chaleckis *et al*., 2016) In the current study, severity of CAD is linked with older age (beta=0.23 and p=0.02) and lower BMI (beta -0.22, p=0.02).

The relationship between BMI and CAD is controversial. Although some previous researches have indicated that obesity is a risk factor for CAD (Poirier *et al*., 2006; Must *et al*., 1999). Recently some contradictory findings have surfaced. Bhattachariya *et al*. (2014) reported significantly lower BMI in CAD patients as compared to controls. This corroborates our findings of inverse relationship of BMI and severity of CAD (Bhattachariya *et al*., 2014). According to a meta-analysis by Wang *et al*. (2015) short term mortality in CAD patients is inversely

related to BMI which further validates our findings.

Conclusions

In CAD patients, serum BCAA levels are affected by age, male gender and presence of diabetes mellitus. The severity of CAD is related to BMI and age. High BCAA levels are not linked with the severity of CAD either in diabetics or in non-diabetics.

Statement of conflict of interest

The authors have declared no conflict of interest.

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