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ORIGINAL ARTICLE

Serum levels of Branched Chain Amino Acids and its Correlation with Coronary Artery disease severity in diabetics and non-diabetics

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ABSTRACT

Background: Branched chain amino acids are classified as essential amino acids, their role in metabolic disease has recently been established. The current research sort to identify the correlation of BCAAs with severity of coronary artery disease in diabetics and non-diabetics.
Aim: To measure and compare blood levels of BCAAs in the following groups of subjects: Diabetics and Non diabetics undergoing angiography under the clinical suspicion of coronary artery disease. Secondly, to correlate serum BCAA levels with Coronary Artery Disease severity by using Gensini Score in each group.
Methods: 119 patients who underwent angiography were recruited from cardiology department Mayo hospital, Lahore. They were allocated into two groups, diabetic and non-diabetic on the basis of HBA1c. 5 ml blood was collected, serum was separated and BCAA levels were measured by using internationally available calorimetric kit. Results were described as mean ± SD. Normality of continuous variables were assessed by using Shapiro Wilks test. Variables which were not normally distributed were log transformed.
Results: Over all 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). Factors determining the likely diabetes in CAD patients were male gender (p=0.013, Odds ratio 4.92s), BCAA levels (p=0.03, Odds ratio 1.05), age (p=0.01, odds ratio=1.08) and Gensini Score (p=0.05, odds ratio=1.013).
Conclusions In CAD patient's serum BCAA level are affected by age, male gender and diabetes status. Secondly the severity CAD is mainly affected by BMI and age. However the correlation between Gensini score and BCAA is not significant.
Keywords: Coronary artery disease, branched chain amino acids, diabetic, serum level

INTRODUCTION

Cardiovascular disease (CVD) is now becoming the foremost reason for morbidity and mortality all over the world. There are multiple risk factors which enhance the probability of coronary artery disease, including hypertriglyceridemia[1], smoking[2,3], sedentary life style, male gender, family history and diabetes[(4)]. Actually, a huge percentage of cardiovascular events happen in persons with risk lower than projected with reference to the above mentioned risk factors. Therefore researchers are much more concerned to identify novel biomarkers of CAD. For instance many researches have been conducted and shown a strong association between BCAAs and CAD[5].

BCAAs consist of valine, Leucine, Isoleucine and others[6]. These are essential amino acids[6]. Body can't synthesize these amino acids therefore they are supplied by diet. Diet rich of BCAAs include meat and dairy products. There is also evidence that BCAA may be produced by the gut micro biota[7].

BCAAs activates mammalian target of Rapamycin (mTOR); mTOR is classified biochemically a serine threonine kinase which detects the nutrition and energy status of the cell and its milieu[8]. There are two types of mTOR complexes, mTORC 1 and mTORC 2. BCAAs activates mTORC 1 which in turn activates P70-S6 kinase cascade, this results in phosphorylation of serine/ threonine residues, which suppresses insulin receptor substrate 1(IRS-1) that transmits insulin signals to intracellular pathway. As a result patient develops insulin resistance and subsequent type 2 diabetes.

Increased levels of BCAAs are associated with insulin resistance (9, 10)and diabetes[11]. Increased levels of BCAAs are also positively associated with risk factors of coronary artery disease(CAD)[(12)] including body mass index(BMI), blood pressure, carotid intima media thickness(cIMT), obesity ,fasting blood glucose, serum triglycerides, , apo B, apo CII, apo CIII and negatively associated with HDL C and apo AI[12,13].

Several published articles have demonstrated that circulating levels of BCAAs are directly proportional to the risk of cardiovascular disease[14,15]. The subjects in all these studies included both non-diabetics and diabetics. The present study sought to investigate the association of BCAA with CAD severity in diabetics and non-diabetics separately. We thus designed a research in which we compared serum levels of BCAA in diabetics and non-diabetics underwent angiography and correlated the Coronary Artery Disease Severity with serum BCAA levels. Coronary artery disease severity is measured by using Gensini scoring system[(16).] Gensini scoring system is widely used to assess the severity of CAD.

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Hypothesis: Increased levels of serum BCAAs in patients are associated with development of coronary artery disease.
Objectives: To measure and compare serum levels of Branched Chain Amino Acids in the following groups of subjects:
Non diabetics undergoing angiography under the clinical suspicion of coronary artery disease
Diabetics undergoing angiography under the clinical suspicion of coronary artery disease
To correlate serum BCAA levels with Coronary Artery Disease severity by using Gensini Score in each groups

OPERATIONAL DEFINITIONS

Non diabetics: Glycated hemoglobin (HbA1c) ≤48mmol/mol (<6.5%)(75).

Diabetics: Glycated hemoglobin (HbA1c) ≥48mmol/mol (≥ 6.5%)

Coronary Artery Disease Severity Score: Gensini scoring system will be used to identify the severity of coronary artery disease. According to this scoring system the stenosis in the * lumen of coronary arteries is assigned a score[16].

"1 to 25% stenosis, score 1
26 to 50% stenosis, score 2
51 to 75% stenosis, score 4
76 to 90% stenosis, score 8
90 to 99% stenosis, score 16
100% stenosis(total occlusion), score 32

There after multiplied by a constant number according to the location of lesions along the coronary arteries. A multiplication constant of 5 points are assigned for left main coronary artery (LMCA) lesions. Whereas 2.5 points are assigned for left circumflex (LCX) or proximal left anterior descending (LAD) artery lesions.

Regarding LCX and LAD, lesions involving mid segments and distal segments are given points 1.5 and 1 respectively.
The lesions involving first obtuse marginal (OM) and diagonal branches, intermediate and posterior descending arteries (PDA) are generally multiplied with constant 1.
While lesions in second OM and diagonal branches are assigned the multiplication constant 0.5.
Gensini score <25 shows mild atherosclerosis and score >25 shows severe atherosclerosis.

MATERIALS AND METHODS

This cross sectional comparative study was finished within 12 months after the approval of synopsis and purchase of kits and consumables conducted in Cardiology Department Mayo Hospital King Edward Medical University, Lahore after permission from Ethical Review Committee. Sampling technique used was mpm probability convenient sampling. Sample size of 112 patients (56 patients in each group) is estimated by using 95% confidence level, 90% power of test with expected mean value of diabetic group as 183.6±35.5 and non diabetic group as 162.3±35.5 using the following formula[20].
$n= \sigma 2(Z1-\alpha+ Z1-\beta)2$
$(\mu_o- \mu a)2$ Where
σ2 = variance, SD=35.5
Z1-α=confidence level=95%=1.96 Z1-β=power of test 90%
μ_o= population mean #1=183.6 μa= population mean #2=162.3
Inclusion criteria:
Age 18 to 65 years
Both genders will be included
People already undergone angiography under the clinical suspicion of coronary artery disease
Diabetics and non-diabetics will be differentiated using HBA1c as mentioned in operational definitions
Exclusion criteria:
Age<18 or >65 years
People receiving lipid lowering drugs in preceding 6 months[21].
Diabetic patients with HbA1C < 6.5
Patient processing: Informed consent was taken from all participants. The patients who underwent angiography d/t clinical suspicion of coronary artery disease(CAD) were assessed according to inclusion and exclusion criteria. A total of 119 patients were recruited from the cardiology department of Mayo Hospital Lahore. The patients were interviewed and Performa was filled. Age was calculated from date of birth. Drug history was recorded for the last 6 months. Height in meters and weight in kg were recorded by a standard height weight machine.

After informed consent 5 ml blood was drawn from the patients by venipuncture from brachial vein with the help of a disposable syringe using sterile technique. 2 ml blood was poured into CBC vial containing EDTA for HBA1c and 3 ml blood was poured into serum vial for BCAA measurement. CBC vial was inverted several times so that EDTA completely mixed with blood.

Serum vial which contained 3 ml of blood was centrifuged for 5 minutes at 2500 RPM and serum separated. Serum was extracted with the help of micro pippete and poured into micro centrifuge tube (eppendorf). Each eppendorf was properly labeled according to the number assigned to the patients. Serum samples were stored at -20°C.
Descriptive Statistics: All data was entered in SPSS-20. Quantitative continuous variables like age, BCAA levels, BMI and HBA1c were presented as mean ± SD, while Gensini score which was an ordinal variable was presented as median ±IQR. Shapiro Wilk's test was used to determine normal distribution for continuous quantitative variables. (As age, BCAA levels, BMI and HBA1c were not normally distributed they were transformed into their natural logs for further analysis by multiple regression models. Qualitative variables like gender and diabetes status were presented as frequency and percentages).

Pie Chart was used to represent gender distribution graphically. Bar Charts were constructed to visually depict the differences in HbA1C, BCAA, BMI and age differences between diabetics and non-diabetics.
Analytical statistics: Age, HbA1C, Gensini score, BCAA levels were compared in diabetics and non-diabetics by independent sample t-test. Gender Distribution between the two groups was compared by Chi Square test.
Regression Analysis: As BCAA levels, and CAD severity are multifactorial, multiple regression analysis was used to assess the relationship with other variables instead of simple correlation tests like Spearman correlation test which are used when there are no confounders[22].

Factors affecting Serum BCAA levels in all patients of CAD were assessed by using multiple linear regression modeling. Reliability of the model was assessed by ANOVA and relevance was measured by R^2*(see footnote) change. As a further sensitivity analysis, the analysis was repeated in diabetics and non-diabetics separately.

The likely of diabetes in CAD patients was determined by multiple logistic regression analysis. Presence of diabetes was the dependent variable whereas BCAA, gender, BMI, Age and Gensini score were entered as risk factors in the model. p value ≤0.05 was taken as significant.

*R^2 change here refers to the predictive value of the regression model and nor r^2 which is the correlation coefficient used in Pearsons and Spearman's correlation tests

RESULTS

The cohort was composed of 119 patients (As extra reactions were available in the kits we did additional sampling). Their characteristics are summarized in table 1. Total number of diabetic patients were 55, among them 41 were males and 14 females. Total number of non-diabetic patients were 64, 59 were males and 5 females.
1. Gender distribution, Median Gensini scores, mean age and mean HBA1c levels were significantly different between the two groups at p=0.01, 0.02, 0.01 and <0.001 respectively. However there was no significant difference between diabetic and non-diabetic subjects as far as serum BCAA levels (p=0.710) and BMI (p=0.30) were concerned.

The effect of age, BMI, Gender, Gensini score and diabetic status of the subjects on serum BCAA levels was assessed by mulitple linear regression as table 2. The model was statistically significant (ANOVA p<0.001) and adjusted R2 revealed that our proposed risk factors accounted for 0.18 of changes in BCAA levels. Over all three factors affected
P values here reflect differences in the two groups which were adjusted in regression analysis: BCAA levels; age, gender and diabetic status. The results showed that age of patients was negatively associated (Beta= -0.32) with BCAA levels at p <0 .001. Gender was another significant factor associated with BCAA levels at p=<0.001. Women tended to have lower BCAA levels as compared to men (Beta=-0.32). Diabetes status of patients were also linked with BCAA levels, at p=03, Beta=-0.20. On the other hand Gensini score (p=06, Beta=-0.04) and BMI (p=0.41, beta=-0.07) showed no significant association with serum BCAA levels.

As a sensitivity analysis the regression model was repeated for diabetic and non-diabetic patients separately. For diabetic subjects the regression model was statistically significant at p=0.04 with Adjusted R2 change of 0.12. When only diabetics were considered male gender came out to be the only significant risk factor for raised serum BCAA levels (p=0.01, beta -0.37). Effect of age (p=0.087, beta –o.23), BMI (p=0.57, beta -0.57) and Gensini score (p=0.95,beta -0.067) did not reach statistical significance.

Table 1: Patient Characteristics

Patient Characteristic	Diabetic Patients (n=55)	Non diabetics (n=64)	Significance (p)
Gender	41 men 14 women	59 men 5 women	0.01
Gensini Score (Median ±IQR*)	49.50±41.00	33.00±38.80	0.02
Age in years(Mean±SD**)	51.93±6.13	47.41±10.16	0.01
HbA1C (%)***	8.96±1.69	5.58±0.35	<0.001
BCAA**** in mM/uL	31.73±11.08	30.99±10.63	0.71
BMI***** in kg/m2	24.48±4.78	23.65±4.00	0.30

*IQR Inter quartile range; **SD=Standard deviation; ***percentage of glycated Hemoglobin;
****BCAA= serum branched Chain amino acids; *****BMI= Body Mass Index

Table 2 Factors affecting Serum BCAA levels in all patients of CAD

Model	Unstandardized Coefficients		Standardized Coefficients	T	Sig.
	B	Std. Error	Beta		
(Constant)	6.342	.776		8.175	.000
Natural Log of age (years)	-.508	.140	-.318	-3.621	.000
Natural log of BMI (kg/m2)	-.136	.164	-.073	-.831	.408
Gender of patient	-.281	.078	-.318	-3.589	.000
Calculated Gensini Score	.000	.001	-.043	-.483	.630
Diabetic and Non Diabetic Patients	-.131	.059	-.201	-2.228	.028

a. Dependent Variable: lnbcaa; ANOVA <0.001; Adjusted R2=18

Figure 1: Mean BCAA levels in diabetic and non-diabetic CAD patients

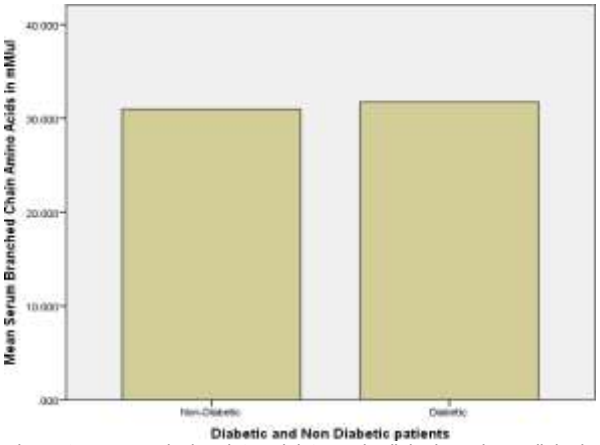
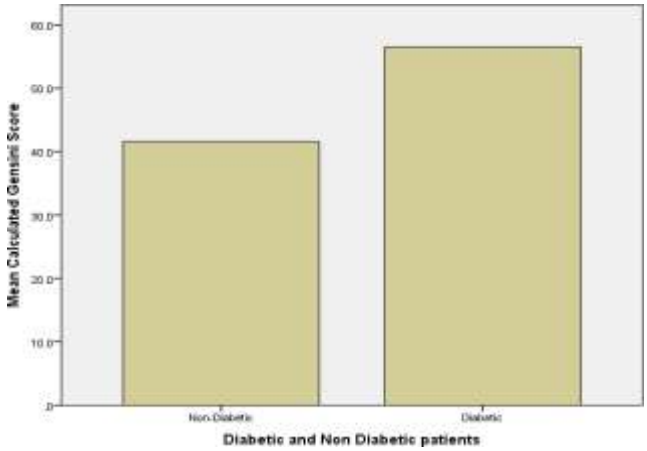


Figure 2: Mean calculated Gensini Score in diabetic and non-diabetic CAD patients



DISCUSSION

The results of the current study are focused on the following main themes

1. Factors affecting serum BCAA levels
2. Risk factor for coronary artery disease severity as assessed by Gensini score and
3. Factors determining the likelihood of diabetes in patients of CAD.

To the best of the author's knowledge the current research is the first ever in Pakistan on any aspect related to Branched chain amino acids and their role in metabolism and disease. In the current study raised BCAA levels were found to be associated with younger age (p<0.001), male gender (p<0.001) and presence of diabetes (p=0.03). Our results are similar to previous studies. Alfaqih et al in 2018 reported that serum BCAA levels were highly significantly associated with diabetes status at p<0.001[23].

Study by Nakamura et al has shown that levels of Branched chain amino acids valine, Leucine and isoleucine are higher in patients with higher fasting insulin levels and HbA1C as compared to controls[24]. This is similar to our findings in which we found that BCAA levels were significantly higher in patients of diabetes even after adjusting for age, BMI, gender and severity of CAD.

The current study unveils a statistically significant association of Diabetes with serum BCAA levels in the cohort of CAD patients. This is backed up by previous researches. However the causal relationship between diabetes and BCAA remains vague. A recent study by Mahendran et al the investigators used a genetic risk score (GRSs) of the identified genetic variants linked to circulating BCAA levels or IR as instrumental variables to study the causal effect of BCAA concentrations on IR and vice versa[(25)]. They concluded that it was in fact increased insulin resistance which led to increased levels of BCAA. However further studies are required to elucidate this relationship.

Researchers have proven the positive relationship between a BCAA and their general metabolic well-being[17,18]. Even though there are some proposed beneficial outcomes of BCAAs on metabolic and general health, an increase in the levels of BCAAs is associated with an increasing risk of insulin resistance (IR) and T2DM in patients and in animal models[19]. This seemingly contradictory role of BCAAs in metabolism opens way for more research[26].

Several studies have reported on the association of BCAA with CAD. E-g Yang et al have shown that raised BCAA levels are associated risk of CAD[27]. Similarly Shah et al has shown increased blood levels of BCAAs in acute coronary syndrome patients in comparison with healthy subjects[28]. In the current study the risk of CAD with increased BCAA levels was not assessed per se but the association of BCAA levels with severity of CAD was evaluated. In the current study there was a mild direct relationship between BCAA levels (Beta=+0.036) and Gensini score but this relationship did not reach a statistical significance (p=0.71). The said study did not control for lipid lowering medication and thus their results are likely to be confounded by other factors. In the current study all patients on lipid lowering were excluded from the study. This could account for the differences in results. There was no relationship between BCAA levels and BMI in our cohort. Other researchers have shown a direct positive correlation of BMI with BCAA levels[27]. However Yang et al reported 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.

In the current study serum BCAA levels were also positively associated with male gender (beta=+0.32, p<0.001) and increasing age (beta=0.32, <=0.001). Our results are congruent with published literature. Pitkanen et al has reported that men have higher levels of essential amino acids as compared to women[29]. This is hypothesized to be related to differences in the muscle mass and protein metabolism[30]. The same study also reports decreased BCAA levels with increasing age which is also in line

with our findings[29]. These findings have recently been validated with further new studies[31,32]. In the current study severity of CAD as indicated by Gensini score was positively associated with age (beta=0.23 and p=0.02) and inversely associated with BMI (beta -0.22, p=0.02). In the current cohort there were no significant correlations with serum BCAA (as discussed above), diabetes status or gender.

The relationship between BMI and coronary artery disease is controversial. Although some previous researches indicated obesity is a risk factor for CAD [33, 34], recently some contradictory findings have surfaced. Bhattachariya et al reported significantly lower BMI in CAD as compared to controls. This supports our findings of inverse relationship of BMI and severity of CAD[35]. A latest meta-analysis by Wang et al shows that short term mortality in CAD patients is inversely related to BMI which further validates our findings. It has been shown that diabetics are more likely to have CAD as compared to controls in subjects from CATHGEN bio repository, USA [35]. The same relationship has been shown in Chinese population as well[27]. This is similar to our findings where diabetic patients of CAD tended to have a higher Gensini score than non-diabetics however this association failed to reach statistical significance probably because of low sample size.

It has been shown recently that BMI may have an inverse relationship with risk of diabetes however this relationship was not shown to be significant[23] The same study however showed a direct relationship with increase waist circumference and diabetes risk. This strengthens our argument that BMI alone may not be a valid and sufficient indicator of body adiposity and total lipid content.

There were several limitations to our study. First the current study is only limited to one center. However Mayo Hospital is the largest hospital of Pakistan and one of the largest tertiary care hospitals of South East Asia. Patients are referred here from all over the province.[36] Therefore data from this center is most likely to be representative of the status of cardiovascular patients in the Punjab. Second, several risk factors for coronary artery disease were missed in patients data collection including smoking history, dietary history and genetic risk factors. However the focus of the current study was the role of BCAA in CAD. Therefore data collection was focused around factors affecting BCAA levels. Nevertheless it is proposed that future studies should incorporate all risk factors associated with CAD when studying BCAA levels. Third genetic analysis to study variation in genes of BCAA metabolic pathway was not conducted. Although this would have offered a most comprehensive examination of serum BCAA levels determinants, this was beyond the scope of the current study.

It is recommended that the role BCAA in development of CAD in diabetics and non- diabetics be further explored via a multidisciplinary approach. Genetic analysis documenting the polymorphisms and mutations in the genes involved in metabolism of BCAA are necessary to develop a comprehensive model predicting BCAA levels in CAD patients. Furthermore a prospective cohort study is recommended in which risk of CAD is assessed with reference to BCAA levels over a long term follow-up. This will aid in establishing causality relationship between BCAA and CAD.

CONCLUSIONS

In CAD patient's serum BCAA level are affected by age, male gender and diabetes status. Secondly the severity CAD is mainly affected by BMI and age. High serum levels of branched chain amino acids are not significantly correlated with coronary artery disease in both diabetic and non-diabetic groups of patients.

REFERENCES

1. Kushner PA, Cobble ME. Hypertriglyceridemia: the importance of identifying patients at risk. Postgrad Med. 2016;6:6.
2. Ambrose JA, Barua RS. The pathophysiology of cigarette smoking and cardiovascular disease: an update. Journal of the American college of cardiology. 2004;43(10):1731-7.
3. Choi BG, Rha SW, Park T, Choi SY, Byun JK, Shim MS, et al. Impact of Cigarette Smoking: a 3-Year Clinical Outcome of Vasospastic Angina Patients. Korean Circ J. 2016;46(5):632-8.
4. Reusch JE. Diabetes, microvascular complications, and cardiovascular complications: what is it about glucose? The Journal of clinical investigation. 2003;112(7):986-8.
5. Ruiz-Canela M, Toledo E, Clish CB, Hruby A, Liang L, Salas-Salvadó J, et al. Plasma branched-chain amino acids and incident cardiovascular disease in the PREDIMED trial. Clinical chemistry. 2016:clinchem. 2015.251710.
6. Zheng Y, Li Y, Qi Q, Hruby A, Manson JE, Willett WC, et al. Cumulative consumption of branched-chain amino acids and incidence of type 2 diabetes. Int J Epidemiol. 2016;13.
7. Bucci M. Gut microbiome: Branching into metabolic disease. Nature Chemical Biology. 2016;12(9):657-.
8. Weichhart T. Mammalian target of rapamycin: a signaling kinase for every aspect of cellular life. Methods Mol Biol. 2012;821:1-14.
9. Zhao X, Han Q, Liu Y, Sun C, Gang X, Wang G. The Relationship between Branched-Chain Amino Acid Related Metabolomic Signature and Insulin Resistance: A Systematic Review. J Diabetes Res. 2016;2794591(10):25.
10. Yoon MS. The Emerging Role of Branched-Chain Amino Acids in Insulin Resistance and Metabolism. Nutrients. 2016;8(7).
11. Newgard C. Interactions of branched-chain amino acids and lipids in metabolic disease. The FASEB Journal. 2016;30(1 Supplement):383.3-.3.
12. Yang R, Dong J, Zhao H, Li H, Guo H, Wang S, et al. Association of branched-chain amino acids with carotid intima-media thickness and coronary artery disease risk factors. PLoS One. 2014;9(6).
13. Bhattacharya S, Granger CB, Craig D, Haynes C, Bain J, Stevens RD, et al. Validation of the association between a branched chain amino acid metabolite profile and extremes of coronary artery disease in patients referred for cardiac catheterization. Atherosclerosis. 2014;232(1):191-6.
14. Ruiz-Canela M, Toledo E, Clish CB, Hruby A, Liang L, Salas-Salvadó J, et al. Plasma branched-chain amino acids and incident cardiovascular disease in the PREDIMED trial. Clinical chemistry. 2016;62(4):582-92.
15. Yang RY, Wang SM, Sun L, Liu JM, Li HX, Sui XF, et al. Association of branched-chain amino acids with coronary artery disease: A matched-pair case-control study. Nutr Metab Cardiovasc Dis. 2015;25(10):937-42.
16. Ucar FM, Acar B, Gul M, Ozeke O, Aydogdu S. The Association between Platelet/Lymphocyte Ratio and Coronary Artery Disease Severity in Asymptomatic Low Ejection Fraction Patients. Korean Circ J. 2016;46(6):821-6.
17. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nature Reviews Endocrinology. 2014;10(12):723.
18. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E, et al. Metabolite profiles and the risk of developing diabetes. Nature medicine. 2011;17(4):448.
19. Giesbertz P, Daniel H. Branched-chain amino acids as biomarkers in diabetes. Current Opinion in Clinical Nutrition & Metabolic Care. 2016;19(1):48-54.
20. Shah SH, Bain JR, Muehlbauer MJ, Stevens RD, Crosslin DR, Haynes C, et al. Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. Circulation: Cardiovascular Genetics. 2010:CIRCGENETICS. 109.852814.
21. Yang P, Hu W, Fu Z, Sun L, Zhou Y, Gong Y, et al. The positive association of branched- chain amino acids and metabolic dyslipidemia in Chinese Han population. Lipids in health and disease. 2016;15(1):120.
22. Licht MH. Multiple regression and correlation. 1995.
23. Alfaqih MA, Abu-Khdair Z, Saadeh R, Saadeh N, Al-Dwairi A, Al-Shboul O. Serum branched chain amino acids are associated with type 2 diabetes mellitus in Jordan. Korean journal of family medicine. 2018;39(5):313.
24. Nakamura H, Jinzu H, Nagao K, Noguchi Y, Shimba N, Miyano H, et al. Plasma amino acid profiles are associated with insulin, C-peptide and adiponectin levels in type 2 diabetic patients. Nutrition & diabetes. 2014;4(9):e133.
25. Mahendran Y, Jonsson A, Have CT, Allin KH, Witte DR, Jørgensen ME, et al. Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. Diabetologia. 2017;60(5):873-8.
26. Yoon M-S. The emerging role of branched-chain amino acids in insulin resistance and metabolism. Nutrients. 2016;8(7):405.
27. Yang R, Wang S, Sun L, Liu J, Li H, Sui X, et al. Association of branched-chain amino acids with coronary artery disease: A matched-pair case–control study. Nutrition, Metabolism and Cardiovascular

Diseases. 2015;25(10):937-42.
28. Shah SH, Bain JR, Muehlbauer MJ, Stevens RD, Crosslin DR, Haynes C, et al. Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. Circulation: Genomic and Precision Medicine. 2010:CIRCGENETICS. 109.852814.
29. Pitkänen H, Oja S, Kemppainen K, Seppä J, Mero A. Serum amino acid concentrations in aging men and women. Amino acids. 2003;24(4):413-21.
30. Tipton KD. Gender differences in protein metabolism. Current Opinion in Clinical Nutrition & Metabolic Care. 2001;4(6):493-8.
31. Calvani R, Picca A, Marini F, Biancolillo A, Gervasoni J, Persichilli S, et al. A Distinct Pattern of Circulating Amino Acids Characterizes Older Persons with Physical Frailty and Sarcopenia: Results from the BIOSPHERE Study. Nutrients. 2018;10(11):1691.
32. Chaleckis R, Murakami I, Takada J, Kondoh H, Yanagida M. Individual variability in human blood metabolites identifies age-related differences. Proceedings of the National Academy of Sciences. 2016;113(16):4252-9.
33. Poirier P, Giles TD, Bray GA, Hong Y, Stern JS, Pi-Sunyer FX, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss: an update of the 1997 American Heart Association Scientific Statement on Obesity and Heart Disease from the Obesity Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation. 2006;113(6):898-918.
34. Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH. The disease burden associated with overweight and obesity. Jama. 1999;282(16):1523-9.
35. Bhattacharya S, Granger CB, Craig D, Haynes C, Bain J, Stevens RD, et al. Validation of the association between a branched chain amino acid metabolite profile and extremes of coronary artery disease in patients referred for cardiac catheterization. Atherosclerosis. 2014;232(1):191-6.
36. Mayo Hospital Lahore. [cited2019 23/01.2019]; Available from: http://www.mayohospital.gop.pk/index.php.