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Amino Acids Profile and Vitamin D Measurement in Hypertension in Egyptian Population

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Authors' contributions

This work was carried out in collaboration among all authors. Author THS designed the study and supervised the chemical analysis of all parameters. Author HHA helped in samples collection and clinical evaluation of the patients. Author AF performed the statistical analysis. Author SAA made the chemical analysis of some parameters. Author MFM made the chemical analysis of some parameters, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author MEF made the chemical analysis of some parameters, managed the analyses of the study and revised the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Objective: To investigate the association between plasma free amino acids (PFAAs) profile changes, vitamin D concentration and hypertension and evaluate the clinical utility of this association for nascent hypertension before the development of complications.
Methods: 70 subjects were enrolled in this study; 50 of them were hypertensive (25 were with uncontrolled hypertensive and 25 were with controlled hypertensive), the other 20 subjects were healthy controls.
Results: Circulating levels of Branched chain amino acids (BCAAs); (valine, leucine, and isoleucine), Aromatic amino acids (AAAs); (phenylalanine, tyrosine, and tryptophan), homocysteine, aspartic, ornithine, asparagine and lysine were elevated significantly in both uncontrolled and controlled hypertensive subgroups in comparison with control group. On the

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contrary, the results showed marked decline in the concentration of threonine, serine, methionine, and arginine amino acids in the two hypertensive subgroups compared to control group. Moreover, there was a marked decrease of vitamin D level in hypertensive population in comparison with control.

Conclusion: There is obvious association between PFAAs profile changes, hypovitaminosis D and hypertension.

Keywords: Hypertension; branched chain amino acids; aromatic chain amino acids; homocysteine; hypovitaminosis D.

1. INTRODUCTION

Hypertension leads to one death every eight deaths according to the world health organization estimation; therefore, hypertension is considered the third leading killer worldwide [1]. Universally, hypertension results in one billion hypertensive cases and four million deaths every year [1]. It is epidemiologically expected that the significant increase in poor health outcomes be directly related to the burden of high blood pressure globally. Worldwide spread of hypertension is estimated to be higher by 15-20% by 2025. Good management of high blood pressure is fundamental to any scheme designed to handle high blood pressure at the public level, but at the same time management and control is potentially costly [1]. Therefore, nowadays the new attitude is the early diagnosis of hypertension before the development of cardiac diseases and other complications with different biomarkers.

Free plasma amino acids (FPAAs) level is recently introduced as promising biomarkers for evaluation and expectation of early Hypertension but still under research [2]. Dietary BCCAs clustered with AAAs and proline displayed a positive association with incidence of hypertension [3]. Mangge et al. [4] also found that unrelated to Body Mass index (BMI) classification, BCAAs particularly Val and Leu, were proposed as a metabolic risk marker in cardiac diseases. Mels et al. [5] found that serine, glycine, alanine, histidine, and methionine were more abundant in the black group who experienced more arterial stiffness and were more vulnerable to be hypertensive. BCAAs in combination with plasma phenylalanine in the same cluster, displayed a direct correlation with systolic and diastolic BP [6]. According to Hsu and Tain [7] fetal programming in pregnant women is affected by the impairment of tryptophan metabolism, leading to the development of hypertension in adult offspring. On the other hand, glycine, tyrosine, methionine and alanine showed very different results in different studies concerning the relation between their plasma levels and risk for hypertension [8,9,10]. Onyemelukwe and Maiha, [11] reported that hyperhomocysteinaemia is more prevalent in North-Western Nigerian hypertensives than normal controls. They also noticed that plasma of patients taking anti-hypertensive drugs contains lower homocysteine than those who are not receiving anti-hypertensive medications. Several studies tried to investigate the cause of increased plasma homocysteine level and postulated that a deficiency in vitamin B12 could affect the plasma homocysteine [12,13] or folate deficiency [14,13] or by severe renal dysfunction [15].

Data form numerous studies support an association between hypertension and serum hypovitaminosis D [16,17,18]. Possible mechanisms linked insufficiency of vitamin D with BP have been postulated such as, insulin resistance [19], regulatory effects on the renin–angiotensin–aldosterone system (RAAS) [20]. Hence, the current study also examined the relationship between hypertension and plasma 25-OH vitamin D.

The central aims of our study were to investigate the role of measuring plasma free amino acids and total plasma 25-OH vitamin D as potential early biomarkers of nascent hypertension before the development of complication and correlate free plasma amino acids level with lipid profile in our locality.

2. SUBJECTS AND METHODS

Subjects: The current study is classified as a case control study with 70 subjects divided into two groups. Group (1): included 50 hypertensive patients (11 males and 39 females) who were chosen indiscriminately from cardiology Hospital, Assiut University between December 2018 and June 2019. Their ages ranged between 26 and 77, divided into 2 subgroups, subgroup1A: included 25 patients (6 males and 19 females)

with uncontrolled hypertension at time of sampling with no medication, subgroup1B: include 25 patients (5 males and 20 females) with controlled hypertension under medication. Group (2): control group included 20 completely healthy subjects (4 males and 16 females); with age and sex matched to the patient's group.

The diagnosis of hypertensive patients using a mercury sphygmomanometer and the Korotkoff sound technique. The procedure is carried out through measuring blood pressure two times on the right arm, with an interval half a minute at least and with a minimum resting period 15 minutes. The accuracy of this technique is 2 mm Hg, and then we calculate the average of two readings as the actual pressure of subjects. The start of the first sound is an indication to systolic blood pressure (SBP) and its disappearance refer to the diastolic blood pressure. Hypertension is known as systolic blood pressure>140, or diastolic blood pressure > 90 [21].

Participants' personal data were gathered by a well-formulated survey. All participants were informed of the aim of the study before filling the questionnaire. The questionnaire was filled by those who read and write and by the researcher for those who did not read or write and included the following data: personal data. Medical history including duration of Hypertension, medication used, and presence of other complications associated with Hypertension.

Exclusion criteria: Subjects who have history of coronary artery diseases, cerebral vascular accidents, diabetes mellitus and cancers. In addition, lactating and pregnant women were excluded, and those who refuse to be included in the study.

Sample collection: Six milliliters venous blood samples (of antecubital vein) were collected from each patient and control and were divided into 3 tubes: Two milliliters of blood were collected in a tube containing Heparin for Amino acid profile assessment. The second two milliliters were gathered in a tube having potassium EDTA (ethylene diamine tetra acetic acid) for measurement of total homocysteine level and total vitamin D. The third two milliliters were collected on plain test tube for lipid profile assessment. The tubes were inverted gently to mix the contents. The tubes were centrifuged at high-speed run (3000 rpm) for around 10 minutes. Then the serum and plasma were isolated and kept at -20°C until time of analysis. Randomly, ten milliliters urine samples were obtained for estimation of microalbumin/creatinine ratio, which were stored at -20°C to be analyzed lately.

Other investigations: The following investigations were done for all participant to exclude any complications of Hypertension: ECG, funds examination, ankle brachial index.

Methods: High performance liquid chromatography was used to measure plasma free amino acids using Sykam Amino Acid Analyzer S 433 provided by Sykam GmbH. Germany CAT. NO. 1120 001. For preparing free amino acids samples from plasma, we use sulfosalicylic acid followed by centrifugation for precipitation. Free amino acids remaining in the supernatant were stored at -20°C to be analyzed lately, using high performance liquid chromatography specifically through ion exchange separation technique. Column used for separation and analysis was cation separation column LCAK06/Na. size: 150 mm x 4.6mm. Catalog No. 51 12 007. Asymmetry: 0.8-1.5, and column pressure: 40-75bar. Specification range: MET Efficiency: >20000, while resolution THR/SER>1300. For total homocysteine level measurement, we used Human Homocysteine ELISA kit, supplied by SinoGeneClon Biotech Co., Ltd, China (catalogue No: SG-10387). For the quantitative measurement of total plasma 25-OH vitamin D, we used Total 25-OH vitamin D EIA kit, supplied by Epitope Diagnostics, Inc. San Diego, CA 92121, USA (Catalogue No: KT-715).

Serum total cholesterol was determined by enzymatic colorimetric technique provided by spectrum-diagnostic, Egypt CAT.no.230003 (Caraway, 1976). The serum high-density lipoprotein cholesterol was estimated by enzymatic colorimetric precipitation method supplied by spectrum-diagnostic, Egypt catalog no. 266002 (Warnick & Wood, 1995). The serum triglycerides were estimated by enzymatic colorimetric method supplied by spectrum diagnostics, Egypt catalog no. 314003 (Bucolo G, 1973). Low-density lipoprotein was estimated by calculation. Determination of microalbumin/creatinine ratio in the urine was carried out by DRG®Micro-Albumin ELISA (EAI-2361) kit supplied by DRG International, Inc., US (Gaines-Das, 1988) for microalbuminuria, and Spectrum Diagnostics Creatinine-Jaffè, supplied by Egyptian Company for Biotechnology (S.A.E), (catalogue No: 234 001) for creatinine.

Statistical Analysis: Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests and showed non-parametric distribution. Chi-square test and fisher exact test used to compare between categorical variables while comparing between continuous variables in more than two groups in non-related samples was done by Kruskal Wallis test; Mann Whitney was used to compare between two groups in non-related samples. Correlation coefficients by Pearson correlation test. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

3. RESULTS

The results of all participants regarding ECG, funds examination, and ankle brachial index results were within normal variations. Figs. 1 and 2 show no significant difference among groups in age and sex. Fig. 3 shows that 60% of subgroup 1A (uncontrolled hypertensive patients) were negative and 40% had mild while no moderate cases for microalbuminuria test. As for subgroup 1B (controlled hypertensive patients), 80% were negative, 12% had mild, and 8% were moderate for microalbuminuria test. On the other hand, all control cases were negative for microalbuminuria test. There was a significant difference between control and hypertensive subgroups in microalbuminuria test (p=0.003).

Table 1 shows the lipid profile results in all groups. There was insignificant difference in total cholesterol level between hypertensive subgroups (1A and 1B) and control. For triglycerides level there was insignificant difference between hypertensive subgroups (1A and 1B), whereas there was a significant difference between subgroup 1A and control, and between subgroup 1B and control. High-density lipoprotein results show that there was nonsignificant difference between hypertensive subgroups (1A and 1B), while there was a highly significant difference between subgroup 1A and control, besides a significant difference between subgroup 1B and control. For low-density lipoprotein level, there was nonsignificant difference between hypertensive subgroups (1A and 1B); in contrast, there was a significant difference between subgroup 1A and control group, in addition to a highly significant difference between subgroup 1B and control. Very low-density results show insignificant difference between hypertensive subgroups (1A and 1B), while there was a significant difference between subgroup 1A and control group, along with significant difference between subgroup 1B and control.

Amino acids concentration in the study populations are shown in Figs. 4-10 and Table 2. Plasma free amino acids (PFAA) levels differed significantly between diseased and non-diseased subjects for hypertension.

There was a significant increase in BCAAs; valine, isoleucine, and leucine, as illustrated in Figs. 4, 5 and 6 respectively, in hypertensive subgroups compared to control group. Fig. 4 shows a significant increase in valine level in subgroup 1A in comparison with subgroup 1B, and significant increase in subgroup 1A and 1B in comparison with control group. Fig. 5 shows a significant elevation in isoleucine levels in study subgroups1A compared to subgroup1B, besides a significant elevation in subgroup 1A compared to control group, and a significant elevation in isoleucine level in subgroup 1B compared to control group. Fig. 6 shows a significant increase in leucine level in subgroup 1A compared to

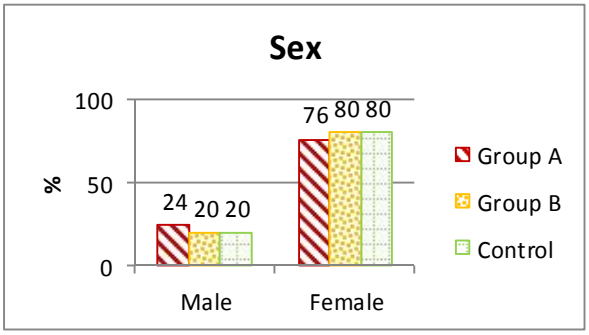

Fig. 1. Graphical presentation of sex ratio
Group A: Uncontrolled hypertensive patients.
Group B: Controlled hypertensive patients

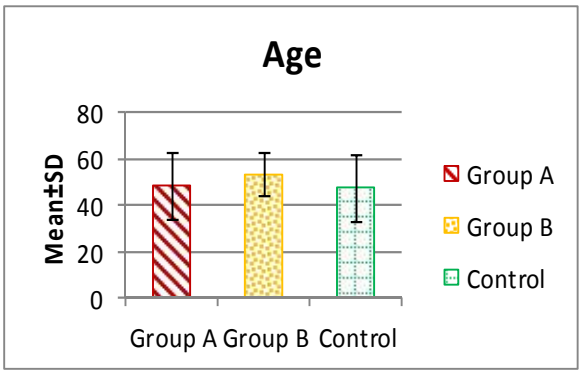

Fig. 2. Graphical presentation of age ratio
Group A: Uncontrolled hypertensive patients.
Group B: Controlled hypertensive patients

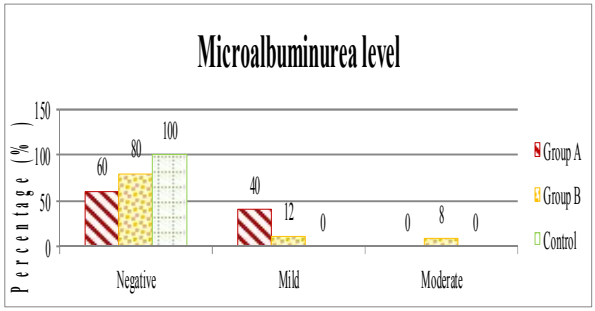

Fig. 3. Graphical presentation of microalbuminurea level
Group A: Uncontrolled hypertensive patients.
Group B: Controlled hypertensive patients

subgroup 1B, in addition to a significant increase in subgroup 1A in comparison with control group, but there was insignificant difference between subgroup 1B and control group.

Figs. 7, 8, and 9 show plasma levels of AAA; Tyrosine, Phenylalanine, and Tryptophan, respectively. Figs. 7 and 8 show a significant elevation in both tyrosine and phenylalanine levels in subgroup 1A compared to subgroup 1B, besides a significant elevation in subgroup 1A compared to control group, and also they show a significant elevation in subgroup 1B compared to control group. Fig. 9 shows a highly significant elevation in tryptophan level in subgroup 1B compared to subgroup 1A, in addition to highly significant elevation in subgroup 1B compared to control group. It also shows a significant elevation in tryptophan level in subgroup 1A compared to control group.

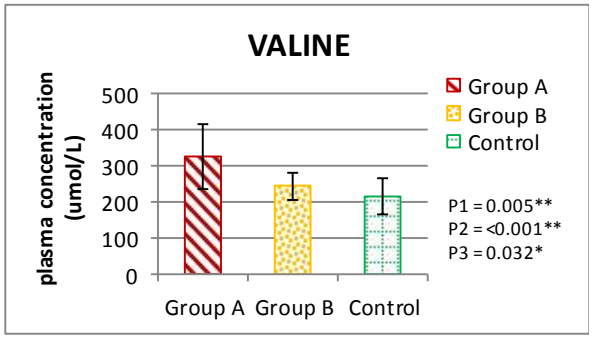

Fig. 4. Graphical presentation of valine level
*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

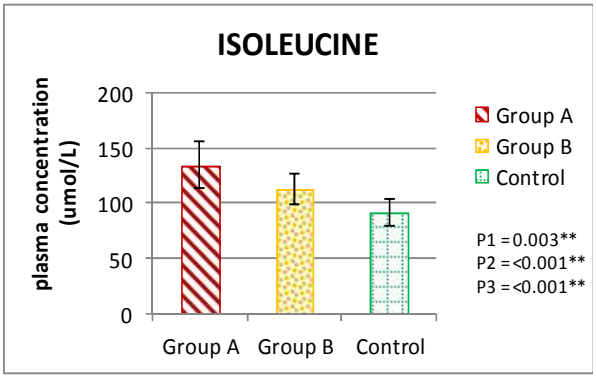

Fig. 5. Graphical presentation of isoleucine level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

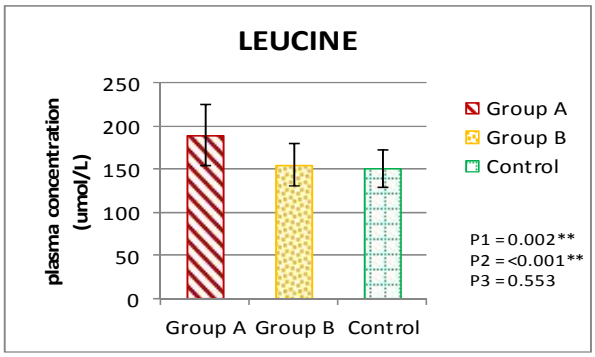

Fig. 6. Graphical presentation of leucine level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

Fig. 10 shows a significant elevation in plasma level of Homocysteine in subgroup 1A compared to subgroup 1B, and a remarkable significant elevation in subgroup 1A compared to control group, also there was a significant difference between subgroup 1B and control group.

Table 2 shows plasma level of various amino acids in all groups. There was a significant increase in Aspartic acid in hypertensive subgroups (1A and 1B) compared to control group. Table 2 also shows a significant increase in ornithine in subgroups 1A and 1B in comparison with control group. The data in Table 2 shows significant elevation in Asparagine in study subgroups (1A and 1B) in comparison with control group. Table 2 also shows significant increase in lysine in study subgroups (1A and 1B) in comparison with control group, contrarily, lysine plasma level was higher in subgroup 1B compared to subgroup 1A with significant difference. The level of Alanine as shown in Table 2 is significantly higher in subgroup 1A compared to both; subgroup 1B and control group.

According to the data in Table 2, the level of histidine was significantly elevated in subgroup 1A compared to subgroup 1B and to control group, while it was significantly higher in control group compared to subgroup 1B. Table 2 also shows a significant increase in Glycine level in subgroup 1A compared to 1B, and it shows insignificant increase in Glycine level in subgroup 1A compared to control group. On the other hand, a significant decrease in plasma levels of Threonine, Serine, Methionine, and Arginine were observed in hypertensive subgroups (1A and group 1B) compared to control group as shown in Table 2.

Fig. 11 illustrates a significant deficiency of plasma vitamin D level in hypertensive patients (subgroup 1A and 1B) compared to control.

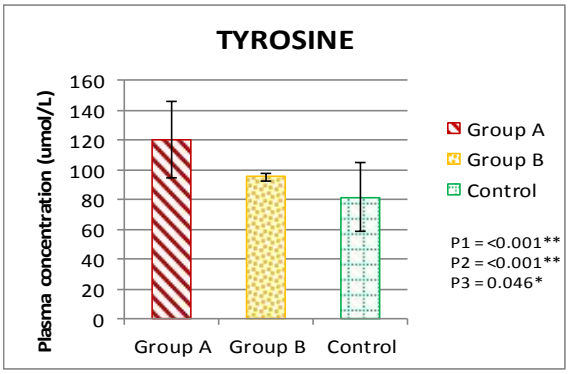

Fig. 7. Graphical presentation of tyrosine level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

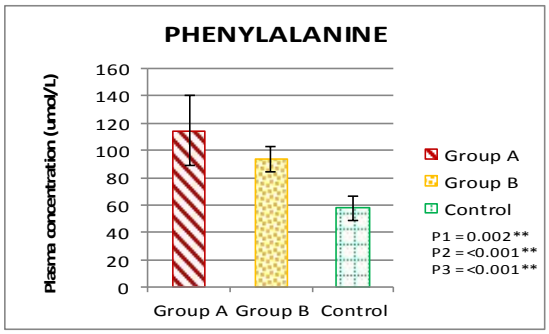

Fig. 8. Graphical presentation of phenylalanine level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

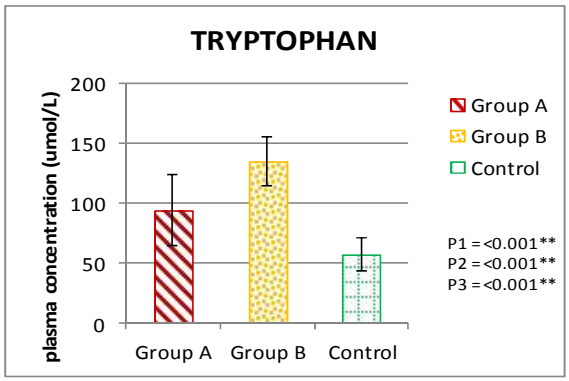

Fig. 9. Graphical presentation of tryptophan level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

Fig. 12 shows weak positive correlation between ornithine and TRG (r=0.421, p= 0.036) in subgroup A, and Fig. 13 shows weak positive correlation between ornithine and VDL (r= 0.414, p=0.040) in the same subgroup in addition, Fig. 14 shows moderate positive correlation between tyrosine and HDL (r= 0.507, p= 0.010) in subgroup A.

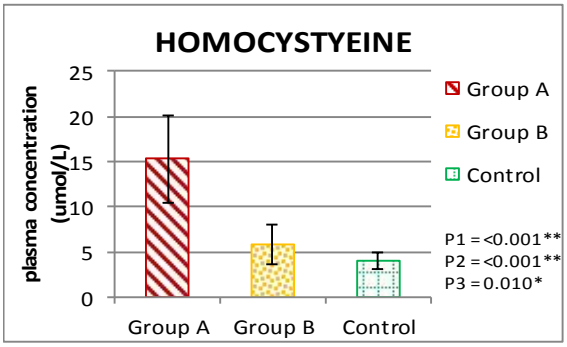

Fig. 10. Graphical presentation of homocysteine level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

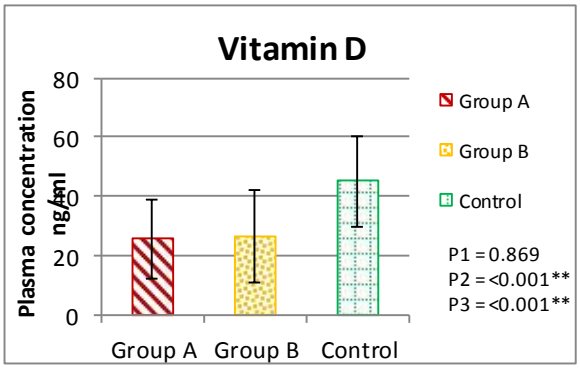

Fig. 11. Graphical presentation of vitamin D level

*Group A: Uncontrolled hypertensive patients. Group B: Controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

Table 1. Comparison between Lipid profiles in different groups

	Group A (n=25)	Group B (n=25)	Control (n=20)	P_1	P_2	P3
	Mean±SD	Mean±SD	Mean±SD			
T. cholest (mm/l)	232.14±108.2	240.56±137.19	170.55±21.91	0.961	0.057	0.136
TRG	69.48±52.61	80.4±52.84	120.3±32.97	0.368	0.001**	0.005**
HDL	45.12±10.15	48.33±9.53	56.5±6.21	0.239	<0.001**	0.002**
LDL	182.05±112.95	185.4±132.76	87.28±22.94	0.936	0.004**	0.024*
VLDL	13.9±10.64	15.72±10.86	24.06±6.58	0.402	0.001**	0.005**

*Data represented as mean±SD. P value <0.05 is a significant value. P_1: Group A vs Group B. P_2: Group A vs Control. P_3: Group B vs Control. T.cholest (total cholesterol). TRG (triglycerides). HDL (high-density lipoprotein). LDL (low-density lipoprotein). VLDL (very low-density lipoprotein). *P<0.05, **P<0.01 and***P<0.001*

4. DISCUSSION

The present study showed that circulating levels of BCAA, AAA, aspartic, ornithine, asparagine and lysine are elevated significantly in both uncontrolled and controlled hypertensive subgroups compared to control group. The results also showed that Homocysteine amino acid is associated with hypertension and it was higher in hypertensive patients than control group. Contrarily, the results showed marked decrease in plasma concentration of threonine, serine, methionine and arginine amino acids in the two subgroups of hypertensive patients compared to control group.

Table 2. Plasma concentration of amino acids in hypertensive subgroups and control

	Group A (n=25)	Group B (n=25)	Control (n=20)	P_1	P_2	P_3
	Mean±SD	Mean±SD	Mean±SD			
Aspartic	21.08±5.65	18.48±5.01	8.12±4.41	0.061	<0.001**	<0.001**
Ornithine	183.59±46.13	163.6±9.75	118.55±75.54	0.985	0.001**	0.004**
Asparagine	168.97±66.2	178.59±54.19	80.93±30.12	0.455	<0.001**	<0.001**
Lysine	293.03±53.7	327.23±24.73	157.25±33.59	0.041*	<0.001**	<0.001**
Alanine	372.23±100.09	240.39±48.42	240.9±41.24	<0.001**	<0.001**	0.982
Glycine	313.12±75.08	193.41±17.69	293.82±70.23	<0.001**	0.411	<0.001**
Histdine	109.88±28.38	67.59±5.64	82.66±12.56	<0.001**	0.001**	0.001**
Threonine	139.04±34.02	105.15±4.73	158.51±37.23	0.002**	0.047*	<0.001**
Serine	152.6±7.88	105.5±7.4	161.14±30.93	<0.001**	0.010*	<0.001**
Methionine	47.61±12.59	35.15±2.59	78.88±15.34	<0.001**	<0.001**	<0.001**
Argnine	119.45±42.98	78.23±9.12	187.35±196.45	0.001**	0.909	<0.001**

*Data represented as mean±SD. P value <0.05 is a significant value. Group A: uncontrolled hypertensive. Group B: controlled hypertensive patients. P_1: Group A vs Group B. P_2: Group A vs Control. P_3: Group B vs Control. *P<0.05, **P<0.01 and***P<0.001*

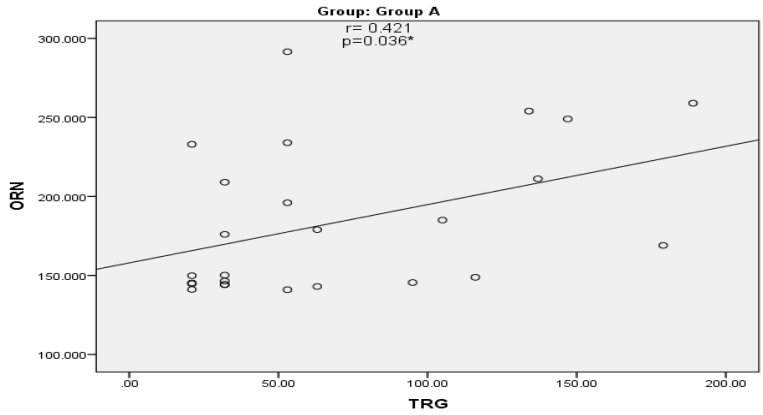

Fig. 12. Graphical presentation of correlation between ornithine and TRG

*Group A: uncontrolled hypertensive. ORN: ornithine. TRG: triglycerides. VDL: very low-density lipoprotein. (r) is person's correlation coefficient: P value <0.05 is a significant value, *P<0.05, **P<0.01 and***P<0.001*

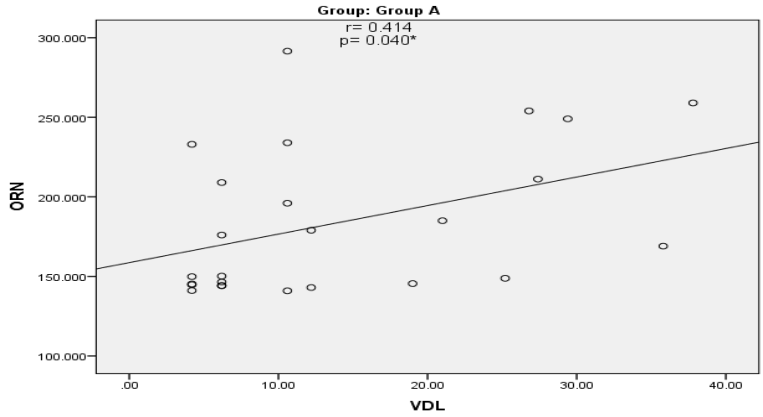

Fig. 13. Graphical presentation of correlation between ornithine and VLDL

*Group A: uncontrolled hypertensive. ORN: ornithine. TRG: triglycerides. VDL: very low-density lipoprotein. (r) is person's correlation coefficient: P value <0.05 is a significant value, *P<0.05, **P<0.01 and***P<0.001*

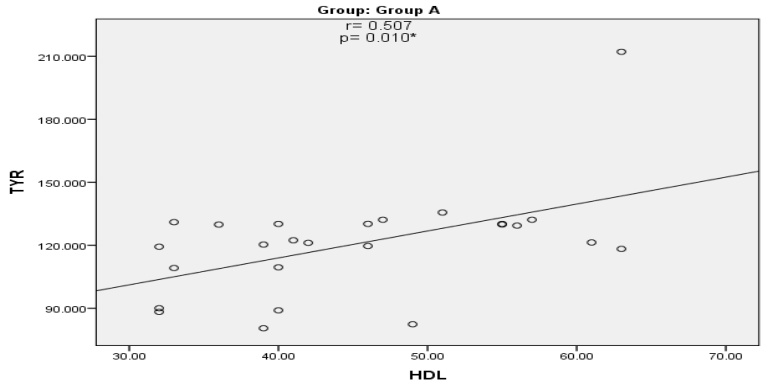

Fig. 14. Graphical presentation of correlation between tyrosine and HDL

*Group A: uncontrolled hypertensive. ORN: ornithine. TRG: triglycerides. VDL: very low-density lipoprotein. (r) is person's correlation coefficient: P value <0.05 is a significant value, *P<0.05, **P<0.01 and***P<0.001*

The results of BCAAs, phenylalanine and tyrosine are in accordance with Teymoori et al. [3] who in their study found that dietary BCCAs clustered with AAAs and proline showed a positive association with increased incidence of hypertension. These outcomes also are in accordance with Yamaguchi et al. [22] and Yang et al. [23] who found a positive relationship between BCCAs, AAAs and hypertension. Flores-Guerrero et al. [24] reported the usefulness of BCCAAs plasma level as a strong biomarker for the incidence of hypertension [24]. Although BCAAs levels in serum and plasma were directly related to hypertension, results from some studies, which based on dietary records, were inconsistent [6]. Batch et al. [25] and Soleimani et al. [26] showed that accumulation of BCAAs and their byproducts due to changes in BCAAs metabolism, are related to significant metabolic alterations such as insulin resistance that is linked to rising risk of high blood pressure. In the same context Martin et al. [27] found that, the attachment of bioactive peptide to the angiotensin converting enzymes (which is essential in blood pressure control) can be affected by hydrophobic or bulky residues, which exist in BCAAs and AAAs. Marta et al. [28] noticed obvious differences between subjects who were positive for metabolic syndrome (MS) and those who were negative for it, in terms of amino acids profile. Plasma concentration of individual amino acids; isoleucine, phenylalanine, leucine and valine, in addition the total concentration of BCAAs and AAAs were significantly greater in the metabolic syndrome positive group in comparison with the metabolic syndrome negative group.

According to Yamaguchi et al. [22] results there was elevation in the concentrations of BCAAs & AAAs in MS diseased compared to the MS non-diseased. Mangge et al. [4] also found that unrelated to Body Mass index (BMI) classification, BCAAs particularly Val and Leu, were proposed as a cardiometabolic risk marker. Yamakado et al. [29] mentioned that the amino acids levels act as an early biomarker for nascent hypertension within four years, their results also showed elevation in the concentrations of BCCAs and AAAs in those people who already developed hypertension compared to people who did not develop hypertension.

Pozefsky et al. [30] suggested that rising in BCAAs levels in lifestyle-related diseases is due to decline in insulin activity and using of amino acids in muscles which in turn decreased uptake of BCAAs in muscles. The key enzyme in BCAAs oxidative catabolism in visceral adipose tissue and liver; branched-chain alpha-keto acid dehydrogenase, was found to be significantly reduced in terms of abundance and/or activity in both patients and rodent animal who have insulin resistance [31,32]. Subjects with obesity or insulin resistance have a high proportion of BCAAs and their metabolic products, which resulted from partial catabolism [33,34]. Newgard, [35] suggested that the reason for rising in BCCAs level in insulin resistant and obese cases is the decrease in their breakage in adipose tissue. This decline may be explained as follows down regulation of enzymes that catabolize BCCAs due to the repression of peroxisome proliferator-activated receptor-γ (PPAR-γ) [31].

Magnusson et al. [36] proposed that high concentrations of BCAAs and AAAs in plasma raised susceptibility of high blood pressure by expected intermediate metabolites. Joyner et al. [37] postulated that elevation in blood pressure by stimulating the sympathetic system and intensify the vascular tone occurred due to metabolites of dietary AAAs. McCormack et al. [38] mentioned that BCAAs together with their metabolites have adverse effects on insulin resistance; consequently, they could influence blood pressure, closer to the relationship between high blood pressure and insulin resistance, which was suggested earlier by Soleimani et al. [26]. Other studies conducted by Tovar et al. [39] and Wessels et al. [40] assumed that threonine and tryptophan or glutamic acid entry inside the brain can be affected by elevated serum concentrations of BCAAs and serine, respectively, which eventually decline the biosynthesis of beneficial neurotransmitters for blood pressure.

Some studies postulated that tyrosine, serving as an initiator for biosynthesis of noradrenaline; regulate the amount of noradrenaline and consequently affects the sympathetic tone of blood vessels. Administration of tyrosine in rats lead to decrease in blood pressure and this action is attributed to the effect of catecholamine on α-receptors [41,42].

Most phenylalanine is hydroxylated into tyrosine, and the changes in tyrosine levels potentially affect blood pressure. Nonetheless, phenylalanine by itself can affect the production of tetrahydrobiopterin (BH4), which acts as a cofactor for hydroxylation reaction of aromatic amino acids that related to endothelium relaxation [43]. In the availability of large number of AAAs, BH4 oxidation can cause changes to its vasoactive features, which may lead to harmful consequences on the endothelium [44]. Serotonin (a monoaminergic neurotransmitter) biosynthesis requires Tryptophan as an initiator; serotonin receptors are found on adrenergic nerves at the level of the sympathetic vascular junction, possibly illustrating underlying mechanism of 5-hydroxy tryptamine effect on the vascular tone [45]. According to Hsu and Tain [7] fetal programming in pregnant women is affected by the impairment of tryptophan metabolism, leading to the development of hypertension in adult offspring. Reduction in animals' blood pressure was induced after administration of tryptophan [46]. Furthermore, peptides, which contain tryptophan that resulted from breakdown of food protein by enzymes, may suppress angiotensin-converting enzyme through intervention with the renin angiotensin axis, although still human studies-based evidence are needed [47].

Relating to methionine amino acid, its plasma concentration was markedly decreased in hypertensive subjects in comparison with control group. Ogawa et al. [48] postulated that the decrease of methionine plasma level in essential hypertension occurred because methionine is enzymatically converted to homocysteine, then homocysteine and serine are transformed into cystathionine, which in turn is converted to cysteine. Cysteine is eventually converted via hypotaurine to taurine, which has been shown to exert an antihypertensive effect. These results differed from results based on dietary records, where Systolic and diastolic blood pressure were augmented in association with increased dietary methionine [10]. Methionine is an indispensable amino acid; and homocysteine is one of its metabolic byproducts, when elevated, it may influence the endothelial function stimulating the production of asymmetrical dimethylarginine (ADMA), eventually lead to inhibition of nitric oxide synthesis [49]. Therefore, methionine affects blood pressure indirectly through elevation in homocysteine levels, as displayed in dietary supplementation-based studies with methionine in both humans and animals [50,51].

The present study showed significant reduction of arginine levels in both hypertensive groups compared to control group. Arginine is considered one of those amino acids, which is well known to have vasogenic features [52]. The beneficial influence of L-arginine on blood pressure supposed to be due to various mechanisms. One of them is due augmentation of NO production and improving its bioavailability in vascular smooth muscle cell by L-arginine, which in turn exhibit antihypertensive activities and essential to maintain vascular homeostasis [53,54]. Moreover, L-arginine has been illustrated to enhance insulin resistance [55,56], which has a great influence on the etiology of high blood pressure related to metabolic syndrome [57,58]. Many researchers illustrated the beneficial effects of supplementation of L-arginine in diet, for example decreasing both systolic and diastolic blood pressures levels [59,60]. On the other hand, studies concentrating specifically on arginine from diet, discarding supraphysiological intake, did not show any relationship between arginine and blood pressure [61,62]. Likewise, in

a Dutch elderly male population, dietary arginine did not associate with blood pressure [62].

Alanine findings in the current study are in accordance with Stamler et al. [63] who found a positive relation between dietary alanine and blood pressure in the INTERMAP study that represented as percent of total protein ingestion. Also, Tuttle et al. [10] in a cohort study (THIS-DIET study) as daily ingestion in absolute value, who found the same results. Mels et al. [5] found that, alanine, and histidine were more abundant in the black group who experienced more arterial stiffness and were more vulnerable to be hypertensive. In addition, Holmes et al. [64] found positive association of SBP and DBP with alanine. Yamakado et al. [29] results show elevation in the concentrations of alanine, histidine, and ornithine in population who developed hypertension compared to population who did not develop hypertension. According to Yamaguchi et al. [22] plasma free concentrations of alanine and ornthine are elevated in hypertensive diseased population compared to non-diseased population. Virdis et al. [65] has postulated that alanine and methionine raise the risk of hypertension.

Teymoori et al. [3] found that ingestion of serine and threonine (alcoholic amino acids) in addition to BCAAs and AAAs elevated the risk of high blood pressure to 83% due to synergistic effects and intervention among these different groups of amino acids.

Homocysteine results in the present study are in line with Rodrigo et al. [66] who has reported a direct relationship between essential hypertension development and hyperhomocysteinemia. Yang et al. [67] found a direct relationship between homocysteine and serum creatinine and urea nitrogen level in elder hypertensive males. The biochemical mechanisms, which explain the association between vascular diseases and hyperhomocysteinemia are still ambiguous, however some studies have postulated that, the bioavailability of nitric oxide (NO) is limited by homocysteine [68], elevated oxidative stress [69]. In addition, it changes the elastic features of the wall of the vessels [70]. Other mechanisms suggested by previous studies postulated that homocysteine could develop high blood pressure by increasing arterial stiffness [71], reducing vasodilation [72] and increasing resistance to insulin [73].

Homocysteine increases oxidative stress that causes injury to the vascular endothelium, which impairs the vasomotor regulation that depends on endothelium; these explanation makes the association between oxidative stress and hyperhomocysteinemia biologically reasonable [74].

It suggested that homocysteine has a pathological role in multiorgan damage; however, the pathophysiological mechanism through which it performs this damage effects still unclear, may be associated with impairment of vascular endothelial and the function of smooth muscle cell [70]. Also, it is postulated that hyperhomocysteinemia contributes to the damage of target organ, for example glomerular damage, related to high blood pressure [75]. On the other hand, one of the major risk factors for arterial vascular disease is mild hyperhomocysteinemia [76].

Hyperhomocysteinemia could diminish resistant vessels responses to vasodilators that depend on endothelium, therefore, partially interpret the adverse effects which lead to high blood pressure. Supplementation with folic acid, vitamin B12 and B6 decrease hyperhomocysteinemia, which in turn contribute to diminish blood pressure in subjects who have essential hypertension. In addition, the additional detrimental vascular effects, which are triggered by the elevated level of homocysteine, are supposed to be prevented by vitamin B and folic acid administration [66].

The obvious contribution of homocysteine-lowering treatment in reduction of both systolic and diastolic blood pressure elevated the possible casual role of homocysteine in the pathogenesis of hypertension [77,78].

Positive correlation between PFAA and dyslipidemia in our study is in accordance with Yamakado et al. [29] who postulated that PFAA is a marker, which directly reflects metabolic disturbances related to dyslipidemia. In our study, we found positive correlation between ornithine and triglycerides. Ornithine is the substrate or ornithine decarboxylase enzyme which is the rate limiting enzyme in the synthesis of polyamines. Leon et al. [79] found an interesting association between ornithine decarboxylase, polyamines and triglycerides synthesis and storage. Fukushima et al. [80], explained that - in non-diabetic Japanese population- individual BCAA and total BCAA

concentrations were correlated with HDL-C levels and serum triglycerides, however LDL-C level had weak association with BCAA in males and females. Moreover, they found that the risk of metabolic dyslipidemia elevated simultaneously with the increase of each BCAA and total BCAA. Also, Li et al. [81] established a relationship between high tyrosine and low HDL-cholesterol in association with type 2 diabetes mellitus in Chinese people.

Hypovitaminosis D results in hypertensive patients in the current study are in accordance with some previous studies, which evidenced the same results [16,17,18]. Vitamin D was postulated to regulate the renin-angiotensin-aldosterone system (RAAS) at the molecular and pathophysiological levels in human studies according to Forman et al. [82] and Santoro et al. [83]. Moreover, since it is proved that any alteration of (RAAS) is related directly to the hypertension development [84], so vitamin D deficiency might relate to hypertension. In addition, Wu et al. [19] interpreted the association between vitamin D and hypertension by vitamin D contribution in insulin resistance, which is related to hypertension also.

5. CONCLUSION

Our study showed that BCAA, AAA, homocysteine, aspartic, ornithine, asparagine and lysine amino acids are elevated significantly during hypertension and could be used as early predictive marker for hypertension after more research. On the other hand, our results showed significant decrease in the level of threonine, serine, methionine and arginine amino acids. Also there is a significant deficiency of plasma vitamin D level in hypertensive patients. These observations may help to understand the associating disturbance of metabolism during hypertension that may help in finding helpful dietary recommendations to hypertensive patients.

ETHICAL APPROVAL AND CONSENT

The ethical committee of faculty of Medicine, Assiut University has sanctioned the current study under IRB no (17100855). All participants in the study (patients and control) have given an informed consent.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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