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Orgınal Article

The Role of Amino Acids in Knee Osteoarthritis

Diz Osteoartritinde Amino Asitlerin Rolü

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Highlights

- No study in which all amino acids were examined, in the literatüre.
- Norvaline, leucine, isoleucine, alllo-isoleucine, cystathionine, phenylalanine, MHIS1, arginine, alanine, cystine, valine, threonine and tryptophan levels were low in knee OA.
- These amino acids may have an important role in the etiogenesis and pathogenesis of knee OA.
- These AAs can be used as potential systemic serum biomarkers.

Abstract

Background: Osteoarthritis is an important public health problem and the most common musculoskeletal disease in the World. The pathogenesis and etiology of osteoarthritis is still unclear. We aimed to make an amino acids analysis that will contribute to the pathogenesis, diagnosis and treatment of knee osteoarthritis.

Materail and Methods: The study included according to the radiological grading scale of Kellgren-Lawrence, 30 patients at Grade 1-2; Group 1 (Grade 1-2), 30 patients at Grade 3-4; Group 2 (Grade 3-4), 30 healthy controls; Group 3. We compared between groups age, sex, body mass index, Western Ontario and McMaster Universities, Short form-36 findings, and plasma-free amino acid levels.

Results:A comparison of the serum norvaline, leucine, isoleucine, allo-isoleucine, cystathionine, phenylalanine, 1-methyl hystidine, arginine, alanine, cystine, valine, threonine, and tryptophane levels of the knee osteoarthritis and control groups compared a statistically significant difference (p=0.001, p=0.001, p=0.001, p=0.001, p=0.044, p=0.003, p=0.001, p=0.035, p=0.010, p=0.011, p=0.001, p=0.001, p=0.003).

Conclusions

We consider that, norvaline leucine; isoleucine, allo-isoleucine, cystathionine, phenylalanine, 1-methyl hystidine, arginine, alanine, cystine, valine, threonine, and tryptophane amino acids could be, as potential systemic serum biomarkers for diagnosis of knee osteoarthritis.

Key words: Knee Osteoarthritis, Amino Acid Levels, Kellgren-Lawrence

ÖZ

Amaç: Osteoartrit dünyada en sık görülen kas iskelet sistemi hastalığı olup önemli bir halka sağlığı sorunudur. Osteoartritin patogenezi ve etiyolojisi hala belirsizdir. Diz osteoartritinin patogenezine, tanı ve tedavisine katkı sağlayacak bir Amino asit analizi yapmayı amaçladık.**Gereç ve Yöntem:** Çalışmaya, Kellgren-Lawrence radyolojik evreleme ölçeğine göre grup 1, evre 1-2 olan 30 hasta; grup 2, evre 3-4 olan 30 hasta; grup 3, 30 sağlıklı kontrol dahil edildi. Gruplar arasında yaş, cinsiyet, vücut kitle indeksi, Western Ontario ve McMaster Üniversiteleri Osteoartrit İndeksi, Short form-36 ve plazma serbest amino asit düzeylerini karşılaştırıldı.**Bulgular:** Diz osteoartritli ve kontrol gruplarının serum norvalin, lösin, izolösin, allo-izolösin, sistatiyonin, fenilalanin, 1-metil histidin, arginin, alanin, sistin, valin, treonin ve triptofan düzeyleri karşılaştırıldığında istatistiksel olarak anlamlı fark bulundu (p=0.001, p=0.001, p=0.001, p=0.001, p=0. 044, p=0,003, p=0,001, p=0,035, p=0,010, p=0,011, p=0,001, p=0,001, p=0,003).**Sonuçlar:** Norvalin, lösin izolösin, allo-izolösin, sistatiyonin, fenilalanin, 1-metil histidin, arginin, alanin, sistin, valin, treonin ve triptofan amino asitlerinin, diz osteoartritinin tanısında potansiyel sistemik serum biyobelirteçleri olabileceğini düşünüyoruz.

Anahtar Kelimeler: Diz Osteoartriti, Amino Asit, Kellgren-Lawrence

Introduction

Osteoarthritis (OA) is a degenerative disease that results in progressive cartilage destruction in heavily loaded joints as a result of certain genetic, mechanical, and biochemical factors (1). It is an important public health problem and the most common musculoskeletal disease in the world (2). It is most commonly observed in the knee joint and causes pain and dismobility (3,4). The incidence of OA increases by age. Particularly, It is more common in women over 50 years old than men (5). In the USA, it is predicted that 14 million people, including 3 millions of ethnic minorities, have a symptomatic knee OA (6).

The pathogenesis and etiology of OA is still unclear. Some genetic and environmental factors such as natural immune system, occupational exposure, obesity, ethnic origin and physical activity play a significant role in OA pathogenesis (7). There is no efficient approach to stop, eliminate or delay OA's progress.

Metabolism has an important effect on the development and progression of many diseases (8). In recent years, metabolomics (Lipids, small peptides, amino acids (AA), vitamins and nucleic acids) analyses have been performed to determine OA phenotypes and to examine biochemical indicators (9,10). Analysing metabolisms of body AAs under physiological and pathological conditions contributes to defining pathological mechanisms of some diseases and diagnosing and treating them (11).

We aimed to analyze serum free AA that will contribute to the diagnosis, prevention and treatment of knee OA. This study examines the relationship between radiological severity and serum free AA levels in patients with knee OA.

Materials and Methods

Sample , Ethics

The study was approved by the Ethics Committee of Clinical Research of Harran University (HRU/20.06.25). Written consent was obtained from all participants.

This study were include 60 patients with bilateral knee pain who were diagnosed with primary knee OA according to American College of Rheumatology (ACR) criteria and 30 healty controls. Patients were evaluated according to the radiologically according to the Kellgren-Lawrence (K-L) scale divided into two groups and healty controls. Group 1 included patients KL grade 1-2, group 2 included KL grade 3-4 and group 3 included healty controls.

The patients who had primary knee OA but were younger than 40 years or older than 60 years, the patients with systemic inflammatory or autoimmune diseases, the patients who have had glucocorticoid injection into their knee joint in the last three months or hyaluronic acid injection in the last six months, the patients who underwent total knee arthroplasthy or other knee surgeries, and the patients with malignancies exclude in the study.

Data collection

Age and gender were recorded. Body mass index (BMI) was calculated. Clinical severity was determined according to the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), while overall quality of life was measured using the Short Form-36 (SF-36) questionnaire.

5-ml blood samples were taken from patient groups and healthy controls into edta tubes. The collected samples were taken to a biochemistry laboratory in cool-packs. They were centrifuged at 5000 rpm for 10 mins. The supernatant body in the tubes was gently transferred into Eppendorf tubes through pipettes. Each plasma sample was first named and numbered and then transfused into numbered tubes. The samples were kept at -80ºC till the day the study was conducted.

JASEM amino acid kits were used in the study. To analyse plasma-free AA profile, the samples removed from -80ºC refrigerator were cooled at room temperature. Then, 50µl was taken into sterile Eppendorf tubes. Onto these tubes, 50µl of Internal Standard solution in amino acid kits were added. Each tube was carefully vortexed at 5 seconds. Later, 700µl of Reagent-1 in kits were added into vortexed tubes. The tubes were carefully re-vortexed at 15 seconds. The re-vortexed tubes were centrifuged at 3000 rpm for 5 mins. The supernatant body of the samples was gently taken from centrifuged tubes and transfused into HPLC vials through sterile pipettes. Then, for analysis, they were placed onto the tray in HPLC part of LC-MS/MS device (Shimadzu 8045, Japan) and scanned. As mobile phase, Mobile Phase-A and Mobile Phase-B in AA kits were used. Restek LC Columns was used as column.

Statistical analysis

SPSS 22.0 software (SPSS® for Windows Chicago, IL, USA) was used for statistical analysis. An evaluation of the distribution of the numerical data was performed using the Shapiro-Wilk test. When the distribution of the numerical data was normal or skewed, an independent-sample t-test or Mann-Whitney U test was used,

respectively. A Chi-Square test was used to compare categorical data. Results with a p value of <0.05 were considered statistically significant.

Results

Average ages and BMI of the groups are presented in **Table 1**. Average ages of group 1, group 2 and group 3 are 52.26±5.43; 53.20±4.87; 45.56±3.92 years. BMI of the groups are 33.15±5.86 kg/m^2; 35.37±6.11 kg/m^2; 27.64±4.67 kg/m^2, respectively. There was a statistically significant difference age and BMI were lower in the control group when compared to patient groups. (p<0.005, p<0.005).

Group 1 include 27 (90%) women and 3 (10%) men. Group 2 included 26 (86.67%) women and 4 (13.33%) men. Group 3 had 10 (33.33%) women and 20 (66.67%) men. While the ratio of women was higher in patient groups, the ratio of men was higher in healthy controls. There was a statistically significant difference (p<0.005). Comparisons of gender among groups are presented in **Table 1**.

Table 1. Age, BMI and gender in groups

	Group 1	Group 2	Group 3	p
Age(years)	52.26±5.43	53.20±4.87	45.56±3.92	**0.001**
BMI(kg/m^2)	33.15±5.86	35.37±6.11	27.64±4.67	**0.001**
Gender				
Female	27(90%)	26(86.67%)	10(33.33%)	**0.001**
Male	3(10%)	4(13.33%)	20(66.67%)	

WOMAC index and SF-36 data are presented in Table 2. WOMAC index analysis was not conducted for healthy controls. However, WOMAC score was found to be 49.33±22.28 in Group 1 and 57.75±17.19 in group 2. WOMAC scores weren't, significant difference between group 1 and group 2 (p=0.139). The difference between the groups was significant according to the findings of subgroups of SF-36 such as physical role limitations, emotional role limitations, social functionality, physical functionality, vitality, general mental health, pain and general health perception (p<0.005, p<0.005, p< 0.005, p<0.005, p<0.005, p<0.005, p<0.050, p<0.050 respectively). It was found that SF-36 findings were higher in healthy controls than in patient groups with relatively lower scores.

Table 2.WOMAC total score and SF-36 subgroup scores

	Group 1	Group 2	Group 3	p
WOMAC	49.33±22.28	57.75±17.19	-	0.139
SF-36 Physical functioning	54, 75±24, 84	33, 16±19, 27	86, 00±26, 40	**0.001**
SF-36 Vitality	43, 66±20, 59	40, 16±15, 72	64, 33±22, 15	**0.001**
SF-36 Mental health	55, 86 ±1, 71	49, 73±17, 40	68, 26±18, 28	**0.001**
SF-36 Pain	45, 32 ± 22, 57	32, 42±22, 71	77, 41±22, 17	**0.001**
SF-36 General health perception	39,16 ± 17.37	34,75±15,26	62, 66±14, 12	**0.001**
SF-36Role limitations (emotional problems)	37.77 ± 45.26	25.55±35.75	88.88±28.14	**0.001**
SF-36 Role limitations (physical problems)	39.16±42.89	25.83±33.78	85.83 ±29.12	**0.001**
SF-36 Social functioning	63.81±23.29	55.75±27.66	87.50±19.42	**0.001**

The analysis on AA levels in knee osteoarthritis which are significant presented in **Table 3**. The analysis on AA levels in knee osteoarthritis which are significant presented in **Table 3**. Regarding levels of norvaline, leucine, isoleucine, allo-isoleucine, cystathionine, phenylalanine, MHIS1, arginine, alanine, cystine, valine, threonine, and tryptophane, significant differences were observed among groups (p<0.001, p<0.001, p<0.001, p<0.001, p=0.044, p=0.003, p<0.00, p=0.035, p=0.010, p=0.011, p<0.001, p<0.001, p=0.006, respectively). When the difference among groups was examined, it was seen that levels of all parameters were lower in patient groups than those of healthy controls. However, for other AAs examined in the study, statistically significant difference was not observed among groups. Findings for these AAs are also presented in **Table 4**.

Table 3. AA's, which is a significant difference between groups

	Group 1	Group 2	Group 3	p
Norvaline	4.53±1.07	4.34±1.14	7.27±2.52	**0.001[a]**
Leucine	190.12±53.23	185.72±43.25	268.27±99.76	**0.001[b]**
Isoleucine	61.63±24.04	63.29±22.60	95.49±38.14	**0.001[c]**
Allo-isoleucine	22.81±8.01	22.88±7.61	34.84±14.00	**0.001[d]**
Cystathionine	0.87±0.45	0.93±0.47	1.25±0.76	**0.044[e]**
Phenylalanine	86.48±27.81	86.51±17.07	109.84±31.80	**0.003[f]**
Mhıs1	7.23±3.67	7.18±3.18	13.69±9.15	**0.001[g]**
Arginine	51.17±30.97	53.65±35.78	80.82±50.23	**0.035[h]**
Alanine	381.05±117.42	424.48±120.16	498.55±166.49	**0, 010[ı]**
Cystine	35.92±18.44	37.59±24.56	50.62±18.09	**0, 011[i]**
Valine	231.48±69.43	228.89±64.88	328.14±100.19	**0, 001[j]**
Threonine	133.00±40.96	118.41±59.95	198.12±97.09	**0.001[k]**
Tryptophane	65.20±19.84	67.20±17.62	83.42±27.46	**0.006[l]**

Abberivations:[a]significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.001), [b]significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.001), [c]significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.001), [d] significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.001), [e]significant difference was observed between group 3 and group 1 and group 2 (p=0.034, p=0.070), [f] significant difference was observed between group 3 and group 1 and group 2 (p=0.005, p=0.001), [g] significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.001), [h] significant difference was observed between group 3 and group 1 and group 2 (p=0.007, p<0.00) [ı] significant difference was observed between group 3 and group 1 and group 2 (p=0.003, p=0.001), [i] significant difference was observed between group 3 and group 1 and group 2 (p=0.023, p=0.031), [j] significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.001). [k] significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p<0.00), [l] significant difference was observed between group 3 and group 1 and group 2 (p=0.001, p=0.002)

Table 4. AAs with no significant difference between groups

	Group 1	Group 2	Group 3	p
Aspartic acid	18.95±7.29	16.11±6.75	21.41±10.13	0.071
Carnosine	0.84±0.82	0.88±1.08	2.53±4.72	0.530
Ethanolamine	11.30±3.95	11.01±3.45	10.68±5.73	0.703
Gaba	0.26±0.15	0.24±0.11	0.27±0.16	0.984
Homositrulin	2.03±1.88	1.98±1.29	2.71±1.93	0.212
Trans_4_OHProline	6.20±2.82	6.79±3.30	6.06±2.13	0.699
Orthophosphorylethanolamine	12.52±10.11	12.96±12.01	10.59±8.85	0.754
Glutamine	749.18±172.47	720.81±117.62	800.12±203.56	0.187
Glutamic acid	73.63±36.65	80.37±28.87	89.19±51.68	0.428
Glycine	200.26±147.57	223.52±93.79	216.59±138.12	0.352
Histidine	134.19±62.17	137.09±57.66	161.97±64.62	0.168
Lysine	201.78±41.29	208.48±49.04	243.69±76.51	0.053
Methionine	35.74±9.80	34.95±6.93	43.49±17.16	0.172
Ornithine	184.41±54.96	192.97±57.33	205.99±91.92	0.802
Proline	419.72±222.51	450.03±175.31	471.36±246.74	0.427
Serine	133.03±55.57	130.04±43.50	146.05±72.88	0.813
Taurine	44.70±16.95	46.75±18.10	46.39±22.80	0.878
4-OH Proline	7.79±3.78	8.82±4.13	7.38±2.77	0.334
5-OH-Lysine	1.20±1.75	1.04±0.58	1.26±1.11	0.388
Asparagine	90.86±24.36	94.50±23.49	95.80±29.60	0.889
Citrulline	43.64±16.50	42.21±13.69	51.23±18.06	0.162
Tyrosine	123.83±39.37	130.09±30.83	143.42±44.78	0.123
MHIS3	10.63±10.70	8.30±9.05	16.11±17.81	0.070

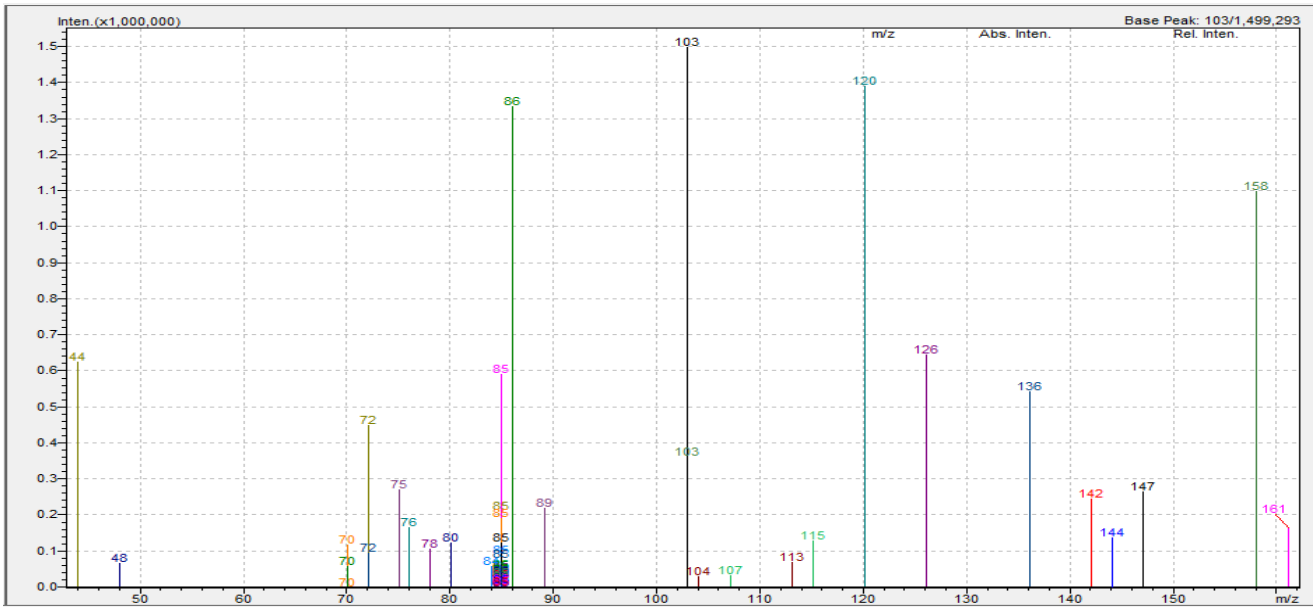

Figure 1.LC-MS/MS analysis parameters

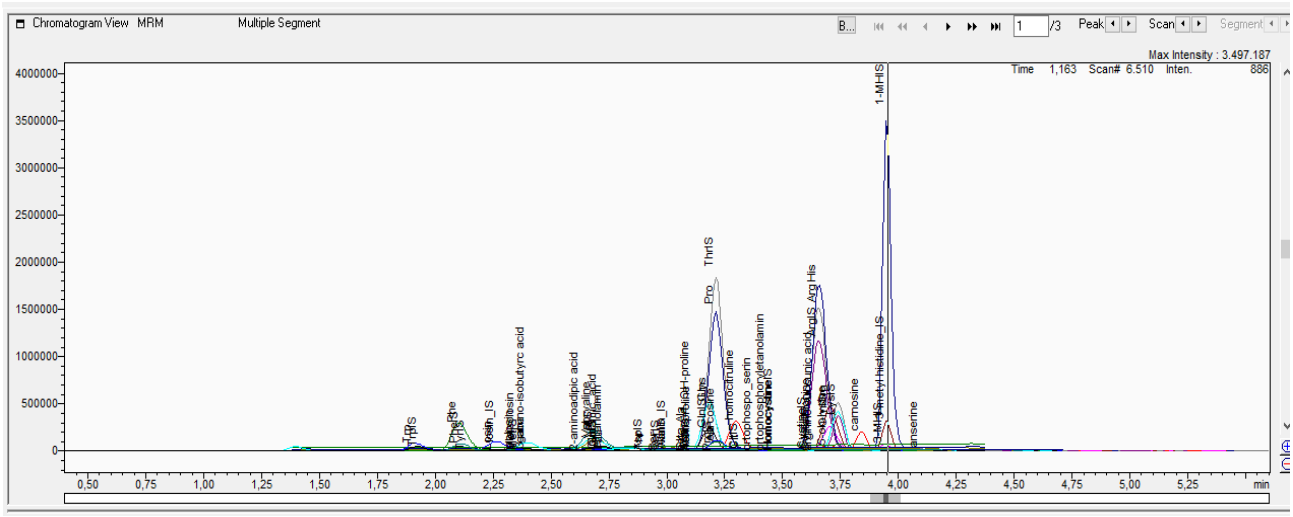

Figure 2.LC-MS/MS analysis parameters peak image

Discussion

In this study, serum norvaline, leucine, isoleucine, alllo-isoleucine, cystathionine, phenylalanine, 1-methyl histidine (MHIS1), arginine, alanine, cystine, valine, threonine and tryptophan levels were low in knee osteoarthritis. It can be thought that the deficiency of these amino acids may be effective in the etiology and pathology of knee OA.

Comparison of our data with other studies is difficult because of the small number of studies on AA in knee OA.

In a recent study, amino acids such as arginine, asparagine, leucine, phenylalanine and serine were high (12). We found the opposite in our study. This may be due to the fact that the other study included hip osteoarthritis. This may be due to the difference in the pathophysiology of hip OA and knee OA.

The study suggested that combined supplementation of allopurinol and L-arginine significantly inhibited inflammatory markers and these supplements could be very effective in OA cases (13). It has been reported that OA patients had lower arginine concentrations in their plasma, arginine / asymmetric dimethyl arginine and higher symmetrical dimethylarginine concentrations compared to healthy controls (14). Similarly, our study showed that lower levels of arginine in the patient groups. The Arginine supplementation can be effective.

Carnitine is a quaternary ammonium cation synthesized in the liver and kidney from the amino acids lysine and methionine; in a study concluded that supplementation improved the clinic in women with OA (15). We

did not observe any difference between the study groups for lysine and methionine amino acid levels. The lack of difference does not mean that it is not be used as a supplement.

A study reported that ratio of the branched-chain amino acids (BCAA; valine, leucine and isoleucine) to histidine were associated with knee OA and that valine and isoleucine levels increased in knee OA (16). Another study showed that increased cytokine levels resulting from an increase in BCAA concentration increase the rate of joint collagen degradation leading to OA (17). In contrast to the studies in literature, the concentrations of BCAAs such as valine, leucine and isoleucine were significantly decreased in patients with knee OA when compared to healthy controls in our study. This can be due to the differences between the material and method in the studies.

It is thought that the increase in glycine, proline and leucine concentrations increases the synthesis of type II collagen and thus the joint cartilage matrix is regenerated (18). In our study, glycine, proline and leucine levels were higher in the healthy group. This result supports the literature.

Threonine, homoserine, and allotreonine concentrations are increase after mechanical overload (19). In our study, glycine, serine and threonine concentrations was increase in patient groups with higher body mass index compared to healthy controls. However, glycine and serine concentrations was not statistically significant difference between the patient groups and the control group. However, plasma threonine concentration was found to be lower in both patient groups compared to the control group.

While 4-OH proline, glycine and histidine levels decreased, tryptophan levels increased in OA patients compared to healthy controls in a study (20). However, 4-OH proline, glycine and histidine levels did not change in our study, tryptophan levels were found to be lower in the patient groups. This is due to the differences between the material and method in the studies.

Kinurenic acid which is a metabolite of tryptophan, is thought to contribute to chronic inflammation in OA (21). Contrary to the study in literature, plasma tryptophane levels were found to be lower in patients with knee OA in our study.

Glutamate and aspartate levels in synovial fluid were 54 and 28 times higher in patients with synovitis than healthy controls. Therefore, it is considered that glutamate and aspartate contribute to arthritis pathogenesis in humans (22). Further studies indicate that glutamate and aspartate have significant impacts on activating inflammatory mediators such as (RANTES) protein, IL-8, TNF-α, which are expressed and secreted in synovial liquid of patients with inflammatory arthropathies including active rheumatoid arthritis, acute gout and symptomatic OA (23). In our study, although plasma aspartic acid and glutamate concentration was lower in patient groups than control group, the difference was not meaningful. This can be differences only plasma free AA levels were examined in our study. However, a combined study on the levels of synovial liquid or intra-articular tissues could have presented more useful findings.

Aging is the most powerful risk factor for OA. But, it is not an enough cause for OA (24). In our study, average age was higer in patient groups when compared to control group, confirming other studies in literature which argue that aging increases osteoarthritis.

There is a relationship between the incidence and prevalence of knee OA with BMI (25). Similarly, another study showed that obese women with a BMI of 30 to 35 kg / m2 had a four-fold higher risk of OA than non-obese women (26). Consistent with other studies in the literature, our study showed higher BMI in patient groups.

The OA is more common in men younger than 50 years old, its prevalence increases in women older than 50 years old (27). Therefore, knee OA prevalence is higher in women than men (6). However, the incidence and prevalence of OA become equal for both men and women at around 80 years old (28). Chu et al. found that knee OA prevalence is higher in women in all grades of the disease (29). In our study, gender distribution was observed difference between control group and patient groups which confirmed previous studies. Whereas the ratio of women was higher in patient groups, the number of men was higher in control group. Thus, findings confirm the claim that aging increases knee OA risk in women.

A significant correlation has been demonstrated between WOMAC scores and the radiological severity of knee OA. It has been suggested that there are significant differences in function, pain, and flexibility among patients with the same radiographic scores (30). In another study of 114 patients with knee OA, it was argued that K-L grading could not be associated with WOMAC pain sub-scoring (31). Similarly, in our study, no meaningful relation was found between radiological severity of knee OA and WOMAC score. These findings suggest that radiological severity of knee OA is not always compatible with clinical severity and symptoms.

Patients with severe knee OA had lower mean quality of life subscales (32). In a similar study, patients with knee OA were found to have lower scores on all sub-dimensions of SF-36 compared to healthy controls (33). In our study, SF-36 scores were found to be higher in the control group than in the patient group. According to

the findings of SF-36 sub-dimensions such as physical role limitations, emotional role limitations, social functionality, physical functionality, vitality, general mental health, pain and general health perception, the difference between the patient groups and the control group was found to be significant. Higher scores on SF-36 sub-dimensions indicate better health (34). Our findings confirming the studies in the literature also show that knee OA affects the quality of life. Thus, the quality of life can be increased and kept at an optimum level by determining the risk factors that cause the progression of OA, taking measures against it and organizing effective treatment.

Study limitations

There are some limitations in our study. For example; plasma free AA levels may be affected, such as comorbidities of patients, diet and medical treatments. Therefore, the findings of our study may be affected by these factors. In addition, only AA level in plasma was examined in our study. However, a combined study on the levels of synovial fluid or intra-articular tissues may provide more useful findings. Since our sample set is not very large, it lacks the high statistical power that can be provided by a larger population.

Conclusions

Norvaline, leucine, isoleucine, allo-isoleucine, cystathionine, phenylalanine, MHIS1, arginine, alanine, cysteine, valine, threonine and tryptophan levels were low in knee OA. Therefore, we conclude that these amino acids may have an important role in the etiogenesis and pathogenesis of knee OA. In conclusion, we think that these AAs can be used as potential systemic serum biomarkers and may be useful as dietary supplements in knee OA.

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Ethical Approval: *The study protocol was approved by* The Ethics Committee of Clinical Research of Harran University *no:* HRU/20.06.25

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