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RESEARCH ARTICLE

Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

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Abstract

BACKGROUND: Amino acids are important for proliferation and maintenance of tumor cells. Breast cancer patients were found to have significant changes in the number of amino acids, which are assumed to be correlated with the molecular subtypes of breast cancer. Therefore, current study was conducted to analyze plasma amino acids in breast cancer patients with luminal A and B subtypes.

METHODS: Breast cancer and control subjects were recruited, and venous blood was collected for the measurement of plasma amino acids. Total 19 plasma amino acids were measured using reverse-phase high-performance liquid chromatography with C18 column. Mean comparison for normally distributed and homogeneous data was further analzyed using independent sample T-test, with $p<0.05$ was considered as significant.

RESULTS: From total 19 amino acids, only 7 amino acids; cysteine, glutamic acid, histidine, ornithine, threonine, tyrosine, valine, were statistically different between the healthy control and breast cancer subjects. Eventhough no amino acids was found to be statistically different between breast cancer subjects with luminal A and B subtypes, but some amino acids were found to be significantly different when correlated to various breast cancer risk factors.

CONCLUSION: Amino acid profile of patients with Luminal A and B subtypes of breast cancer differs compared to healthy controls and is also correlated with breast cancer risk factors. Increase in cysteine level in Luminal A subtype patients and decrease of alanine and leucine in Luminal B subtype patients can be used as a biomarker.

KEYWORDS: amino acid, plasma, breast cancer, risk factor, biomarker

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Introduction

Breast cancer is the most common type of cancer in women.(1-3) Based on data of the International Agency for Research on Cancer, breast cancer was ranked as the second highest incidence cancer in the world. Breast cancer was the leading cause of cancer death among women.(4,5) Around 2.3 million cases were recorded, representing the fifth cause of cancer-related mortality. Breast cancer cases in Asia were higher than those in any other continent, especially in the South East Asian region.(6) By 2020, breast cancer continued as the most common cancer in women (30.8%) and the leading cause of death in Indonesia (15.3%).(7,8)

Development of breast cancer is influenced by several risk factors such as age, genetic and family history, *BRCA* mutation, first menstrual history, low parity, hormone usage history and hormone replacement therapy. The incidence of breast cancer also increases in the group of women aged >40 years.(8) Obesity has been reported to be associated with the development of breast cancer as well. Aromatization of adrenal androgen into estrogen at adipose tissue affected the development of breast cancer.(9,10)

Amino acids, essential nutrients in all living cells, are important for the proliferation and maintenance of tumor cells. Since tumor cells proliferate more rapidly, they need more amount of amino acids than the normal cells.(11) Interestingly, breast cancer cells limit the use of amino acids for cell proliferation based on amino acid availability, which depends on estrogenic receptor status.(12) Compared to the control group, breast cancer patients were found to have significant changes in the number of amino acids. An increase in the branched-chain group of essential and non-essential amino acids was reported, namely leucine, phenylalanine, aspartic acid, taurine, and lysine, among others.(10,13)

Tumor-dependent increase of serum amino acid levels has been reported to be correlated with molecular subtypes of breast cancer.(14) Therefore it is crucial to investigate further the amino acid in order to find potential biomarker for breast cancer. Current study was conducted to analyze plasma amino acids of breast cancer patients with luminal A and B subtypes.

Methods

Study Design and Subject Recruitment

Patients of Dr. Cipto Mangunkusumo National Central General Hospital in January to March 2020, aged ≥18-year-old with complete medical, histopathological and immunohistochemical results for breast cancer were recruited. All study subjects read, comprehended, and signed the written informed consents. This research protocol was approved by the Ethical Committee of Faculty of Medicine, Universitas Indonesia (#20-08-0877).

Amino Acid Profiling

For examination of amino acid, subjects fasted for at least 8 hours and then 2.5 mL of venous blood was collected and processed to obtain plasma. For the measurement of amino acids (alanine, arginine, aspartic acid, citrulline, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine and valine), the plasma was separated and analyzed using reverse-phase high-performance liquid chromatography (HPLC) (Waters 2695, Framingham, MA, USA) with C18 column. The solvent were 0.1M ammonium acetate pH 6.8 in acetonitrile, methanol, and water in composition of 44:10:46, respectively.(13,15)

Statistical Analysis

Data analysis was performed with SPSS version 25.0 (IBM Corporation, Armonk, New York, USA). Normality test was performed by using Shapiro-Wilk test. Normally distributed and homogeneous data were further analyzed for mean comparison with independent sample T-test. A $p<0.05$ was considered as significant.

Results

Subject Characteristics

Twenty-eight breast cancer and 29 healthy women were included in this study. Breast cancer subjects were characterized by breast cancer subtype, breast cancer stage, age, age of menarche, parity and family cancer history (Table 1). Most breast cancer subjects were having luminal A and B subtypes, T2 and T3 stages, age of ≥40 years, age of menarche of ≥12 years, multiparity and no family cancer history. Meanwhile, most healthy control subjects were having age of ≥40 years, age of menarche of ≥12 years, multiparity and no family cancer history as well.

Amino Acid Profiles of Healthy Control and Breast Cancer Subjects

Amino acid profile distribution of 28 healthy control subjects was normal and homogeneous for 13 amino acids (alanine, arginine, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, ornithine, phenylalanine, serine, threonine, tyrosine, valine) (Table 2). Based on these 13 amino acids of healthy control subjects, the amino acid profile distribution of 29 breast cancer subjects was further analyzed. Twelve amino acids were found normally distributed and homogeneous (alanine, arginine, cysteine, glutamic acid, histidine, isoleucine, leucine, ornithine, serine, threonine, tyrosine, valine) (Table 3).

Among the normally distributed and homogeneous 12 amino acids of breast cancer subjects, 7 amino acids (cysteine, glutamic acid, histidine, ornithine, threonine, tyrosine, valine) were found to be statistically different between the healthy control and breast cancer subjects

Table 1. Characteristics of breast cancer and control subjects.

Characteristics	Breast Cancer (n=29)				Healthy Controls (n=28)
	HER2 Positive Subtype (n=4)	Luminal A Subtype (n=10)	Luminal B Subtype (n=13)	Triple Negative Subtype (n=2)	
Breast Cancer Stage					
T2	3 (75%)	4 (40%)	4 (30.8%)	2 (100%)	
T3	1 (25%)	5 (50%)	7 (53.8%)	0 (0%)	
T4	0 (0%)	1 (10%)	2 (15.4%)	0 (0%)	
Age (year)					
<40	0 (0%)	1 (10%)	3 (23.1%)	0 (0%)	11 (39.3%)
≥40	4 (100%)	9 (90%)	10 (76.9%)	2 (100%)	17 (60.7%)
Age of Menarche (year)					
<12	1 (25%)	0 (0%)	2 (15.4%)	0 (0%)	8 (28.6%)
≥12	3 (75%)	10 (100%)	11 (84.6%)	2 (100%)	20 (71.4%)
Parity					
0-1 parity	1 (25%)	4 (40%)	1 (7.7%)	0 (0%)	10 (35.7%)
Multiparity	3 (75%)	6 (60%)	12 (92.3%)	2 (100%)	18 (64.3%)
Family Cancer History					
No	4 (100%)	8 (80%)	11 (84.6%)	0 (0%)	18 (64.3%)
Yes	0 (0%)	2 (20%)	2 (15.4%)	2 (100%)	10 (35.7%)

(Figure 1). However, these 7 amino acids were not statistically different between breast cancer subjects with luminal A and B subtypes (Figure 2). When the 7-amino-acids data of breast cancer subjects with luminal A and B subtypes were correlated with cancer stage, the glutamic acid was found to be statistically different between T2 and T3 of breast cancer subjects with luminal B subtype (Figure 3).

Table 2. Distribution and normality test of control subjects (n=28).

No.	Amino Acid	Distribution (Range)	*p*- value Normality Test	mean±SD
1	Alanine	298-841	0.459*	507.79±130.91
2	Arginine	88-206	0.756*	144.14±30.18
3	Aspartic Acid	2-46	0.017	-
4	Citrulline	11-86	0.002	-
5	Cysteine	20-74	0.064*	43.43±14.98
6	Glutamic Acid	53-140	0.471*	85.93±21.22
7	Glycine	151-527	0.173*	292.43±83.497
8	Histidine	66-152	0.862*	105.89±20.91
9	Isoleucine	42-145	0.659*	89.54±23.50
10	Leucine	96-262	0.179*	156.54±36.91
11	Lysine	136-356	0.013	-
12	Methionine	17-206	0.000	-
13	Ornithine	57-220	0.449*	125.50±37.40
14	Phenylalanine	58-128	0.031	-
15	Proline	77-657	0.001	-
16	Serine	59-151	0.736*	103.68±24.64
17	Threonine	88-270	0.601*	162.61±48.60
18	Tyrosine	49-133	0.066*	75.89±19.02
19	Valine	198-399	0.713*	290.79±49.02

*Normality test with Saphiro-Wilk. Data is distributed normally if $p>0.05$.

Table 3. Distribution and normality test of breast cancer subjects (n=29).

No.	Amino Acid	Distribution (Range)	*p*- value Normality Test	mean±SD
1	Alanine	154-665	0.825*	444.55±115.55
2	Arginine	91-211	0.524*	142.66±27.40
3	Cysteine	20-113	0.387*	58.62±18.53
4	Glutamic Acid	49-101	0.157*	73.69±15.72
5	Glycine	162-623	0.001	-
6	Histidine	41-115	0.972*	79.86±17.56
7	Isoleucine	50-136	0.158*	86.24±22.75
8	Leucine	75-222	0.455*	137.72±37.05
9	Ornithine	33-133	0.897*	85.14±25.09
10	Serine	64-132	0.159*	96.76±20.08
11	Threonine	71-197	0.530*	133.72±32.68
12	Tyrosine	23-96	0.184*	59.03±16.26
13	Valine	149-326	0.461*	229.24±49.10

*Normality test with Saphiro-Wilk. Data is distributed normally if $p>0.05$.

Amino Acid Profiles of Breast Cancer Subjects with Cancer Risk Factors

When correlated with age, the ornithine was found statistically different between age of <40 and ≥40 years of breast cancer subjects with luminal B subtype (Figure 4A). When correlated with age of menarche, the glutamic acid was found statistically different between age of menarche of <12 and ≥12 years of breast cancer subjects with luminal B subtype (Figure 4B). When correlated with parity, the glutamic acid, histidine and valine were found statistically different between 0-1 parity and multiparity of breast cancer subjects with luminal A subtype (Figure 4C). When correlated with family cancer history, the glutamic acid was found statistically different between breast-cancer-luminal-A-subtype subjects with and without family cancer history (Figure 5A). In addition, valine was found statistically different between breast-cancer-luminal-B-subtype subjects with and without family cancer history (Figure 5B).

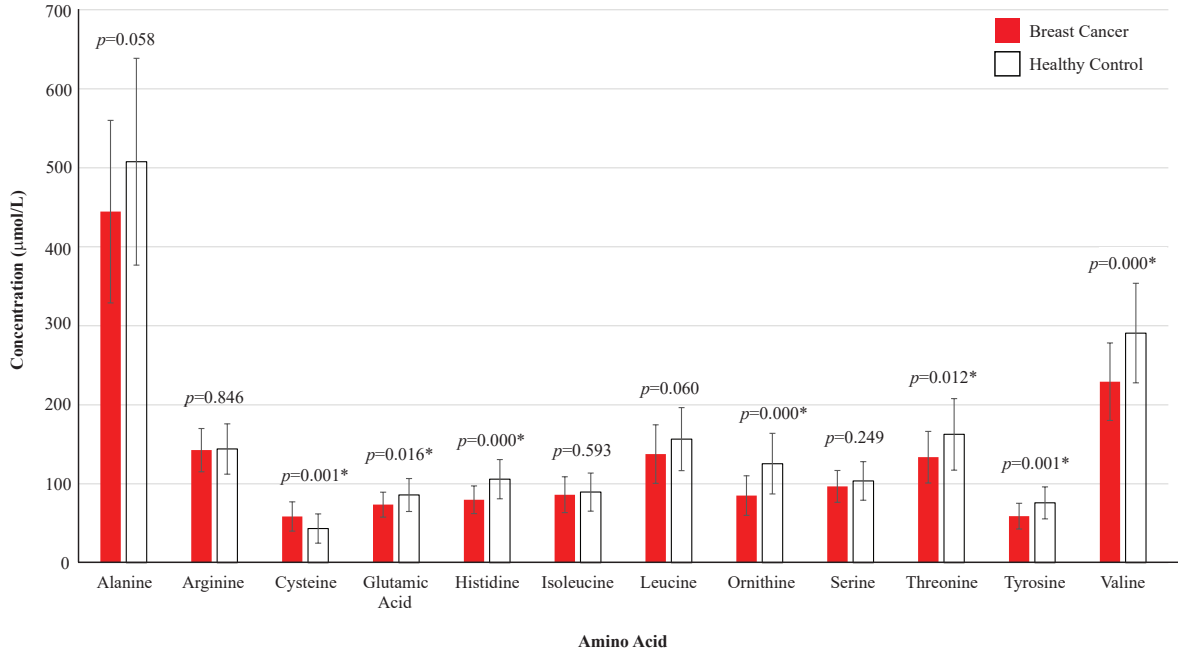

Figure 1. Mean comparison of 12 amino acids between breast cancer (n=29) and control (n=28) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

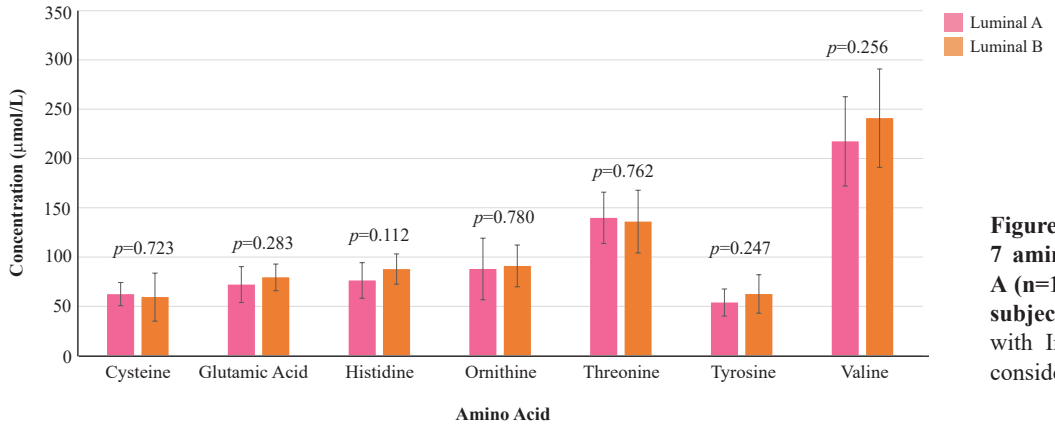

Figure 2. Mean comparison of 7 amino acid between Luminal A (n=10) and Luminal B (n=13) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

Discussion

Amino acids are essential nutrients in all living cells and are important for the proliferation and maintenance of tumor cells.(11) Oncogenesis depends on amino acids, the building blocks for protein synthesis, as well as energy sources and metabolites. Due to their accelerated growth, cancer cells will also require a greater quantity of amino acids than normal cells.(11,16) There was a statistically significant difference between subjects with breast cancer and healthy controls in terms of the amino acid cystine ($p=0.001$).

Cystine is an amino acid derived from homocysteine that plays a role in nucleotide methylation and DNA synthesis. As an inhibitor of the methyltransferase enzyme, plasma concentrations of cystine rise during folic acid deficiency. This renders ineffective processes of DNA methylation and regulation of gene expression, which contribute to oncogenesis at the genetic level and

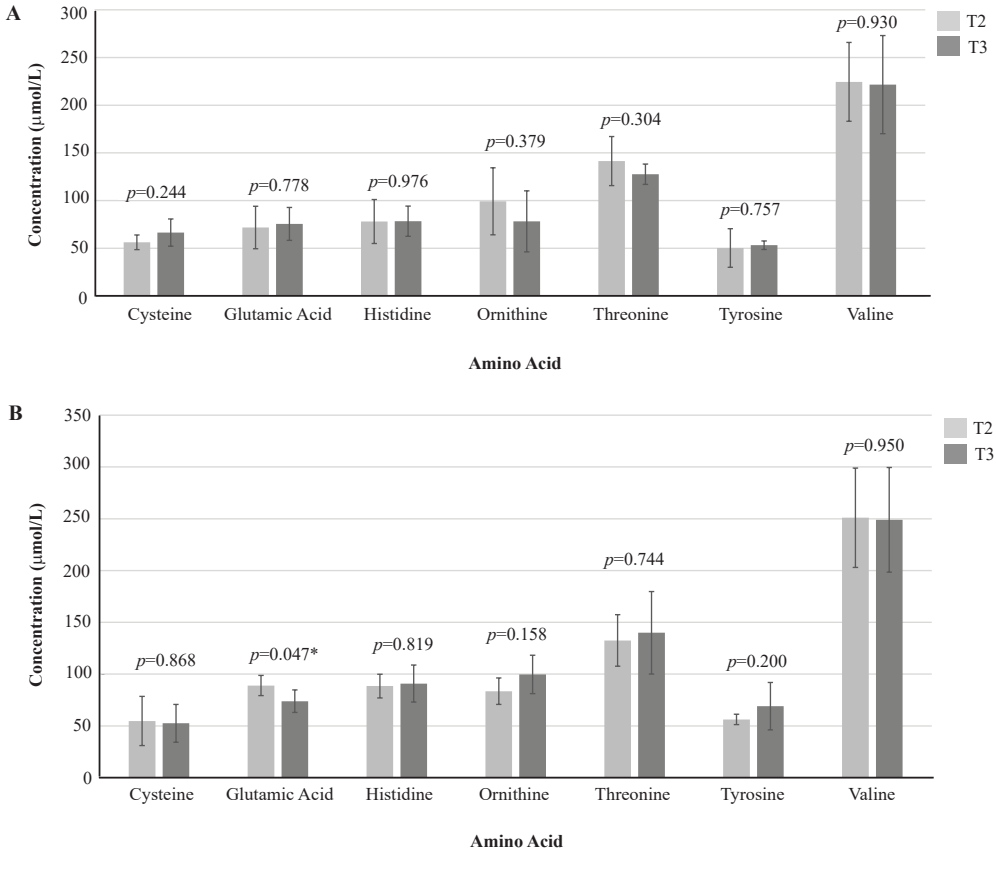

Figure 3. Mean comparison of 7 amino acid between T2 and T3 cancer stage. A: T2 cancer stage (n=4) *vs.* T3 cancer stage (n=5) in Luminal A subjects. B: T2 cancer stage (n=4) *vs.* T3 cancer stage (n=7) in Luminal B subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

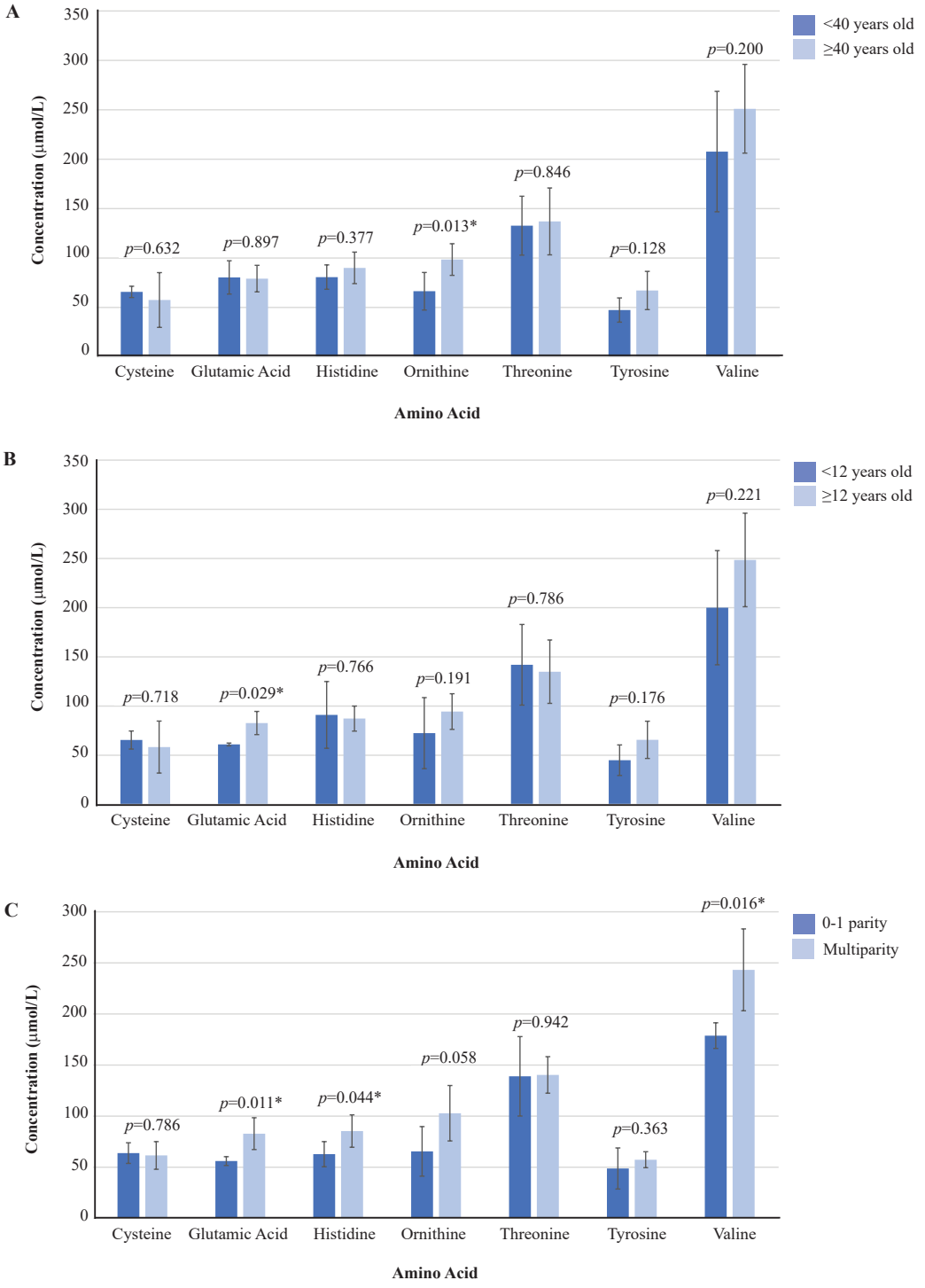

Figure 4. Mean comparison of 7 amino acid based on various risk factors (age, age of menarche, and parity). A: Based on age <40 years old (n=3) *vs.* age ≥40 years old (n=10) in Luminal B subjects. B: Based on age of menarche <12 years old (n=2) *vs.* age of menarche ≥12 years old (n=11) in Luminal B subjects. C: Based on 0-1 parity (n=4) *vs.* multiparity (n=6) in Luminal A subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

initiate cancer.(17) Increased cystine proteinases such as cathepsin B and L activities have been observed as well in a variety of human and animal malignant tumors, which may be due to changes in their expression, activation and processing, intracellular trafficking, as well as declining regulation of these proteinases due to decreased expression and activity of their endogenous inhibitors.(18)

Through the production of alpha-ketoglutarate, alanine plays a role in the formation of extracellular matrix in metastatic breast cancer.(19) The breast cancer

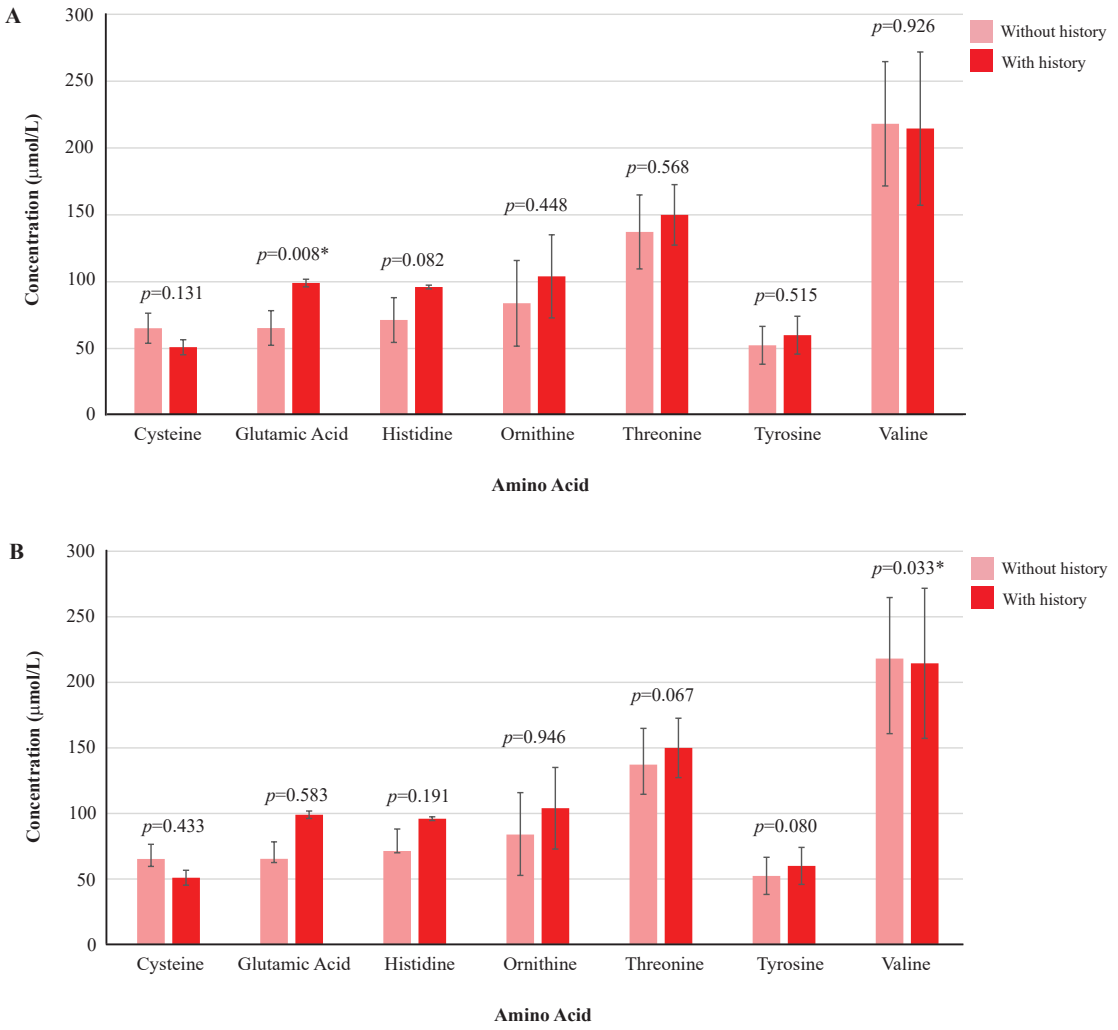

Figure 5. Mean comparison of 7 amino acid between subjects with and without family Ca history. A: Family Ca history in Luminal A subjects (No=8; Yes=2). B: Family Ca history in Luminal B subjects (No=11; Yes=2). *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

proliferative pathway inhibits the enzyme GPT2 (alanine transaminotransferase-2) that produces alanine from glutamate and pyruvate.(20) This is associated with a decrease in the average amount of alanine in breast cancer subject compared to healthy controls.

Significantly decreased in breast cancer subjects than the healthy control was found in this study. The lower level of leucine level might be due to highly expressed of leucine aminopeptidase 3 (LAP3) in breast cancer tissues. LAP3 is an exopeptidase that catalyzes the hydrolysis of leucine residues at the amino terminus of a protein or peptide substrate.(21,22) LAP3 is also implicated in breast tumor cell proliferation, migration, invasion, and angiogenesis. It enhances breast cancer cell motility and invasion by activating many signaling pathways.(22)

The amino acid profile is not only associated with breast cancer incidence, but also with breast cancer risk factors.(23) The multiparities risk factor was significant for the increasing of glutamic acid and histidine levels in breast cancer subject with luminal B. The age risk factor was significant for the increasing of ornithine level in breast cancer subject with luminal B. As for the age of menarche, glutamic acid level was significant increased in breast cancer subject with luminal B.

In this current study, we found that breast cancer subjects with luminal A and B did not show significant difference for several amino acids. This study lacks of research samples from each research subject, so further research is needed to be conducted with a larger sample size to obtain a clear significance between the amino acid profile of breast cancer and its risk factors. Further research at the cellular level is also necessary to determine specifically the changes in amino acid profiles due to cancer.

Conclusion

The amino acid profile of patients with Luminal A and B subtypes of breast cancer differs compared to healthy controls and is also correlated with breast cancer risk factors. An increase in cysteine level in Luminal A subtype patients and the decrease of alanine and leucine in Luminal B subtype patients can be used as a biomarker.

Authors Contribution

SSP, AR, R, NS, and H were involved in the conceptualization of the study. SSP and AR were involved in the preparation of study methodology. SSP, RIP, AR, and FS conducted the formal analysis. SSP and FS prepared the original draft and manuscript revision. SSP, NS, and H supervised the study. All authors read and approved the final manuscript.

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