Print ISSN: 2085-3297, Online ISSN: 2355-9179

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 3
June 2023

Published by:

PERI
The Prodia Education & Research Institute

Secretariat: Prodia Tower 9th Floor
Jl. Kramat Raya No.150,
Jakarta 10430, Indonesia
Tel.: +62-21-3144182
Fax.: +62-21-3144181
E-mail: Secretariat@InaBJ.org
Website: www.InaBJ.org

#200/M/KPT/2020

Included in:
Scopus DOAJ DIRECTORY OF OPEN ACCESS JOURNALS
EMERGING SOURCES CITATION (Web of Science) Clarivate Analytics

REVIEW ARTICLES

RESEARCH ARTICLES

Print ISSN: 2085-3297, Online ISSN: 2355-9179

The Indonesian BIOMEDICAL JOURNAL

Volume 15 Number 3, June 2023

Contact Address
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Tel.: +62-21-3144182, ext. 3872
Fax.: +62-21-3144181
WhatsApp No.: +62 877-3616-3117
E-mail: Secretariat@InaBJ.org
Website: www.InaBJ.org

Focus & Scope
The Indonesian Biomedical Journal (InaBJ) is an open access, peer-reviewed journal that encompasses all fundamental and molecular aspects of basic medical sciences, emphasizing on providing the molecular studies of biomedical problems and molecular mechanisms.

InaBJ is dedicated to publish original research and review articles covering all aspects in biomedical sciences. The editors will carefully select manuscript to present only the most recent findings in basic and clinical sciences. All professionals concerned with biomedical issues will find this journal a most valuable update to keep them abreast of the latest scientific development.

Section Policies
Review Article
Review Article should consist of no more than 10,000 words, not including the words in abstract, references, table, figure, and figure legend. The manuscript should have no more than eight figures and/or tables in total and no more than 250 references. Only invited authors are allowed to submit review article.

Research Article
Research Article should consist of no more than 3,500 words, not including the words in abstract, references, table, figure, and figure legend. The manuscript should have no more than six figures and/or tables in total and no more than 40 references.

Peer Review Process
All manuscripts submitted to InaBJ will be selected and double-blind peer-reviewed by two or more reviewers to present valuable and authentic findings in biomedical sciences. At least, an external reviewer will be included as the reviewer in each manuscript reviewing process.

Author can suggest reviewer/s that not having publication together within five years and should not be member/s of the same research institution. However, reviewers will be selected independently by Section Editor based on their expertise, specialties, and independencies to fit the topic. Section Editor will ensure that the reviewers will be not from the same institution as the author.

Manuscript will be reviewed comprehensively, including appropriate title; content reflecting abstract; concise writing; clear purpose, study method and figures and/or tables; and summary supported by content. Supplementary data will also be sent to reviewer. The reviewing process will take generally 2-3 months depends on sufficiency of information provided.

Decisions are ultimately made by the Section Editor based on the peer-reviewing results. Therefore, Section Editor will consider thoroughly, if necessary Section Editor can invite another one or more reviewer/s to conclude the final decision.

Publication Frequency
InaBJ is published bimonthly (in February, April, June, August, October, and December).

Open Access Policy
InaBJ provides immediate open access to its content on the principle that making research freely available to the public supports a greater global exchange of knowledge.

Print ISSN: 2085-3297, Online ISSN: 2355-9179

Content

The Indonesian Biomedical Journal
Volume 15 Number 3, June 2023

REVIEW ARTICLE

RESEARCH ARTICLE

RESEARCH ARTICLE

The Indonesian Biomedical Journal, Vol.15, No.3, June 2023, p.194-295 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

Sonar Soni Panigoro[1], Arif Kurniawan[1], Ramadhan[1], Ninik Sukartini[2], Herqutanto[3], Rafika Indah Paramita[4,5,6], Ferry Sandra[7,*]

[1]Surgical Oncology Division, Department of Surgery, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[3]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[4]Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[5]Bioinformatics Core Facilities - IMERI, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[6]Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[7]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

Received date: Oct 25, 2022; Revised date: Jun 6, 2023; Accepted date: Jun 8, 2023

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

Indones Biomed J. 2023; 15(3): 269-76

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).

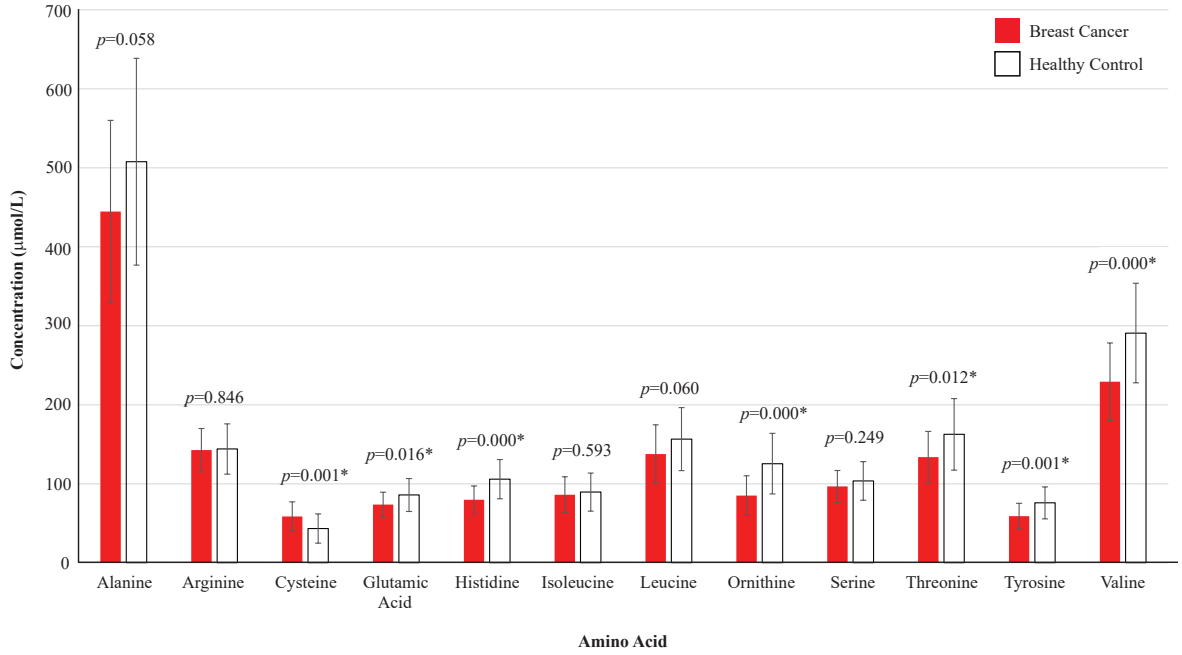

Figure 1. Mean comparison of 12 amino acids between breast cancer (n=29) and control (n=28) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

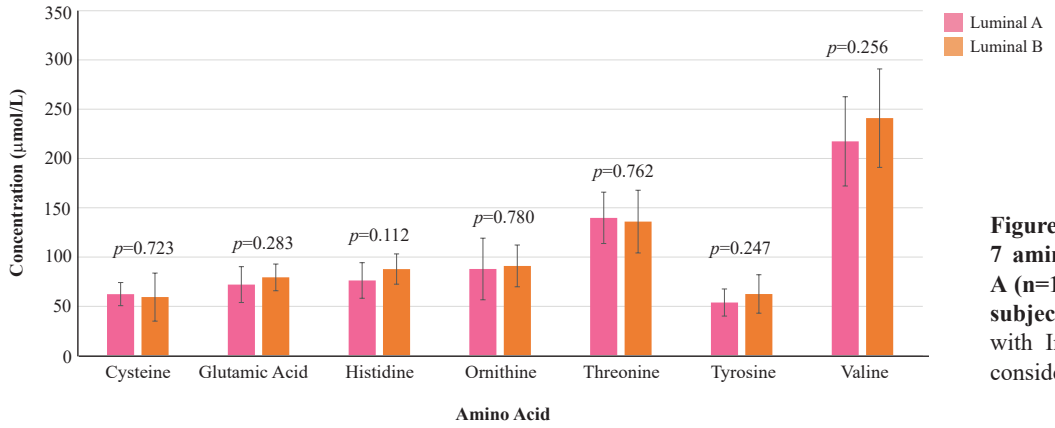

Figure 2. Mean comparison of 7 amino acid between Luminal A (n=10) and Luminal B (n=13) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

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

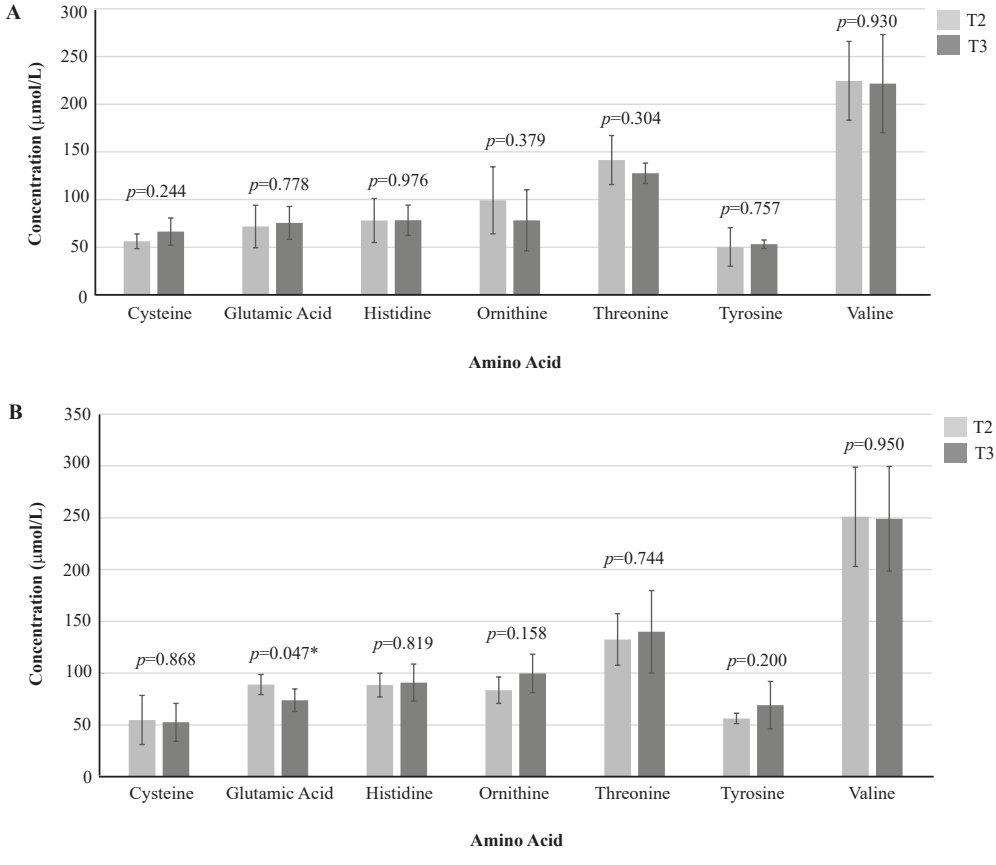

Figure 3. Mean comparison of 7 amino acid between T2 and T3 cancer stage. A: T2 cancer stage (n=4) *vs.* T3 cancer stage (n=5) in Luminal A subjects. B: T2 cancer stage (n=4) *vs.* T3 cancer stage (n=7) in Luminal B subjects. *Mean comparison test with Independent T-test. Data is considered significant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

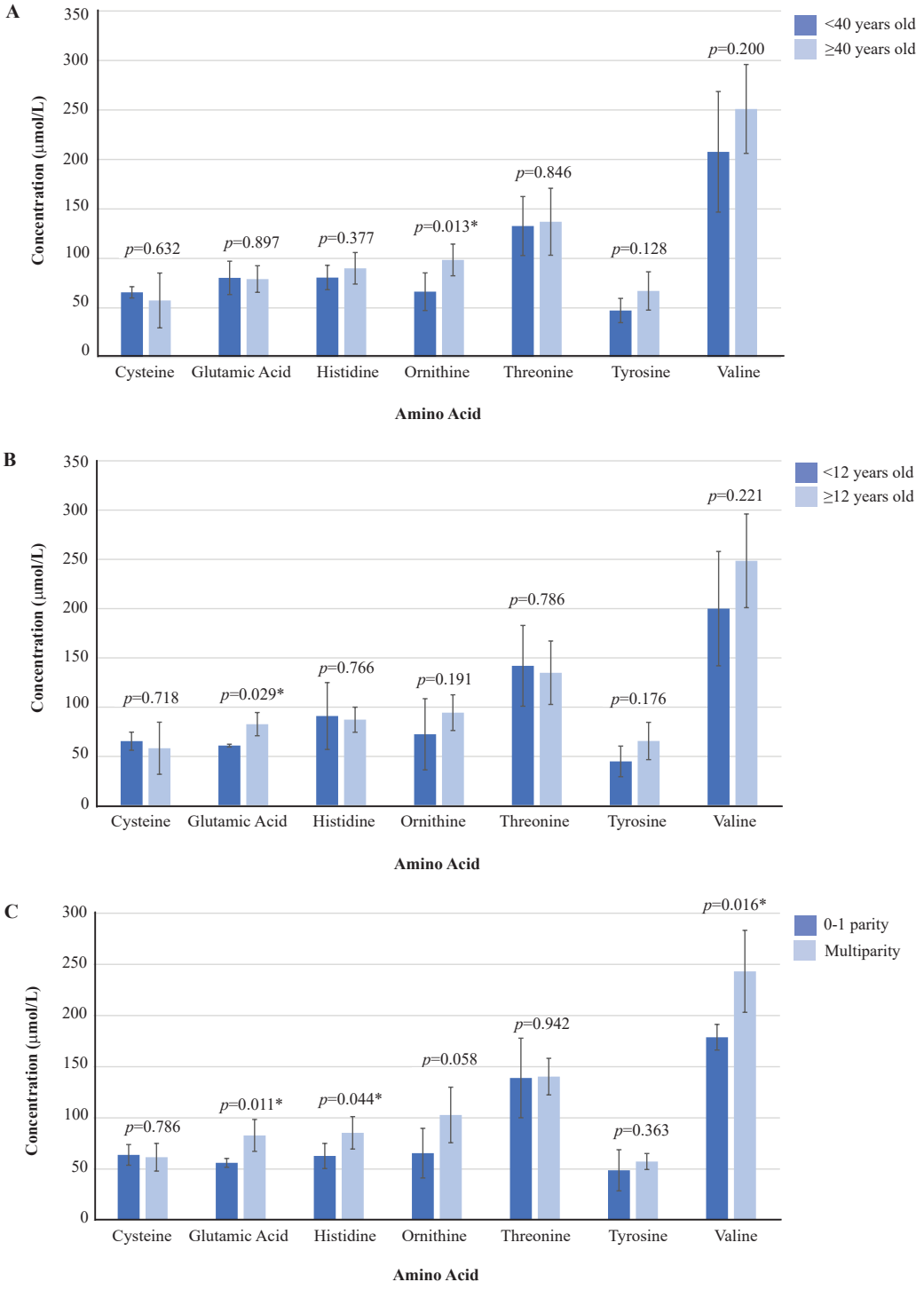

Figure 4. Mean comparison of 7 amino acid based on various risk factors (age, age of menarche, and parity). A: Based on age <40 years old (n=3) *vs.* age ≥40 years old (n=10) in Luminal B subjects. B: Based on age of menarche <12 years old (n=2) *vs.* age of menarche ≥12 years old (n=11) in Luminal B 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

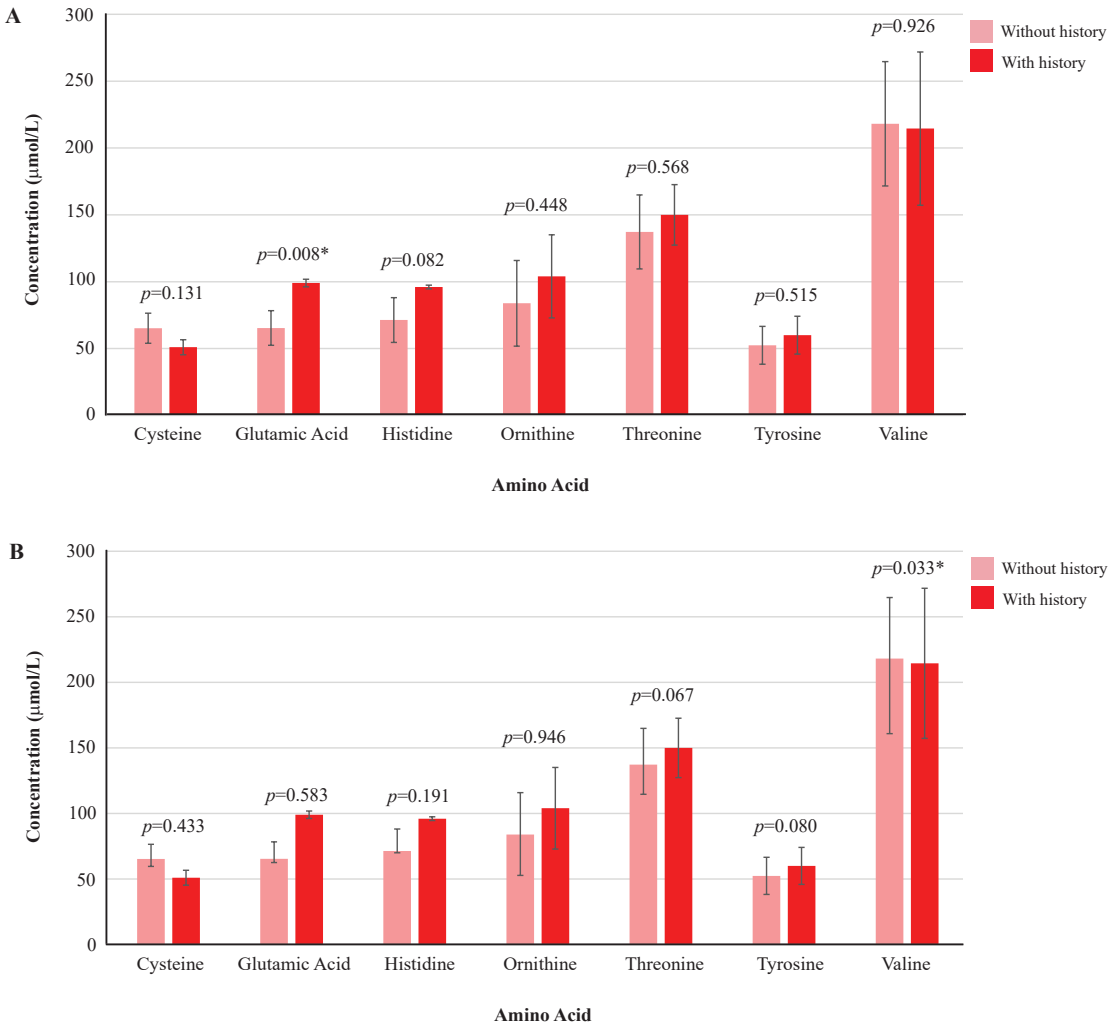

Figure 5. Mean comparison of 7 amino acid between subjects with and without family Ca history. A: Family Ca history in Luminal A subjects (No=8; Yes=2). B: Family Ca history in Luminal B subjects (No=11; Yes=2). *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

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.

References

1. Savitri M, Bintoro UY, Sedana MP, Diansyah MN, Romadhon PZ, Amrita PNA, *et al.* Circulating plasma miRNA-21 as a superior biomarker compared to ca 15-3: assessment in healthy age matched subjects and different stage of breast cancer patients. Indones Biomed J. 2020; 12(2): 157-64.
2. Purwanto I, Heriyanto DS, Ghozali A, Widodo I, Dwiprahasto I, Aryandono T, *et al.* Basal-like subgroup is associated with younger age, increased expression of androgen receptor, and worse prognosis, while non-basal-like subtype is associated with higher BMI in triple-negative breast cancer patients. Indones Biomed J. 2020; 12(4): 349-54.
3. Widowati W, Jasaputra DK, Sumitro SB, Widodo MA, Afifah E, Rizal R, *et al.* Direct and indirect effect of TNFα and IFNγ toward apoptosis in breast cancer cells. Mol Cell Biomed Sci. 2018; 2(2): 60-9.
4. Abdihalim TS, Idris AAA. Mucin level as a potential biomarker for breast cancer diagnosis. Mol Cell Biomed Sci. 2022; 6(3): 117-20.
5. Ilyan T, Retnoningrum D, Hendrianingtyas M, Widyaningrum D, Rachmawati B. Association of 25-hydroxyvitamin D, cyclooxygenase-2 and prostaglandin E2 serum levels in breast cancer patients. Indones Biomed J. 2021; 13(4): 426-32.
6. Remelia M, Bela B, Widyaningtyas ST, Antarianto RD, Mazfufah NF, Pawitan JA. The use of cell-penetrating peptide for delivery of recombinant transcription factor DNA into primary human fibroblast. Mol Cell Biomed Sci. 2023; 7(1): 28-37.
7. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3): 209-49.
8. Kraeima J, Siesling S, Vliegen IM, Klaase JM, IJzerman MJ. Individual risk profiling for breast cancer recurrence: towards tailored follow-up schemes. Br J Cancer. 2013; 109(4): 866-71.
9. Retnowarnadi A, Kresno SB, Arif M. Association of obesity and breast cancer risk: The role of estrogen, tumor necrosis factor-alpha, and adiponectin as risk factors (preliminary study). Indones Biomed J. 2009; 1(1): 45-52.
10. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr. 2006; 136(1): 227S-31S.
11. Cha Y, Kim ES, Koo J. Amino acid transporters and glutamine metabolism in breast cancer. Int J Mol Sci. 2018; 19(3): 907. doi: 10.3390/ijms19030907.
12. Saito Y, Soga T. Amino acid transporters as emerging therapeutic targets in cancer. Cancer Sci. 2021; 112(8): 2958-65.
13. Barnes T, Bell K, DiSebastiano KM, Vance V, Hanning R, Russell C, *et al.* Plasma amino acid profiles of breast cancer patients early in the trajectory of the disease differ from healthy comparison groups. Appl Physiol Nutr Metab. 2014; 39(6): 740-4.
14. Poschke I, Mao Y, Kiessling R, De Boniface J. Tumor-dependent increase of serum amino acid levels in breast cancer patients has diagnostic potential and correlates with molecular tumor subtypes. J Transl Med. 2013; 11(1): 1–9. doi: 10.1186/1479-5876-11-290.
15. Laposata M. Laposata's Laboratory Medicine Diagnosis of Disease in Clinical Laboratory. 3rd Edition. Columbus: McGraw-Hill Education; 2018.
16. Geck RC, Toker A. Nonessential amino acid metabolism in breast cancer. Adv Biol Regul. 2016; 62: 11-7.
17. Yang L, Wang Y, Cai H, Wang S, Shen Y, Ke C. Application of metabolomics in the diagnosis of breast cancer : a systematic review. J Cancer. 2020; 11(9): 2540-51.
18. Rudzińska M, Parodi A, Soond SM, Vinarov AZ, Korolev DO, Morozov AO, *et al.* The role of cysteine cathepsins in cancer progression and drug resistance. Int J Mol Sci. 2019; 20(14): 3602. doi: 10.3390/ijms20143602.
19. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in cancer. Br J Cancer. 2020; 122: 150-6.
20. Mitra D. Investigating the Role of Alanine Aminotransferase 2 in Breast Cancer [Dissertation]. Heidelberg: University of Heidelberg; 2018.
21. Tsujimoto M, Goto Y, Maruyama M, Hattori A. Biochemical and enzymatic properties of the M1 family of aminopeptidases involved in the regulation of blood pressure. Heart Fail Rev. 2008; 13(3): 285-91.
22. Fang C, Zhang J, Yang H, Peng L, Wang K, Wang Y, *et al.* Leucine aminopeptidase 3 promotes migration and invasion of breast cancer cells through upregulation of fascin and matrix metalloproteinases-2/9 expression. J Cell Biochem. 2019; 120(3): 3611-20.
23. Lieu EL, Nguyen T, Rhyne S, Kim J. Amino acids in cancer. Exp Mol Med. 2020; 52(1): 15-30.

Digital Receipt

This receipt acknowledges that Turnitin received your paper. Below you will find the receipt information regarding your submission.

The first page of your submissions is displayed below.

Submission author:	Ferry Sandra
Assignment title:	SIJALI 8
Submission title:	InaBJ V15N3A8 - Amino Acid Profile of Luminal A and B Subty...
File name:	V15N3A8.pdf
File size:	1.5M
Page count:	8
Word count:	4,358
Character count:	22,277
Submission date:	17-Jan-2024 08:43AM (UTC+0700)
Submission ID:	2272238295

The Indonesian Biomedical Journal, Vol.15, No.3, June 2023, p.194-295 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

Sonar Soni Panigoro[1], Arif Kurniawan[1], Ramadhan[1], Ninik Sukartini[2], Herqutanto[3], Rafika Indah Paramita[4,5,6], Ferry Sandra[7,*]

[1]Surgical Oncology Division, Department of Surgery, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[3]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[4]Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[5]Bioinformatics Core Facilities - IMERI, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[6]Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[7]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

Received date: Oct 25, 2022; Revised date: Jun 6, 2023; Accepted date: Jun 8, 2023

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 analyzed 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

Indones Biomed J. 2023; 15(3): 269-76

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)

InaBJ V15N3A8 - Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

by Ferry Sandra

Submission date: 17-Jan-2024 08:43AM (UTC+0700)
Submission ID: 2272238295
File name: V15N3A8.pdf (1.5M)
Word count: 4358
Character count: 22277

The Indonesian Biomedical Journal, Vol.15, No.3, June 2023, p.194-295 Print ISSN: 2085-3297, Online ISSN: 2355-9179

RESEARCH ARTICLE

Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

Sonar Soni Panigoro[1], Arif Kurniawan[1], Ramadhan[1], Ninik Sukartini[2], Herqutanto[3], Rafika Indah Paramita[4,5,6], Ferry Sandra[7,*]

[1]Surgical Oncology Division, Department of Surgery, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo Central General Hospital, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[2]Department of Clinical Pathology, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[3]Department of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[4]Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[5]Bioinformatics Core Facilities - IMERI, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[6]Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jl. Salemba Raya No.6, Jakarta 10430, Indonesia
[7]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No. 260, Jakarta 11440, Indonesia

*Corresponding author. Email: ferry@trisakti.ac.id

Received date: Oct 25, 2022; Revised date: Jun 6, 2023; Accepted date: Jun 8, 2023

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

Indones Biomed J. 2023; 15(3): 269-76

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).

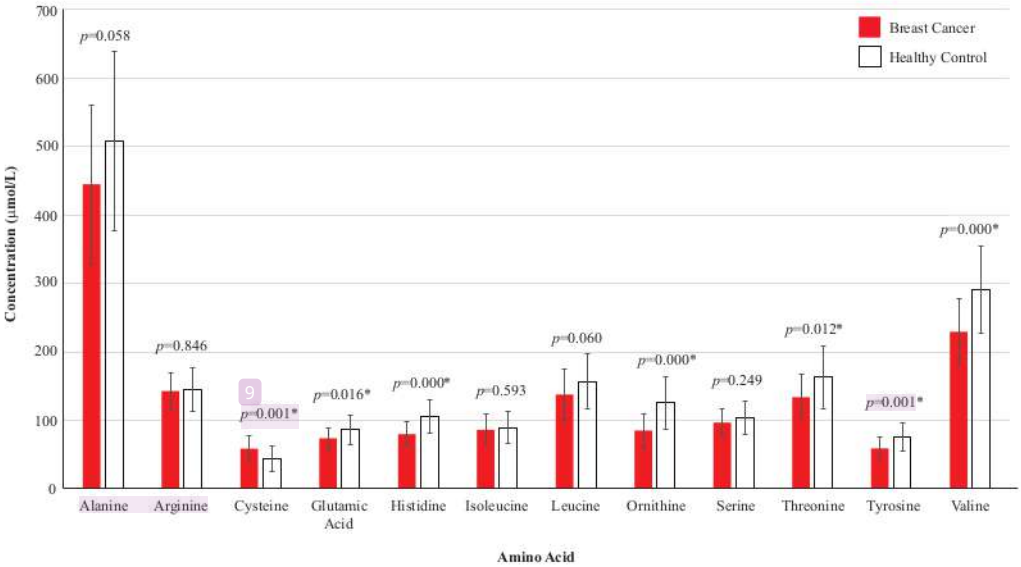

Figure 1. Mean comparison of 12 amino acids between breast cancer (n=29) and control (n=28) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

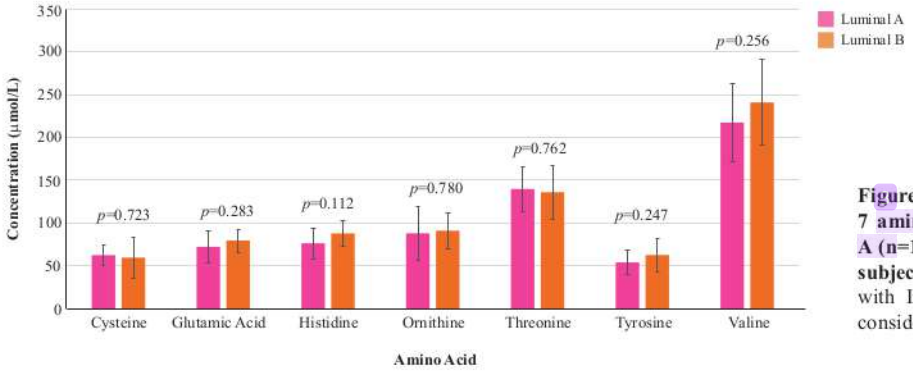

Figure 2. Mean comparison of 7 amino acid between Luminal A (n=10) and Luminal B (n=13) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

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

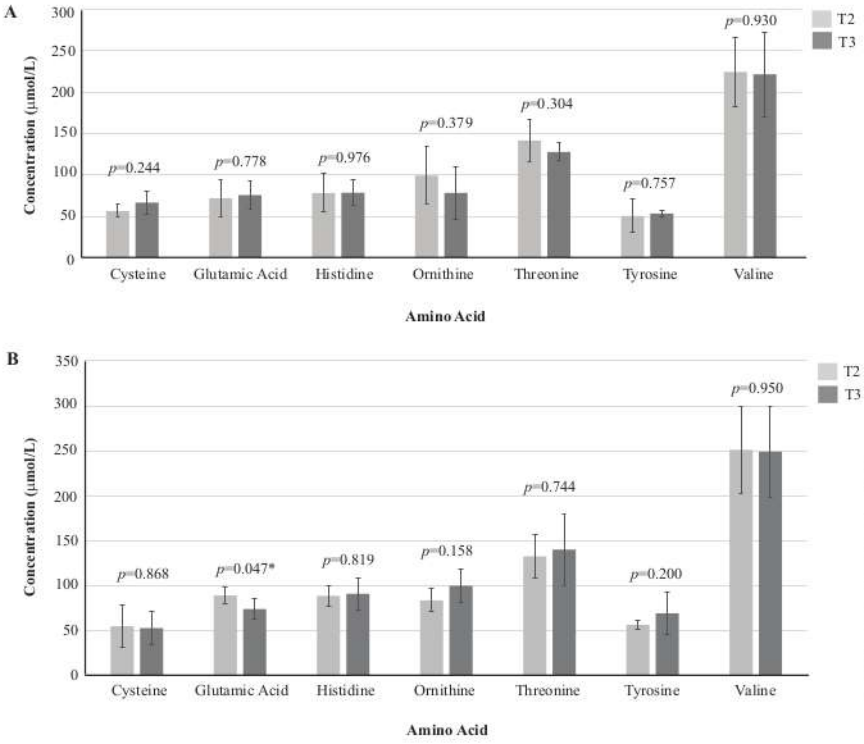

Figure 3. Mean comparison of 7 amino acid between T2 and T3 cancer stage. A: T2 cancer stage (n=4) *vs.* T3 cancer stage (n=5) in Luminal A subjects. B: T2 cancer stage (n=4) *vs.* T3 cancer stage (n=7) in Luminal B subjects. *Mean comparison test with Independent T-test. Data is considered significant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

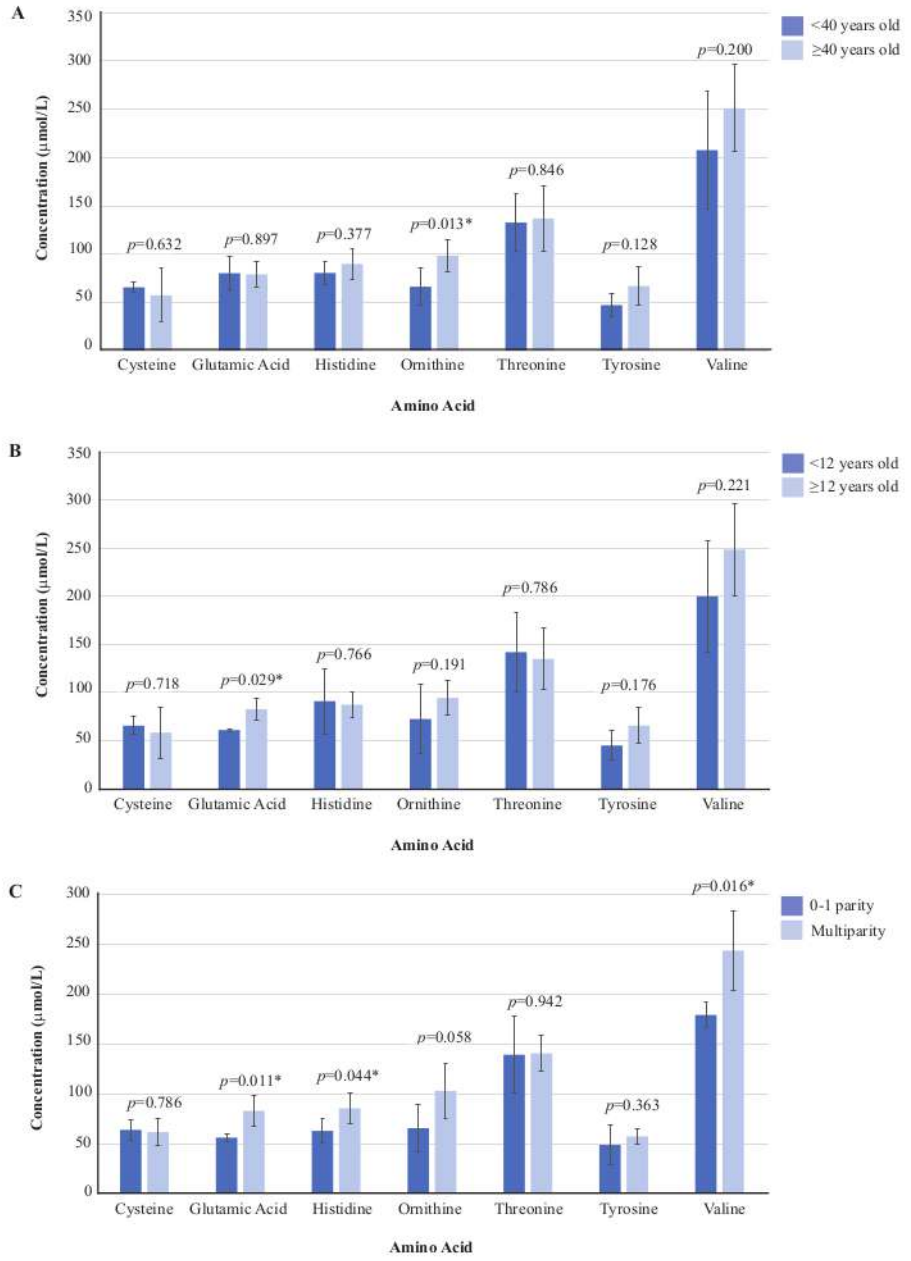

Figure 4. Mean comparison of 7 amino acid based on various risk factors (age, age of menarche, and parity). A: Based on age <40 years old (n=3) *vs.* age ≥40 years old (n=10) in Luminal B subjects. B: Based on age of menarche <12 years old (n=2) *vs.* age of menarche ≥12 years old (n=11) in Luminal B 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

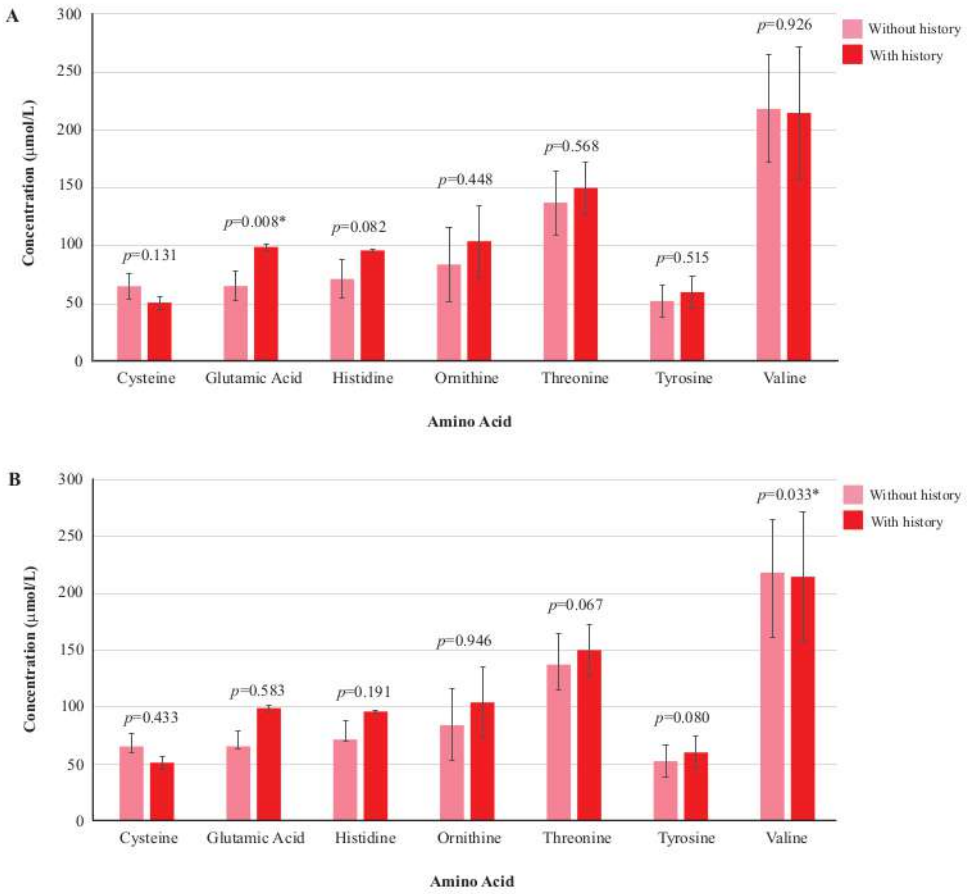

Figure 5. Mean comparison of 7 amino acid between subjects with and without family Ca history. A: Family Ca history in Luminal A subjects (No=8; Yes=2). B: Family Ca history in Luminal B subjects (No=11; Yes=2). *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

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.

References

1. Savitri M, Bintoro UY, Sedana MP, Diansyah MN, Romadhon PZ, Amrita PNA, *et al.* Circulating plasma miRNA-21 as a superior biomarker compared to ca 15-3: assessment in healthy age matched subjects and different stage of breast cancer patients. Indones Biomed J. 2020; 12(2): 157-64.
2. Purwanto I, Heriyanto DS, Ghozali A, Widodo I, Dwiprahasto I, Aryandono T, *et al.* Basal-like subgroup is associated with younger age, increased expression of androgen receptor, and worse prognosis, while non-basal-like subtype is associated with higher BMI in triple-negative breast cancer patients. Indones Biomed J. 2020; 12(4): 349-54.
3. Widowati W, Jasaputra DK, Sumitro SB, Widodo MA, Afifah E, Rizal R, *et al.* Direct and indirect effect of TNFα and IFNγ toward apoptosis in breast cancer cells. Mol Cell Biomed Sci. 2018; 2(2): 60-9.
4. Abdihalim TS, Idris AAA. Mucin level as a potential biomarker for breast cancer diagnosis. Mol Cell Biomed Sci. 2022; 6(3): 117-20.
5. Ilyan T, Retnoningrum D, Hendrianingtyas M, Widyaningrum D, Rachmawati B. Association of 25-hydroxyvitamin D, cyclooxygenase-2 and prostaglandin E2 serum levels in breast cancer patients. Indones Biomed J. 2021; 13(4): 426-32.
6. Remelia M, Bela B, Widyaningtyas ST, Antarianto RD, Mazfufah NF, Pawitan JA. The use of cell-penetrating peptide for delivery of recombinant transcription factor DNA into primary human fibroblast. Mol Cell Biomed Sci. 2023; 7(1): 28-37.
7. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, *et al.* Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3): 209-49.
8. Kraeima J, Siesling S, Vliegen IM, Klaase JM, IJzerman MJ. Individual risk profiling for breast cancer recurrence: towards tailored follow-up schemes. Br J Cancer. 2013; 109(4): 866-71.
9. Retnowarnadi A, Kresno SB, Arif M. Association of obesity and breast cancer risk: The role of estrogen, tumor necrosis factor-alpha, and adiponectin as risk factors (preliminary study). Indones Biomed J. 2009; 1(1): 45-52.
10. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr. 2006; 136(1): 227S-31S.
11. Cha Y, Kim ES, Koo J. Amino acid transporters and glutamine metabolism in breast cancer. Int J Mol Sci. 2018; 19(3): 907. doi: 10.3390/ijms19030907.
12. Saito Y, Soga T. Amino acid transporters as emerging therapeutic targets in cancer. Cancer Sci. 2021; 112(8): 2958-65.
13. Barnes T, Bell K, DiSebastiano KM, Vance V, Hanning R, Russell C, *et al.* Plasma amino acid profiles of breast cancer patients early in the trajectory of the disease differ from healthy comparison groups. Appl Physiol Nutr Metab. 2014; 39(6): 740-4.
14. Poschke I, Mao Y, Kiessling R, De Boniface J. Tumor-dependent increase of serum amino acid levels in breast cancer patients has diagnostic potential and correlates with molecular tumor subtypes. J Transl Med. 2013; 11(1): 1–9. doi: 10.1186/1479-5876-11-290.
15. Laposata M. Laposata's Laboratory Medicine Diagnosis of Disease in Clinical Laboratory. 3rd Edition. Columbus: McGraw-Hill Education; 2018.
16. Geck RC, Toker A. Nonessential amino acid metabolism in breast cancer. Adv Biol Regul. 2016; 62: 11-7.
17. Yang L, Wang Y, Cai H, Wang S, Shen Y, Ke C. Application of metabolomics in the diagnosis of breast cancer : a systematic review. J Cancer. 2020; 11(9): 2540-51.
18. Rudzińska M, Parodi A, Soond SM, Vinarov AZ, Korolev DO, Morozov AO, *et al.* The role of cysteine cathepsins in cancer progression and drug resistance. Int J Mol Sci. 2019; 20(14): 3602. doi: 10.3390/ijms20143602.
19. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in cancer. Br J Cancer. 2020; 122: 150-6.
20. Mitra D. Investigating the Role of Alanine Aminotransferase 2 in Breast Cancer [Dissertation]. Heidelberg: University of Heidelberg; 2018.
21. Tsujimoto M, Goto Y, Maruyama M, Hattori A. Biochemical and enzymatic properties of the M1 family of aminopeptidases involved in the regulation of blood pressure. Heart Fail Rev. 2008; 13(3): 285-91.
22. Fang C, Zhang J, Yang H, Peng L, Wang K, Wang Y, *et al.* Leucine aminopeptidase 3 promotes migration and invasion of breast cancer cells through upregulation of fascin and matrix metalloproteinases-2/9 expression. J Cell Biochem. 2019; 120(3): 3611-20.
23. Lieu EL, Nguyen T, Rhyne S, Kim J. Amino acids in cancer. Exp Mol Med. 2020; 52(1): 15-30.

InaBJ V15N3A8 - Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

ORIGINALITY REPORT

8%	5%	9%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Sonar Soni Panigoro, Rafika Indah Paramita, Kristina Maria Siswiandari, Fadilah Fadilah. "Targeted Sequencing of Germline Breast Cancer Susceptibility Genes for Discovering Pathogenic/Likely Pathogenic Variants in the Jakarta Population", Diagnostics, 2022 Publication	2%
2	www.ncbi.nlm.nih.gov Internet Source	2%
3	html.rhhz.net Internet Source	1%
4	www.amcaid.com Internet Source	1%
5	Baowen Yuan, Simon Schafferer, Qiuqiong Tang, Matthias Scheffler et al. "A plasma metabolite panel as biomarkers for early primary breast cancer detection", International Journal of Cancer, 2019 Publication	1%

6	k-ris.keio.ac.jp Internet Source	1 %
7	link.springer.com Internet Source	1 %
8	www.polskieaminokwasy.pl Internet Source	<1 %
9	Pant, Nisha. "Plasma Free Amino Acid Profile in Oral Submucous Fibrosis (OSF) Using High Performance Liquid Chromatography (HPLC)", Rajiv Gandhi University of Health Sciences (India), 2023 Publication	<1 %
10	www.researchsquare.com Internet Source	<1 %

Exclude quotes	On	Exclude matches	< 15 words
Exclude bibliography	On		

InaBJ V15N3A8 - Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

GRADEMARK REPORT

FINAL GRADE

/15

GENERAL COMMENTS

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2022190 Editor Decision Round 1 - Resubmit for Review

Secretariat of InaBJ <secretariatinabj@gmail.com> Wed, Dec 7, 2022 at 9:15 AM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Profile of Plasma Amino Acid of Breast Cancer Patients and It's Relation to Breast Cancer Risk Factors**".

Our decision is: **Resubmit for Review.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **December 23, 2022**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: https://inabj.org/index.php/ibj/author/submissionReview/2109, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,
--
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Phone. +62-21-3144182 ext. 3872
Fax. +62-21-3144181
https://www.inabj.org

3 attachments

Round 1 Reviewer 1 - Manuscript.docx
168K

Round 1 Reviewer 2 - F09 Manuscript Review Form.pdf
321K

Round 1 Reviewer 2 - Manuscript.docx
165K

Profile of Plasma Amino Acid of Breast Cancer Patients and ~~It's~~ Its Relation to Breast Cancer Risk Factors

ABSTRACT

Background: Breast cancer ranks second as ~~the~~ cancer with the highest incidence in the world. For nucleotide synthesis and DNA methylation, cancer cells require amino acids and will utilize greater amounts than normal cells. This study ~~was~~ aimed to analyze the plasma amino acids profile ~~on~~ of breast cancer patients and on its risk factors.

Methods: Venous blood from subjects ~~were~~ was taken, and then centrifuged at 2500 rpm for 10 minutes to obtain plasma. The blood plasma was analyzed using a liquid chromatography technique to quantify the amino acid level.

Results: There was a statistically significant difference between amino acid levels from subjects with breast cancer compared to healthy controls, i.e. increasing of cystine and decreasing of valine, lysine, histidine, alanine, ornithine, tyrosine, glutamic acid, methionine, and proline. ~~From~~ this study also suggests that several amino acids are associated with breast cancer risk factors, i.e. decreasing of alanine in patients with older age, multiple parities and patients with familial cancer history, and decreasing of ornithine in older age of menarche.

Conclusion: The amino acid profile of breast cancer patients differs from ~~that of~~ healthy controls and is correlated with breast cancer risk factors as well. Increasing ~~of~~ cysteine levels can be used as a biomarker of breast cancer because it was found significantly higher in breast cancer patients than in healthy controls, especially in luminal A patients.

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

Comment [NN1]: No correlation between these sentences. The paragraph can be started with general characteristic of cancer cells.

INTRODUCTION

Cancer is known as a complex disease ~~which~~ that can be caused by several factors, such as environmental, lifestyle, genetic, and clinical. Its increasing prevalence ~~that increased~~, lead cancer to the list of chronic debilitating disease.[1] The most common type of cancer which ~~be~~ is found ~~on~~ in women is breast cancer.[2] Based on data from the International Agency for Research on Cancer, breast cancer ranks second as the cancer with the highest incidence in the world. Breast cancer also ranks first in the incidence of cancer in women. Globally, an estimated 18.1 million people are affected. The reported breast cancer mortality rate ranges from 626,679 (6.6%), second only to lung cancer. In Indonesia alone, breast cancer has the highest prevalence of 0.5%, or 61,682 cases per year.[3,4]

In its development, breast cancer is influenced by several risk factors such as age, genetic and family history of breast cancer, BRCA gene mutations, younger first menstrual history, low parity, a history of hormone use and hormone replacement therapy. Obesity can be associated with the development of breast cancer as well. It can be affected by the act ~~from~~ of aromatization of adrenal androgen into estrogen at adipose tissue.[5] The incidence of breast cancer also increases in the group of women aged >40 years.[4]

For nucleotide synthesis and DNA methylation, cancer cells require and utilize amino acids ~~and will utilize~~ in greater amounts than normal cells. Compared to the control group, breast cancer patients were found to have significant changes in ~~a~~ the number of amino acids. In its report, Barnes et al. described an increase in the branched-chain group of essential and non-essential amino acids ~~in a report. These amino acids consist of~~ namely leucine, phenylalanine, aspartic acid, taurine, and lysine, among others.[6,7] Additionally, almost all amino acids undergo fluctuating changes in blood, cells, and plasma. The most significant value was observed ~~was~~ valine and isoleucine with value (230±15) in the control group and

Comment [NN2]: This paragraph can be started with the general characteristic of cancer cell and its proliferation.

Also, make it relatables with previous paragraph.

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

p, (182±12) compared to in breast cancer patients (182±12), and 48.8± 4.2 vs 40.1± 2.8 for isoleucine.[8]

In addition to the role of histidine via histidine-rich glycoprotein (HRG), which is a binding plasma protein that modulates cell immunity, cell adhesion, angiogenesis, and thrombosis, other mechanisms relating to the development of breast cancer have also been attributed to essential amino acids. HRG RNA expression was significantly elevated in all subtypes of breast cancer, with the basal subtype and stage II having the highest levels.[9,10]

With In regard to the role of the anti-cancer amino acid lysine, the opposite results were also reported. L-Lysine a-oxidase catalyzed by the conversion of L-lysine into a-keto-E-aminocaproic acid, H_2O_2, and ammonia with oxygen consumption exhibited significant anticancer effects on in vitro cell cultures or several in vivo tumor models. It has been demonstrated that the combination of lysine and other micronutrients inhibits the expression and invasion of matrix metalloproteinase (MMP) in several cancers, including breast cancer.[11,12] In general, amount of MMP expressions promote hallmarks of cancer progression, including invasion, metastasis, angiogenesis, and correlates with patient survival.[13] There is currently no report on the study of amino acids in Indonesian breast cancer patients. Therefore, this study was aimed to analyze the plasma amino acids profile on of breast cancer patients and on its risk factors.

Comment [NN3]: the role of histidine wasn't discussed previously

Formatted: Font color: Black, English (U.S.), Strikethrough

METHODS

Patients' characterization

This research was conducted from July 2020 to September 2020 at Cipto Mangunkusumo National Hospital Jakarta (RSCM) and. This research was previously approved by the Faculty of Medicine Universitas Indonesia Ethical Committee (approval number: 20-08-

0877). The inclusion criteria were at least 18-year-old women with breast cancer confirmed by histopathology and immunohistochemistry who came to RSCM between January to March 2020. Patients with incomplete histopathology and medical record information were excluded from this study. At least 18-year-old women healthy were recruited as healthy control. All subjects were able to read, comprehend, and sign was written agreements.

Amino acid profiling

For examination of amino acid, subjects were asked to fast for at least 8 hours and then 2.5 ml of venous blood was ~~taken~~ drawn, which was collected in a sterile container containing heparin anticoagulant. Heparinized blood was then centrifuged at 2500 rpm for 10 minutes to obtain plasma. Plasma was stored at 80°C until examination was carried out.

For the measurement of plasma aspartic acid, glutamate, serine, asparagine, glycine, Glutamic acid, taurine, histidine, alanine, arginine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, ornithine, and lysine, amino acids were analyzed using high-performance liquid chromatography (Waters 2695; Reverse-phase HPLC separation; octadecyl (C18) columns, 25 cm in length (5 µm)). Singletons were used to run samples, external standards, and internal standards. The external standards were utilized at concentrations of 50, 100, and 250 mol/L.[7,14]

Statistical analysis

Data analysis ~~were~~ was done with SPSS version 25.0. Using *independent samples t-test* for both variables, a bivariate analysis was performed to determine the difference in mean between two unpaired samples and ANOVA with p-value of 0.05 to determine the difference among three groups. In contrast, if a p-value of 0.05 is obtained, there is a statistically significant difference between the two or three samples. If the significance value is greater than 0.05, however, there is no significant difference between the two samples. If the data is neither normal nor homogeneous, the *Mann-Whitney U* or Kruskal-Wallis test is

Comment [NN4]: this term is more appropriate

Formatted: Strikethrough

Comment [NN5]: samples

Comment [NN6]: this explanation is not necessary

performed to determine whether there is a statistically significant difference between the two or three groups.

RESULTS

Patients' characteristics

Characteristics of breast cancer research subjects were divided by age, stage of breast cancer and the characteristics of breast cancer risk factors, including age ~~risk factors~~, parity and age of menarche. From a total of 40 research subjects with breast cancer, the average age of the subjects was 52.08 years while in healthy women as controls, the average age was 41.65 years. Among 40 Breast cancer research subjects in this study, 45% had stage T2, and 42.5% had luminal A. Table 1 shows the data on the characteristics of the subjects in this study.

Amino acid profiles of breast cancer patients

The distribution of data from 80 samples of research subjects was normal and homogeneous for 14 amino acids, but was not normal and ~~was~~ not homogeneous for the remaining of 5 amino acids namely ~~.~~ Glutamic acid, isoleucine, methionine, phenylalanine, and proline~~ are the five amino acids~~. Both samples of amino acid data, which are normally distributed and homogeneous, are subjected to a parametric test using an *independent sample t-test*. Due to the abnormal and non-homogeneous nature of the data, non-parametric tests ~~utilizing the~~ *Mann-Whitney U* ~~test~~ were conducted to determine whether there were significant differences between the two samples. The amino acid profiles of breast cancer patients and healthy controls are displayed in Table 2.

Based on the result, there was a statistically significant difference between amino acid levels ~~from~~ of subjects with breast cancer and controls, in which ~~with~~ breast cancer patients having higher levels of cystine and phenylalanine than controls. In addition to the increase in

Formatted: Strikethrough

Comment [NN7]: Rephrase this sentence

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

cystine and phenylalanine levels, there was also a significant decrease in valine, lysine, histidine, alanine, ornithine, tyrosine, Glutamic acid, methionine, and proline levels in breast cancer patients compared to healthy controls.

We also added some analysis on amino acid profile of breast cancer patients based on their subtype and breast cancer stages (Table 3-7). In HER2 positive samples, ornithine, valine, tyrosine, proline, and phenylalanine were significantly decrease than in healthy control. On the other side, patients with Luminal A and B, had lower level of ornithine, histidine, lysine, valine, proline, methionine and phenylalanine. Cystine had significantly higher level in patients with luminal A ~~significantly~~ compared to healthy control. Contrast with triple negative result, aspartic acid and threonine showed lower level significantly than healthy control. In stages comparison, cystine showed lower levels significantly in higher stages.

Comment [NN8]: it is not clear. wether the amino acids are significantly decreasing or only lower than in healthy control

Amino acid profiles of breast cancer risk factors

To determine the role of amino acids in breast cancer risk factors, an *independent sample t-test* or *Mann-Whitney U* test was performed between amino acids and each risk factor in breast cancer research subjects (Table 8). These results suggest that several amino acids are associated with breast cancer risk factors. Age is a known risk factor with a significant mean value for alanine (p=0.025). The parity risk factor was also significant for threonine (p=0.032), alanine (p=0.022), and Glutamic acid (p=0.025). The significance of the risk factor for menarche for the amino acid ornithine is 0.015. The family history factors showed lower level as well of lysine (p=0.040), leucine (p=0.002), alanine (p=0.010), isoleucine (p=0.021), and methionine (p=0.028).

Comment [NN9]: I think it is better to present this results in the form of table

DISCUSSIONS

Amino acids are organic compounds containing an amino group (-NH) and a carboxyl group (-COOH); they are the fundamental structural units of proteins. Amino acids are categorized into essential amino acids and non-essential amino acids. Amino acids are the primary molecules required for protein synthesis and have multiple functions within the cell. As amino acids are essential for nucleotide synthesis and DNA methylation, they play a role in cancer cell proliferation. 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.[15,16]

Comment [NN10]: what about their roles in normal cells?

There was a statistically significant difference between subjects with breast cancer and controls in terms of the amino acid cystine, with breast cancer patients having higher levels of cystine than controls. From our study, the mean of cysteine level ~~was~~ increase in breast cancer patients (62.95 ± 22.22) compared to healthy control (45.51 ± 15.24), especially in luminal A type ($p<0.001$). 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 in ~~the presence of~~ folic acid deficiency. This renders ineffective ~~the~~ processes of DNA methylation and regulation of gene expression, which contribute to oncogenesis at the genetic level and 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.[18–20]

Formatted: Strikethrough

Formatted: Strikethrough

Formatted: Strikethrough

There were some significant decreases in some amino acids levels in breast cancer patients compared to healthy controls. In line with previous study, patients with breast cancer had decreased plasma levels of valine due to the excessive consumption of breast cancer cells by uncontrolled cell growth. The decrease in valine levels in breast cancer is attributable to a

shift in the concentration of energy on tumor cell proliferation, resulting in an increase in anaerobic glycolysis, which inhibits the TCA cycle.[21]

Comment [NN11]: I suggest to move this paragraoh earlier, prior discussion on more specific amino acids.

L-lysine is an essential amino acid that must be obtained through the consumption of food. L-Lysine, a lysine derivative, is known to be effective as an anti-cancer agent. According to reports, lysine can function as an anti-tumor agent by inhibiting MMP.[11,12] Lysine methylation and lysine acetylation play a role in cancer cell proliferation, differentiation, migration, and signal transduction via metabolic regulation. This correlates with lower lysine levels in breast cancer compared to healthy controls. Low lysine levels indicate high methylation and acetylation of lysine in breast cancer cells during cancer cell proliferation.[22]

The correlation between histidine and breast cancer is explained by several pathways. Histidine-rich glycoprotein (HRG), a plasma protein that binds numerous ligands and modulates immunity, cell adhesion, angiogenesis, and thrombosis, is converted into a plasma protein.[9,10] Aspartic acid and oxaloacetate in the TCA cycle are linked to the decrease in histidine levels in breast cancer cells.[22] Aspartic acid consumption by cancer cells has decreased aspartic acid levels in the blood. Histidine concentrations in the blood decrease when aspartic acid concentrations fall. In addition to histidine, proline is involved in feeding the TCA cycle via the urea cycle and ROS formation via proline dehydrogenase (PRODH). Previous studies reported in line with our study that histidine and proline levels were lower in breast cancer patients than controls.[7,21]

Through the production of alpha-ketoglutarate, alanine plays a role in the formation of extracellular matrix in metastatic breast cancer.[23] The breast cancer proliferative pathway inhibits the enzyme GPT2 (alanine transaminotransferase-2) that produces alanine from glutamate and pyruvate.[24] This is associated with a decrease in the average amount of alanine in breast cancer as compared to healthy controls.

198 The most abundant amino acid in plasma is Glutamic acid. It has been demonstrated that
199 Glutamic acid plays a role in cancer cell proliferation by providing carbon and nitrogen for
200 biosynthetic reactions. Multiple amino acid transporters, including ASCT2, are responsible
201 for Glutamic acid uptake by cancer cells. In some tumors, Glutamic acid absorption is also
202 significantly increased.[9,25] Glutamic acid levels have decreased in breast cancer due to its role
203 in cancer cell proliferation via glutaminolysis, which reduces blood Glutamic acid levels.[21]
204 There are several amino acids that has significant difference in abundance between
205 stages of breast cancer as well (Table 7). ANOVA test between stages of breast cancer found
206 that serine, cystine, histidine, and leucine are significantly different between stages.
207 Contradict to our study, all three genes involved in the L: -serine biosynthesis pathway,
208 phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and
209 phosphoserine phosphatase (PSPH), were reported to be upregulated in the highly metastatic
210 variant, according to genome-wide gene expression profiling of this isogenic cell line pair.
211 High PHGDH and PSAT1 expression in primary breast cancer was related with lower
212 relapse-free and overall survival, as well as malignant phenotypic characteristics of breast
213 cancer.[26,27] Meanwhile, we found lower level of serine in stage T4.
214 In this study, we found that leucine level is significantly lower in stage T3 and T4 of
215 breast cancer. There is a report of leucine aminopeptidase 3 (LAP3), an exopeptidase that
216 catalyzes the hydrolysis of leucine residues at the amino terminus of a protein or peptide
217 substrate, to be highly expressed in breast cancer tissues.[28,29] LAP3 is also implicated in
218 breast tumor cell proliferation, migration, invasion, and angiogenesis. It enhances breast
219 cancer cell motility and invasion by activating many signaling pathways.[29]
220 The amino acid profile is not only associated with breast cancer incidence, but also with
221 breast cancer risk factors. Age is a known risk factor with a significant mean value for
222 alanine, as well seen in our study, that patients with age above 40 years old has higher level

of Alanine. In previous study reported that Alanine levels increased with age and reached a maximum between the ages of 40 and 55. This relationship was not linear, but rather U-shaped and inverted.[30] The parity risk factor was also significant for threonine (p=0.032), alanine (p=0.022), and Glutamic acid (p=0.025). The significance of the risk factor for menarche for the amino acid ornithine is 0.015. Ornithine decarboxylase (ODC), according to Deng et al., regulates estrogen receptor alpha expression and breast cancer cell growth.[31]

The limitation of this study is the lack of research samples from each research subject, so further research is needed 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 needed to determine specifically the changes in amino acid profiles due to cancer.

CONSCLUSION

Formatted: Strikethrough

The amino acid profile of breast cancer patients differs from that of healthy controls and is correlated with breast cancer risk factors as well. Increasing of cysteine level can be used as biomarker of breast cancer because it was found significantly higher in breast cancer patients than healthy controls, especially in patients with luminal A ~~patients than healthy controls~~.

REFERENCES

1. Meiliana A, Dewi NM, Wijaya A. Red meats and processed meat as the carcinogenic foods and phytochemical-chemoprevention. Indones Biomed J. 2019;11(3):225–39.
2. Purwanto I, Heiyanto DS, Ghozali A, Widodo I, Dwiprahasto I, Aryandono T, et al. Basal-like subgroup is associated with younger age, increased expression of androgen receptor, and worse prognosis, while non-basal-like subtype is associated with higher BMI in triple-negative breast cancer patients. Indones Biomed J. 2020;12(4):349–54.

3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
4. Kraeima J, Siesling S, Vliegen IMH, Klaase JM, IJzerman MJ. Individual risk profiling for breast cancer recurrence: towards tailored follow-up schemes. Br J Cancer. 2013;109(4):866–71.
5. Retnowarnadi A, Kresno SB, Arif M. Association of Obesity and Breast Cancer Risk: The Role of Estrogen, Tumor Necrosis Factor-alpha, and Adiponectin as Risk factors (preliminary study). Indones Biomed J. 2009;1(1):45.
6. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr. 2006;136(1 Suppl):227S–31S.
7. Barnes T, Bell K, DiSebastiano KM, Vance V, Hanning R, Russell C, et al. Plasma amino acid profiles of breast cancer patients early in the trajectory of the disease differ from healthy comparison groups. Appl Physiol Nutr Metab. 2014;39(6):740–4.
8. Proenza AM, Oliver J, Palou A, Roca P. Breast and lung cancer are associated with a decrease in blood cell amino acid content. J Nutr Biochem. 2003;14(3):133–8.
9. Eissa S, Azzazy HME, Matboli M, Shawky SM, Said H, Anous FA. The prognostic value of histidine-rich glycoprotein RNA in breast tissue using unmodified gold nanoparticles assay. Appl Biochem Biotechnol. 2014;174(2):751–61.
10. Matboli M, Eissa S, Said H. Evaluation of histidine-rich glycoprotein tissue RNA and serum protein as novel markers for breast cancer. Med Oncol. 2014;31(4):897.
11. Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Inhibition of matrix metalloproteinase-2 secretion and invasion by human ovarian cancer cell line SK-OV-

3 with lysine, proline, arginine, ascorbic acid and green tea extract: Nutrients inhibit ovarian cancer. J Obstet Gynaecol Res. 2006;32(2):148–54.

12. Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. In vitro and in vivo antitumorigenic activity of a mixture of lysine, proline, ascorbic acid, and green tea extract on human breast cancer lines MDA-MB-231 and MCF-7. Med Oncol. 2005;22(2):129–38.

13. Wulandari F, Ikawati M, Kirihata M, Kato JY, Meiyanto E. Curcumin analogs, PGV-1 and CCA-1.1 exhibit anti-migratory effects and suppress MMP9 expression on WiDr cells. Indones Biomed J. 2021;13(3):271–80.

14. Laposata M. Laposata's laboratory medicine diagnosis of disease in clinical laboratory third edition. 3rd ed. Columbus, OH: McGraw-Hill Education; 2019.

15. Cha Y, Kim ES, Koo J. Amino acid transporters and Glutamic acid metabolism in breast cancer. Int J Mol Sci. 2018;19(3):907.

16. Geck RC, Toker A. Nonessential amino acid metabolism in breast cancer. Adv Biol Regul. 2016;62:11–7.

17. Yang L, Wang Y, Cai H, Wang S, Shen Y, Ke C. Application of metabolomics in the diagnosis of breast cancer: a systematic review. J Cancer. 2020;11(9):2540–51.

18. SLOANE BF, MOIN K, LAH TT. Regulation of Lysosomal Endopeptidases in Malignant Neoplasia. Biochemical and Molecular Aspects of Selected Cancers. 1994 Jan 1;411–66.

19. Berquin IM, Sloane BF. Cysteine proteases and tumor progression. Perspectives in Drug Discovery and Design. 1995 Jul;2(3):371–88.

20. Buck MR, Karustis DG, Day NA, Honn K v., Sloane BF. Degradation of extracellular-matrix proteins by human cathepsin B from normal and tumour tissues. Biochemical

Journal [Internet]. 1992 Feb 15 [cited 2022 Nov 7];282(1):273–8. Available from: /biochemj/article/282/1/273/36721/Degradation-of-extracellular-matrix-proteins-by

21. Varela A, Puebla-Pérez AM, Delgado-Saucedo JI, Rodr\'\iguez-Arévalo F, Zúñiga-González GM, Figuera LE, et al. Increased homocysteine plasma levels in breast cancer patients of a Mexican population. Exp Oncol. 2018;40(2):114–8.

22. Cappelletti V, Iorio E, Miodini P, Silvestri M, Dugo M, Daidone MG. Metabolic footprints and molecular subtypes in breast cancer. Dis Markers. 2017;2017:1–19.

23. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in cancer. Br J Cancer. 2020;122(2):150–6.

24. Mitra D. Investigating the role of alanine aminotransferase 2 in breast cancer. Heidelberg University Library; 2018.

25. Juhász C, Nahleh Z, Zitron I, Chugani DC, Janabi MZ, Bandyopadhyay S, et al. Tryptophan metabolism in breast cancers: molecular imaging and immunohistochemistry studies. Nucl Med Biol. 2012;39(7):926–32.

26. Pollari S, Käkönen SM, Edgren H, Wolf M, Kohonen P, Sara H, et al. Enhanced serine production by bone metastatic breast cancer cells stimulates osteoclastogenesis. Breast Cancer Res Treat [Internet]. 2011 Jan [cited 2022 Nov 7];125(2):421–30. Available from: https://pubmed.ncbi.nlm.nih.gov/20352489/

27. Luo J. Cancer's sweet tooth for serine. Breast Cancer Research [Internet]. 2011 Nov 28 [cited 2022 Nov 7];13(6):1–3. Available from: https://breast-cancer-research.biomedcentral.com/articles/10.1186/bcr2932

28. Tsujimoto M, Goto Y, Maruyama M, Hattori A. Biochemical and enzymatic properties of the M1 family of aminopeptidases involved in the regulation of blood pressure. Heart Fail Rev [Internet]. 2008 Sep 13 [cited 2022 Nov 7];13(3):285–91. Available from: https://link.springer.com/article/10.1007/s10741-007-9064-8

29. Fang C, Zhang J, Yang H, Peng L, Wang K, Wang Y, et al. Leucine aminopeptidase 3 promotes migration and invasion of breast cancer cells through upregulation of fascin and matrix metalloproteinases-2/9 expression. J Cell Biochem [Internet]. 2019 Mar 1 [cited 2022 Nov 7];120(3):3611–20. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jcb.27638

30. Elinav E, Ben-Dov IZ, Ackerman E, Kiderman A, Glikberg F, Shapira Y, et al. Correlation between serum alanine aminotransferase activity and age: an inverted U curve pattern. Am J Gastroenterol. 2005;100(10):2201–4.

31. Deng W, Jiang X, Mei Y, Sun J, Ma R, Liu X, et al. Role of ornithine decarboxylase in breast cancer. Acta Biochim Biophys Sin (Shanghai). 2008;40(3):235–43.

TABLES

Table 1. Subjects' characteristics

Characteristics	Breast cancer patients	Healthy controls
Age	52.08 ± 8.42 y.o	41.65 ± 12.46 y.o
Breast Cancer Stage		
T4	5	
T3	17	
T2	18	
Molecular Subtype		
HER2 positive	5	
Luminal A	17	
Luminal B	16	
Triple Negative	2	
Risk factors		
Age: <40 years old	18	18
>40 years old	22	22
0-1 parity	10	13
Multiparity	30	27
Age of menarche: <12 y.o	31	10
>12 y.o	9	30

339 **Table 2.** Amino acid profiles of breast cancer patients and healthy control

Amino Acid	Breast cancer patients	Healthy control	*p-value**
	Mean ± SD	Mean ± SD	
Aspartic acid	14.30 ± 8.34	17.12 ± 12.57	0.752
Arginine	142.52 ± 26.18	144.07 ± 29.52	0.804
Serine	98.77 ± 24.02	108.46 ± 29.87	0.116
Glycine	286.02 ± 94.56	300.50 ± 94.72	0.508
Cystine	62.95 ± 22.22	45.51 ± 15.24	**0.000**
Valin	235.85 ± 56.35	301.70 ± 60.58	**0.000**
Lysine	167.40 ± 43.14	210.58 ± 52.88	**0.000**
Leucine	148.25 ± 61.10	163.92 ± 49.72	0.069
Histidine	80.30 ± 18.59	106.84 ± 21.46	**0.000**
Threonine	142.47 ± 48.68	156.65 ± 49.48	0.191
Citrulline	32.17 ± 17.18	26.58 ± 13.04	0.117
Alanine	448.70 ± 117.89	511.70 ± 139.08	**0.032**
Ornithine	88.15 ± 28.39	130.10 ± 44.76	**0.000**
Tyrosine	64.60 ± 24.18	76.61 ± 22.28	**0.014**
	Median (IQR)	**Median (IQR)**	**
Glutamic acid	74.00 (28.25)	87.00 (37.25)	**0.026**
Isoleucine	80.50 (39.50)	94.00 (39.25)	0.355
Methionine	22.50 (6.00)	29.00 (13.00)	**0.000**
Phenylalanine	88.00 (38.50)	69.00 (20.50)	**0.000**
Proline	129.00 (105.00)	242.00 (199.00)	**0.000**

340 * Utilizing *independent sample t-test,* significant at *p-value<0.05*
341 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*

342 **Table 3.** Amino acid profiles of HER2 breast cancer vs control

Amino Acid	HER2	Control	p-value
	Mean ± SD	Mean ± SD	*
Aspartic Acid	16.8±3.42	17.13±12.57	0.898
Serine	96.8±31.85	108.46±29.88	0.473
Threonine	179.4±87.69	154.82±50.15	0.572
Glycine	314.8±75.3	300.5±94.72	0.713
Cystine	54±11.85	45.51±15.25	0.196
Citrulline	28.4±4.51	26.59±13.05	0.542
Alanine	438.2±65.5	511.7±139.08	0.076
Ornithine	73.4±12.93	130.1±44.76	**<0.001**
Histidine	84.4±18.5	106.85±21.47	0.054
Lysine	207.2±35.75	210.59±52.88	0.857
Arginine	144.8±28.04	144.08±29.52	0.959
Valine	227.2±46.93	301.7±60.59	**0.018**
Tyrosine	58.6±14.36	76.62±22.28	**0.044**
Leucine	165.6±50.78	163.93±49.73	0.947
	Median (IQR)	**Median (IQR)**	**
Glutamic Acid	16 (25)	13 (33.75)	**0.043**
Proline	105 (56)	235.5 (186.25)	**<0.001**
Isoleucine	102 (46)	94 (37.75)	0.873
Methionine	25 (3)	29 (12.5)	0.516
Phenylalanine	64 (6)	88 (37.5)	**0.001**

343 * Utilizing *independent sample t-test,* significant at *p-value<0.05*
344 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*
345

346 **Table 4.** Amino acid profiles of luminal A breast cancer vs control

Amino Acid	Luminal A	Control	p-value
	Mean ± SD	Mean ± SD	*
Aspartic Acid	15.94±9.85	17.13±12.57	0.951
Serine	96.71±24.7	108.46±29.88	0.134
Threonine	140.88±35.17	154.82±50.15	0.241
Glycine	277.71±74.62	300.5±94.72	0.343
Cystine	73.24±20.73	45.51±15.25	**<0.001**
Citrulline	29.82±14.35	26.59±13.05	0.432
Alanine	488.12±111.66	511.7±139.08	0.503
Ornithine	92.35±31.29	130.1±44.76	**0.001**
Histidine	76.41±20	106.85±21.47	**<0.001**
Lysine	170.71±42.53	210.59±52.88	**0.005**
Arginine	143.18±23.75	144.08±29.52	0.904
Valine	232.06±61.74	301.7±60.59	**<0.001**
Tyrosine	62.47±26.19	76.62±22.28	0.063
Leucine	170.18±79.81	163.93±49.73	0.768
	Median (IQR)	**Median (IQR)**	**
Glutamic Acid	69 (31)	13 (33.75)	0.116
Proline	135 (127)	235.5 (186.25)	**0.008**
Methionine	23 (5)	94 (37.75)	**0.005**
Isoleucine	102 (78)	29 (12.5)	0.638
Phenylalanine	76 (23)	88 (37.5)	**0.006**

347 ***** Utilizing *independent sample t-test,* significant at *p-value<0.05*
348 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*
349

Table 5. Amino acid profiles of luminal B breast cancer vs control

Amino Acid	Luminal B	Control	p-value
	Mean ± SD	Mean ± SD	*
Aspartic Acid	12.38±8.07	17.13±12.57	0.101
Serine	104.75±21.04	108.46±29.88	0.605
Threonine	141.5±40.76	154.82±50.15	0.312
Glycine	294.69±119.67	300.5±94.72	0.864
Cystine	56.88±23.65	45.51±15.25	0.091
Citrulline	36.38±21.83	26.59±13.05	0.110
Alanine	424±132.4	511.7±139.08	**0.035**
Ornithine	91.44±27.94	130.1±44.76	**<0.001**
Histidine	86.63±13.97	106.85±21.47	**<0.001**
Lysine	155.75±39.43	210.59±52.88	**<0.001**
Arginine	144.5±28.07	144.08±29.52	0.960
Valine	246.31±53.78	301.7±60.59	**0.002**
Tyrosine	69.56±26.18	76.62±22.28	0.354
Leucine	125.19±27.07	163.93±49.73	**<0.001**
	Median (IQR)	Median (IQR)	**
Glutamic Acid	78.5±14.63	13 (33.75)	0.200
Proline	149.56±86.52	235.5 (186.25)	**0.003**
Methionine	23.44±15.97	94 (37.75)	**<0.001**
Isoleucine	81.25±20.9	29 (12.5)	0.080
Phenylalanine	73.38±16.23	88 (37.5)	**0.001**

***** Utilizing *independent sample t-test,* significant at *p-value<0.05*

** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*

354 **Table 6.** Amino acid profiles of triple negative breast cancer vs control

Amino Acid	Triple Negative	Control	p-value
	Mean ± SD	Mean ± SD	*
Aspartic Acid	5±2.83	17.13±12.57	**0.013**
Serine	73.5±10.61	108.46±29.88	0.059
Threonine	71.5±0.71	154.82±50.15	**<0.001**
Glycine	215.5±75.66	300.5±94.72	0.368
Cystine	46.5±10.61	45.51±15.25	0.921
Citrulline	28±22.63	26.59±13.05	0.945
Alanine	337.5±31.82	511.7±139.08	**0.005**
Ornithine	63±24.04	130.1±44.76	0.171
Histidine	52.5±16.26	106.85±21.47	0.138
Lysine	133±50.91	210.59±52.88	0.283
Arginine	116±35.36	144.08±29.52	0.469
Valine	206±80.61	301.7±60.59	0.346
Tyrosine	58±1.41	76.62±22.28	**0.001**
Leucine	103±9.9	163.93±49.73	**0.002**

355 ***** Utilizing *independent sample t-test, significant at p-value<0.05*
356 ** Utilizing the *Mann-Whitney U test, significant at p-value<0.05*
357

358

359 **Table 7.** Amino acid profiles of breast cancer patients according to stages

Amino Acid	**Stages**			**p-value**
	T2	**T3**	**T4**	*
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	
Aspartic Acid	18.24±8.04	12.18±8.2	9.2±5.12	0.086
Serine	91.83±24.03	110.18±22.38	85±12.79	**0.026**
Threonine	144.78±53.31	149.65±44.69	109.8±38.19	0.270
Glycine	289.44±80.46	304.59±111.12	210.6±40.69	0.145
Cystine	66.56±17.03	65.76±26.4	40.4±8.32	**0.048**
Citrulline	30.72±12.73	34.65±21.65	29±16.36	0.732
Alanine	488±105.65	407.76±123.48	446.4±114.03	0.131
Ornithine	86.11±27.22	86.41±25.44	101.4±43.28	0.549
Histidine	74.72±18.05	88.88±16.13	71.2±19.77	**0.036**
Lysine	179.94±42.39	158.59±44.27	152.2±36.72	0.246
Arginine	139.83±23.28	146.82±29.08	137.8±29.35	0.678
Valine	227.67±54.02	234.41±51.52	270.2±78.47	0.333
Methionine	24.17±5.11	23.29±15.49	20±3.08	0.749
Tyrosine	61.17±25.67	63.24±20.88	81.6±27.2	0.241
Isoleucine	108.67±54.25	83.53±27.46	85±25.35	0.189
Leucine	174.94±76.65	125.06±34.3	131±29.27	**0.039**
Phenylalanine	72.11±14.21	72.59±15.57	75.4±15.39	0.909
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**
Glutamic Acid	64 (24.75)	78 (26)	74 (13)	0.199
Proline	133 (77)	133 (136)	111 (34)	0.289
Methionine	24.5 (5.11)	20 (6)	20 (2)	**0.042**

Amino Acid	Stages			p-value
	T2	T3	T4	*
	Mean ± SD	Mean ± SD	Mean ± SD	
Isoleucine	103.5 (49.75)	121 (28)	79 (18)	0.335
Phenylalanine	67 (18.75)	71 (18)	74 (21)	0.859

* Utilizing *ANNOVA,* significant at *p-value<0.05*
** Utilizing the Kruskal-Wallis test, significant at *p-value<0.05*

Table 8. Amino acid profiles of breast cancer risk factors

Amino Acid	Age		Parities		Menarche Age		Family History	
	>40 y.o	**<40 y.o**	**0-1**	**Multiparities**	**<12 y.o**	**>12 y.o**	**Yes**	**No**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Aspartic acid	13.29 ± 8.13	18.66 ± 12.66	16.72 ± 12.91	15.32 ± 9.82	14.84 ± 9.87	15.88 ± 10.91	14.13±12.45	14.52±7.3
Arginine	143.79 ± 31.71	142.69 ± 22.37	140.36 ± 28.58	144.41 ± 28.70	146.69 ± 25.40	142.64 ± 28.30	131.75±23.17	145.25±26.53
Serine	102.31 ± 27.29	105.11 ± 27.70	100.57 ± 28.19	104.63 ± 27.18	112.69 ± 37.21	101.75 ± 24.92	92.75±16.78	100.28±25.51
Glycine	294.55 ± 106.29	291.25 ± 78.60	285.23 ± 78.38	295.96 ± 100.01	338.69 ± 117.29	270.75±79.92	289.84±98.66	289.84±98.66
Cystine	57.11 ± 21.91	50.85 ± 19.32	51.95 ± 24.31	55.20 ± 19.69	51.61 ± 18.30	59.13±17.64	63.91±23.38	63.91±23.38
Valin	263.00 ± 68.57	275.83 ± 65.08	266.90 ± 68.41	269.48 ± 66.92	286.84 ± 90.08	265.26 ± 61.70	207.25±42.31	243±57.68
Lysine	183.38 ± 57.15	195.42 ± 46.14	185.47 ± 46.46	189.89 ± 54.96	202.30 ± 59.71	186.04 ± 51.14	**136.75±41.78 (p=0.040)**	175.06±40.56
Leucine	152.56 ± 59.14	160.38 ± 52.18	148.27 ± 40.70	159.05 ± 60.73	169.30 ± 76.53	153.52 ± 51.33	**110.88±22.88 (p=0.002)**	157.59±64.25
Histidine	94.84 ± 24.12	91.60 ± 24.05	89.19 ± 22.47	94.93 ± 24.53	100.76 ± 24.92	79.13±22.52	80.59±17.89	100.28±25.51
Threonine	143.38 ± 43.14	157.14 ± 55.93	**170.30* ± 53.15 (p=0.032)**	142.17 ± 46.16	164.92 ± 50.59	146.27 ± 48.80	114.5±41.04	149.47±48.46
Citrulline	29.36 ± 17.54	29.48 ± 12.56	27.04 ± 12.57	30.27 ± 16.37	27.23 ± 18.69	29.84 ± 14.85	39.63±28.33	30.31±13.13
Alanine	**450.45* ± 126.55**	516.55 ± 131.00	**534.72* ± 142.77**	459.51 ± 122.65	494.07 ± 165.52	477.50 ± 125.77	**366.5±79.79**	469.25±117.76

Amino Acid	Age		Parities		Menarche Age		Family History	
	>40 y.o	**<40 y.o**	**0-1**	**Multiparities**	**<12 y.o**	**>12 y.o**	**Yes**	**No**
	(p=0.025)		**(p=0.022)**				**(p=0.010)**	
Ornithine	107.25 ± 46.60	110.88 ± 37.83	105.61 ± 44.09	110.03 ± 42.53	135.07 ± 54.41	**103.69* ± 38.45** **(p=0.015)**	86.38±29.93	88.59±28.48
Tyrosine	68.20 ± 22.34	73.45 ± 25.74	71.14 ± 25.54	70.31 ± 23.89	78.92 ± 24.40	68.87 ± 23.63	64.13±24.68	64.72±24.46
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Glutamic acid	80.50 (29.00)	73.50 (40.25)	**66.50** (28.75)** **(p=0.025)**	84.50 (32.00)	78.00 (28.50)	78.00 (33.00)	74 (25.75)	76 (26.25)
Isoleucine	82.50 (32.25)	99.00 (45.75)	84.00 (44.50)	91.00 (39.25)	91.00 (44.50)	87.00 (39.00)	**66.5 (13.25)** **(p=0.021)**	91 (45.5)
Methionine	23.50 (9.00)	26.00 (12.00)	26.00 (15.50)	24.00 (9.25)	26.00 (20.50)	24.00 (9.50)	**19 (3.75)** **(p=0.028)**	23 (5.25)
Phenylalanine	77.00 (26.00)	83.50 (31.00)	84.00 (28.00)	79.00 (25.50)	88.00 (50.00)	79.00 (24.00)	68.5 (17.25)	69 (19.75)
Proline	171.00 (148.00)	176.00 (200.00)	162.50 (225.50)	176.00 (149.50)	195.50 (174.75)	165.00 (157.00)	188.5(104.75)	115 (63.75)

***** Utilizing *independent sample t-test,* significant at *p-value<0.05*

****** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Manuscript Review Form

Reviewer	:	Reviewer 2
Manuscript #	:	**M2022190**
Manuscript Title	:	**Profile of Plasma Amino Acid of Breast Cancer Patients and It's Relation to Breast Cancer Risk Factors**

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?		✓
	Notes: There are numerous studies about plasma amino acid profile in breast cancer patients since 2012. This study claims that this amino acid profile represents Indonesian patients, however, this study conducted only for 3 months (July to September 2020), collecting "only" 40 breast cancer samples.		
2.	Are the title and abstract of the manuscript appropriate?		✓
	Notes: A manuscript's title shoud represent the main findings on a study. This manuscript's title does not represent its main finding, which is the difference of amino acid profile between breast cancer and control groups. The abstract should be rewritten, making it more consice.		
3	Do the title and abstract reflect the study result/content?	✓	
	Notes:		
4.	Is the significance of the study well explained at the Background?		✓
	Notes: The authors claim that there is no current report on amino acid analysis in Breast cancer patients in Indonesia. Once again, this study should represent the whole Indonesian population and the background should be able to explain the important of conducting this study for Indonesia.		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?		
	Notes: Methods should be represented in detail. Grouping should be explained in detail. Number of subjects recruited for this study should be explained as well. Data collected from medical records should be explained in detail.		

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

6.	Are the results, ideas, and data presented in this manuscript important enough for publication?	✓	
	Notes: Yes, they are important.However, the authors need to present it in more consice and stronger sentences.		
7.	Are all figures and tables necessarily presented?		✓
	Notes: Too many tables were put in this manuscript, which makes it too complicated. Tabel 3 – 5 can be represented in one bar graph, presenting all amino acid data for each breast cancer subtype. Table 8 should be revised		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?		✓
	Notes: I do not see a logical flow of argument in the discussion. The authors did explain each amino acid in detail but fail to represent the big picture of amino acid profile in breast cancer patients.		
9.	Are the conclusions and interpretations valid and supported by the data?	✓	
	Notes: Both can be written in a better way		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?		✓
	Notes: This manuscript was written in a good English with only minor mistakes here and there. However, it needs to be explained in more comprehensive way, explaining about all amino acids identified as a profile for breast cancer patients.		

Specific Reviewer's Comments and Suggestions:

(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Reviewer's Recommendation (Please tick only one option)	√
Accept Submission (No significant alterations suggested)	
Revisions Required (Suggest changes to the manuscript as specified in this review)	
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	✓
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

Further Reviewer's Comments Regarding Disposition of the Manuscript:

Date and Sign:
December 05, 2022

Reviewer 2

Profile of Plasma Amino Acid of Breast Cancer Patients and It's Relation to Breast Cancer Risk Factors

Comment [MH1]: A title should represents the major findings of a study.

ABSTRACT

Background: Breast cancer ranks second as the cancer with the highest incidence in the world. For nucleotide synthesis and DNA methylation, cancer cells require amino acids and will utilize greater amounts than normal cells. This study ~~was~~ aimed to analyze the plasma amino acids profile on breast cancer patients and on its risk factors.

Methods: Venous blood from subjects were taken, and then centrifuged at 2500 rpm for 10 minutes to obtain plasma. The blood plasma was analyzed using a liquid chromatography technique to quantify the amino acid level.

Results: There was a statistically significant difference between amino acid level from subjects with breast cancer, i.e. increasing of cystine and decreasing of valine, lysine, histidine, alanine, ornithine, tyrosine, glutamic acid, methionine, and proline, compared to healthy controls. From this study also suggest that several amino acids are associated with breast cancer risk factors, i.e. decreasing of alanine in older age, multiple parity and patients with familial cancer history, and decreasing of ornithine in older age of menarche.

Conclusion: The amino acid profile of breast cancer patients differs from that of healthy controls and correlated with breast cancer risk factors as well. Increasing of cysteine level can be used as biomarker of breast cancer because it was found significantly higher in breast cancer patients, especially in luminal A patients than healthy controls.

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

INTRODUCTION

Cancer is known as complex disease which can be caused by several factors, such as environmental, lifestyle, genetic, and clinical. Its prevalence that increased, lead cancer to the list of chronic debilitating disease.[1] The most common type of cancer which be found on woman is breast cancer.[2] Based on data from the International Agency for Research on Cancer, breast cancer ranks second as the cancer with the highest incidence in the world. Breast cancer also ranks first in the incidence of cancer in women. Globally, an estimated 18.1 million people are affected. The reported breast cancer mortality rate ranges from 626,679 (6.6%), second only to lung cancer. In Indonesia alone, breast cancer has the highest prevalence of 0.5%, or 61,682 cases per year.[3,4] In its development, breast cancer is influenced by risk factors such as age, genetic and family history of breast cancer, BRCA gene mutations, younger first menstrual history, low parity, a history of hormone use and hormone replacement therapy. Obesity can be associated with development of breast cancer as well. It can be affected by the act from aromatization of adrenal androgen into estrogen at adipose tissue.[5] The incidence of breast cancer also increases in the group of women aged >40 years.[4]

Comment [MH2]: Please use the updated data

For nucleotide synthesis and DNA methylation, cancer cells require amino acids and will utilize greater amounts than normal cells. Compared to the control group, breast cancer patients were found to have significant changes in a number of amino acids. Barnes et al. described an increase in the branched chain group of essential and non-essential amino acids in a report. These amino acids consist of leucine, phenylalanine, aspartic acid, taurine, and lysine, among others.[6,7] Additionally, almost all amino acids undergo fluctuating changes in blood, cells, and plasma. The most significant value observed was valine (230±15) in the control group, compared to in breast cancer patients (182±12), and 48.8± 4.2 vs 40.1± 2.8 for isoleucine.[8]

In addition to the role of histidine via histidine-rich glycoprotein (HRG), which is a binding plasma protein that modulates cell immunity, cell adhesion, angiogenesis, and thrombosis, other mechanisms relating to the development of breast cancer have also been attributed to essential amino acids. HRG RNA expression was significantly elevated in all subtypes of breast cancer, with the basal subtype and stage II having the highest levels.[9,10] In regard to the role of the anti-cancer amino acid lysine, the opposite results were also reported. L-Lysine a-oxidase catalyzed by the conversion of L-lysine into a-keto-E-aminocaproic acid, H_2O_2, and ammonia with oxygen consumption exhibited significant anticancer effects on in vitro cell cultures or several in vivo tumor models. It has been demonstrated that the combination of lysine and other micronutrients inhibits the expression and invasion of matrix metalloproteinase (MMP) in several cancers, including breast cancer.[11,12] In general, amount of MMP expressions promote hallmarks of cancer progression, includes invasion, metastasis, angiogenesis, and correlate with patient survival.[13] There is currently no report on the study of amino acids in Indonesian breast cancer patients. Therefore, this study was aimed to analyze the plasma amino acids profile on breast cancer patients and on its risk factors.

METHODS

Patients' characterization

Comment [MH3]: Recruiting subjects

This research was conducted from July 2020 to September 2020 at Cipto Mangunkusumo National Hospital Jakarta (RSCM). This research was approved by the Faculty of Medicine Universitas Indonesia Ethical Committee (approval number: 20-08-0877). The inclusion criteria were at least 18-year-old women with breast cancer confirmed by histopathology and immunohistochemistry who came to RSCM between January to March 2020. Patients with incomplete histopathology and medical record information were exclude from this study. At

least 18-year-old women healthy were recruited as healthy control. All subjects were able to read, comprehend, and sign written agreements.

Amino acid profiling

For amino acid examination, subjects were asked to fast for at least 8 hours and then 2.5 ml of venous blood was taken, which was collected in a sterile container containing heparin anticoagulant. Heparinized blood was then centrifuged at 2500 rpm for 10 minutes to obtain plasma. Plasma was stored at 80°C until examination was carried out.

For the measurement of plasma aspartic acid, glutamate, serine, asparagine, glycine, Glutamic acid, taurine, histidine, alanine, arginine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, ornithine, and lysine, amino acids were analyzed using high-performance liquid chromatography (Waters 2695; Reverse-phase HPLC separation; octadecyl (C18) columns, 25 cm in length (5 μm)). Singletons were used to run samples, external standards, and internal standards. The external standards were utilized at concentrations of 50, 100, and 250 mol/L.[7,14]

Statistical analysis

Data analysis were done with SPSS version 25.0. Using *independent samples t-test* for both variables, a bivariate analysis was performed to determine the difference in mean between two unpaired samples and ANOVA to determine the difference among three groups. In contrast, if a p-value of 0.05 is obtained, there is a statistically significant difference between the two or three samples. If the significance value is greater than 0.05, however, there is no significant difference between the two samples. If the data is neither normal nor homogeneous, the *Mann-Whitney U* or Kruskal-Wallis test is performed to determine whether there is a statistically significant difference between the two or three groups.

RESULTS

Patients' characteristics

Characteristics of breast cancer research subjects were divided by age, stage of breast cancer and the characteristics of breast cancer risk factors, including age risk factors, parity and age of menarche. From a total of 40 research subjects with breast cancer, the average age of the subjects was 52.08 years while in healthy women as controls, the average age was 41.65 years. Breast cancer research subjects in this study 45% had stage T2, and 42.5% had luminal A. Table 1 shows the data on the characteristics of the subjects in this study.

Amino acid profiles of breast cancer patients

The distribution of data from 80 samples of research subjects was normal and homogeneous for 14 amino acids, but was not normal and was not homogeneous for the remaining 5 amino acids. Glutamic acid, isoleucine, methionine, phenylalanine, and proline are the five amino acids. Both samples of amino acid data, which are normally distributed and homogeneous, are subjected to a parametric test using an *independent sample t-test*. Due to the abnormal and non-homogeneous nature of the data, non-parametric tests utilizing the *Mann-Whitney U* test were conducted to determine whether there were significant differences between the two samples. The amino acid profiles of breast cancer patients and healthy controls are displayed in Table 2.

Based on the result, there was a statistically significant difference between amino acid level from subjects with breast cancer and controls, with breast cancer patients having higher levels of cystine and phenylalanine than controls. In addition to the increase in cystine and phenylalanine levels, there was a significant decrease in valine, lysine, histidine, alanine, ornithine, tyrosine, Glutamic acid, methionine, and proline levels in breast cancer patients compared to healthy controls.

We also added some analysis on amino acid profile of breast cancer patients based on their subtype and breast cancer stages (Table 3-7). In HER2 positive samples, ornithine,

valine, tyrosine, proline, and phenylalanine were significantly decrease than in healthy control. On the other side, patients with Luminal A and B, had lower level of ornithine, histidine, lysine, valine, proline, methionine and phenylalanine. Cystine had higher level in luminal A significantly compared to healthy control. Contrast with triple negative result, aspartic acid and threonine showed lower level significantly than healthy control. In stages comparison, cystine showed lower levels significantly in higher stages.

Amino acid profiles of breast cancer risk factors

To determine the role of amino acids in breast cancer risk factors, an *independent sample t-test* or *Mann-Whitney U* test was performed between amino acids and each risk factor in breast cancer research subjects (Table 8). These results suggest that several amino acids are associated with breast cancer risk factors. Age is a known risk factor with a significant mean value for alanine (p=0.025). The parity risk factor was also significant for threonine (p=0.032), alanine (p=0.022), and Glutamic acid (p=0.025). The significance of the risk factor for menarche for the amino acid ornithine is 0.015. The family history factors showed lower level as well of lysine (p=0.040), leucine (p=0.002), alanine (p=0.010), isoleucine (p=0.021), and methionine (p=0.028).

DISCUSSIONS

Amino acids are organic compounds containing an amino group (-NH) and a carboxyl group (-COOH); they are the fundamental structural units of proteins. Amino acids are categorized into essential amino acids and non-essential amino acids. Amino acids are the primary molecules required for protein synthesis and have multiple functions within the cell. As amino acids are essential for nucleotide synthesis and DNA methylation, they play a role in cancer cell proliferation. 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.[15,16]

There was a statistically significant difference between subjects with breast cancer and controls in terms of the amino acid cystine, with breast cancer patients having higher levels of cystine than controls. From our study, the mean of cysteine level was increase in breast cancer patients (62.95 ± 22.22) compared to healthy control (45.51 ± 15.24), especially in luminal A type ($p<0.001$). 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 in the presence of folic acid deficiency. This renders ineffective the processes of DNA methylation and regulation of gene expression, which contribute to oncogenesis at the genetic level and 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.[18–20]

There were some significant decreases in some amino acids levels in breast cancer patients compared to healthy controls. In line with previous study, patients with breast cancer had decreased plasma levels of valine due to the excessive consumption of breast cancer cells by uncontrolled cell growth. The decrease in valine levels in breast cancer is attributable to a shift in the concentration of energy on tumor cell proliferation, resulting in an increase in anaerobic glycolysis, which inhibits the TCA cycle.[21]

L-lysine is an essential amino acid that must be obtained through the consumption of food. L-Lysine, a lysine derivative, is known to be effective as an anti-cancer agent. According to reports, lysine can function as an anti-tumor agent by inhibiting MMP.[11,12] Lysine methylation and lysine acetylation play a role in cancer cell proliferation,

differentiation, migration, and signal transduction via metabolic regulation. This correlates with lower lysine levels in breast cancer compared to healthy controls. Low lysine levels indicate high methylation and acetylation of lysine in breast cancer cells during cancer cell proliferation.[22]

The correlation between histidine and breast cancer is explained by several pathways. Histidine-rich glycoprotein (HRG), a plasma protein that binds numerous ligands and modulates immunity, cell adhesion, angiogenesis, and thrombosis, is converted into a plasma protein.[9,10] Aspartic acid and oxaloacetate in the TCA cycle are linked to the decrease in histidine levels in breast cancer cells.[22] Aspartic acid consumption by cancer cells has decreased aspartic acid levels in the blood. Histidine concentrations in the blood decrease when aspartic acid concentrations fall. In addition to histidine, proline is involved in feeding the TCA cycle via the urea cycle and ROS formation via proline dehydrogenase (PRODH). Previous studies reported in line with our study that histidine and proline levels were lower in breast cancer patients than controls.[7,21]

Through the production of alpha-ketoglutarate, alanine plays a role in the formation of extracellular matrix in metastatic breast cancer.[23] The breast cancer proliferative pathway inhibits the enzyme GPT2 (alanine transaminotransferase-2) that produces alanine from glutamate and pyruvate.[24] This is associated with a decrease in the average amount of alanine in breast cancer as compared to healthy controls.

The most abundant amino acid in plasma is Glutamic acid. It has been demonstrated that Glutamic acid plays a role in cancer cell proliferation by providing carbon and nitrogen for biosynthetic reactions. Multiple amino acid transporters, including ASCT2, are responsible for Glutamic acid uptake by cancer cells. In some tumors, Glutamic acid absorption is also significantly increased.[9,25] Glutamic acid levels have decreased in breast cancer due to its role in cancer cell proliferation via glutaminolysis, which reduces blood Glutamic acid levels.[21]

There are several amino acids that has significant difference in abundance between stages of breast cancer as well (Table 7). ANOVA test between stages of breast cancer found that serine, cystine, histidine, and leucine are significantly different between stages. Contradict to our study, all three genes involved in the L: -serine biosynthesis pathway, phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH), were reported to be upregulated in the highly metastatic variant, according to genome-wide gene expression profiling of this isogenic cell line pair. High PHGDH and PSAT1 expression in primary breast cancer was related with lower relapse-free and overall survival, as well as malignant phenotypic characteristics of breast cancer.[26,27] Meanwhile, we found lower level of serine in stage T4.

In this study, we found that leucine level is significantly lower in stage T3 and T4 of breast cancer. There is a report of leucine aminopeptidase 3 (LAP3), an exopeptidase that catalyzes the hydrolysis of leucine residues at the amino terminus of a protein or peptide substrate, to be highly expressed in breast cancer tissues.[28,29] 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.[29]

The amino acid profile is not only associated with breast cancer incidence, but also with breast cancer risk factors. Age is a known risk factor with a significant mean value for alanine, as well seen in our study, that patients with age above 40 years old has higher level of Alanine. In previous study reported that Alanine levels increased with age and reached a maximum between the ages of 40 and 55. This relationship was not linear, but rather U-shaped and inverted.[30] The parity risk factor was also significant for threonine ($p=0.032$), alanine ($p=0.022$), and Glutamic acid ($p=0.025$). The significance of the risk factor for menarche for the amino acid ornithine is 0.015. Ornithine decarboxylase (ODC), according to Deng et al., regulates estrogen receptor alpha expression and breast cancer cell growth.[31]

The limitation of this study is the lack of research samples from each research subject, so further research is needed 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 needed to determine specifically the changes in amino acid profiles due to cancer.

CONSCLUSION

The amino acid profile of breast cancer patients differs from that of healthy controls and correlated with breast cancer risk factors as well. Increasing of cysteine level can be used as biomarker of breast cancer because it was found significantly higher in breast cancer patients, especially in luminal A patients than healthy controls.

REFERENCES

1. Meiliana A, Dewi NM, Wijaya A. Red meats and processed meat as the carcinogenic foods and phytochemical-chemoprevention. Indones Biomed J. 2019;11(3):225–39.
2. Purwanto I, Heiyanto DS, Ghozali A, Widodo I, Dwiprahasto I, Aryandono T, et al. Basal-like subgroup is associated with younger age, increased expression of androgen receptor, and worse prognosis, while non-basal-like subtype is associated with higher BMI in triple-negative breast cancer patients. Indones Biomed J. 2020;12(4):349–54.
3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
4. Kraeima J, Siesling S, Vliegen IMH, Klaase JM, IJzerman MJ. Individual risk profiling for breast cancer recurrence: towards tailored follow-up schemes. Br J Cancer. 2013;109(4):866–71.

5. Retnowarnadi A, Kresno SB, Arif M. Association of Obesity and Breast Cancer Risk: The Role of Estrogen, Tumor Necrosis Factor-alpha, and Adiponectin as Risk factors (preliminary study). Indones Biomed J. 2009;1(1):45.

6. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr. 2006;136(1 Suppl):227S–31S.

7. Barnes T, Bell K, DiSebastiano KM, Vance V, Hanning R, Russell C, et al. Plasma amino acid profiles of breast cancer patients early in the trajectory of the disease differ from healthy comparison groups. Appl Physiol Nutr Metab. 2014;39(6):740–4.

8. Proenza AM, Oliver J, Palou A, Roca P. Breast and lung cancer are associated with a decrease in blood cell amino acid content. J Nutr Biochem. 2003;14(3):133–8.

9. Eissa S, Azzazy HME, Matboli M, Shawky SM, Said H, Anous FA. The prognostic value of histidine-rich glycoprotein RNA in breast tissue using unmodified gold nanoparticles assay. Appl Biochem Biotechnol. 2014;174(2):751–61.

10. Matboli M, Eissa S, Said H. Evaluation of histidine-rich glycoprotein tissue RNA and serum protein as novel markers for breast cancer. Med Oncol. 2014;31(4):897.

11. Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. Inhibition of matrix metalloproteinase-2 secretion and invasion by human ovarian cancer cell line SK-OV-3 with lysine, proline, arginine, ascorbic acid and green tea extract: Nutrients inhibit ovarian cancer. J Obstet Gynaecol Res. 2006;32(2):148–54.

12. Roomi MW, Ivanov V, Kalinovsky T, Niedzwiecki A, Rath M. In vitro and in vivo antitumorigenic activity of a mixture of lysine, proline, ascorbic acid, and green tea extract on human breast cancer lines MDA-MB-231 and MCF-7. Med Oncol. 2005;22(2):129–38.

13. Wulandari F, Ikawati M, Kirihata M, Kato JY, Meiyanto E. Curcumin analogs, PGV-1 and CCA-1.1 exhibit anti-migratory effects and suppress MMP9 expression on WiDr cells. Indones Biomed J. 2021;13(3):271–80.

14. Laposata M. Laposata's laboratory medicine diagnosis of disease in clinical laboratory third edition. 3rd ed. Columbus, OH: McGraw-Hill Education; 2019.

15. Cha Y, Kim ES, Koo J. Amino acid transporters and Glutamic acid metabolism in breast cancer. Int J Mol Sci. 2018;19(3):907.

16. Geck RC, Toker A. Nonessential amino acid metabolism in breast cancer. Adv Biol Regul. 2016;62:11–7.

17. Yang L, Wang Y, Cai H, Wang S, Shen Y, Ke C. Application of metabolomics in the diagnosis of breast cancer: a systematic review. J Cancer. 2020;11(9):2540–51.

18. SLOANE BF, MOIN K, LAH TT. Regulation of Lysosomal Endopeptidases in Malignant Neoplasia. Biochemical and Molecular Aspects of Selected Cancers. 1994 Jan 1;411–66.

19. Berquin IM, Sloane BF. Cysteine proteases and tumor progression. Perspectives in Drug Discovery and Design. 1995 Jul;2(3):371–88.

20. Buck MR, Karustis DG, Day NA, Honn K v., Sloane BF. Degradation of extracellular-matrix proteins by human cathepsin B from normal and tumour tissues. Biochemical Journal [Internet]. 1992 Feb 15 [cited 2022 Nov 7];282(1):273–8. Available from: /biochemj/article/282/1/273/36721/Degradation-of-extracellular-matrix-proteins-by

21. Varela A, Puebla-Pérez AM, Delgado-Saucedo JI, Rodr\'\iguez-Arévalo F, Zúñiga-González GM, Figuera LE, et al. Increased homocysteine plasma levels in breast cancer patients of a Mexican population. Exp Oncol. 2018;40(2):114–8.

22. Cappelletti V, Iorio E, Miodini P, Silvestri M, Dugo M, Daidone MG. Metabolic footprints and molecular subtypes in breast cancer. Dis Markers. 2017;2017:1–19.

297 23. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in
298 cancer. Br J Cancer. 2020;122(2):150–6.
299 24. Mitra D. Investigating the role of alanine aminotransferase 2 in breast cancer.
300 Heidelberg University Library; 2018.
301 25. Juhász C, Nahleh Z, Zitron I, Chugani DC, Janabi MZ, Bandyopadhyay S, et al.
302 Tryptophan metabolism in breast cancers: molecular imaging and
303 immunohistochemistry studies. Nucl Med Biol. 2012;39(7):926–32.
304 26. Pollari S, Käkönen SM, Edgren H, Wolf M, Kohonen P, Sara H, et al. Enhanced serine
305 production by bone metastatic breast cancer cells stimulates osteoclastogenesis. Breast
306 Cancer Res Treat [Internet]. 2011 Jan [cited 2022 Nov 7];125(2):421–30. Available
307 from: https://pubmed.ncbi.nlm.nih.gov/20352489/
308 27. Luo J. Cancer's sweet tooth for serine. Breast Cancer Research [Internet]. 2011 Nov
309 28 [cited 2022 Nov 7];13(6):1–3. Available from: https://breast-cancer-
310 research.biomedcentral.com/articles/10.1186/bcr2932
311 28. Tsujimoto M, Goto Y, Maruyama M, Hattori A. Biochemical and enzymatic properties
312 of the M1 family of aminopeptidases involved in the regulation of blood pressure.
313 Heart Fail Rev [Internet]. 2008 Sep 13 [cited 2022 Nov 7];13(3):285–91. Available
314 from: https://link.springer.com/article/10.1007/s10741-007-9064-8
315 29. Fang C, Zhang J, Yang H, Peng L, Wang K, Wang Y, et al. Leucine aminopeptidase 3
316 promotes migration and invasion of breast cancer cells through upregulation of fascin
317 and matrix metalloproteinases-2/9 expression. J Cell Biochem [Internet]. 2019 Mar 1
318 [cited 2022 Nov 7];120(3):3611–20. Available from:
319 https://onlinelibrary.wiley.com/doi/full/10.1002/jcb.27638

30. Elinav E, Ben-Dov IZ, Ackerman E, Kiderman A, Glikberg F, Shapira Y, et al. Correlation between serum alanine aminotransferase activity and age: an inverted U curve pattern. Am J Gastroenterol. 2005;100(10):2201–4.

31. Deng W, Jiang X, Mei Y, Sun J, Ma R, Liu X, et al. Role of ornithine decarboxylase in breast cancer. Acta Biochim Biophys Sin (Shanghai). 2008;40(3):235–43.

TABLES

Table 1. Subjects' characteristics

Characteristics	Breast cancer patients	Healthy controls
Age	52.08 ± 8.42 y.o	41.65 ± 12.46 y.o
Breast Cancer Stage		
T4	5	
T3	17	
T2	18	
Molecular Subtype		
HER2 positive	5	
Luminal A	17	
Luminal B	16	
Triple Negative	2	
Risk factors		
Age: <40 years old	18	18
>40 years old	22	22
0-1 parity	10	13
Multiparity	30	27
Age of menarche: <12 y.o	31	10
>12 y.o	9	30

333 **Table 2.** Amino acid profiles of breast cancer patients and healthy control

Amino Acid	Breast cancer patients	Healthy control	*p-value**
	Mean ± SD	Mean ± SD	
Aspartic acid	14.30 ± 8.34	17.12 ± 12.57	0.752
Arginine	142.52 ± 26.18	144.07 ± 29.52	0.804
Serine	98.77 ± 24.02	108.46 ± 29.87	0.116
Glycine	286.02 ± 94.56	300.50 ± 94.72	0.508
Cystine	62.95 ± 22.22	45.51 ± 15.24	**0.000**
Valin	235.85 ± 56.35	301.70 ± 60.58	**0.000**
Lysine	167.40 ± 43.14	210.58 ± 52.88	**0.000**
Leucine	148.25 ± 61.10	163.92 ± 49.72	0.069
Histidine	80.30 ± 18.59	106.84 ± 21.46	**0.000**
Threonine	142.47 ± 48.68	156.65 ± 49.48	0.191
Citrulline	32.17 ± 17.18	26.58 ± 13.04	0.117
Alanine	448.70 ± 117.89	511.70 ± 139.08	**0.032**
Ornithine	88.15 ± 28.39	130.10 ± 44.76	**0.000**
Tyrosine	64.60 ± 24.18	76.61 ± 22.28	**0.014**
	Median (IQR)	**Median (IQR)**	**
Glutamic acid	74.00 (28.25)	87.00 (37.25)	**0.026**
Isoleucine	80.50 (39.50)	94.00 (39.25)	0.355
Methionine	22.50 (6.00)	29.00 (13.00)	**0.000**
Phenylalanine	88.00 (38.50)	69.00 (20.50)	**0.000**
Proline	129.00 (105.00)	242.00 (199.00)	**0.000**

334 * Utilizing *independent sample t-test,* significant at *p-value<0.05*
335 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*

336 **Table 3.** Amino acid profiles of HER2 breast cancer vs control

Amino Acid	HER2	Control	p-value
	Mean ± SD	Mean ± SD	*
Aspartic Acid	16.8±3.42	17.13±12.57	0.898
Serine	96.8±31.85	108.46±29.88	0.473
Threonine	179.4±87.69	154.82±50.15	0.572
Glycine	314.8±75.3	300.5±94.72	0.713
Cystine	54±11.85	45.51±15.25	0.196
Citrulline	28.4±4.51	26.59±13.05	0.542
Alanine	438.2±65.5	511.7±139.08	0.076
Ornithine	73.4±12.93	130.1±44.76	**<0.001**
Histidine	84.4±18.5	106.85±21.47	0.054
Lysine	207.2±35.75	210.59±52.88	0.857
Arginine	144.8±28.04	144.08±29.52	0.959
Valine	227.2±46.93	301.7±60.59	**0.018**
Tyrosine	58.6±14.36	76.62±22.28	**0.044**
Leucine	165.6±50.78	163.93±49.73	0.947
	Median (IQR)	**Median (IQR)**	**
Glutamic Acid	16 (25)	13 (33.75)	**0.043**
Proline	105 (56)	235.5 (186.25)	**<0.001**
Isoleucine	102 (46)	94 (37.75)	0.873
Methionine	25 (3)	29 (12.5)	0.516
Phenylalanine	64 (6)	88 (37.5)	**0.001**

337 ***** Utilizing *independent sample t-test,* significant at *p-value<0.05*
338 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*
339

340 **Table 4.** Amino acid profiles of luminal A breast cancer vs control

Amino Acid	Luminal A	Control	p-value
	Mean ± SD	**Mean ± SD**	*
Aspartic Acid	15.94±9.85	17.13±12.57	0.951
Serine	96.71±24.7	108.46±29.88	0.134
Threonine	140.88±35.17	154.82±50.15	0.241
Glycine	277.71±74.62	300.5±94.72	0.343
Cystine	73.24±20.73	45.51±15.25	**<0.001**
Citrulline	29.82±14.35	26.59±13.05	0.432
Alanine	488.12±111.66	511.7±139.08	0.503
Ornithine	92.35±31.29	130.1±44.76	**0.001**
Histidine	76.41±20	106.85±21.47	**<0.001**
Lysine	170.71±42.53	210.59±52.88	**0.005**
Arginine	143.18±23.75	144.08±29.52	0.904
Valine	232.06±61.74	301.7±60.59	**<0.001**
Tyrosine	62.47±26.19	76.62±22.28	0.063
Leucine	170.18±79.81	163.93±49.73	0.768
	Median (IQR)	**Median (IQR)**	**
Glutamic Acid	69 (31)	13 (33.75)	0.116
Proline	135 (127)	235.5 (186.25)	**0.008**
Methionine	23 (5)	94 (37.75)	**0.005**
Isoleucine	102 (78)	29 (12.5)	0.638
Phenylalanine	76 (23)	88 (37.5)	**0.006**

341 ***** Utilizing *independent sample t-test,* significant at *p-value<0.05*
342 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*
343

344 **Table 5.** Amino acid profiles of luminal B breast cancer vs control

Amino Acid	Luminal B	Control	p-value
	Mean ± SD	**Mean ± SD**	*
Aspartic Acid	12.38±8.07	17.13±12.57	0.101
Serine	104.75±21.04	108.46±29.88	0.605
Threonine	141.5±40.76	154.82±50.15	0.312
Glycine	294.69±119.67	300.5±94.72	0.864
Cystine	56.88±23.65	45.51±15.25	0.091
Citrulline	36.38±21.83	26.59±13.05	0.110
Alanine	424±132.4	511.7±139.08	**0.035**
Ornithine	91.44±27.94	130.1±44.76	**<0.001**
Histidine	86.63±13.97	106.85±21.47	**<0.001**
Lysine	155.75±39.43	210.59±52.88	**<0.001**
Arginine	144.5±28.07	144.08±29.52	0.960
Valine	246.31±53.78	301.7±60.59	**0.002**
Tyrosine	69.56±26.18	76.62±22.28	0.354
Leucine	125.19±27.07	163.93±49.73	**<0.001**
	Median (IQR)	**Median (IQR)**	**
Glutamic Acid	78.5±14.63	13 (33.75)	0.200
Proline	149.56±86.52	235.5 (186.25)	**0.003**
Methionine	23.44±15.97	94 (37.75)	**<0.001**
Isoleucine	81.25±20.9	29 (12.5)	0.080
Phenylalanine	73.38±16.23	88 (37.5)	**0.001**

345 * Utilizing *independent sample t-test,* significant at *p-value<0.05*
346 ** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*
347

348 **Table 6.** Amino acid profiles of triple negative breast cancer vs control

Amino Acid	Triple Negative	Control	p-value
	Mean ± SD	Mean ± SD	*
Aspartic Acid	5±2.83	17.13±12.57	**0.013**
Serine	73.5±10.61	108.46±29.88	0.059
Threonine	71.5±0.71	154.82±50.15	**<0.001**
Glycine	215.5±75.66	300.5±94.72	0.368
Cystine	46.5±10.61	45.51±15.25	0.921
Citrulline	28±22.63	26.59±13.05	0.945
Alanine	337.5±31.82	511.7±139.08	**0.005**
Ornithine	63±24.04	130.1±44.76	0.171
Histidine	52.5±16.26	106.85±21.47	0.138
Lysine	133±50.91	210.59±52.88	0.283
Arginine	116±35.36	144.08±29.52	0.469
Valine	206±80.61	301.7±60.59	0.346
Tyrosine	58±1.41	76.62±22.28	**0.001**
Leucine	103±9.9	163.93±49.73	**0.002**

349 ***** Utilizing *independent sample t-test, significant at p-value<0.05*
350 ** Utilizing the *Mann-Whitney U test, significant at p-value<0.05*
351

352

353 **Table 7.** Amino acid profiles of breast cancer patients according to stages

Amino Acid	Stages			p-value
	T2	T3	T4	*
	Mean ± SD	Mean ± SD	Mean ± SD	
Aspartic Acid	18.24±8.04	12.18±8.2	9.2±5.12	0.086
Serine	91.83±24.03	110.18±22.38	85±12.79	**0.026**
Threonine	144.78±53.31	149.65±44.69	109.8±38.19	0.270
Glycine	289.44±80.46	304.59±111.12	210.6±40.69	0.145
Cystine	66.56±17.03	65.76±26.4	40.4±8.32	**0.048**
Citrulline	30.72±12.73	34.65±21.65	29±16.36	0.732
Alanine	488±105.65	407.76±123.48	446.4±114.03	0.131
Ornithine	86.11±27.22	86.41±25.44	101.4±43.28	0.549
Histidine	74.72±18.05	88.88±16.13	71.2±19.77	**0.036**
Lysine	179.94±42.39	158.59±44.27	152.2±36.72	0.246
Arginine	139.83±23.28	146.82±29.08	137.8±29.35	0.678
Valine	227.67±54.02	234.41±51.52	270.2±78.47	0.333
Methionine	24.17±5.11	23.29±15.49	20±3.08	0.749
Tyrosine	61.17±25.67	63.24±20.88	81.6±27.2	0.241
Isoleucine	108.67±54.25	83.53±27.46	85±25.35	0.189
Leucine	174.94±76.65	125.06±34.3	131±29.27	**0.039**
Phenylalanine	72.11±14.21	72.59±15.57	75.4±15.39	0.909
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**
Glutamic Acid	64 (24.75)	78 (26)	74 (13)	0.199
Proline	133 (77)	133 (136)	111 (34)	0.289
Methionine	24.5 (5.11)	20 (6)	20 (2)	**0.042**

Amino Acid	Stages			p-value
	T2	T3	T4	*
	Mean ± SD	Mean ± SD	Mean ± SD	
Isoleucine	103.5 (49.75)	121 (28)	79 (18)	0.335
Phenylalanine	67 (18.75)	71 (18)	74 (21)	0.859

354 * Utilizing *ANNOVA,* significant at *p-value<0.05*
355 ** Utilizing the Kruskal-Wallis test, significant at *p-value<0.05*
356

Table 8. Amino acid profiles of breast cancer risk factors

Amino Acid	Age		Parities		Menarche Age		Family History	
	>40 y.o	**<40 y.o**	**0-1**	**Multiparities**	**<12 y.o**	**>12 y.o**	**Yes**	**No**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Aspartic acid	13.29 ± 8.13	18.66 ± 12.66	16.72 ± 12.91	15.32 ± 9.82	14.84 ± 9.87	15.88 ± 10.91	14.13±12.45	14.52±7.3
Arginine	143.79 ± 31.71	142.69 ± 22.37	140.36 ± 28.58	144.41 ± 28.70	146.69 ± 25.40	142.64 ± 28.30	131.75±23.17	145.25±26.53
Serine	102.31 ± 27.29	105.11 ± 27.70	100.57 ± 28.19	104.63 ± 27.18	112.69 ± 37.21	101.75 ± 24.92	92.75±16.78	100.28±25.51
Glycine	294.55 ± 106.29	291.25 ± 78.60	285.23 ± 78.38	295.96 ± 100.01	338.69 ± 117.29	270.75±79.92	289.84±98.66	289.84±98.66
Cystine	57.11 ± 21.91	50.85 ± 19.32	51.95 ± 24.31	55.20 ± 19.69	51.61 ± 18.30	59.13±17.64	63.91±23.38	63.91±23.38
Valin	263.00 ± 68.57	275.83 ± 65.08	266.90 ± 68.41	269.48 ± 66.92	286.84 ± 90.08	265.26 ± 61.70	207.25±42.31	243±57.68
Lysine	183.38 ± 57.15	195.42 ± 46.14	185.47 ± 46.46	189.89 ± 54.96	202.30 ± 59.71	186.04 ± 51.14	**136.75±41.78 (p=0.040)**	175.06±40.56
Leucine	152.56 ± 59.14	160.38 ± 52.18	148.27 ± 40.70	159.05 ± 60.73	169.30 ± 76.53	153.52 ± 51.33	**110.88±22.88 (p=0.002)**	157.59±64.25
Histidine	94.84 ± 24.12	91.60 ± 24.05	89.19 ± 22.47	94.93 ± 24.53	100.76 ± 24.92	79.13±22.52	80.59±17.89	100.28±25.51
Threonine	143.38 ± 43.14	157.14 ± 55.93	**170.30* ± 53.15 (p=0.032)**	142.17 ± 46.16	164.92 ± 50.59	146.27 ± 48.80	114.5±41.04	149.47±48.46
Citrulline	29.36 ± 17.54	29.48 ± 12.56	27.04 ± 12.57	30.27 ± 16.37	27.23 ± 18.69	29.84 ± 14.85	39.63±28.33	30.31±13.13
Alanine	**450.45* ± 126.55**	516.55 ± 131.00	**534.72* ± 142.77**	459.51 ± 122.65	494.07 ± 165.52	477.50 ± 125.77	**366.5±79.79**	469.25±117.76

Amino Acid	Age		Parities		Menarche Age		Family History	
	>40 y.o	**<40 y.o**	**0-1**	**Multiparities**	**<12 y.o**	**>12 y.o**	**Yes**	**No**
	(p=0.025)		**(p=0.022)**				**(p=0.010)**	
Ornithine	107.25 ± 46.60	110.88 ± 37.83	105.61 ± 44.09	110.03 ± 42.53	135.07 ± 54.41	**103.69* ± 38.45** **(p=0.015)**	86.38±29.93	88.59±28.48
Tyrosine	68.20 ± 22.34	73.45 ± 25.74	71.14 ± 25.54	70.31 ± 23.89	78.92 ± 24.40	68.87 ± 23.63	64.13±24.68	64.72±24.46
	Median (IQR)	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Glutamic acid	80.50 (29.00)	73.50 (40.25)	**66.50** (28.75)** **(p=0.025)**	84.50 (32.00)	78.00 (28.50)	78.00 (33.00)	74 (25.75)	76 (26.25)
Isoleucine	82.50 (32.25)	99.00 (45.75)	84.00 (44.50)	91.00 (39.25)	91.00 (44.50)	87.00 (39.00)	**66.5 (13.25)** **(p=0.021)**	91 (45.5)
Methionine	23.50 (9.00)	26.00 (12.00)	26.00 (15.50)	24.00 (9.25)	26.00 (20.50)	24.00 (9.50)	**19 (3.75)** **(p=0.028)**	23 (5.25)
Phenylalanine	77.00 (26.00)	83.50 (31.00)	84.00 (28.00)	79.00 (25.50)	88.00 (50.00)	79.00 (24.00)	68.5 (17.25)	69 (19.75)
Proline	171.00 (148.00)	176.00 (200.00)	162.50 (225.50)	176.00 (149.50)	195.50 (174.75)	165.00 (157.00)	188.5(104.75)	115 (63.75)

***** Utilizing *independent sample t-test,* significant at *p-value<0.05*

****** Utilizing the *Mann-Whitney U test,* significant at *p-value<0.05*

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2022190 Editor Decision Round 1 - Resubmit for Review

Ferry Sandra <ferry@trisakti.ac.id> Tue, May 23, 2023 at 9:20 AM
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Good day. Thank you for your comprehensive review of manuscript M2022190 that was previously titled "Profile of Plasma Amino Acid of Breast Cancer Patients and It's Relation to Breast Cancer Risk Factors". I have made a major revision to the manuscript and all the comments from the reviewers have been addressed accordingly. Herein I attached the revised manuscript, hopefully you can find it well.

Thank you.

Regards,
Ferry Sandra
[Quoted text hidden]
--
Ferry Sandra, D.D.S., Ph.D.
Head of Medical Research Center
Universitas Trisakti

Round 1 Revision from Author.docx
1458K

Distribution and Profiling of Amino Acids in Breast Cancer Patients

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 Luminal 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

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.(4-6) Around 2.3 million cases were recorded by Global Cancer Observatory in 2020, 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.(7) By 2020, breast cancer continued as the most common cancer in women (30.8%) and the leading cause of death in Indonesia (15.3%).(8,9)

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.(9) 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.(10,11)

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.(12) Interestingly, breast cancer cells limit the use of amino acids for cell proliferation based on amino acid availability, which depends on estrogenic receptor status.(13) 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.(11,14)

Tumor-dependent increase of serum amino acid levels has been reported to be correlated with molecular subtypes of breast cancer.(15) 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.(14,16)

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-value<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 statistically different between the healthy control and breast cancer subjects (Figure 1). However, the 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 statistically different between T2 and T3 of breast cancer subjects with luminal B subtype (Figure 3).

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).

DISCUSSIONS

Amino acids are essential nutrients in all living cells and are important for the proliferation and maintenance of tumor cells.(12) 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.(12,17) 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 initiate cancer.(18) 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.(19)

Through the production of alpha-ketoglutarate, alanine plays a role in the formation of extracellular matrix in metastatic breast cancer.(20) The breast cancer proliferative pathway inhibits the enzyme GPT2 (alanine transaminotransferase-2) that produces alanine from glutamate and pyruvate.(21) This is associated with a decrease in the average amount of alanine in breast cancer subject compared to healthy controls.

In this study, we found that leucine level is significantly decreased in breast cancer subjects than the healthy control. 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.(22,23) 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.(23)

The amino acid profile is not only associated with breast cancer incidence, but also with breast cancer risk factors.(24) 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 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 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 needed to determine specifically the changes in amino acid profiles due to cancer.

CONCLUSION

The amino acid profile of patients with Luminal A and Luminal 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.

REFERENCES

1. Savitri M, Bintoro UY, Sedana MP, Diansyah MN, Romadhon PZ, Amrita PNA, et al. Circulating plasma miRNA-21 as a superior biomarker compared to ca 15-3: assessment in healthy age matched subjects and different stage of breast cancer patients. Indones Biomed J. 2020; 12(2): 157-64

2. Purwanto I, Heriyanto DS, Ghozali A, Widodo I, Dwiprahasto I, Aryandono T, et al. Basal-Like Subgroup is Associated with Younger Age, Increased Expression of Androgen Receptor, and Worse Prognosis, while Non-basal-like Subtype is Associated with Higher BMI in Triple-Negative Breast Cancer Patients. Vol. 12. 2020.

3. Widowati W, Jasaputra DK, Sumitro SB, Widodo MA, Afifah E, Rizal R, et al. Direct and Indirect Effect of TNFα and IFNγ Toward Apoptosis in Breast Cancer Cells. Mol Cell

Biomed Sci. 2018;2(2):60.

4. Abdihalim TS, Idris AAA. Mucin Level as a Potential Biomarker for Breast Cancer Diagnosis. Mol Cell Biomed Sci. 2022;6(3):117.

5. Ilyan T, Retnoningrum D, Hendrianingtyas M, Widyaningrum D, Rachmawati B. Association of 25-hydroxyvitamin D, Cyclooxygenase-2 and Prostaglandin E2 Serum Levels in Breast Cancer Patients. Indones Biomed J. 2021; 13(4): 426-32.

6. Abdihalim TS, Idris AAA. Mucin Level as a Potential Biomarker for Breast Cancer Diagnosis. Mol Cell Biomed Sci. 2022; 6(3): 117-20.

7. Remelia M, Bela B, Widyaningtyas ST, Antarianto RD, Mazfufah NF, Pawitan JA. The Use of Cell-penetrating Peptide for Delivery of Recombinant Transcription Factor DNA into Primary Human Fibroblast. Mol Cell Biomed Sci. 2023; 7(1): 28-37.

8. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.

9. Kraeima J, Siesling S, Vliegen IMH, Klaase JM, Ijzerman MJ. Individual risk profiling for breast cancer recurrence : towards tailored follow-up schemes [Internet]. Vol. 109, British Journal of Cancer. Nature Publishing Group; 2013. Available from: http://dx.doi.org/10.1038/bjc.2013.401

10. Retnowarnadi A, Kresno SB, Arif M. Association of Obesity and Breast Cancer Risk: The Role of Estrogen, Tumor Necrosis Factor-alpha, and Adiponectin as Risk factors (preliminary study). Indones Biomed J [Internet]. 2009 Apr 1;1(1):45. Available from: https://inabj.org/index.php/ibj/article/view/82

11. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr. 2006;136(1):227–31.

198 12. Cha Y, Kim ES, Koo J. Amino Acid Transporters and Glutamine Metabolism in
199 Breast Cancer. Int J Mol Sci [Internet]. 2018 Mar 19;19(3):907. Available from:
200 http://www.mdpi.com/1422-0067/19/3/907
201 13. Saito Y, Soga T. Amino acid transporters as emerging therapeutic targets in cancer.
202 Cancer Sci. 2021;112(8):2958–65.
203 14. Barnes T, Bell K, DiSebastiano KM, Vance V, Hanning R, Russell C, et al. Plasma
204 amino acid profiles of breast cancer patients early in the trajectory of the disease
205 differ from healthy comparison groups. Appl Physiol Nutr Metab. 2014;39(6):740–4.
206 15. Poschke I, Mao Y, Kiessling R, De Boniface J. Tumor-dependent increase of serum
207 amino acid levels in breast cancer patients has diagnostic potential and correlates with
208 molecular tumor subtypes. J Transl Med. 2013;11(1):1–9.
209 16. Laposata M. Concepts in Laboratory Medicine Ranges Used in the Interpretation of
210 Test Results. In: Laposata's laboratory medicine diagnosis of disease in clinical
211 laboratory third edition. 3rd Editio. Columbus: McGraw-Hill Education; 2019.
212 17. Geck RC, Toker A. Nonessential amino acid metabolism in breast cancer. Adv Biol
213 Regul [Internet]. 2016;62:11–7. Available from:
214 http://dx.doi.org/10.1016/j.jbior.2016.01.001
215 18. Yang L, Wang Y, Cai H, Wang S, Shen Y, Ke C. Application of metabolomics in the
216 diagnosis of breast cancer : a systematic review. 2020;11.
217 19. Rudzińska M, Parodi A, Soond SM, Vinarov AZ, Korolev DO, Morozov AO, et al.
218 The Role of Cysteine Cathepsins in Cancer Progression and Drug Resistance. Int J
219 Mol Sci [Internet]. 2019 Jul 23;20(14):3602. Available from:
220 https://www.mdpi.com/1422-0067/20/14/3602
221 20. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in
222 cancer. Br J Cancer [Internet]. 2020;(October 2019). Available from:

http://dx.doi.org/10.1038/s41416-019-0620-5

21. Mitra D. Investigating the role of alanine aminotransferase 2 in breast cancer. University of Heidelberg; 2018.

22. Tsujimoto M, Goto ÆY. Biochemical and enzymatic properties of the M1 family of aminopeptidases involved in the regulation of blood pressure. 2008.

23. Wang Y, Zhao X, Liu H, Dou C, Shi L, Zhao C, et al. Leucine aminopeptidase 3 promotes migration and invasion of breast cancer cells through upregulation of fascin and matrix metalloproteinases - 2 / 9 expression. 2018.

24. Lieu EL, Nguyen T, Rhyne S, Kim J. Amino acids in cancer. Exp Mol Med [Internet]. 2020;52(1):15–30. Available from: http://dx.doi.org/10.1038/s12276-020-0375-3

Tables and Figures

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%)

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 $p>0.05$.

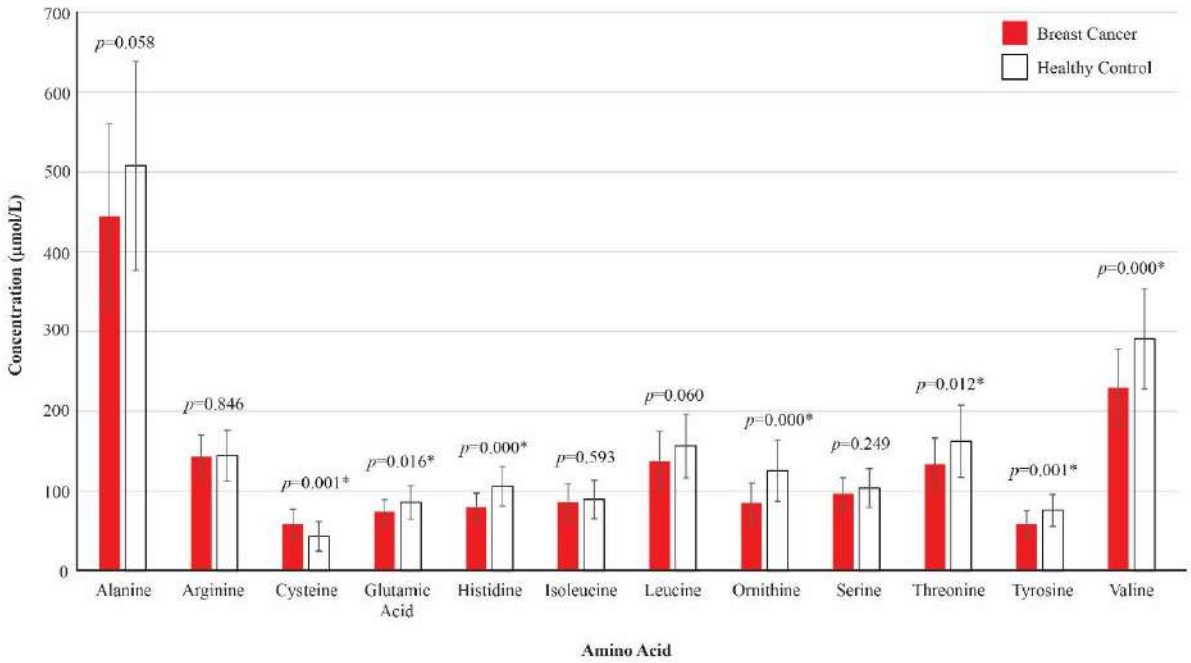

Figure 1. Mean comparison of 12 amino acids between breast cancer (n=29) and control (n=28) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

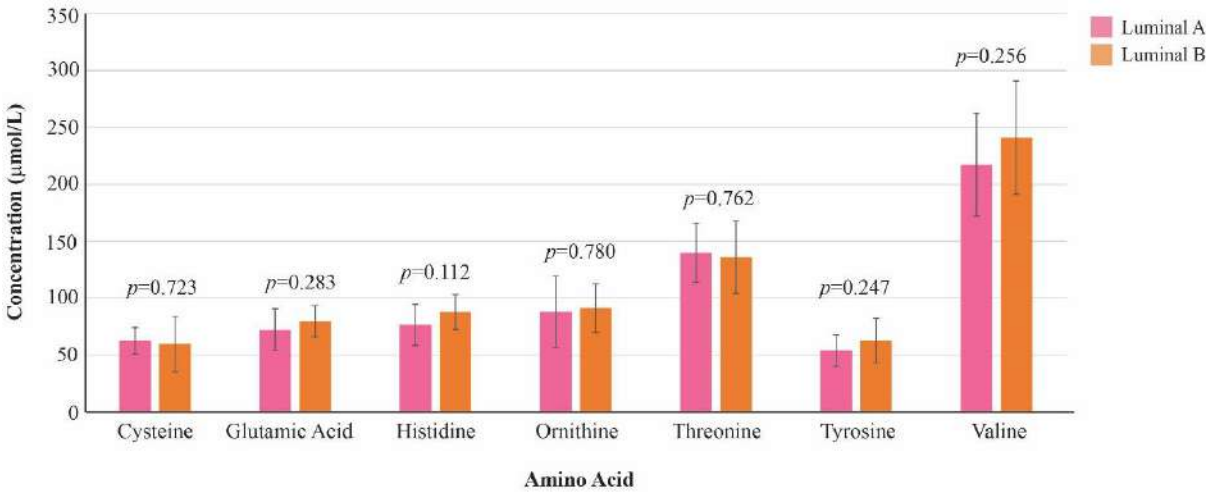

Figure 2. Mean comparison of 7 amino acid between Luminal A (n=10) and Luminal B (n=13) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

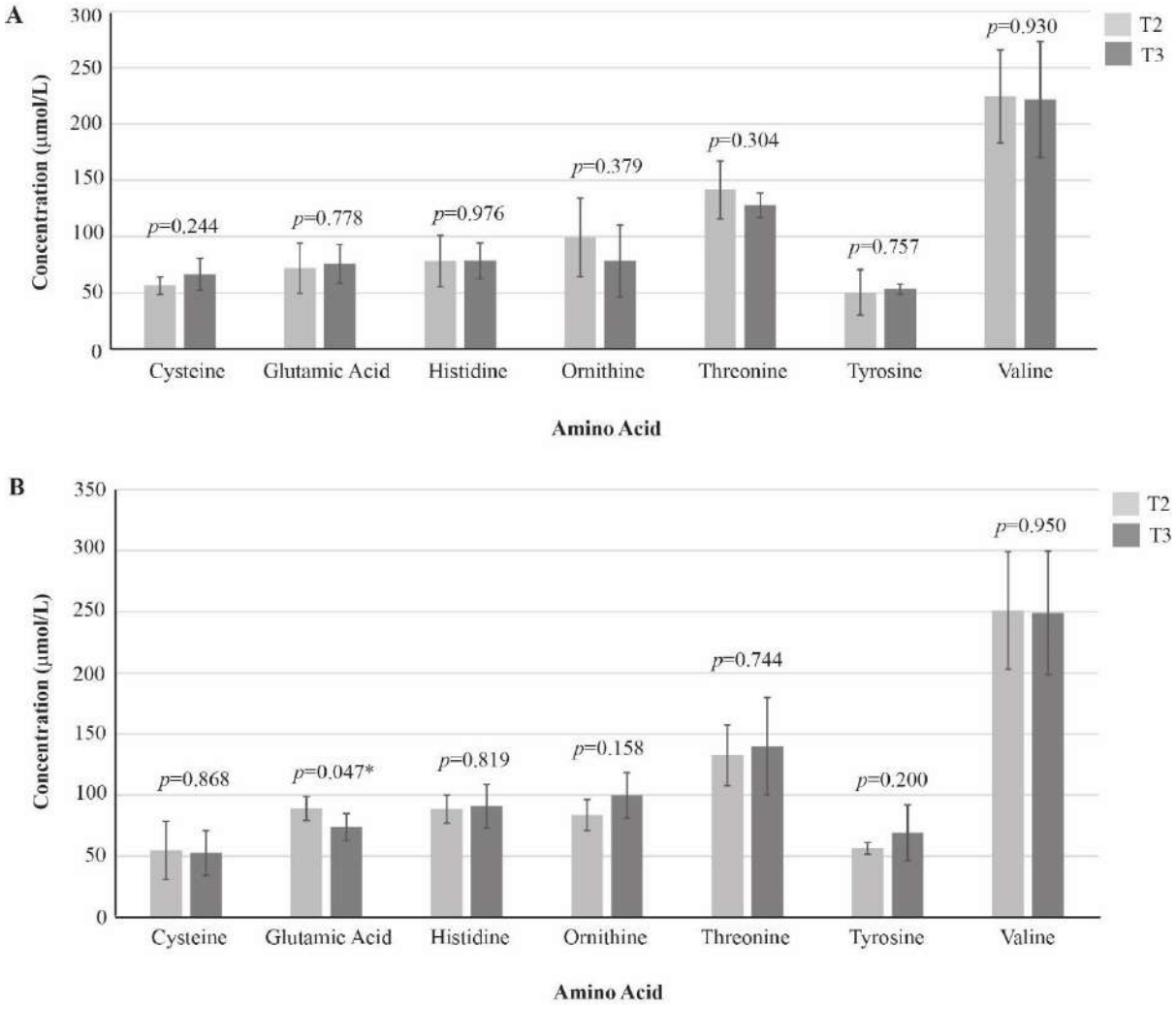

Figure 3. Mean comparison of 7 amino acid between T2 and T3 cancer stage. A: T2 cancer stage (n=4) vs T3 cancer stage (n=5) in Luminal A subjects. B: T2 cancer stage (n=4) vs T3 cancer stage (n=7) in Luminal B subjects. *Mean comparison test with Independent T-test. Data is considered significant if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

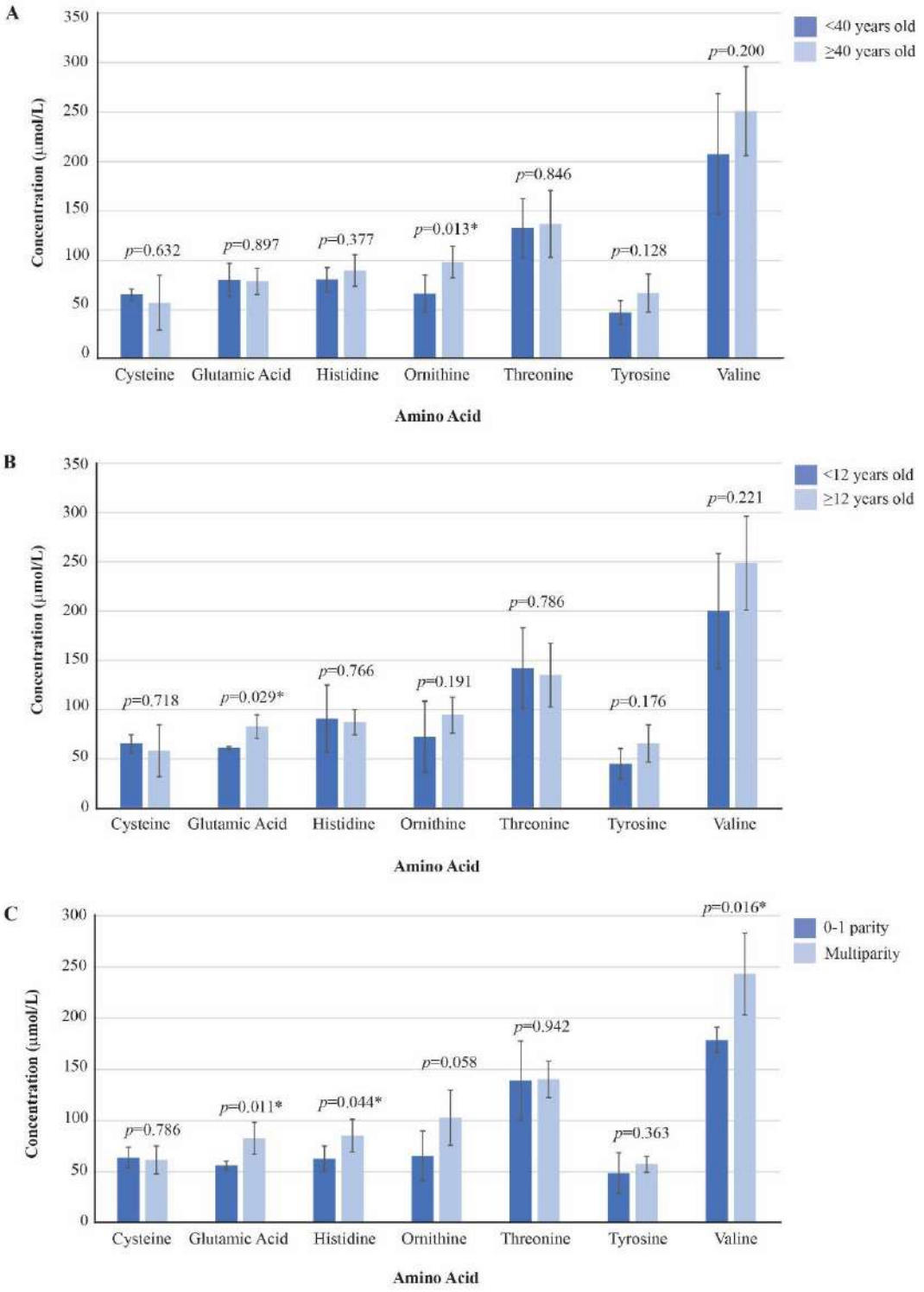

Figure 4. Mean comparison of 7 amino acid based on various risk factors (age, age of menarche, and parity). A: Based on age <40 years old (n=3) vs age >=40 years ol (n=10) in Luminal B subjects. B: Based on age of menarche <12 years old (n=2) vs age of menarche >=12 years old (n=11) in Luminal B subjects. 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 $p<0.05$.

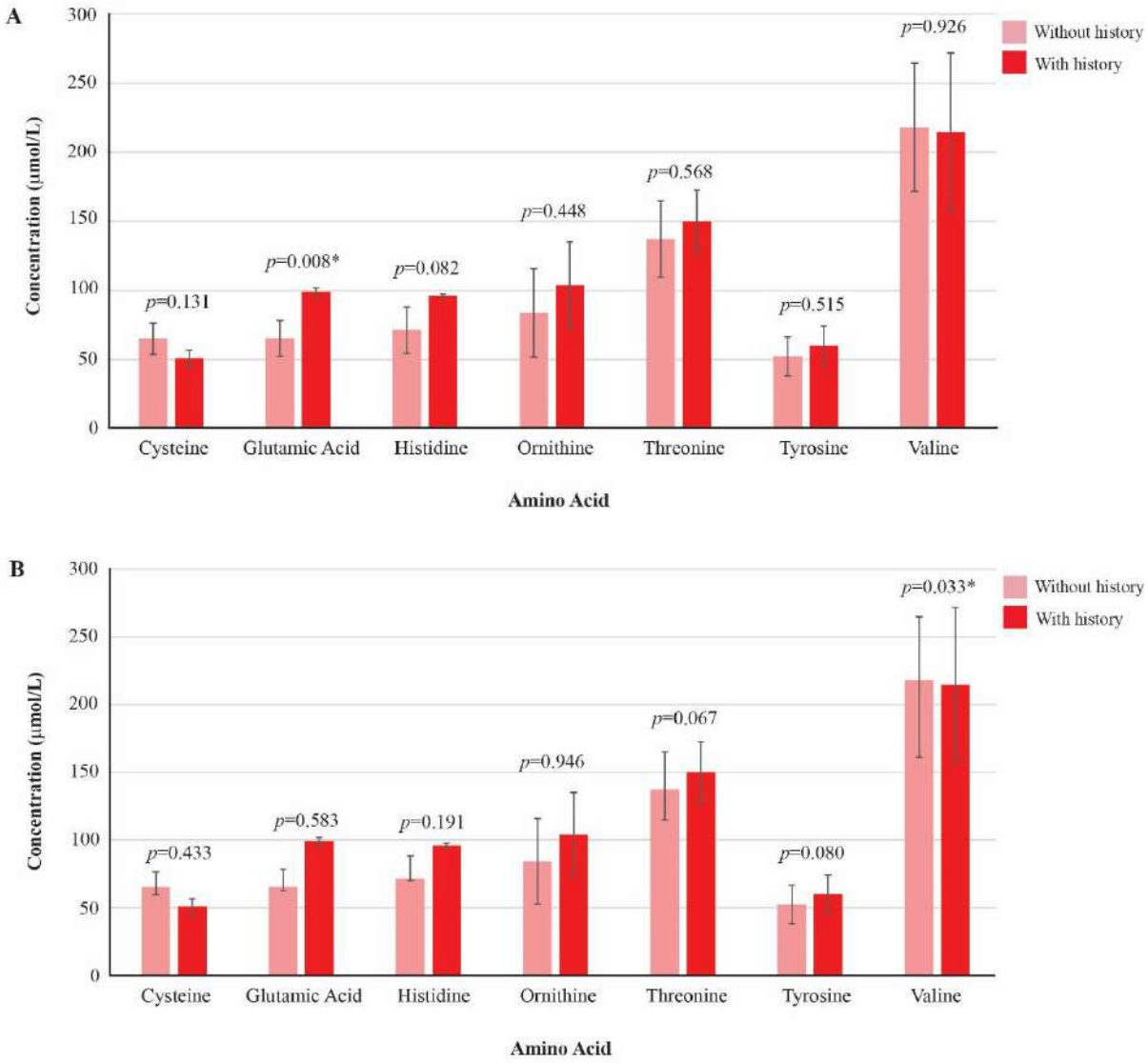

Figure 5. Mean comparison of 7 amino acid between subjects with and without family Ca history. A: Family Ca history in Luminal A subjects (No=8; Yes=2). B: Family Ca history in Luminal B subjects (No=11; Yes=2). *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2022190 Editor Decision Round 2 - Revisions Required

Secretariat of InaBJ <secretariatinabj@gmail.com> Tue, Jun 6, 2023 at 8:16 AM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Distribution and Profiling of Amino Acids in Breast Cancer Patients**".

Our decision is: **Revisions Required.**

Find the file attached to see detailed comments from reviewers. Please make sure you read all the comments and revise the manuscript based on the suggestions given.

Revise this manuscript thoroughly before **June 13, 2023**. Mark/highlighted the revised part of the manuscript, so that the editor will notice the changes.

When you are done, you can upload it in: https://inabj.org/index.php/ibj/author/submissionReview/2109, or simply send us an email of your revised manuscript and response letter.

Please let us know when you have received this email. If you have any questions, do not hesitate to contact us. Thank you for your attention. We wish you a nice day.

Best Regards,

--
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Phone. +62-21-3144182 ext. 3872
Fax. +62-21-3144181
https://www.inabj.org

Round 2 Reviewer 2 - F09 Manuscript Review Form.pdf
186K

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Manuscript Review Form

Reviewer	:	Reviewer 2
Manuscript #	:	M2022190
Manuscript Title	:	Distribution and Profiling of Amino Acids in Breast Cancer Patients

No.	Manuscript Components	Yes	No
1.	Does this manuscript present new ideas or results that have not been previously published?		✓
	Notes: I believe the authors can explore deeper to find new ideas regarding this study.		
2.	Are the title and abstract of the manuscript appropriate?		✓
	Notes: Please choose a more specific title.		
3	Do the title and abstract reflect the study result/content?		✓
	Notes: The authors have done a wonderful job writing this manuscript. It would be better if the title represents the molecular type or breast cancer investigated in this study. The reason why only luminal A and B breast cancer investigated in this study should be added in the abstract.		
4.	Is the significance of the study well explained at the Background?	✓	
	Notes: It could be better		
5.	Are the research study methods technically correct, accurate, and complete enough to be reproduced/cited by other scientists?	✓	
	Notes:		

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

6.	Are the results, ideas, and data presented in this manuscript important enough for publication?	✓	
	Notes:		
7.	Are all figures and tables necessarily presented?	✓	
	Notes:		
8.	Is there a logical flow of argument in the Discussion which elucidate all the presented/obtained data?	✓	
	Notes: A discussion about the possibility of a group of amino acids observed in this study involved in a pathway or immunological response should be added.		
9.	Are the conclusions and interpretations valid and supported by the data?	✓	
	Notes: It would be better if the authors add something about pathway involved in pathogenesis of breast cancer based on the amino acid profile.		
10.	Is the manuscript clear, comprehensible, and written in a good English structure?	✓	
	Notes: Much better than the previous version		

Specific Reviewer's Comments and Suggestions:
(These comments may be in addition to or in lieu of reviewer comments inserted into the text of the manuscript. Use as many lines as needed.)

The Indonesian Biomedical Journal

Prodia Tower 9th Floor, Jl. Kramat Raya No. 150, Jakarta 10430 - Indonesia
Tel.: +62-21-3144182 ext.872, Fax.: +62-21-3144181
Email: Secretariat@InaBJ.org, Website: www.InaBJ.org

Reviewer's Recommendation (Please tick only one option)	√
Accept Submission (No significant alterations suggested)	
Revisions Required (Suggest changes to the manuscript as specified in this review)	✓
Resubmit for Review (Major revisions should be made and suggestions as specified in this review must be addressed. Revised manuscript should be resubmitted to the reviewer for further review)	
Decline Submission (Do not encourage a rewrite, manuscript is totally rejected)	

Further Reviewer's Comments Regarding Disposition of the Manuscript:

Date and Sign:
June 6th, 2023

Reviewer 2

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2022190 Editor Decision Round 2 - Revisions Required

Ferry Sandra <ferry@trisakti.ac.id> Tue, Jun 6, 2023 at 3:20 PM
To: Secretariat of InaBJ <secretariatinabj@gmail.com>

Dear Secretariat of The Indonesian Biomedical Journal,

Please find the revision of the manuscript M2022190 that was previously entitled "Distribution and Profiling of Amino Acids in Breast Cancer Patients" as well as the response letter addressing the reviewer's questions. We have revised the manuscript accordingly. Thank you.

Regards,
Ferry Sandra

[Quoted text hidden]

2 attachments

Round 2 Revision from Author.docx
1457K

Round 2 Response Form from Author.xlsx
12K

Amino Acid Profile of Luminal A and B Subtypes Breast Cancer

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

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.(4-6) Around 2.3 million cases were recorded by Global Cancer Observatory in 2020, 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.(7) By 2020, breast cancer continued as the most common cancer in women (30.8%) and the leading cause of death in Indonesia (15.3%).(8,9)

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.(9) 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.(10,11)

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.(12) Interestingly, breast cancer cells limit the use of amino acids for cell proliferation based on amino acid availability, which depends on estrogenic receptor status.(13) 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.(11,14)

Tumor-dependent increase of serum amino acid levels has been reported to be correlated with molecular subtypes of breast cancer.(15) 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.(14,16)

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-value<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 statistically different between the healthy control and breast cancer subjects (Figure 1). However, the 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 statistically different between T2 and T3 of breast cancer subjects with luminal B subtype (Figure 3).

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).

DISCUSSIONS

Amino acids are essential nutrients in all living cells and are important for the proliferation and maintenance of tumor cells.(12) 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.(12,17) 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 initiate cancer.(18) 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.(19)

Through the production of alpha-ketoglutarate, alanine plays a role in the formation of extracellular matrix in metastatic breast cancer.(20) The breast cancer proliferative pathway inhibits the enzyme GPT2 (alanine transaminotransferase-2) that produces alanine from glutamate and pyruvate.(21) This is associated with a decrease in the average amount of alanine in breast cancer subject compared to healthy controls.

In this study, we found that leucine level is significantly decreased in breast cancer subjects than the healthy control. 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.(22,23) 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.(23)

The amino acid profile is not only associated with breast cancer incidence, but also with breast cancer risk factors.(24) 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 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 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 needed 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.

REFERENCES

1. Savitri M, Bintoro UY, Sedana MP, Diansyah MN, Romadhon PZ, Amrita PNA, et al. Circulating plasma miRNA-21 as a superior biomarker compared to ca 15-3: assessment in healthy age matched subjects and different stage of breast cancer patients. Indones Biomed J. 2020; 12(2): 157-64

2. Purwanto I, Heriyanto DS, Ghozali A, Widodo I, Dwiprahasto I, Aryandono T, et al. Basal-Like Subgroup is Associated with Younger Age, Increased Expression of Androgen Receptor, and Worse Prognosis, while Non-basal-like Subtype is Associated with Higher BMI in Triple-Negative Breast Cancer Patients. Vol. 12. 2020.

3. Widowati W, Jasaputra DK, Sumitro SB, Widodo MA, Afifah E, Rizal R, et al. Direct and Indirect Effect of TNFα and IFNγ Toward Apoptosis in Breast Cancer Cells. Mol Cell

Biomed Sci. 2018;2(2):60.

4. Abdihalim TS, Idris AAA. Mucin Level as a Potential Biomarker for Breast Cancer Diagnosis. Mol Cell Biomed Sci. 2022;6(3):117.

5. Ilyan T, Retnoningrum D, Hendrianingtyas M, Widyaningrum D, Rachmawati B. Association of 25-hydroxyvitamin D, Cyclooxygenase-2 and Prostaglandin E2 Serum Levels in Breast Cancer Patients. Indones Biomed J. 2021; 13(4): 426-32.

6. Abdihalim TS, Idris AAA. Mucin Level as a Potential Biomarker for Breast Cancer Diagnosis. Mol Cell Biomed Sci. 2022; 6(3): 117-20.

7. Remelia M, Bela B, Widyaningtyas ST, Antarianto RD, Mazfufah NF, Pawitan JA. The Use of Cell-penetrating Peptide for Delivery of Recombinant Transcription Factor DNA into Primary Human Fibroblast. Mol Cell Biomed Sci. 2023; 7(1): 28-37.

8. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.

9. Kraeima J, Siesling S, Vliegen IMH, Klaase JM, Ijzerman MJ. Individual risk profiling for breast cancer recurrence : towards tailored follow-up schemes [Internet]. Vol. 109, British Journal of Cancer. Nature Publishing Group; 2013. Available from: http://dx.doi.org/10.1038/bjc.2013.401

10. Retnowarnadi A, Kresno SB, Arif M. Association of Obesity and Breast Cancer Risk: The Role of Estrogen, Tumor Necrosis Factor-alpha, and Adiponectin as Risk factors (preliminary study). Indones Biomed J [Internet]. 2009 Apr 1;1(1):45. Available from: https://inabj.org/index.php/ibj/article/view/82

11. Kimball SR, Jefferson LS. Signaling pathways and molecular mechanisms through which branched-chain amino acids mediate translational control of protein synthesis. J Nutr. 2006;136(1):227–31.

12. Cha Y, Kim ES, Koo J. Amino Acid Transporters and Glutamine Metabolism in Breast Cancer. Int J Mol Sci [Internet]. 2018 Mar 19;19(3):907. Available from: http://www.mdpi.com/1422-0067/19/3/907
13. Saito Y, Soga T. Amino acid transporters as emerging therapeutic targets in cancer. Cancer Sci. 2021;112(8):2958–65.
14. Barnes T, Bell K, DiSebastiano KM, Vance V, Hanning R, Russell C, et al. Plasma amino acid profiles of breast cancer patients early in the trajectory of the disease differ from healthy comparison groups. Appl Physiol Nutr Metab. 2014;39(6):740–4.
15. Poschke I, Mao Y, Kiessling R, De Boniface J. Tumor-dependent increase of serum amino acid levels in breast cancer patients has diagnostic potential and correlates with molecular tumor subtypes. J Transl Med. 2013;11(1):1–9.
16. Laposata M. Concepts in Laboratory Medicine Ranges Used in the Interpretation of Test Results. In: Laposata's laboratory medicine diagnosis of disease in clinical laboratory third edition. 3rd Editio. Columbus: McGraw-Hill Education; 2019.
17. Geck RC, Toker A. Nonessential amino acid metabolism in breast cancer. Adv Biol Regul [Internet]. 2016;62:11–7. Available from: http://dx.doi.org/10.1016/j.jbior.2016.01.001
18. Yang L, Wang Y, Cai H, Wang S, Shen Y, Ke C. Application of metabolomics in the diagnosis of breast cancer : a systematic review. 2020;11.
19. Rudzińska M, Parodi A, Soond SM, Vinarov AZ, Korolev DO, Morozov AO, et al. The Role of Cysteine Cathepsins in Cancer Progression and Drug Resistance. Int J Mol Sci [Internet]. 2019 Jul 23;20(14):3602. Available from: https://www.mdpi.com/1422-0067/20/14/3602
20. Vettore L, Westbrook RL, Tennant DA. New aspects of amino acid metabolism in cancer. Br J Cancer [Internet]. 2020;(October 2019). Available from:

http://dx.doi.org/10.1038/s41416-019-0620-5

21. Mitra D. Investigating the role of alanine aminotransferase 2 in breast cancer. University of Heidelberg; 2018.

22. Tsujimoto M, Goto ÆY. Biochemical and enzymatic properties of the M1 family of aminopeptidases involved in the regulation of blood pressure. 2008.

23. Wang Y, Zhao X, Liu H, Dou C, Shi L, Zhao C, et al. Leucine aminopeptidase 3 promotes migration and invasion of breast cancer cells through upregulation of fascin and matrix metalloproteinases - 2 / 9 expression. 2018.

24. Lieu EL, Nguyen T, Rhyne S, Kim J. Amino acids in cancer. Exp Mol Med [Internet]. 2020;52(1):15–30. Available from: http://dx.doi.org/10.1038/s12276-020-0375-3

Tables and Figures

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%)

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 $p>0.05$.

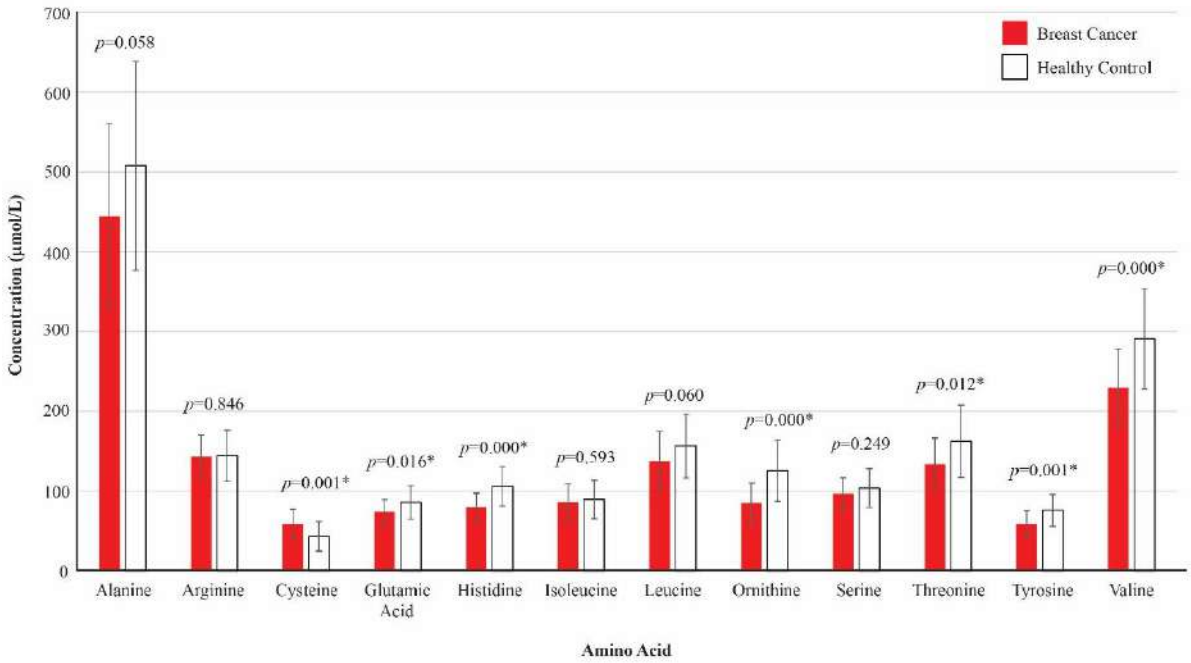

Figure 1. Mean comparison of 12 amino acids between breast cancer (n=29) and control (n=28) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

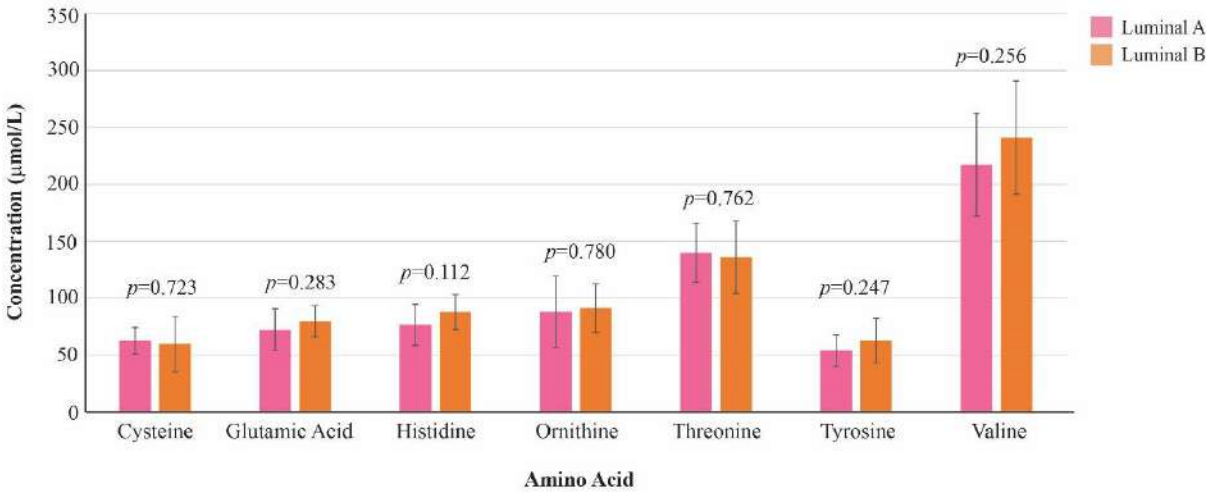

Figure 2. Mean comparison of 7 amino acid between Luminal A (n=10) and Luminal B (n=13) subjects. *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

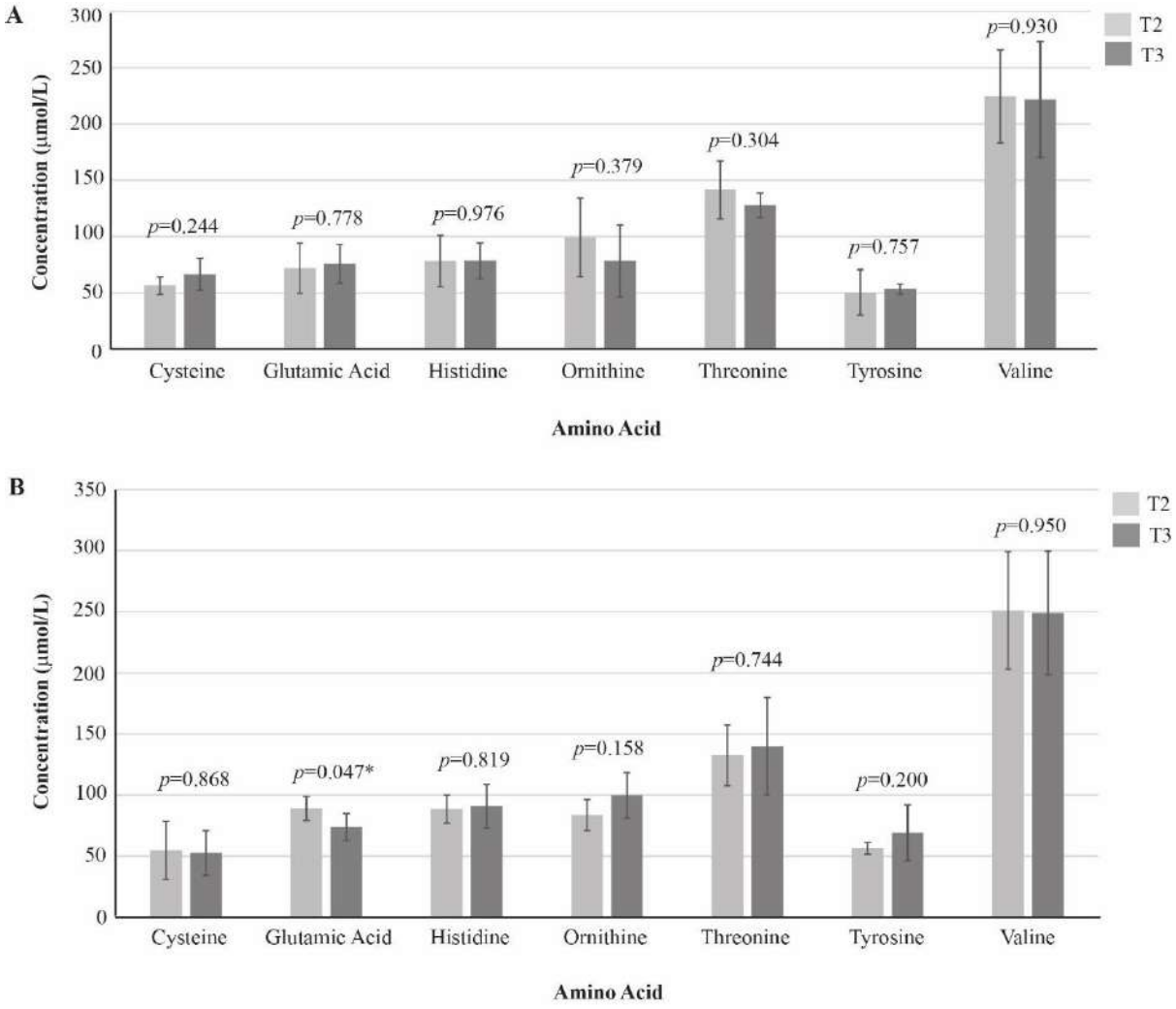

Figure 3. Mean comparison of 7 amino acid between T2 and T3 cancer stage. A: T2 cancer stage (n=4) vs T3 cancer stage (n=5) in Luminal A subjects. B: T2 cancer stage (n=4) vs T3 cancer stage (n=7) in Luminal B subjects. *Mean comparison test with Independent T-test. Data is considered significant if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

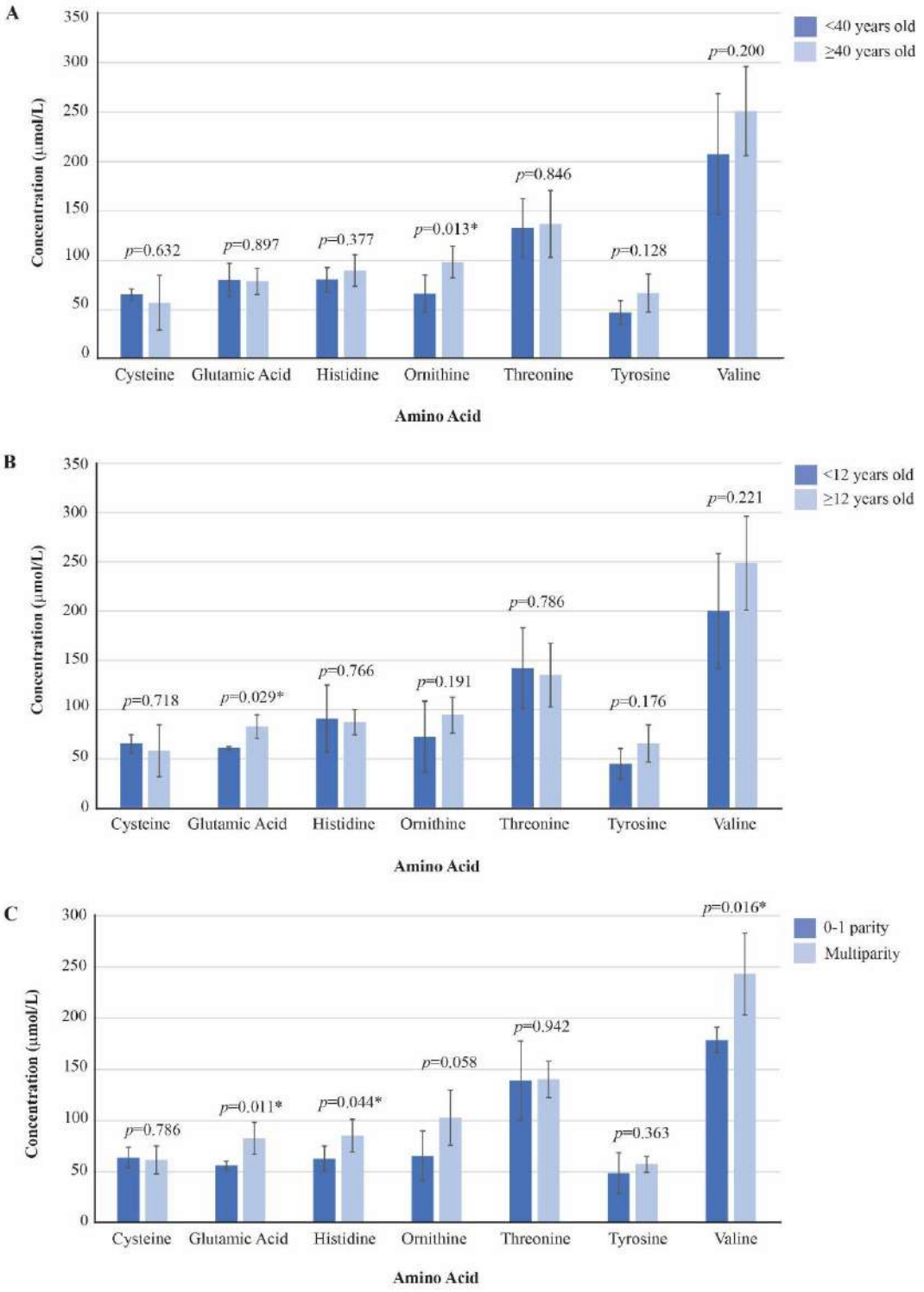

Figure 4. Mean comparison of 7 amino acid based on various risk factors (age, age of menarche, and parity). A: Based on age <40 years old (n=3) vs age >=40 years ol (n=10) in Luminal B subjects. B: Based on age of menarche <12 years old (n=2) vs age of menarche >=12 years old (n=11) in Luminal B subjects. 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 $p<0.05$.

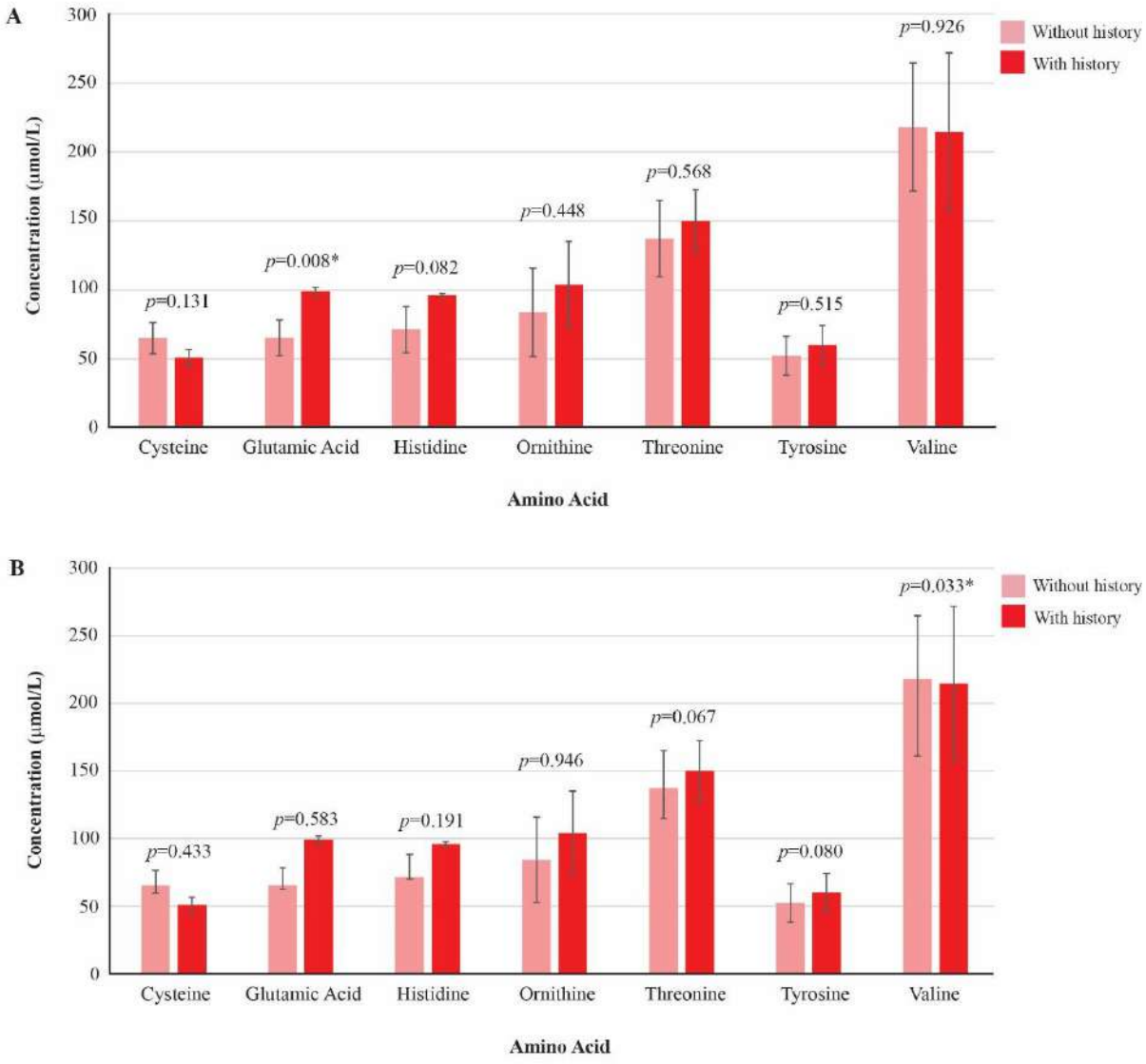

Figure 5. Mean comparison of 7 amino acid between subjects with and without family Ca history. A: Family Ca history in Luminal A subjects (No=8; Yes=2). B: Family Ca history in Luminal B subjects (No=11; Yes=2). *Mean comparison test with Independent T-test. Data is considered significant if $p<0.05$.

Response Form for Reviewer's Comments

Corresponding Author : Ferry Sandra
Manuscript Code : M2022190
Manuscript Ttile : Distribution and Profiling of Amino Acids in Breast Cancer Patients

No. (Reviewer and comments number code)	**Comments** (Comments/question from reviewer or editor)	**Author's Response** (Please write your response regarding the comment here)	**Line Number** (Please write the line number of the said revision)
#1	Please choose a more specific title.	We have revised the manuscript title to be more specific: "Amino Acid Profile of Luminal A and B Subtypes Breast Cancer"	1
#2	The authors have done a wonderful job writing this manuscript. It would be better if the title represents the molecular type or breast cancer investigated in this study. The reason why only luminal A and B breast cancer investigated in this study should be added in the abstract.	We already revised the title. However, we can not add more background about why we reported only luminal A and B, since we chose to investigate luminal A and B after we found more subjects with these subtypes compared to subjects with HER2 or triple negative subjects. Hence, that was the reason why we chose luminal A and B, so that the analysis results can be representatif with the subjects we recruited.	-
#3	A discussion about the possibility of a group of amino acids observed in this study involved in a pathway or immunological response should be added. It would be better if the authors add something about pathway involved in pathogenesis of breast cancer based on the amino acid profile.	The discussion about pathway involved is already written in line 118-138. However, we did not discuss further because we did not examined other parameters.	118-138

UNIVERSITAS TRISAKTI

Ferry Sandra <ferry@trisakti.ac.id>

[InaBJ] M2022190 Editor Decision - Manuscript Accepted

Secretariat of InaBJ <secretariatinabj@gmail.com> Thu, Jun 8, 2023 at 2:41 PM
To: ferry@trisakti.ac.id

Dear Dr. Ferry Sandra,

Good day. We have reached a decision regarding your submission to The Indonesian Biomedical Journal, "**Amino Acid Profile of Luminal A and B Subtypes Breast Cancer**."

Our decision is to: **Accept Manuscript.**

Your manuscript will be sent to our publisher for typesetting and you should receive the proofreading in due course.

Congratulations on your interesting research, and thank you for allowing us to publish this valuable material. Please let us know once you have read this email. We wish you a nice day.

Best Regards,

--
Secretariat of The Indonesian Biomedical Journal
Prodia Tower 9th Floor
Jl. Kramat Raya No.150, Jakarta 10430, Indonesia
Phone. +62-21-3144182 ext. 3872
Fax. +62-21-3144181
https://www.inabj.org