Gender Differentiation in Tryptophan Metabolism in Postmortem Cases

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Abstract

Tryptophan (TRP) is an essential amino acid and is metabolized via kynurenine and serotonin pathway. Both pathways are significant role in many diseases and have been studied as biomarkers for differentiate and clarify in many diseases including forensic works. Metabolism in the body might be affected by both intrinsic and extrinsic factors. This study aimed to compare postmortem blood level of TRP and its metabolites in male and female to be applied in gender identification in forensic medicine cases. Blood samples were collected from male group (n=11) and female group (n=11) and the level of the TRP and its metabolites were identified by HPLC-DAD. The values of the TRP, metabolites and metabolite ratios did not different between the groups. While, the value of ratio of picolinic acid/ quinolinic acid different significantly (p <0.05) among the groups which might be applied for gender identification in postmortem cases. In conclusion, we found that gender have no effect on the TRP and its metabolites. By the way, the effect of gender should be expanded sample size and develop to gender identification method.

Keywords: *tryptophan metabolism; postmortem; gender; picolinic acid; quinolinic acid*

Introduction

Tryptophan (TRP) is an essential amino acid and contained in a variety of including rice, eggs, meat, cheese and bananas[1]. After intake TRP into the body, TRP is metabolized to give many bioactive molecules by using kynurenine and serotonin pathway. Kynurenine pathway has been believed as a keystone to initiate many diseases including cardiovascular diseases, diabetes, neurodegenerative diseases and cancer. The TRP and its metabolites levels in blood and urine have been used as a biomarker for diagnosis. For forensic medicine, the TRP and its metabolites correlated with coronary artery disease among sudden unexpected death[2]. Quinolinic acid (QUIN) and picolinic acid (PIC) level were changed significantly among suicide victims and non-suicide victims[3-5]. There are many factors might affect the metabolism including dietary profile, age, hormone and gender[6-9]. However, studies the effects of those factors on the TRP metabolisms are still limit. This study was aimed to find out effect of gender on the TRP metabolism by using postmortem blood sample to let us understand a different and might be applied in gender identification in forensic medicine cases.

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Materials and Methods

Subject and study design

Twenty-two cases (11 cases of female and 11 cases of male) who died and underwent autopsy at Department of Forensic medicine, Faculty of Medicine, Chiang Mai University were included in this study. The decomposed subjects, postmortem interval more than 24 hours and blood could not be collected were excluded. We selected cases with the same cause of death in the both groups to reduce confounding factors that might affect on TRP metabolism. Written informed consent was obtained

from direct relatives. The study protocol was approved by the Research Ethics Committee Faculty of Medicine, Chiang Mai University (FOR-2562-06504).

Collection and specimen preparation

Femoral blood approximately 5 mL was collected and contained in sodium fluoride tube. The samples were stored at -80 °C before analysis. Two milliliters of the blood sample were mixed with two milliliters of acetonitrile. The sample was shaken for 5 minutes and centrifuged at 5,500 rpm for 5 minutes. The organic layer was collected and the blood sample was re-extracted twice. The supernatants were combined and evaporated under nitrogen gas. The residue was reconstituted with 20mM sodium acetate buffer pH.6.4 before analysis with HPLC-DAD[2].

Measurement of Tryptophan and Its Metabolites

The levels of TRP, KYN, KYNA, 3HK, 3HAA, QUIN, PIC, 5HTRP and HIAA in whole blood were measured by applied the method of Santisukwongchote *et al*.[2]. The instrument for analysis was an Agilent LC 1260 infinity binary pump system. Chromatographic separation was carried out by using Lichrocart 55-4 purosphere STAR RP-18 (5 µm, C_{18}, 250x4.6 mm). The column was operated at a constant temperature of 25 °C. The flow rate was 0.7 mL/ min and gradient elution was used. The mobile phase consisted of sodium acetate buffer pH.5.5 (A) and acetonitrile (B). The following condition of elution was applied: 0-6 min, 100% A; 6-20 min, 100-80% A; 20-35 min, 100% A. The total run time was 35 minutes and injection volumes were 10 µL. The identification of the chromatographic peak was achieved by comparing the retention times and spectral characteristic (λ=200-400 nm) of the eluting peaks with the standards.

Statistical Analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 22.0). Descriptive statistics were shown as mean ± S.E. Differentiation of blood TRP and its metabolites level between male and female group were compared using Mann-Whitney U test. A *p*-value of less than 0.05 was considered statistically significant.

Result and Discussion

Tryptophan and its metabolites were found in all twenty-two blood samples. Mean value of the TRP, its metabolites and some ratios that reflex to enzyme activities in the TRP catabolism are shown in Table 1. There was no significantly difference in age between male group and female group (p=0.792). Mean value of the TRP, KYN, 3HK, 3HAA, QUIN, PIC, KYNA, 5HTRP and HIAA level did not different between the male and female group. Some ratios of the TRP metabolites represented some enzyme activities in the TRP catabolite pathway such as KYN/TRP indicated an indoleamine 2, 3-dioxygease (IDO) activity and 5HTRP/TRP indicated a tryptophan hydroxylase (TPH) activity. Our study also revealed that the IDO and the TPH activity were no significantly difference between the groups which like as a previous study that gender have no effect on plasma TRP metabolites[6].

Table 1. Tryptophan and its metabolites level in whole blood

Metabolites	Range	Total (n=22)	Male (n=11)	Female (n=11)	p-value
TRP (mM)	2.16-4.53	3.35±0.57	2.84±0.88	3.83±0.74	0.200
KYN (mM)	0.51-1.04	0.77±0.13	0.72±0.19	0.82±0.18	0.491
3HK (mM)	0.24-0.51	2.67±1.17	3.60±2.17	1.73±0.91	0.279
3HAA (mM)	14.56-31.35	22.95±4.04	18.44±3.64	27.47±7.15	0.450
QUIN (mM)	13.22-21.30	17.26±1.94	18.31±3.52	16.21±1.79	0.870
PIC (mM)	13.99-25.36	19.68±2.73	17.14±5.04	22.21±2.17	0.061
KYNA (mM)	0.32-0.82	0.57±0.12	0.67±0.23	0.47±0.06	0.870
5HTRP (mM)	2.55-3.54	3.04±0.24	3.17±0.35	2.92±0.34	0.491

Cont... **Table 1. Tryptophan and its metabolites level in whole blood**

HIAA (mM)	2.46-5.02	3.74±0.61	3.88±0.81	3.61±0.96	0.622
KYN/TRP	0.19-0.55	0.37±0.87	0.47±0.16	0.26±0.06	0.309
3HK/KYN	0.79-10.96	5.88±2.44	6.73±4.05	5.02±2.93	0.309
3HAA/3HK	23.07-119.14	71.11±23.10	39.15±16.88	103.01±41.85	0.341
QUIN/3HAA	0.75-2.25	1.69±0.75	1.28±0.28	1.73±0.67	0.491
PIC/3HAA	0.58-3.25	1.92±0.64	1.08±0.31	2.75±1.22	0.450
KYNA/KYN	0.71-1.49	1.10±0.19	1.29±0.31	0.91±0.21	0.533
5HTRP/TRP	1.01-2.68	1.84±0.40	2.45±0.65	1.24±0.41	0.061
HIAA/5HTRP	0.93-1.79	1.36±0.21	1.30±0.23	1.42±0.36	0.974
PIC/QUIN	0.88-1.49	1.19±0.15	0.90±0.20	1.47±0.18	0.011*
Age (year)	34.11-46.71	40.41	40.27	40.55	0.792

*significantly different between male group and female group (p<0.05). The range of total cases are presented as 95% CI. Other values are presented as mean ± S.E. A non-parametric Mann-Whitney U-test was used for comparing between groups.

The PIC/QUIN ratio represented as antioxidant to oxidant substance or neuroprotective to neurotoxic substance. The results showed that the PIC/QUIN in female group was higher than in male group significantly (p = 0.011). This result might be occurred from different of sex hormone. Female has higher level of estrogen than male and estrogen hormone acts as antioxidant properties[7]. Then, estrogen might have some effect on the TRP catabolism. For application, the PIC/QUIN might be applied in gender identification by using blood. The others sample including tissues, skeletal remains the PIC/QUIN ratio should be study and should expand a sample size in further.

Conclusion

In postmortem samples, gender had no effected on the TRP, its metabolites and enzyme activities. But gender might affect on the PIC/QUIN ratio through hormone differentiation. The different value might be applied to gender identification in forensic medicine cases.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

QUIN = Quinolinic acid, TRP = Tryptophan, KYN = Kynurenine, 3HK = 3-Hydroxykynurenine, 3HAA = 3-Hydroxyanthranilic acid, PIC = Picolinic acid, 5HTRP = L-5-Hydroxytryptophan (5HTRP), 5HT = 5-hydroxytryptamine or Serotonin, HIAA = 5-hydroxyindole acetic acid, IDO = indoleamine2, 3-dioxygenase, KMO = kynurenine 3- monooxygenase, 3-HAO = 3- hydroxyanthranilate oxygenase, ACMSD = 2-amino-3-hydroxymuconic-6-semialdehyde, KATs = kynurenine aminotransferase, TPH = Tryptophan hydroxylase

References

1. Kapalka, G. Substances involved in neurotransmission. In *Nutritional and Herbal therapies for children and adolescent*, 2018; 10.1016/b978-0-12-374927-7.00004-2pp. 71-99.
2. Santisukwongchote, K.; Amornlertwatana, Y.; Sastraruji, T.; Jaikang, C. Possible Use of Blood Tryptophan Metabolites as Biomarkers for Coronary Heart Disease in Sudden Unexpected Death. *Metabolites* 2019, *10*, doi:10.3390/metabo10010006.

3. Bryleva, E.Y.; Brundin, L. Kynurenine pathway metabolites and suicidality. *Neuropharmacology* 2017, *112*, 324-330, doi:10.1016/j.neuropharm.2016.01.034.
4. Brundin, L.; Bryleva, E.Y.; Thirtamara Rajamani, K. Role of Inflammation in Suicide: From Mechanisms to Treatment. *Neuropsychopharmacology* 2017, *42*, 271-283, doi:10.1038/npp.2016.116.
5. Savitz, J. The kynurenine pathway: a finger in every pie. *Mol Psychiatry* 2019, 10.1038/s41380-019-0414-4, doi:10.1038/s41380-019-0414-4.
6. Brundin, L.; Sellgren, C.M.; Lim, C.K.; Grit, J.; Palsson, E.; Landen, M.; Samuelsson, M.; Lundgren, K.; Brundin, P.; Fuchs, D., et al. An enzyme in the kynurenine pathway that governs vulnerability to suicidal behavior by regulating excitotoxicity and neuroinflammation. *Transl Psychiatry* 2016, *6*, e865, doi:10.1038/tp.2016.133.
7. Stepniak, J.; Karbownik-Lewinska, M. 17beta-estradiol prevents experimentally-induced oxidative damage to membrane lipids and nuclear DNA in porcine ovary. *Syst Biol Reprod Med* 2016, *62*, 17-21, doi:10.3109/19396368.2015.1101510.
8. Fukuwatari, T.; Shibata, K. Nutritional aspect of tryptophan metabolism. *Int J Tryptophan Res* 2013, *6*, 3-8, doi:10.4137/IJTR.S11588.
9. Jayawickrama, G.S.; Nematollahi, A.; Sun, G.; Gorrell, M.D.; Church, W.B. Inhibition of human kynurenine aminotransferase isozymes by estrogen and its derivatives. *Sci Rep* 2017, *7*, 17559, doi:10.1038/s41598-017-17979-7.