Assessment of Blood Plasma Free-amino Acid Levels in Infertile Men

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Abstract. Background/Aim: The objective of this study was to investigate the significance of the blood levels of free amino acids (AAs) in infertile men. Patients and Methods: Ninety-three men who underwent examinations for infertility were included. The concentrations of 20 AAs were measured and compared in four groups (normospermia, obstructive azoospermia, oligozoospermia, non-obstructive azoospermia) based on semen analysis and clinical parameters. Results: When the 93 men were divided into normospermia, obstructive azoospermia, oligozoospermia, and non-obstructive azoospermia groups, no significant differences were found in the concentrations of the 20 AAs between them. We then compared 49 men diagnosed with normozoospermia or oligozoospermia according to the median sperm motility and morphology abnormalities rates (30% and 20%, respectively). Men with low motility rates had significantly lower levels of tryptophan and alanine, and men with high abnormal morphology rates had significantly lower levels of aspartate and glutamate. Conclusion: AAs are probably involved in the pathogenesis of male infertility, particularly oligozoospermia.

Amino acids (AAs) are used for protein synthesis and have been shown to play important roles in a wide variety of physiological events. It is well documented that free AAs are present in the blood (1), and an imbalance in their levels has been shown to be associated with the development and progression of various diseases (2). For example, several studies have reported close relationships between changes in the profile of free AAs in the blood and metabolic syndrome, including diabetes mellitus, hypertension, and fatty liver (3, 4). An imbalance in AAs has been observed in a high proportion of patients with several malignant tumors, such as lung, colorectal, breast, and prostate cancers (5-7).

Several studies have shown the important roles of the metabolic pathways involving AAs in the regulation of parameters associated with semen quality (8-10). For example, metabolic disorders involving AAs are thought to be involved in the structural and functional alterations of spermatozoa in men with severe oligoasthenospermia (8). Furthermore, a few human studies and several animal models have shown the significant roles of free-AAs on sperm quality (11-15). However, the significance of free AA levels in infertile men remains unknown.

Therefore, we measured the blood levels of free AAs in 93 men, who were examined for male infertility, to determine whether clinical features in these men were affected by the profile of free AAs.

Patients and Methods

Patients. This study was approved by the institutional review board of Hamamatsu University School of Medicine (No. 15-241), and written informed consent was obtained from all participants. We consecutively enrolled 93 men who visited our institution between January 2011 and December 2018 with a chief complaint of infertility. We excluded men with a previous history of treatment for male infertility or malignant disease, those without data on gonadotropins, and those with chromosomal abnormalities, such as Klinefelter syndrome.

Data collection. All data used in this study were retrospectively obtained from medical records (laboratory findings, semen analysis, and pathological findings on testicular biopsy). In this series, an endocrine evaluation consisting of measurements of serum follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone levels in a blood sample was conducted for all participants. In addition, according to the World Health Organization laboratory manual, all semen samples were collected by masturbation after 3 days of sexual abstinence, and evaluated within 1 h of collection to measure the volume, sperm count, motility rate, and abnormal morphology rate using a Makler chamber.

In this study, high-performance liquid chromatography mass spectrometry (HPLC-MS) (16) was used to measure the concentration of free- AAs in the blood at SRL Inc. (Tokyo, Japan).
Table I. Free amino acid (AA) concentrations in the blood according to serum follicle-stimulating hormone (FSH) levels.

|                        | FSH ≤10 IU/ml (n=64)* | FSH >10 IU/ml (n=29)* | p-Value |
|------------------------|------------------------|------------------------|---------|
| Essential AAs          |                        |                        |         |
| Histidine              | 81.5 (43.8-116.4)      | 81.6 (71.7-115.3)      | 0.64    |
| Isoleucine             | 68.1 (45.7-121.8)      | 74.5 (45.3-129.0)      | 0.050   |
| Leucine                | 119.9 (77.1-201.2)     | 125.1 (84.5-210.2)     | 0.19    |
| Lysine                 | 179.9 (118.4-303.1)    | 176.2 (118.5-260.9)    | 0.87    |
| Methionine             | 25.5 (14.7-43.4)       | 25.1 (17.2-47.8)       | 0.68    |
| Phenylalanine          | 60.9 (36.9-79.4)       | 61.2 (49.1-83.7)       | 0.24    |
| Threonine              | 119.6 (73.1-172.7)     | 127.2 (86.5-264.5)     | 0.34    |
| Tryptophan             | 57.54 (40.9-73.1)      | 57.1 (43.9-85.5)       | 0.28    |
| Valine                 | 223.2 (154.2-323.2)    | 232.7 (179.4-326.0)    | 0.13    |
| Non-essential AAs      |                        |                        |         |
| Alanine                | 376.1 (237.8-591.8)    | 349.8 (275.8-592.2)    | 0.51    |
| Arginine               | 96.0 (44.5-154.2)      | 99.9 (60.0-143.6)      | 0.69    |
| Asparagine             | 48.2 (27.6-86.9)       | 48.1 (38.0-89.1)       | 0.64    |
| Aspartic acid          | 4.5 (2.0-12.2)         | 4.6 (2.0-12.7)         | 0.80    |
| Cystine                | 24.9 (11.8-52.4)       | 26.2 (14.3-41.4)       | 0.60    |
| Glutamine              | 562.3 (477.3-820.0)    | 553.6 (437.2-711.3)    | 0.44    |
| Glutamic acid          | 48.8 (16.4-122.5)      | 52.2 (10.6-95.6)       | 0.81    |
| Glycine                | 224.8 (134.3-375.9)    | 218.2 (159.1-419.4)    | 0.96    |
| Proline                | 199.1 (112.1-493.5)    | 186.9 (119.8-364.0)    | 0.43    |
| Serine                 | 112.6 (61.6-171.5)     | 107.9 (88.2-177.9)     | 0.30    |
| Tyrosine               | 57.1 (33.1-108.4)      | 59.4 (41.2-88.9)       | 0.38    |

*Values are presented as median (range).

Nine essential AAs (EAAs), consisting of histidine, isoleucine, leucin, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, and 11 non-EAAs (NEAAs), consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, and tyrosine, were measured.

Statistical analysis. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (17), and a p-value <0.05 was considered statistically significant. Differences between groups were compared using the Mann-Whitney U-test and Kruskal-Wallis test.

Results

The characteristics of the 93 men included in this study were as follows: median age, 36 years (range=25-63 years); median body mass index, 23.4 kg/m² (range=17.0-32.4 Kg/m²); median FSH, 6.9 mIU/ml (range=1.1-80.1 mIU/ml); median LH, 4.9 mIU/ml (range=1.4-25.5 mIU/ml); and median total plasma testosterone 355 ng/dl (range=184-986 ng/dl).

We initially compared the concentrations of AAs in the blood plasma according to serum FSH levels. As shown in Table I, when the 93 men were divided into a group of 64 with serum FSH levels ≤10 IU/ml (68.8%) and a group of 29 with FSH >10 IU/ml (31.2%), there were no significant differences in the concentrations of all 20 AAs between these groups.

We then divided the 93 men into the following four groups: Normospermia group (n=18, 19.4%), sperm count >15×10⁶; Obstructive azoospermia group (n=26, 27.9%), azoospermia with a Johnsen score >7 on testicular biopsy; Oligozoospermia group (n=31, 33.3%), sperm count ≤15×10⁶; and Non-obstructive azoospermia group (n=18, 19.4%), azoospermia with a Johnsen score ≤6. As shown in Table II, there were no significant differences in the concentrations of all 20 AAs between these four groups.

Finally, we compared the concentrations of AAs in the blood of the normospermia and oligozoospermia groups according to median values of sperm motility and abnormal morphology rates (30% and 20%, respectively). Concentrations of tryptophan and alanine in the 25 men with low motility rates (51.0%) were significantly lower than in the 24 men with high motility rates (49.0%); however, there were no significant differences in the concentrations of the remaining 18 AAs between these two groups (Table III). Furthermore, 26 patients with high abnormal morphology rates (53.1%) had low concentrations of aspartic acid and glutamic acid, but not those of the remaining 18 AAs, compared to 23 men with low abnormal morphology rates (46.9%) (Table IV).

Discussion

A number of studies have suggested that AAs play critical roles in a wide variety of pathophysiological events, including male reproduction (2). For example, assessments of the metabolic profile of human sperm cells from men diagnosed with...
idiopathic asthenozoospermia using gas chromatography-mass spectrometry revealed that pathways involved in amino acid and citric acid cycle were disturbed (18). Dietary intervention with functional AAs to alter the abundance and activity of intestinal bacteria was shown to improve reproductive performance in both male and female rats as well as their offspring (13). However, the significance of free AAs in human male reproduction has not yet been well characterized. Therefore, we measured the levels of 20 AAs in the blood plasma using HPLC-MS in 93 men who visited our institution to undergo examinations for infertility, and investigated the association between these outcomes and their clinical features.

We initially compared the concentrations of 20 AAs in the blood of the 93 men according to serum FSH levels and sperm counts; however, there were no significant differences in the concentrations of all 20 AAs. Therefore, FSH levels and sperm count do not influence the balance of the AA concentrations and may not be correlated to spermatogenic function in infertile men. In a previous study, investigating serum samples from non-obstructive azoospermic men, various metabolic pathways, but not those associated with AAs, were shown to be disrupted, suggesting the involvement of serum metabolic disorders in the pathogenesis of male infertility (19).

Considering the important roles of AAs in a wide variety of pathophysiological events, we evaluated our findings,}

### Table II. Free amino acid (AA) concentrations in the blood according to the diagnosis group.

|                      | Normospermia (n=18)* | Obstructive azoospermia (n=26)* | Oligozoospermia (n=31)* | Non-obstructive azoospermia (n=18)* | p-Value |
|----------------------|----------------------|---------------------------------|------------------------|-------------------------------------|---------|
| Essential AAs        |                      |                                 |                        |                                     |         |
| Histidine            | 82.9 (66.0-108.0)    | 81.7 (70.7-107.2)               | 78.6 (43.8-116.4)      | 83.3 (74.2-115.3)                   | 0.22    |
| Isoleucine           | 69.8 (49.0-121.8)    | 68.3 (45.3-106.8)               | 68.4 (45.7-129.0)      | 72.4 (48.2-104.1)                   | 0.52    |
| Leucine              | 119.9 (90.2-201.2)   | 122.1 (92.9-176.0)              | 123.5 (77.1-210.2)     | 128.6 (84.5-178.9)                  | 0.55    |
| Lysine               | 167.2 (128.5-303.1)  | 206.7 (120.1-290.1)             | 178.6 (118.4-257.4)    | 181.4 (118.5-260.9)                 | 0.061   |
| Methionine           | 25.3 (14.7-35.0)     | 25.4 (18.5-43.4)                | 24.2 (15.2-34.6)       | 25.8 (17.2-47.8)                    | 0.31    |
| Phenylalanine        | 61.2 (46.8-79.4)     | 61.6 (51.3-75.5)                | 60.3 (36.9-83.7)       | 60.9 (51.7-78.4)                    | 0.72    |
| Threonine            | 109.5 (73.1-188.8)   | 128.1 (83.1-172.7)              | 118.0 (85.0-171.1)     | 127.4 (89.2-246.5)                  | 0.34    |
| Tryptophan           | 57.5 (45.2-70.6)     | 55.4 (40.9-73.1)                | 57.1 (42.3-70.8)       | 60.5 (47.0-85.5)                    | 0.62    |
| Valine               | 218.8 (175.2-323.2)  | 235.8 (172.8-296.0)             | 219.5 (154.2-326.0)    | 229.4 (180.5-308.3)                 | 0.41    |

*Values are presented as median (range).

|                      |                      |                                 |                        |                                     |         |
|----------------------|----------------------|                                 |                        |                                     |         |
| Non-essential AAs    |                      |                                 |                        |                                     |         |
| Alanine              | 383.7 (301.0-450.2)  | 383.1 (386.6-592.2)             | 360.9 (237.8-501.5)    | 40.24 (275.8-534.7)                 | 0.29    |
| Arginine             | 90.5 (57.9-142.0)    | 104.8 (65.9-154.2)              | 90.0 (44.5-143.6)      | 94.6 (60.0-143.2)                   | 0.094   |
| Asparagine           | 43.5 (27.6-65.3)     | 51.9 (38.5-86.9)                | 48.5 (30.9-59.4)       | 48.7 (38.0-89.1)                    | 0.11    |
| Aspartic acid        | 4.9 (2.4-11.5)       | 4.3 (2.2-11.6)                  | 4.7 (2.0-12.2)         | 4.3 (2.0-12.7)                      | 0.95    |
| Cystine              | 29.7 (14.4-42.6)     | 23.1 (11.8-52.4)                | 26.7 (16.7-47.0)       | 25.9 (12.5-41.4)                    | 0.14    |
| Glutamine            | 555.8 (482.2-641.4)  | 565.0 (494.1-753.6)             | 555.2 (473.7-820.0)    | 560.4 (437.2-711.3)                 | 0.79    |
| Glutamic acid        | 60.7 (20.0-122.5)    | 47.1 (22.8-104.7)               | 56.0 (16.4-119.6)      | 40.4 (10.0-92.7)                    | 0.28    |
| Glycine              | 224.9 (150.5-335.8)  | 232.4 (148.7-375.9)             | 216.0 (134.3-339.0)    | 234.8 (176.6-419.4)                 | 0.32    |
| Proline              | 222.3 (124.2-346.5)  | 199.7 (119.8-493.5)             | 180.6 (112.1-318.3)    | 190.6 (121.3-364.0)                 | 0.33    |
| Serine               | 111.2 (88.2-163.9)   | 110.3 (83.6-171.5)              | 110.0 (61.6-142.6)     | 113.0 (89.0-177.9)                  | 0.66    |
| Tyrosine             | 54.8 (41.2-88.9)     | 57.9 (41.2-86.9)                | 54.0 (33.1-108.4)      | 60.9 (41.4-88.9)                    | 0.26    |

*Values are presented as median (range).

### Table III. Free amino acid (AA) concentrations in the blood according to sperm motility rate.

|                      | Motility rate >30% (n=24)* | Motility rate ≤30% (n=25)* | p-Value |
|----------------------|-----------------------------|-----------------------------|---------|
| Essential AAs        |                             |                             |         |
| Histidine            | 83.0 (63.2-108.0)           | 80.2 (43.8-116.4)           | 0.25    |
| Isoleucine           | 68.9 (46.6-121.8)           | 69.5 (45.7-129.0)           | 0.74    |
| Leucine              | 122.3 (77.1-201.2)          | 120.6 (77.3-210.2)          | 0.56    |
| Lysine               | 175.6 (137.5-303.1)         | 174.5 (118.4-231.3)         | 0.88    |
| Methionine           | 24.3 (16.2-35.0)            | 23.9 (14.7-34.6)            | 0.40    |
| Phenylalanine        | 61.0 (40.7-79.4)            | 57.1 (36.9-83.7)            | 0.48    |
| Threonine            | 110.9 (81.3-171.1)          | 118.6 (73.1-188.8)          | 0.97    |
| Tryptophan           | 58.1 (48.3-70.6)            | 55.0 (42.3-70.8)            | 0.047   |
| Valine               | 219.5 (179.4-323.2)         | 220.9 (154.2-326.0)         | 0.76    |

*Values are presented as median (range).
Table IV. Free amino acid (AA) concentrations in the blood according to sperm abnormal morphology rate.

| AAs          | Abnormal morphology rate ≤20% (n=23)* | Abnormal morphology rate >20% (n=26)* | p-Value |
|--------------|---------------------------------------|----------------------------------------|---------|
| Essential AAs |                                        |                                        |         |
| Histidine    | 378.4 (237.8-501.5)                   | 356.5 (264.3-455.6)                   | 0.083   |
| Arginine     | 87.5 (44.5-143.6)                     | 98.5 (57.2-142.0)                     | 0.40    |
| Asparagine   | 43.3 (30.9-63.2)                      | 48.0 (27.6-65.3)                      | 0.23    |
| Aspartic acid| 5.6 (2.6-12.2)                        | 3.3 (2.0-11.6)                        | 0.002   |
| Glutamine    | 546.9 (482.2-820.0)                   | 566.7 (477.3-683.7)                   | 0.053   |
| Glutamic acid| 74.7 (16.4-122.5)                     | 36.3 (18.2-95.6)                      | 0.009   |
| Cystine      | 29.8 (18.5-47.0)                      | 25.1 (14.4-42.6)                      | 0.20    |
| Valine       | 225.7 (154.2-308.2)                   | 209.0 (167.7-326.0)                   | 0.84    |
| Non-essential AAs |                                    |                                        |         |
| Alanine      | 5.6 (2.6-12.2)                        | 3.3 (2.0-11.6)                        | 0.002   |
| Arginine     | 87.5 (44.5-143.6)                     | 98.5 (57.2-142.0)                     | 0.40    |
| Asparagine   | 43.3 (30.9-63.2)                      | 48.0 (27.6-65.3)                      | 0.23    |
| Aspartic acid| 5.6 (2.6-12.2)                        | 3.3 (2.0-11.6)                        | 0.002   |
| Glutamine    | 546.9 (482.2-820.0)                   | 566.7 (477.3-683.7)                   | 0.053   |
| Glutamic acid| 74.7 (16.4-122.5)                     | 36.3 (18.2-95.6)                      | 0.009   |
| Cystine      | 29.8 (18.5-47.0)                      | 25.1 (14.4-42.6)                      | 0.20    |
| Valine       | 225.7 (154.2-308.2)                   | 209.0 (167.7-326.0)                   | 0.84    |

*Values are presented as median (range).

We showed that despite the lack of significant differences in the concentrations of free AAs according to FSH levels or sperm count, lower levels of alanine and tryptophan, and those of aspartic acid and glutamic acid, were significantly associated with low motility and high abnormal morphology, respectively, in men with either normospermia or oligozoospermia. These findings suggest that an imbalance in free AAs in the blood is involved in the pathogenesis of male infertility, particularly oligozoospermia.

**Conflicts of Interest**

The Authors have no conflicts of interest to declare in relation to this study.

**Authors’ Contributions**

Study conception and design: Takayuki Sugiyama, Hiroshi Terada, Hideaki Miyake; Acquisition of data: Takayuki Sugiyama, Hiroshi Terada; Analysis and interpretation of data: Takayuki Sugiyama, Hideaki Miyake; Drafting of manuscript: Takayuki Sugiyama, Hideaki Miyake.

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**Conclusion**

Several limitations of this study should be mentioned. Firstly, this was a retrospective study including a comparatively small number of men who wished to undergo examinations for infertility. Therefore, it is necessary to perform a prospective large study to confirm our findings. Secondly, the functional significance of an imbalance in free AAs on spermatogenesis should be addressed. Thirdly, despite the use of serum samples in this study, it would be interesting to measure free AA levels in other body fluids. In fact, several studies have analyzed the metabolomic signature of the seminal plasma from infertile men (8, 25, 26). For example, a significant decrease in multiple metabolites, including alanine, aspartic acid, glutamic acid,
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