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· 论 著 ·

乳腺癌患者血清中 20 种氨基酸的检测及在乳腺癌筛查中的意义

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[摘要] **目的** 考察乳腺癌患者与正常体检者血清中氨基酸水平的差异, 并探讨其在乳腺癌筛查中的意义。

方法 招募 2019 年 4—6 月重庆大学附属肿瘤医院病理科确诊的女性乳腺癌患者 59 例作为乳腺癌组, 以正常女性体检者 53 例作为对照组。采用高效液相色谱-质谱法 (HPLC-MS) 检测招募者血清中 20 种氨基酸水平, 分析乳腺癌组与对照组间、乳腺癌不同分子亚型组间、早期乳腺癌组与晚期乳腺癌组间氨基酸水平的差异。采用 ROC 曲线对差异血清氨基酸在乳腺癌筛查中的效能进行评估。**结果** 乳腺癌组血清中丙氨酸 (Ala)、天冬酰胺 (Asn)、瓜氨酸 (Cit)、谷氨酸 (Glu)、组氨酸 (His)、异亮氨酸 (Ile)、亮氨酸 (Leu)、赖氨酸 (Lys)、鸟氨酸 (Orn)、苯丙氨酸 (Phe)、脯氨酸 (Pro)、苏氨酸 (Thr)、酪氨酸 (Tyr)、缬氨酸 (Val) 的水平均高于对照组 ($P < 0.05$), 精氨酸 (Arg) 水平低于对照组 ($P = 0.003$)。ROC 曲线分析表明, 在乳腺癌患者血清中升高的 14 种氨基酸中, Ala 和 Pro 单独筛查乳腺癌的效能较高, AUC 均为 0.75; Ile 单独筛查乳腺癌时有最高的灵敏度, 达 0.82; Lys 单独筛查乳腺癌时有最高的特异度, 达 0.94; 这 14 种氨基酸联合检测时能够提高乳腺癌的筛查效能, AUC 为 0.88, 灵敏度为 0.69, 特异度为 0.94。早期乳腺癌 (TNM 分期 I 期和 II 期) 患者血清 Val 水平高于晚期 (TNM 分期 III 期和 IV 期) 患者 ($P = 0.044$), 其他 19 种氨基酸水平在早期和晚期乳腺癌患者之间差异均无统计学意义 ($P > 0.05$)。20 种血清氨基酸水平在 Luminal A 型、Luminal B 型、三阴性型、人表皮生长因子受体 2 (HER2) 阳性型乳腺癌组间差异均无统计学意义 ($P > 0.05$)。**结论** 乳腺癌患者血清中 15 种氨基酸水平与正常体检者相比存在差异, 有助于乳腺癌筛查; 14 种在乳腺癌患者血清中升高的氨基酸的联合检测有利于提高乳腺癌筛查效能, 是有潜力的生物学标志物。

[关键词] 乳腺肿瘤; 血清; 氨基酸类; 筛查; 高效液相色谱-质谱法; 肿瘤生物标记

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Determination of 20 serum amino acids in breast cancer patients and its significance in breast cancer screening

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[Abstract] **Objective** To investigate the difference of serum amino acid levels between breast cancer patients and normal volunteers, and explore its significance in breast cancer screening. **Methods** Totally 59 breast cancer patients (breast cancer group) diagnosed by the Department of Pathology, Chongqing University Cancer Hospital from Apr. to Jun. 2019 were recruited, and 53 normal healthy women were selected as control group. The levels of 20 amino acids in the sera of recruited people were detected by high performance liquid chromatography-mass spectrometry (HPLC-MS). The differences of amino acid levels were analyzed between breast cancer group and control group, between different molecular subtype groups of breast cancer, and between early and advanced breast cancer groups. Receiver operating characteristic (ROC) curve was used

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to evaluate the effectiveness of different serum amino acids in breast cancer screening. **Results** The levels of alanine (Ala), asparagine (Asn), citrulline (Cit), glutamic acid (Glu), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), ornithine (Orn), phenylalanine (Phe), proline (Pro), threonine (Thr), tyrosine (Tyr) and valine (Val) were higher in the breast cancer group than in the control group (all $P < 0.05$). The level of arginine (Arg) was lower in the breast cancer group than in the control group ($P = 0.003$). ROC curve analysis showed that of the 14 amino acids which were elevated in the sera of breast cancer patients, Ala or Pro alone had better performance in screening breast cancer, and the area under curves (AUCs) were both 0.75; Ile alone had the highest sensitivity, with the AUC of 0.79; Lys alone had the highest specificity, with the AUC of 0.94; and the combined detection of the 14 amino acids improved the performance of breast cancer screening, with the AUC of 0.88, sensitivity of 0.69, and specificity of 0.94. The level of serum Val was higher in early breast cancer patients (TNM stage I and II) than in advanced patients (TNM stage III and IV) ($P = 0.044$), and there was no significant difference in the levels of other 19 amino acids (all $P > 0.05$). No significant difference was found in the 20 amino acids between Luminal A type, Luminal B type, triple-negative, and human epidermal growth factor receptor 2 (HER2) positive breast cancer groups (all $P > 0.05$). **Conclusion** The levels of 15 serum amino acids in breast cancer patients are different from those in normal persons, which is helpful for breast cancer screening. The combination detection of 14 elevated serum amino acids in breast cancer patients can improve the screening performance and may serve as potential biomarkers.

[**Key words**] breast neoplasms; serum; amino acids; screening; high performance liquid chromatography-mass spectrometry; tumor biomarkers

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乳腺癌是女性最常见的恶性肿瘤, 在我国的发病率占所有新发癌症病例的 15%, 且死亡率呈逐年上升趋势, 严重危害女性健康^[1-2]。目前用于乳腺癌筛查的肿瘤标志物主要是癌抗原 15-3 (cancer antigen 15-3, CA15-3), 但其对乳腺癌早期筛查缺乏特异性, 往往在中、晚期乳腺癌患者中呈高表达^[3]。因此, 寻找乳腺癌筛查相对特异的标志物具有重要意义。细胞功能的病理改变常伴有代谢重组, 包括氨基酸代谢的改变^[4]。氨基酸检测对许多遗传性代谢性疾病的筛查非常重要。癌症患者处于高代谢状态和高分解代谢状态, 蛋白的合成和分解较健康人群均有所提高, 从而导致氨基酸浓度的变化和氨基酸代谢异常^[5]。研究表明, 氨基酸在筛查肿瘤方面是一个非常具有潜力的生物标志物, 在肺癌^[6-7]、卵巢癌^[8]、胃癌^[9]、结直肠癌^[10]、胰腺癌^[11]等肿瘤中已有报道, 但在乳腺癌筛查中的应用国内尚缺乏系统性研究。为探讨血清氨基酸在乳腺癌患者与正常人之间水平的差异及其在乳腺癌筛查中的价值, 本实验用高效液相色谱-质谱法 (high performance liquid chromatography-mass spectrometry, HPLC-MS) 对乳腺癌患者和正常人血清中 20 种氨基酸水平进行检测, 分析氨基酸水平的差异, 并对差异血清氨基酸在乳腺癌筛查中的效能进行系统性评估, 以寻找新的乳腺癌筛查生物标志物。

1 资料和方法

1.1 一般资料 招募 2019 年 4—6 月经重庆大学附属肿瘤医院病理科确诊且未经任何治疗的乳腺癌患者 59 例作为乳腺癌组, 均为女性浸润性导管癌患者, 年龄为 44~64 岁 (中位年龄 51 岁)。根据美国癌症联合委员会 (American Joint Committee on Cancer, AJCC) 癌症分期手册第 8 版对患者进行 TNM 分期, 59 例中包括早期乳腺癌患者 26 例 (TNM 分期 I 期 6 例、II 期 20 例)、晚期乳腺癌患者 33 例 (TNM 分期 III 期 23 例、IV 期 10 例)。收集患者临床病理资料并对其进行分子分型, 其中 Luminal A 型 16 例、Luminal B 型 25 例、三阴性型 8 例、人表皮生长因子受体 2 (human epidermal growth factor receptor 2, HER2) 阳性型 10 例。同时招募同期重庆大学附属肿瘤医院健康体检中心正常女性体检者 53 例作为对照组, 年龄为 36~62 岁 (中位年龄 47 岁)。本研究通过重庆大学附属肿瘤医院伦理委员会审批, 所有研究方案均得到招募者同意并签订知情同意书。

1.2 样本采集 于清晨空腹时, 采集招募者前臂肘正中静脉血 3 mL 于未加任何抗凝剂的真空采血管中, 室温静置 0.5 h, 待血液凝固后, 1 200×g 离心 10 min 分离血清 (所有样本离心分离均在样本采集 2 h 内完成), 取 300 μL 上清液于 -80 °C 低温冰箱中冻存待用。

1.3 主要试剂及仪器 Thermo Ultimate 3000 液相色谱仪串联 Thermo TSQ Endura 质谱仪(美国 ThermoFisher Scientific 公司); HPLC-MS 氨基酸标准品和内标(美国剑桥同位素实验室)。

1.4 检测指标 对血清中 20 种氨基酸进行检测,包括 8 种必需氨基酸,即赖氨酸(lysine, Lys)、色氨酸(tryptophan, Trp)、苯丙氨酸(phenylalanine, Phe)、甲硫氨酸(methionine, Met)、苏氨酸(threonine, Thr)、异亮氨酸(isoleucine, Ile)、亮氨酸(leucine, Leu)、缬氨酸(valine, Val); 12 种非必需氨基酸,即精氨酸(arginine, Arg)、组氨酸(histidine, His)、丝氨酸(serine, Ser)、酪氨酸(tyrosine, Tyr)、谷氨酸(glutamic acid, Glu)、鸟氨酸(ornithine, Orn)、丙氨酸(alanine, Ala)、脯氨酸(proline, Pro)、瓜氨酸(citrulline, Cit)、天冬酰胺(asparagine, Asn)、谷氨酰胺(glutamine, Gln)、天冬氨酸(aspartic acid, Asp)。

将沉淀剂与内标储备液按照 13 : 1 的比例混合均匀,另取大于 25 mL 的超纯水,备用。样本准备:取出 V 型 96 孔深孔板,往相应孔中分别加入 40 μL 校准品/空白样本/质控样本(双空白即为 180 μL 超纯水;空白即为 40 μL 超纯水+140 μL 沉淀剂);沉淀:用多道移液器向 96 孔板中加入 140 μL 沉淀剂,封膜涡旋 30 s,重复 2 次,1 200 $\times g$ 4 $^{\circ}\text{C}$ 离心 30 min;稀释:取 20 μL 上清液至相对应的另一块 96 孔板中(-18 $^{\circ}\text{C}$ 可保存 1 d),加入 180 μL 超纯水稀释,封膜涡旋 30 s,重复 2 次后 1 200 $\times g$ 离心 3 min,上机测定。

1.5 实验仪器参数 液相色谱条件:ACQUITY UPLC HSS T3 VanGuard 保护柱(10 nm, 1.8 μm , 2.1 mm \times 5 mm, 美国 Waters 公司), ACQUITY UPLC HSS T3 色谱柱(2.1 mm \times 100 mm, 1.8 μm , 美国 Waters 公司),柱温 45 $^{\circ}\text{C}$,流速 0.4 mL/min,进样量 10 μL ,自动进样器温度 10 $^{\circ}\text{C}$ 。流动相 A 液为含 0.1% 甲酸、0.05% 七氟丁酸的 ddH₂O,流动相 B 液为含 0.1% 甲酸、0.05% 七氟丁酸的乙腈溶液,梯度洗脱条件:0~0.5 min, 98% A; 0.5~1.5 min, 98% A 到 90% A; 1.5~3.5 min, 90% A 到 65% A; 3.5~3.6 min, 65% A 到 5% A; 3.6~4.8 min, 5% A; 4.8~5 min, 5% A 到 98% A; 5~6 min, 98% A。质谱条件:离子源为

电喷雾(electrospray ionization, ESI),离子源喷雾电压为 3.5 kV,鞘气流速为 50 arb,辅助气流速为 15 arb,反向气流速为 1 arb,传输毛细管温度为 350 $^{\circ}\text{C}$,辅助气加热温度为 400 $^{\circ}\text{C}$ 。扫描模式为多反应监测(multiple reaction monitoring, MRM)。

1.6 统计学处理 用 SPSS 23.0 软件进行统计学分析。计量资料如服从正态分布采用 $\bar{x}\pm s$ 表示,正常人与乳腺癌患者和早、晚期乳腺癌患者血清氨基酸水平差异分析采用独立样本 *t* 检验;乳腺癌患者不同分子亚型血清氨基酸水平差异比较采用单因素方差分析,两两比较采用最小显著性差异法。14 种血清氨基酸及其联合筛查效能采用 ROC 曲线进行评估,取约登指数(灵敏度与特异度之和减去 1)最大的一点为最佳截断值,并计算其对应筛查灵敏度和特异度。检验水准(α)为 0.05。

2 结果

2.1 对照组与乳腺癌组血清中 20 种氨基酸水平的比较 乳腺癌组血清中 Ala、Asn、Cit、Glu、His、Ile、Leu、Lys、Orn、Phe、Pro、Thr、Tyr、Val 的水平高于对照组,差异有统计学意义(P 均 <0.05);乳腺癌组血清中 Arg 的水平低于对照组,差异有统计学意义($P=0.003$)。见图 1。

2.2 血清氨基酸在乳腺癌筛查中的效能评估结果 ROC 曲线分析表明,在乳腺癌患者血清中升高的 14 种氨基酸(Ala、Asn、Cit、Glu、His、Ile、Leu、Lys、Orn、Phe、Pro、Thr、Tyr、Val)中,Ala 和 Pro 单独筛查乳腺癌时的效能最高,AUC 均为 0.75;Ile 单独筛查乳腺癌时灵敏度最高,达 0.82;Lys 单独筛查乳腺癌时特异度最高,达 0.94;14 种氨基酸联合检测能够提高乳腺癌患者的筛查效能,AUC 为 0.88,灵敏度为 0.69,特异度为 0.94。见图 2、表 1。

2.3 早期和晚期乳腺癌患者血清中 20 种氨基酸水平比较 早期(TNM 分期 I 期和 II 期)、晚期(TNM 分期 III 期和 IV 期)乳腺癌患者血清氨基酸水平比较结果表明,前者 Val 水平高于后者,差异有统计学意义($P=0.044$);其余 19 种氨基酸水平在早期和晚期乳腺癌患者之间差异均无统计学意义(P 均 >0.05 ,表 2)。

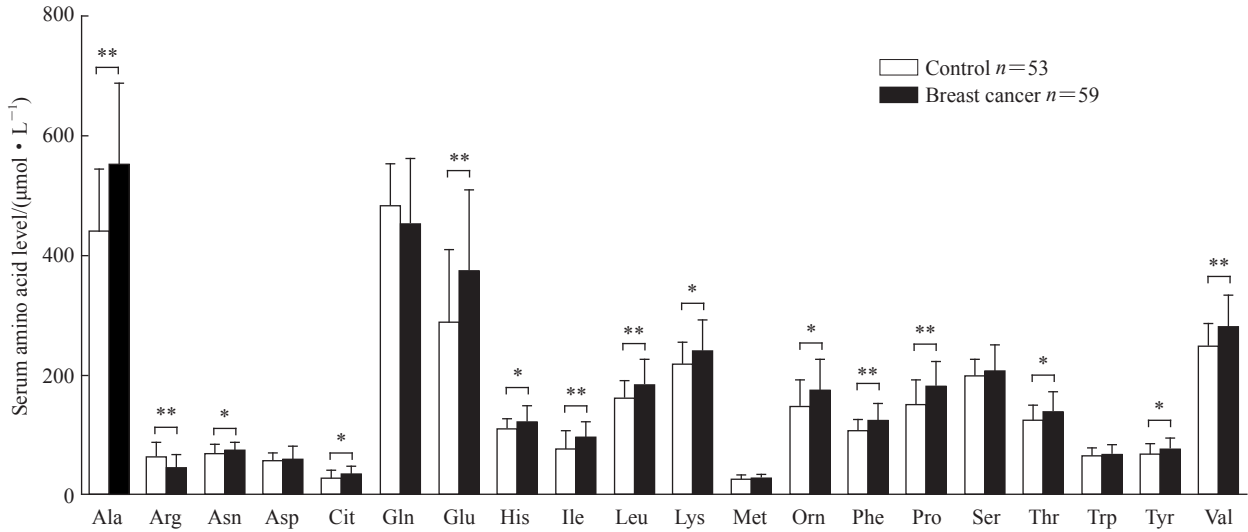


图 1 对照组与乳腺癌组血清氨基酸水平比较

Fig 1 Comparison of serum amino acid levels between control group and breast cancer group

* $P < 0.05$, ** $P < 0.01$. $\bar{x} \pm s$. Ala: Alanine; Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; Cit: Citrulline; Gln: Glutamine; Glu: Glutamic acid; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

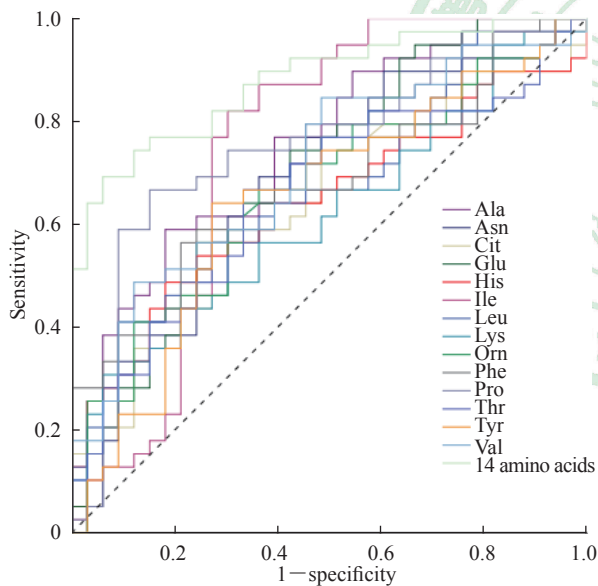


图 2 乳腺癌患者血清中升高的 14 种氨基酸单独及联合检测筛查乳腺癌的 ROC 曲线

Fig 2 ROC curves of 14 elevated serum amino acids alone and their combined detection for screening of breast cancer

ROC: Receiver operating characteristic; Ala: Alanine; Asn: Asparagine; Cit: Citrulline; Glu: Glutamic acid; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Thr: Threonine; Tyr: Tyrosine; Val: Valine.

结果显示 20 种氨基酸在乳腺癌各分子亚型中的水平差异均无统计学意义 (P 均 > 0.05 , 表 3)。

表 1 乳腺癌患者血清中升高的 14 种氨基酸单独及联合检测筛查乳腺癌的效能评估

Tab 1 Screening performance of 14 elevated serum amino acids alone and their combined detection for breast cancer

Amino acid	AUC	Cut-off value/ ($\mu\text{mol} \cdot \text{L}^{-1}$)	Sensitivity	Specificity
Ala	0.75	509.92	0.59	0.82
Asn	0.68	72.17	0.67	0.67
Cit	0.64	26.52	0.77	0.52
Glu	0.70	328.79	0.56	0.76
His	0.64	121.89	0.49	0.82
Ile	0.74	77.46	0.82	0.70
Leu	0.67	193.25	0.41	0.91
Lys	0.62	263.72	0.31	0.94
Orn	0.66	154.83	0.67	0.64
Phe	0.68	119.78	0.56	0.79
Pro	0.75	169.28	0.67	0.85
Thr	0.66	128.36	0.67	0.67
Tyr	0.65	73.92	0.64	0.73
Val	0.71	286.53	0.49	0.88
14 amino acids	0.88	0.76 ^a	0.69	0.94

^a: Prediction probability. AUC: Area under curve; Ala:

Alanine; Asn: Asparagine; Cit: Citrulline; Glu: Glutamic acid; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Thr: Threonine; Tyr: Tyrosine; Val: Valine.

2.4 不同分子亚型的乳腺癌患者血清中 20 种氨基酸水平比较 比较乳腺癌 Luminal A 型、Luminal B 型、三阴性型、HER2 阳性型患者血清氨基酸水平,

表2 早期(TNM分期I~II期)和晚期(TNM分期III~IV期)乳腺癌患者血清中20种氨基酸水平的比较

Tab 2 Levels of 20 serum amino acids in early (TNM stage I-II) and advanced (TNM stage III-IV) breast cancer patients ($\mu\text{mol} \cdot \text{L}^{-1}$), $\bar{x} \pm s$

Amino acid	Early n=26	Advanced n=33	t value	P value	Amino acid	Early n=26	Advanced n=33	t value	P value
Ala	537.72±158.74	563.12±116.31	-0.577	0.567	Lys	249.94±58.53	234.06±43.33	0.974	0.336
Arg	48.56±13.70	45.95±21.69	0.426	0.673	Met	28.35±5.04	27.96±4.31	0.261	0.796
Asn	75.28±13.19	75.74±9.84	-0.126	0.901	Orn	177.30±48.15	173.58±53.31	0.223	0.825
Asp	62.11±16.66	58.96±22.05	0.483	0.632	Phe	127.88±25.52	122.40±27.55	0.629	0.533
Cit	33.56±10.77	34.93±12.54	-0.355	0.725	Pro	185.36±40.84	180.12±38.26	0.410	0.684
Gln	444.73±73.07	460.19±125.49	-0.442	0.661	Ser	211.99±40.32	203.75±42.53	0.608	0.547
Glu	377.40±148.15	375.29±122.29	0.048	0.962	Thr	137.80±30.12	141.67±30.25	-0.393	0.697
His	123.35±22.22	121.33±25.00	0.260	0.797	Trp	69.74±14.22	67.12±14.33	0.563	0.577
Ile	101.97±28.64	93.67±19.19	1.086	0.285	Tyr	78.29±14.67	76.08±17.38	0.416	0.680
Leu	194.87±32.73	178.46±42.89	1.289	0.205	Val	301.30±31.02	268.33±57.53	2.086	0.044

Ala: Alanine; Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; Cit: Citrulline; Gln: Glutamine; Glu: Glutamic acid; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

表3 不同分子亚型乳腺癌患者血清中20种氨基酸水平比较

Tab 3 Levels of 20 serum amino acids in breast cancer patients with different molecular subtypes ($\mu\text{mol} \cdot \text{L}^{-1}$), $\bar{x} \pm s$

Amino acid	Luminal A n=16	Luminal B n=25	Triple-negative n=8	HER2 positive n=10	F value	P value
Ala	526.76±76.96	553.87±167.78	631.50±87.47	512.51±130.77	1.190	0.325
Arg	61.32±22.53	46.33±18.85	39.72±4.06	41.13±16.86	2.491	0.076
Asn	71.91±11.31	74.91±11.51	83.38±8.50	73.75±11.13	1.596	0.208
Asp	57.28±17.34	60.27±14.88	72.54±23.42	53.32±24.73	1.360	0.271
Cit	31.55±14.09	36.18±9.99	34.90±13.29	33.43±12.47	0.282	0.838
Gln	471.76±106.67	473.23±113.04	424.34±47.72	428.58±130.65	0.572	0.637
Glu	335.70±148.10	370.79±127.39	472.55±111.33	346.10±120.67	1.770	0.171
His	119.37±19.68	118.77±22.12	137.98±22.55	117.98±28.37	1.313	0.286
Ile	94.93±22.78	99.37±19.35	112.60±32.93	83.09±16.25	2.377	0.087
Leu	177.30±28.39	184.55±39.30	211.71±14.56	172.66±44.81	1.528	0.224
Lys	226.86±33.02	244.53±52.22	273.43±60.62	220.61±42.32	1.829	0.160
Met	27.83±3.39	28.22±4.36	28.95±6.30	27.57±4.97	0.125	0.945
Orn	155.51±54.87	178.16±44.39	205.57±43.84	163.73±57.41	1.465	0.241
Phe	119.47±26.29	122.56±21.98	143.32±33.27	118.19±25.67	1.527	0.225
Pro	170.10±19.65	180.93±30.91	200.61±48.37	181.07±54.54	0.775	0.516
Ser	191.79±27.15	216.17±34.99	221.41±50.40	194.58±51.63	1.180	0.331
Thr	136.20±19.66	137.90±30.24	152.13±32.06	137.80±37.02	0.449	0.720
Trp	66.96±5.74	68.66±17.53	77.41±8.81	61.37±14.10	1.823	0.161
Tyr	72.70±10.55	77.33±15.41	85.50±17.17	73.57±20.26	0.970	0.418
Val	271.27±35.08	287.18±49.53	295.80±45.18	271.53±69.51	0.453	0.717

HER2: Human epidermal growth factor receptor 2; Ala: Alanine; Arg: Arginine; Asn: Asparagine; Asp: Aspartic acid; Cit: Citrulline; Gln: Glutamine; Glu: Glutamic acid; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Orn: Ornithine; Phe: Phenylalanine; Pro: Proline; Ser: Serine; Thr: Threonine; Trp: Tryptophan; Tyr: Tyrosine; Val: Valine.

3 讨论

氨基酸是合成蛋白质的基本原材料和蛋白质

代谢的产物,氨基酸代谢是调节生长、繁殖和免疫所必需的关键代谢途径^[12]。肿瘤患者因肿瘤炎症刺激产生急性时相反应蛋白的增加及厌食情绪、

吞咽困难、消化不良等会导致机体对蛋白和能量的需求改变, 并伴随代谢紊乱^[4]。代谢组学分析是一种非常有前途的用于肿瘤早期筛查的方法。越来越多的研究表明氨基酸是一类极具潜力的生物标志物, 已被用于恶性肿瘤的筛查和相关发病机制的研究^[4,6-11,13-15], 但在乳腺癌相关研究中仅有少量小样本报道, 尚存在争议。Poschke 等^[16]采用高效液相色谱法对 41 例乳腺癌患者和 9 例乳腺良性病变患者术前、术后 15 种血清氨基酸水平进行了测定, 结果发现与乳腺良性病变患者相比, 乳腺癌患者术前血清中 8 种氨基酸 (Glu、Ser、Gln、Ala、Val、Phe、Ile、Leu) 水平升高。Miyagi 等^[17]研究了 196 例乳腺癌患者和 976 例正常人血浆氨基酸水平, 发现乳腺癌患者血浆 Thr、Pro、Ser、Gly、Ala 和 Orn 水平高于正常人, 而 Gln、Trp、His、Phe 和 Tyr 水平却比正常人低。Gu 等^[18]检测了 28 例乳腺癌和 137 例健康对照者血浆中氨基酸水平, 乳腺癌组 Arg 和 Thr 水平高于健康对照组, 而 Gly、Asp、His、Gln、Pro 水平低于健康对照组。本实验用 HPLC-MS 法对乳腺癌患者和正常人血清中的 20 种氨基酸进行了检测, 发现乳腺癌患者有 14 种血清氨基酸 (Ala、Asn、Cit、Glu、His、Ile、Leu、Lys、Orn、Phe、Pro、Thr、Tyr、Val) 水平高于正常人 (P 均 < 0.05), 仅有 Arg 水平低于正常人 ($P = 0.003$)。各研究结果不一致的原因可能与研究人群来源、样本检测量及检测方法不同有关。但本研究及文献报道的结果均证实乳腺癌患者血液中氨基酸代谢与正常人有差异, 具有用于乳腺癌筛查的潜力。

乳腺癌是女性发病率最高的肿瘤, 严重危害女性健康^[1-2]。高死亡率和较差的预后主要由于滞后的诊断, 因此, 及早地诊断乳腺癌是改善乳腺癌患者整体生存率的有效途径。目前乳腺癌健康筛查主要依靠影像学如超声、钼靶等, 缺乏特异的血清学标志物。本实验对乳腺癌患者血清氨基酸中高于正常人的 14 种差异氨基酸单独及联合检测绘制了 ROC 曲线, 计算筛查最佳截断值及对应的筛查灵敏度和特异度, 并用 AUC 评估其筛查乳腺癌的效能, 结果显示 Ala、Pro 单独筛查乳腺癌的效能较高 (AUC 均为 0.75), Ile 单独筛查乳腺癌时拥有最高灵敏度, Lys 拥有最高特异度。本研究结果显示没有哪一种血清氨基酸单独筛查乳腺癌时同时拥有比较理

想的灵敏度和特异度, 但 14 种氨基酸联合检测时能够提高乳腺癌患者的筛查效能, 因此建议在用血清氨基酸对乳腺癌进行筛查时采用多种氨基酸联合检测。

乳腺癌的分期直接关系到患者的生存预后, 本研究对早期和晚期乳腺癌患者血清氨基酸水平进行了比较, 发现前者血清氨基酸 Val 水平高于后者, 同时 Val 在乳腺癌血清中整体水平高于正常人, 表明血清氨基酸 Val 水平对于早期乳腺癌筛查具有非常大的潜力。

根据乳腺肿瘤组织中 4 个分子 (HER2、雌激素受体、孕激素受体和 Ki-67) 的免疫组织化学染色结果, 可将乳腺癌分为不同的分子亚型 (Luminal A 型、Luminal B 型、三阴性型、HER2 阳性型), 不同分子亚型其临床治疗方案及预后均有所不同^[19-20]。有研究表明血清氨基酸水平在侵袭性最强的乳腺癌亚型 (基底细胞型) 中较侵袭性弱的乳腺癌亚型 (Luminal A 型) 高^[16], 但本研究对乳腺癌 Luminal A 型、Luminal B 型、三阴性型、HER2 阳性型乳腺癌患者血清氨基酸水平的比较结果发现, 在所有分子亚型乳腺癌中 20 种血清氨基酸水平差异均无统计学意义 (P 均 > 0.05)。血清氨基酸在不同分子亚型乳腺癌中的表达差异还需进一步证实。

过去几十年里, 尽管乳腺癌的死亡率随着新的治疗手段的发展而降低, 但没有任何治疗手段对所有分子亚型的乳腺癌都有效^[20]。近年来可用于多种乳腺癌分子亚型治疗的代谢饥饿疗法逐渐走进人们的视野, 这种治疗手段通过去除或限制某些特殊的代谢物以达到有效治疗的目的, 与传统的化学治疗和放射治疗相比不良反应较低, 是一种非常有潜力的治疗策略^[21]。氨基酸失衡疗法是一种非常有前景的代谢饥饿疗法, 通过去除、减少或增加某些特殊氨基酸既能够选择性地抑制肿瘤细胞的生长, 又能改善患者营养状况, 最终达到治疗肿瘤的目的^[22-24]。本研究显示乳腺癌患者血清氨基酸中 14 种血清氨基酸水平高于正常人, 说明正常人中一些非必需氨基酸可能是肿瘤生长的必需氨基酸, 有意识地减少、抑制或去除这些氨基酸的摄入可能有助于乳腺癌治疗。有趣的是, 本研究发现乳腺癌患者血清中非必需氨基酸 Arg 水平低于正常人, 这与 Vissers 等^[25]研究结果类似。然而有研究显示乳

腺癌组织中氨基酸 Arg 水平比乳腺良性组织高,有些肿瘤中由于缺乏精氨酸琥珀酸合成酶无法合成 Arg,因此 Arg 对某些肿瘤来说是必需氨基酸^[26-28]。Arg 代谢与很多其他代谢途径相互作用能够促进肿瘤的生长^[29-31],出现乳腺癌组织中 Arg 水平高而血清中 Arg 水平相对较低的情况,可能由于乳腺癌的生长必需 Arg 参与,而乳腺癌患者体内又缺乏精氨酸琥珀酸合成酶无法合成 Arg,导致血清中 Arg 水平降低。据此推测,通过某些治疗手段耗尽体内 Arg 也许对乳腺癌治疗有利,Arg 可能是乳腺癌新的治疗靶点。

综上所述,血清氨基酸水平在乳腺癌与正常人中存在差异,可用于乳腺癌的筛查,多种血清氨基酸同时检测有利于提高乳腺癌的筛查效能,是非常有潜力的生物标志物。本研究的病例数相对较少,仅进行了探索性研究,血清氨基酸能否用于乳腺癌筛查的临床实践还需要后续大样本验证。

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