医学生化

尿中游离 L-岩藻糖测定的临床应用

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摘要 采用日本宝酒造公司生产的 L-岩藻糖监测试剂盒及质控标准品，用酶分析法在自动生化分析仪上对 86 例正常健康者和 205 例不同肿瘤患者尿中的 UFC 水平进行检测，结果显示，健康组尿中 UFC 水平：$\bar{x}$ = 177.7，s = 56.6，95%，可信限范围是 63.8~ 290.2 μmol/g.cr；肝癌、肝硬化、急慢性肝炎、胰腺癌、食管癌、胃癌、肠癌、肺癌患者尿中 UFC 均值大于 290 μmol/g.cr. 与正常健康组对照，有非常显著性差异；乳腺癌、宫颈癌、卵巢癌患者的 UFC 与健康组比较，有显著性差异. 尿中游离 L-岩藻糖测定方法简便、快速、能自动化、取样方便，并提示尿中 UFC 水平的变化可视为肝癌、急慢性肝炎癌变的特异性指标之一，也是消化系统癌症病人肿瘤标记物试验之一.

关键词 L-岩藻糖，L-岩藻糖脱氢酶，肿瘤，肝脏疾病
学科分类号 R466.1

L-岩藻糖 (L-fucose, L-FC)，又名 6-脱氧半乳糖 (6-deoxygalactose)，是一种以 L-异构体形态天然存在的稀有糖类. 在生物体内，L-FC 主要发现在糖蛋白糖脂等复合肝糖链 (hepatoglycochains) 的非还原端，它在糖结合物的生理和生物学功能中起着重要作用，如细胞转化作用 (致癌作用) 与 L-岩藻糖代谢间存在某些紧密联系. 现已有报道大多数新发现的癌相关的糖抗原含有 L-岩藻糖，如甲胎蛋白 (AFP) 和 CA-199 等肿瘤标志物的糖链附有 L-岩藻糖，在原发性肝癌患者中检测到 α-L 岩藻糖甙酶增高；人类乳腺癌中岩藻糖脂类 (fucolipid) 积累异常丰富及肺癌细胞内岩藻糖酰转移酶 (fucosyltransferase) 高度活跃，并发现肝癌及肝硬变患者随尿排泄的游离 L-岩藻糖量高于正常人[1~3].

1 材料与方法

1.1 材料

1.1.1 试剂：UFC 检测试剂盒及质控标准品 (日本宝酒造公司)；肌酐试剂盒 (国产，中生公司).

1.1.2 仪器：日本 Olympus AU-560；Beckman CX-3，CX-7；日立 7150，7060；美国 Monarch 2000.

1.1.3 标本来源：尿液标本来自北京协和医院，北京医院，北京朝阳医院，中国医学科学院肿瘤医院.

a. 健康组：来自以上各医院已知临床各项指标正常者，年龄 25~ 60 岁，男 36 人，女 50 人.

b. 疾病组：来自上述各医院门诊和住院病人，均由临床诊断标准确诊的患者.

c. 标本收集：随时新鲜尿液，－20℃保存.

1.2 方法

1.2.1 原理：游离 L-岩藻糖测定是利用 L-岩藻糖脱氢酶和 NAD^+ 为试剂的酶分析法，在 340 nm 下测定 NADH 吸光度变化，求出标本中 L-FC 浓度.

L-岩藻糖 + NAD^+ $\xrightarrow{FDH}$ L-岩藻-1，5-内脂 + NADH + H^+

FDH：L-岩藻糖脱氢酶 (L-fucose dehydrogenase)

1.2.2 操作方法：

a. 仪器测定参数由试剂盒说明书提供.

b. 测定结果的判定方法：

在测定尿标本 L-FC 浓度的同时还要测定尿肌酐浓度. 以每 1 克肌酐浓度来计算 UFC 值.

$$UFC/\mu mol \cdot g^{-1}.cr^{-1} = \frac{c(L\text{-}FC)/mmol \cdot L^{-1} \times 10^5}{\rho(肌酐)/mg \cdot dl}$$

2 结 果

2.1 95% 分布范围的确定

健康组共 86 人，经统计 $\bar{x}$ = 177.7 μmol/g.cr，

收稿日期：1998-10-06，修回日期：1999-03-15

s= 56.6, UFC 值频率分布基本接近于正态 (图 1). 以 $\bar{x} \pm 2s$ 表示 95% 分布范围, 故正常人 UFC 的参考范围约 64~ 290 μmol/g.cr.

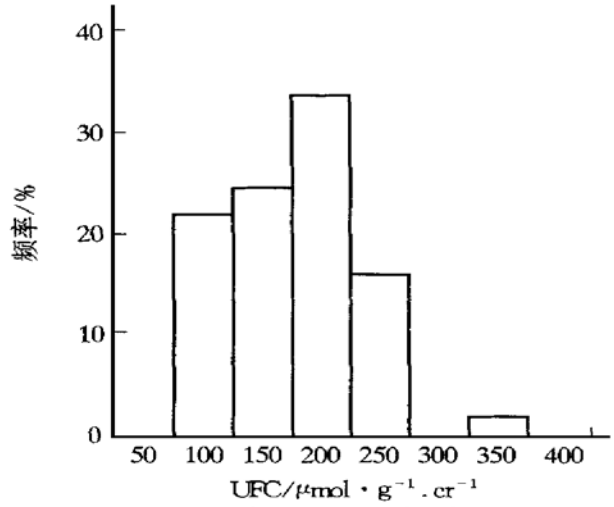

图 1 正常人 UFC 值频率分布

n= 86; M= 177; s= 56.6.

2.2 各疾病组均值与正常人均值显著性测定

运用 POMS 软件, 对各种疾病组 UFC 均值与健康组均值进行团体 t 检验来观察它们有无显著性差别 (表 1).

表 1 各疾病组均值与健康组均值显著性测定

病种	n	M	P	
肝癌	43	473.7	< 0.01	显著性差异
肝硬化	17	324.6	< 0.01	显著性差异
胰腺癌	8	409	< 0.01	显著性差异
食管癌	10	1531.9	< 0.001	非常显著性差异
胃癌	10	813.8	< 0.001	非常显著性差异
肠癌	15	422.2	< 0.01	显著性差异
肺癌	38	436.7	< 0.01	显著性差异
膀胱癌	3	156.6	= 0.5	无显著性差异
乳腺癌	5	510.2	0.01< P< 0.05	显著性差异
宫颈癌	7	648	0.01< P< 0.05	显著性差异
卵巢癌	6	435.6	0.01< P< 0.05	显著性差异
急性肝炎	19	295.8	< 0.01	显著性差异
慢性肝炎	11	278	0.01< P< 0.05	显著性差异
绒癌	3	223	> 0.05	无显著性差异
其他[1)]	10	255	> 0.05	无显著性差异

[1)] 其他: 肾癌, 输卵管癌, 甲状腺癌, 舌癌, 前列腺癌.

2.3 UFC 在疾病组中阳性率分布

我们以 UFC 值大于正常参考范围上限值 (290 μmol/g.cr) 为界限, 对现有数据进行统计 (表 2, 图 2).

表 2 UFC 测试在疾病组中阳性率分布

病种	n	M	UFC> 290 μmol/g.cr 的人数	阳性率/%
肝癌	43	473.7	26	60.4
肝硬化	17	324.8	7	41
胰腺癌	8	409	7	87.5
食管癌	10	1531.9	8	80
胃癌	10	813.8	8	80
肠癌	15	422.2	13	86.6
肺癌	38	436.7	29	76.3
膀胱癌	3	156.6	0	0
乳腺癌	5	510.2	5	100
宫颈癌	7	648	7	100
卵巢癌	6	435.6	5	83
急性肝炎	19	295.8	8	42.1
慢性肝炎	11	278	5	45.4
绒癌	3	223	0	0
其他	10	255	4	40

3 讨 论

从我们的结果来看, 健康组 UFC 水平与文献报道基本一致. 肿瘤病人的 UFC 值明显高于健康组, 尤其是消化系统癌症病人, 在与正常人均值作 t 检验时, 有显著或非常显著的差异, 如胰腺癌阳性率达 87.5%, 这与文献是一致的. 肝脏疾病 UFC 水平与正常人对比有显著差异, 故 UFC 值的变化可视为肝癌和急慢性肝炎的特异性指标之一. 肺癌阳性率为 76.3%, 考虑可能为继发转移癌所引起的. 本实验妇科癌症中乳腺癌、宫颈癌、卵巢癌病人阳性率很高, 和文献报道 26% 不一致, 这可能是病例较少, 有待进一步观察.

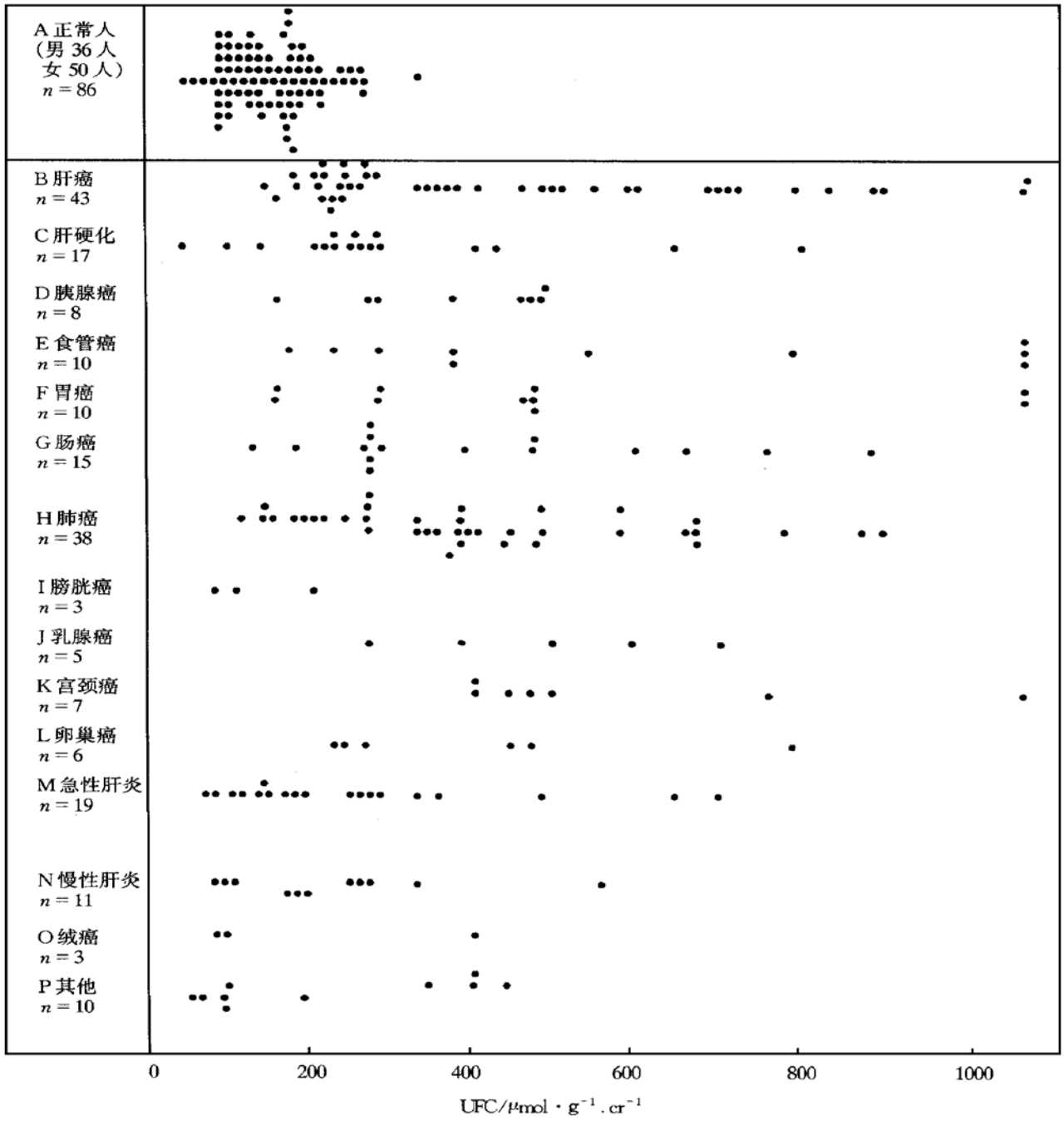

图 2 各种疾病的 UFC 水平散点图

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Clinical Application for Determination of Free L-FC in Urine. SONG Yao-Hong, FENG Tao, SHEN Ying, MA De-Jun, WANG Hui-Zhen (*Chinese Academy of Medical Sciences and Peking Union Medical College Hospital, Beijing* 100730, China).

Abstract L-fucose (L-FC), 6-deoxygalactose, is one of rare sugars which naturally exists in the form of L-isomer. L-fucose plays an important role in their physiological and biological functions. It has been

well known that L-FC is attached to glycochains of tumor markers such as alpha fetoprotein (AFP) and CA-199. Increment of α-L-fucosidase was detected in patients with primary hepatic carcinoma. Fucolipid accumulation in human adenocarcinoma was abnormally increased and fucosyltransferase was highly active in lung cancer cells. In addition, it has been found that patients with liver cancer and cirrhosis excreted free L-fucose via urine to greater extent than normal individuals. The values of UFC were detected in the urine from 86 health people and 205 people with various tumors. The mean value of urinary L-FC in health people was 177.7 μmol/g.cr (s = 56.6, 95% confidence range was 63.8 ~ 290.2 μmol/g.cr). The mean values of UFC in urine obtained from patients with hepatocellular carcinoma, cirrhosis, acute and chronic hepatitis, pancreas carcinoma, gastric cancer, lung cancer, intestinal cancer, esophageal cancer were higher than 290.2 μmol/g.cr, having significant difference from normal individuals. The mean values of UFC in urine obtained from patients with mastadenoma, cervical cancer and ovary cancer have significant difference from normal individuals. The value of L-fucose in urine can be regarded as one of the characteristic indexes for hepatocellular carcinoma and canceration of acute and chronic hepatitis. Detection for L-fucose is one of tumor marker tests for patients with tumor in digestive system.

Key words L-FC, L-fucose dehydrogenase, tumor, liver dieases

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Expression in *Escherichia coli* of anti-Human Tumor Necrosis Factor alpha ScFv Under the Control of T7 Promoter. CHEN Ping, DENG Jian-Bei, CHEN Mei-Hong, Han Hua, Yao Li-Bo, Su Cheng-Zhi (*Department of Biochemistry and Molecular Biology, The Fourth Military Medical University, Xi'an* 710032, China).

Abstract An engineering anti-human TNF-α single-chain antibody (ScFv) gene was cloned into the expression vector pET15b-Etag, and expressed in *E. coli* BL21 (DE3) under the control of T7 promoter. By using 0.1 mmol/L IPTG induction, the amount of the ScFv expression product was more than 38% of total bacterial proteins. Most of them existed in a form of inclusion body. More than 6% of total bacterial proteins were soluable and can be detected in the part of periplasm, which can bind with rhuTNF-α by ELISA and Dot-blotting technique.

Key words hTNF-α, ScFv, high-level expression