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临床经验 CLINICAL PRACTICE

肿瘤M_2型丙酮酸激酶在结直肠癌粪便筛查中的临床价值

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Value of fecal tumor M_2 pyruvate kinase in diagnosis of colorectal cancer

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Abstract

AIM: To evaluate the value of fecal M_2 pyruvate kinase (M_2-PK) as a screening biomarker for colorectal cancer.

METHODS: The tumor fecal M_2-PK was determined by enzyme-linked immunosorbent assay (ELISA) in colorectal cancer patients (n = 80) and healthy controls (n = 80), and the results were comparatively analyzed.

RESULTS: The cut-off value to discriminate patients from controls was established at 166.7 μkat/L for tumor M_2-PK. The mean level of fecal tumor M_2-PK was significantly higher in colorectal cancer patients than that in the normal controls (713.41 μkat/L *vs* 59.55 μkat/L, P < 0.0001), and the overall sensitivity and specificity were 77.5% and 92.5%, respectively. With the progression of colorectal carcinoma, the level of tumor M2-PK as well as the sensitivity was increased (F = 52.984, P < 0.0001). In patients with Dukes A (n = 11), B (n = 37), C (n = 25), and D (n = 7) stages, the mean levels of tumor M_2-PK were 233.53, 522.58, 847.27 and 1998.04 μkat/L, respectively, and the sensitivities were 63.64%, 75.68%, 84% and 84%, respectively.

CONCLUSION: Tumor M_2-PK is detectable in the feces of colorectal cancer patients and correlated with the staging and metastasis of the carcinoma, and it can help to make early diagnosis of colorectal cancer in patients with sub-clinical symptoms.

Key Words: Tumor M_2 pyruvate kinase; Stool screening; Colorectal cancer

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摘要

目的: 评估一种新的肿瘤标志物—肿瘤M_2型丙酮酸激酶(Tumor M_2-PK)在结直肠癌粪便筛查中的诊断价值.

方法: 采集80例结直肠癌患者以及80例正常健康对照者的粪便, 用酶结合免疫吸附测定(ELISA)法检测粪便中Tumor M_2-PK值, 并进行比较分析.

结果: 结肠癌患者粪便中Tumor M_2-PK的整体水平(713.41 μkat/L)显著高于健康人群(59.55 μkat/L)(P<0.0001), 其总体敏感性为77.5%, 总体特异性为92.5%(正常值定为<166.7 μkat/L). 随着结肠癌的进展和转移, Tumor M_2-PK检测值随之升高, 检测的敏感性也随之升高, 11例Dukes A期患者的Tumor M_2-PK平均值为233.53 μkat/L, 敏感性为63.64%; 37例Dukes B期患者为522.58 μkat/L, 敏感性为75.68%; 25例Dukes C期患者为847.27 μkat/L, 敏感性为84%; 7例Dukes D期患者为1998.04 μkat/L敏感性为85.71%.

结论: Tumor M_2-PK在粪便中的检测可以区别大部分的结肠患者和健康人群, 且其敏感性明显高于粪便潜血实验, 他将适用于人群普查和绝大多数有亚临床症状患者的早期诊断.

■背景资料
结肠癌的早期诊断主要依赖结肠镜的广泛开展和特异性的肿瘤标志物检测, 如果能在粪便中找到一种相对敏感的标志物用于结肠癌的早期诊断, 不仅适用于大规模的人群筛查, 而且与血清学标志物相比, 将提高结肠癌诊断的特异性.

■研发前沿
肿瘤M_2型丙酮酸激酶作为一种新的肿瘤标志物, 在高危结肠癌患者粪便中可以方便的检测, 其敏感性显著高于粪便潜血实验, 有较好的临床应用价值.

关键词: 肿瘤M_2丙酮酸激酶; 粪便筛查; 结肠癌

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0 引言

结直肠癌是全球第三大癌症, 每年新增病例约945 000人, 死亡病例约49 200人, 其5 a总体生存率在不发达国家仍低于40%[1-3]. 结肠镜检查虽然是结直肠癌早期诊断的金标准, 但由于技术难度和操作复杂性使其难于进行大规模、定期的人群普查[4-5]. 丙酮酸激酶(pyruvate kinase)是糖酵解途径的关键激酶, 丙酮酸激酶同工酶M_2型在肿瘤细胞中明显过表达, 表现为肿瘤细胞特异性的代谢特征, 因此也称为肿瘤M_2型丙酮酸激酶(Tumor M_2-PK)[6-8]. 临床研究显示, Tumor M_2-PK的浓度在多种实体肿瘤患者的外周血中明显升高[9-13]. 我们通过对结直肠癌患者粪便中Tumor M_2-PK的检测, 评估这一新的肿瘤标志物在结直肠癌人群筛查和早期诊断中的价值.

1 材料和方法

1.1 材料 结肠镜和活检组织病理学诊断为原发性结直肠癌患者共80例, 平均年龄45.6岁, 其中男49例, 女31例. 根据Dukes分期, Dukes A期11例, Dukes B期37例, Dukes C期25例, Dukes D期7例. 80例健康人为对照组.

1.2 方法 所有患者收集手术前粪便标本1份, 并简要记录病史. 手术后记录肿瘤的大小、侵犯深度、淋巴结转移和病理学分型, 并进行Dukes分期. 健康对照人群收集常规粪便一份. 用德国ScheBo®Biotech公司生产的试剂盒, Tumor M_2-PK mAb和相应二抗用夹心法ELISA测定Tumor M_2-PK的浓度. 100 mg粪便标本用10 mL抽提液裂解, 1∶100稀释于样品洗涤液中, 常规方法分别与Tumor M_2-PK抗体和二抗杂交, 过氧化物酶和链霉亲和素染色后, 分光光度计在450 nm处读数.

统计学处理 SPSS11.5统计软件比较数据的差异显著性.

2 结果

2.1 Tumor M_2-PK检测结果 80例结肠癌患者粪便中Tumor M_2-PK的平均值为713.31 μkat/L, 80例健康人的平均值为59.55 μkat/L. 结肠肿瘤患者粪便中的Tumor M_2-PK值明显高于正常人(t = -8.219, P<0.001, 表1). 根据Dukes分期, 11例Dukes A期患者的Tumor M_2-PK平均值为233.53 μkat/L; 37例Dukes B期患者为522.58 μkat/L; 25例Dukes C期患者为847.27 μkat/L; 7例Dukes D期患者为1998.04 μkat/L. 结果发现随着肿瘤的浸润和转移, 晚期结肠癌患者粪便中Tumor M_2-PK值升高明显(F = 52.984, P<0.0001). 组间两两比较发现, Dukes A期患者与对照组间无显著性差异, Dukes B、C和D期患者与对照组间差异显著(表2).

表 1 结肠癌患者与健康对照Tumor M_2-PK水平比较的统计值

分组	n	最小值	最大值	平均值	标准差	标准误
对照组	80	2.00	276.22	59.55	58.37	6.53
结肠癌组	80	7.50	3976.80	713.41[b]	709.18	79.29

[b]P<0.01 *vs* 对照组 .

2.2 粪便中Tumor M_2-PK检测的敏感性 结肠癌患者粪便中Tumor M_2-PK检测的总体敏感性为77.5%, 其中Dukes A期患者为63.64%; Dukes B期为75.68%; Dukes C期为84%; Dukes D期为85.71%. 结果提示, 粪便中Tumor M_2-PK诊断的敏感性与肿瘤分期相关, 在早期结肠癌中其敏感性较低.

3 讨论

肿瘤细胞的代谢状态与正常增生细胞不同, 肿瘤细胞中有氧糖酵解途径增多, 而这一途径的增多与多种因素有关, 包括同工酶结构和活性的改变等. 丙酮酸激酶是糖酵解途径的关键激酶, 其有多种异构体形式存在, 包括丙酮酸激酶L型、R型、M_1型、M_2型和肿瘤M_2型, 这些激酶的表达具有组织特异性. L型主要见于肝脏和正常肾脏的近曲小管; 红细胞主要表达R型; M_1型主要存在于骨骼肌、心脏和脑, M_2型主要表达于肺、正常肾脏的远曲小管、胚胎和未分化或增生的组织. 所有这些异构体都以四聚体形式存在并为其活性状态[14-15]. 丙酮酸激酶同工酶M_2型在肿瘤细胞呈明显过表达, 并转变为二聚体形式存在. 这些二聚体形式的丙酮酸激酶M_2型的浓度在肿瘤细胞中明显升高, 表现为肿瘤细胞特异性的代谢特征, 因此称为肿瘤M_2型丙酮酸激酶(Tumor type M_2 pyruvate kinase, Tumor M_2-PK).

目前, 结肠癌的早期诊断主要依赖结肠镜的广泛开展和特异性的肿瘤标志物检测. 结肠镜检查由于操作复杂, 绝大部分无症状人群难以接受;

表 2 不同分期结肠癌患者Tumor M_2-PK水平比较的统计值

分组	*n*	最小值	最大值	平均值	标准差	标准误	*P*值
对照组	80	2.00	276.22	59.55	58.37	6.53	
Dukes A	11	21.00	501.60	233.53	159.09	47.97	0.172
Dukes B	37	7.50	1512.30	522.58	395.50	65.02	<0.0001
Dukes C	25	79.68	2107.92	847.27	570.57	114.11	<0.0001
Dukes D	7	48.84	3976.80	1998.04	1300.93	491.70	<0.0001

■同行评价
本文观察了肿瘤M_2型丙酮酸激酶在结直肠癌粪便筛查中的临床价值, 研究有较高的临床指导意义, 希望能开展前瞻性研究, 并加以推广.

肿瘤标志物的检测因敏感性和特异性较差, 至今尚无结肠癌特异性的血清学标志物[16-18]. 结直肠作为空腔脏器的特点, 粪便是其主要的分泌物, 如果能在粪便中找到一种相对敏感的标志物用于结肠癌的早期诊断, 一方面适用于大规模的人群筛查, 减轻筛查人群的负担; 同时与血清学标志物相比, 将提高结肠癌诊断的特异性[19-22]. 既往的研究显示, 血清中Tumor M_2-PK的浓度在胃肠道肿瘤中明显升高, 同时在粪便中也可以检测到Tumor M_2-PK浓度的差异[23-24]. 我们假设Tumor M_2-PK只能在胃肠道肿瘤患者的粪便中检测得到, 那么这种肿瘤标志物将非常有利于结肠癌的大规模人群普查. 我们的研究选取经结肠镜和病理活检证实的、不同分期的结肠癌患者, 检测其粪便中Tumor M_2-PK的水平与健康对照人群的差异, 结果显示, 结肠癌患者粪便中Tumor M_2-PK的整体水平(713.41 μkat/L) 显著高于健康人群(59.55 μkat/L)(P<0.0001), 其总体敏感性为77.5%, 总体特异性为92.5%(正常值定为<166.7 μkat/L). 这说明Tumor M_2-PK在粪便中的检测可以区别大部分的结肠患者, 且其敏感性明显高于粪便潜血实验(24%)[25-26]. 但是, 这些总体平均值和敏感性不能作为早期诊断的依据. 因此, 我们进一步分析Tumor M_2-PK的检测值与结肠癌分期的关系. 根据Dukes分期, 随着结肠癌的进展和转移, Tumor M_2-PK检测值随之升高, 检测的敏感性也随之升高(分别为63.64%, 75.68%, 84%和85.71%), 但统计学结果提示, Dukes B, C, D期患者Tumor M_2-PK平均值与对照组间差异显著(P值分别为0.034, <0.0001, <0.0001), 而Dukes A期患者的Tumor M_2-PK平均值与对照组无显著性差异(P = 0.172). 这说明该肿瘤标志物在结肠癌早期诊断中意义不大, 也就是说对于大多数没有临床症状的黏膜下结肠癌高危人群其临床价值较差. 尽管如此, 对于绝大多数Dukes B期以上患者, 其诊断的敏感性较高(>75%). 虽然对粪便中一些癌基因和抑癌基因的检测(如K-ras, p53, APC等基因)将得到更高的特异性, 但由于个体患者的遗传异质性, 单个基因的检测不能提高诊断的敏感性, 而多个基因的检测又将提高检测的费用[27-30]. 因此, 粪便中Tumor M_2-PK的检测将比癌基因和抑癌基因的检测敏感性更高, 他不仅检测手段方便、可靠, 检测标本稳定、容易采集, 而且非常适合大规模的人群普查.

总之, Tumor M_2-PK作为一种新的肿瘤标志物, 在高危结肠癌患者粪便中可以方便的检测, 其敏感性显著高于粪便潜血实验. 在更大规模的临床检验后, 他将适用于人群普查和绝大多数有亚临床症状患者的早期诊断, 但对于很早期结肠癌的筛查仍有局限性, 如果将来配合癌基因和抑癌基因的突变筛查, 将极大的提高结肠癌的早期诊断率[31].

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• 消息 •

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