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肺癌患者血清中 VEGF,TIMP-1 和 MMP-9 水平变化及临床意义

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摘要 目的：研究肺癌患者血清中血管内皮生长因子（VEGF）、组织金属蛋白酶抑制剂 1（TIMP-1）、基质金属蛋白酶 9（MMP-9）水平变化及临床意义。**方法**：选取 2014 年 3 月至 2016 年 3 月来我院治疗的 91 例肺癌患者为病例组，同期选取 40 例健康者为对照组，酶联免疫吸附测定法（ELISA）测定两组血清 VEGF、TIMP-1、MMP-9 水平，分析肺癌患者上述指标与病理特征的关系，并采用 spearman 检验分析相关性。**结果**：病例组血清 VEGF、TIMP-1、MMP-9 水平均高于对照组，差异均具有统计学意义（P<0.05）。肺癌患者血清 VEGF、TIMP-1、MMP-9 水平均与肿瘤体积大小、TNM 分期、淋巴结转移、远处转移有关（P<0.05）。肺癌患者血清 MMP-9 与 TIMP-1 正相关（r=0.337，P<0.05）、血清 MMP-9 与 VEGF 正相关（r=0.312，P<0.05）、血清 TIMP-1 与 VEGF 正相关（r=0.316，P<0.05）。**结论**：血清 VEGF、TIMP-1、MMP-9 相互作用、协同参与肺癌的发生及侵袭转移，可作为肺癌诊断及预后评估的生物学标志物。

关键词：肺癌；MMP-9；TIMP-1；VEGF；生物学标志物

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Changes of the Levels of Serum VEGF, TIMP-1 and MMP-9 in Patients with Lung Cancer and its Clinical Significance

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ABSTRACT Objective: To study the changes of the levels of serum vascular endothelial growth factor (VEGF), tissue inhibitor of metalloproteinases-1(TIMP-1) and matrix metalloprotein-9(MMP-9)in patients with lung cancer and its clinical significance. **Methods:** 91 cases of patients with lung cancer were selected as the case group treated in our hospital from March 2014 to March 2016, and selected 40 healthy people as the control group in the same period, used enzyme linked immunosorbent assay(ELISA) to detect the levels of serum VEGF, TIMP-1, MMP-9 in two groups, then analyzed the relationship between the above indexes and pathological characteristics of patients with lung cancer, and the correlation was analyzed by Spearman test. **Results:** The levels of serum VEGF, MMP-9, TIMP-1 in case group were higher than control group respectively, the differences were statistically significant(P<0.05). The levels of serum VEGF, MMP-9 and TIMP-1 were related to the tumor volume, TNM stage, lymph node metastasis and distant metastasis in patients with lung cancer. Serum MMP-9 correlated with TIMP-1 (r=0.337, P<0.05), serum MMP-9 correlated with VEGF (r=0.312, P<0.05), and serum TIMP-1 correlated with VEGF(r=0.316, P<0.05) in patients with lung cancer. **Conclusion:** Serum VEGF, TIMP-1, MMP-9 are interaction and co participation in the occurrence, invasion and metastasis of lung cancer, which are available as biological markers on diagnosis and prognosis assessment of lung cancer.

Key words: Lung cancer; MMP-9; TIMP-1; VEGF; Biological markers

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前言

肺癌发生于支气管黏膜上皮，近年来肺癌的发病率呈逐年增高的趋势，特别是在一些工业发达的国家和一些工业大城市中，在男性恶性肿瘤中肺癌的发病率已居首位，且女性发病率也呈增高趋势，占女性常见恶性肿瘤的第 2 位或第 3 位[1]。目前的研究来看，肺癌的具体发病原因及机制尚未完全阐明，可能与生活习惯、周身环境、慢性肺部疾病以及遗传等因素相关[2]。肺癌患者早期症状较轻，主要表现为咳嗽、低热、胸部胀痛和痰血，有些患者甚至不表现任何不适症状，因此大多数患者在最终确诊时已到晚期，而即使早期发现并进行手术及药物干预治

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疗的患者,大约两年内近30%的患者出现复发及转移的情况[3]。近年肿瘤标志物研究成为临床研究热点,为癌症的早期发现及治疗提供了重要的参考价值。有文献报道,血管内皮生长因子、基质金属蛋白酶和组织蛋白酶抑制剂在肿瘤新血管形成、肿瘤生成以及侵袭转移中有重要作用[4-6]。本研究通过观察肺癌患者血清血管内皮生长因子(VEGF)、组织金属蛋白酶抑制剂1(TIMP-1)及基质金属蛋白酶9(MMP-9)水平,探讨其与患者病理特征的关系,为临床治疗肺癌患者提供血清学参考,减少治疗的盲从性,从而来提高肺癌的临床治疗水平。

1 材料与方法

1.1 临床资料

选取2014年3月至2016年2月我院收治的经临床和组织病理学检查确诊的91例肺癌患者(病例组),均已排除放化疗、慢性肾脏病、心脏病、高血压以及糖尿病等系统性疾病患者。其中男性62例,女性29例,年龄54～76岁,平均61岁;小细胞肺癌41例,非小细胞肺癌50例;病理分化程度:低分化39例,中分化42例,高分化10例;肿瘤大小:≥ 3 cm 72例,≤ 3 cm 19例;TNM分期:Ⅰ+Ⅱ期58例,Ⅲ+Ⅳ期33例;淋巴结转移55例,无转移36例;远处转移34例,无远处转移57例。另选取体检健康者40例为对照组,其中男性27例,女性13例,年龄53～78岁,平均62岁。

1.2 血清VEGF、TIMP-1、MMP-9指标检测

抽取所有受检者早晨空腹静脉血5 mL,取血后静置1-2 h,4000 rpm离心5 min取上清置于对应标记的离心管中备用。采用酶联免疫吸附法(enzyme linked immunosorbent assay,ELISA)检测VEGF、TIMP-1、MMP-9水平,试剂盒均购自上海基免生物科技股份有限公司。

1.3 统计学方法

应用SPSS 20.0统计软件进行统计学分析。符合正态分布的计量资料用均数± 标准差($\bar{x}$± s)表示,组内比较采用独立样本t检验,多组比较采用方差检验,相关性分析采用spearman检验,以P<0.05为差异有统计学意义。

2 结果

2.1 两组血清VEGF、TIMP-1 、MMP-9水平比较

病例组血清VEGF、TIMP-1、MMP-9水平分别为(217.38± 14.66)pg/mL,(598.43± 12.78)pg/mL,(610.43± 15.56)pg/mL,均显著高于对照组的(104.45± 12.26)pg/mL,(367.76± 12.23)pg/mL,(540.44± 14.54)pg/mL,差异均具有统计学意义(P<0.05)。

2.2 肺癌患者血清中VEGF、TIMP-1、MMP-9水平与病理特征的关系

肺癌患者血清VEGF、TIMP-1、MMP-9表达水平与肿瘤体积、TNM分期、有无淋巴结转移、是否远处转移有关,差异均具有统计学意义(P<0.05),与性别、肺癌病理类型和病理分化程度均无关,差异均无统计学意义(P>0.05)。见表1。

表1 肺癌患者血清VEGF、TIMP-1、MMP-9水平与病理特征的关系

Table 1 The relationship of serum VEGF, MMP-9, TIMP-1 and Pathological Features in patients with lung cancer

Pathological features		n	VEGF(pg/mL)	TIMP-1(pg/mL)	MMP-9(pg/mL)
Gender	Male	62	238.17± 16.46	588.33± 8.24	586.35± 26.32
	Female	29	231.87± 14.33	602.43± 13.18	593.18± 21.23
Pathology types	Small cell carcinoma	41	233.74± 12.53	595.38± 24.52	598.45± 23.87
	Non small cell carcinoma	50	228.67± 10.77	602.53± 23.86	598.89± 25.22
Degree of differentiation	Low	39	217.53± 6.84	606.83± 18.95	605.13± 22.34
	Middle	42	221.17± 12.34	585.73± 18.24	589.86± 22.68
	High	10	225.15± 10.34	593.52± 20.67	598.26± 22.71
Tumor volume (cm)	≤ 3	19	207.47± 11.87*	577.33± 14.84*	568.99± 15.65*
	>3	72	213.54± 6.88	612.92± 22.67	604.73± 22.18
TNM stage	Ⅰ+Ⅱ	58	201.74± 10.56*	572.13± 11.83*	566.34± 16.53*
	Ⅲ+Ⅳ	33	243.78± 9.67	607.34± 16.55	595.72± 15.64
Lymph node metastasis	Yes	55	245.44± 12.36*	637.84± 16.23*	596.41± 18.74*
	No	36	205.33± 16.73	547.38± 14.52	562.15± 15.76
Distant metastasis	Yes	34	241.43± 10.52*	603.52± 15.93*	614.78± 12.47*
	No	57	204.57± 13.38	576.21± 14.36	589.45± 13.98

Note: Compared with the data in the same group, *P<0.05.

2.3 肺癌患者血清VEGF、TIMP-1、MMP-9水平的相关性分析

经spearman相关性检验,肺癌患者血清MMP-9与TIMP-1正相关(r=0.337,P<0.05)、MMP-9与VEGF正相关(r=0.312,P<0.05)、TIMP-1与VEGF正相关(r=0.316,P<0.05)。

3 讨论

肺癌是我国的第一大癌症,其发病率在全球范围内仍呈持续上升的趋势,发病率及死亡率居恶性肿瘤之首[7],肺癌的侵袭与转移是影响患者预后的主要原因,导致肺癌侵袭转移的机制目前尚未阐明。MMP-9 属于基质金属蛋白酶家族,具有降解和重塑细胞外基质的功能。Wang JL[8]研究发现,MMP-9 在非小细胞肺癌患者组织中呈高表达,证实 MMP-9 与肺癌的发病有关。本研究发现无论小细胞或非小细胞肺癌患者的血清 MMP-9 水平均显著高于正常人群,进一步证实了血清 MMP-9 水平异常与肺癌的发生有关。此外本研究中肿瘤 >3cm、TNM Ⅲ+Ⅳ期、淋巴结转移及远处转移的肺癌患者血清 MMP-9 水平均较高,说明血清 MMP-9 与肺癌的侵袭转移有关,与相关研究结果一致[9,10]。TIMP-1 属于组织基质金属蛋白酶抑制家族,主要具有抑制 MMP-9 表达及促细胞增殖和抗凋亡功能。Wang XY[11]研究发现,TIMP-1 与肺癌患者的癌细胞侵袭转移相关。通过研究我们发现 TIMP-1 在肺癌患者血清中的表达显著高于正常人群,且具有统计学意义,且肿瘤 >3 cm、TNM Ⅲ+Ⅳ期、淋巴结转移及远处转移的肺癌患者血清 TIMP-1 水平均较高,说明血清 TIMP-1 与肺癌发生、迁移粘附密切相关,与相关研究结果一致[12,13]。VEGF 作为促血管内皮细胞生成的特异性生长因子,对癌症患者新生血管形成及肿瘤生长和转移起重要作用[14]。本研究发现血清 VEGF 水平在肺癌人群及肺癌肿瘤 >3 cm、TNM Ⅲ+Ⅳ期、淋巴结转移及远处转移的肺癌患者中异常升高,说明血清 VEGF 与肺癌肿瘤新血管形成及肿瘤生长和转移密切相关,与 Horinouchi H 和 Li D 研究结果一致[15,16]。

另外本研究发现肺癌患者血清 VEGF、MMP-9 和 TIMP-1 三者间相互正相关,说明 VEGF、MMP-9 与 TIMP-1 相互作用、协同参与肺癌的新血管形成、发生发展及侵袭转移过程。MMP-9、TIMP-1 和 VEGF 与细胞外基质的降解、细胞间粘附性和肿瘤血管的形成的改变密切相关 [17],MMP-9 作为促血管形成因子不但能够刺激肿瘤新血管的形成,还能增强内皮细胞的迁移能力,对于肿瘤的发生发展具有促进作用;VEGF 除可增强血管的渗透性、诱导血管发生和血管生成及内皮细胞生长、促进细胞迁移、抑制细胞凋亡,还能够激活 MMP-9,促进细胞外基质的降解;TIMP-1 是 MMP-9 的特异性抑制剂,TIMP-1 与 MMP-9 参与肺癌细胞侵袭转移可能与 TIMP-1 和 MMP-9 在组织中表达平衡紊乱及失调相关,打破细胞外基质产生于降解的平衡,而 TIMP-1 具有抑制 MMP-9 活性及促进细胞分裂、增殖生长、调控细胞凋亡及新生血管生成作用[18,19]。有文献报道,在肿瘤新生血管形成初期 VEGF 可诱导血管内皮细胞产生组织因子和基质金属蛋白酶,在它们的共同作用下使凝血酶原转化形成凝血酶,以此来激活明胶酶降解基底膜[20],从而来保证癌细胞的侵袭与转移。这个研究结果提示我们,VEGF 对于早期癌细胞转移具有重要作用。

综上所述,肺癌的发展是众多因素相互作用的结果,血清 VEGF、MMP-9 和 TIMP-1 水平在肺癌患者中均有不同程度的增高,这三者均可单独作为肺癌患者预后的生物学指标,而三者联合检测对于更好的判断肺癌的发生以及预后情况有积极的作用,对于提高肺癌的总体治疗水平有所帮助。

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