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肝细胞生长因子和基质金属蛋白酶-9在过敏性紫癜中表达的意义*

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摘要 目的:探讨肝细胞生长因子(HGF)和基质金属蛋白酶-9(MMP-9)在儿童过敏性紫癜(HSP)中表达的意义,探寻肾脏损害预测的敏感指标。**方法:**选取 HSP 与过敏性紫癜肾炎(HSPN)患儿各 30 例,并选取同期体检的健康儿童 30 名作为对照组,采用酶联免疫吸附试验法(ELISA)检测 3 组血清和尿液中的 HGF 及血清 MMP-9 含量,并采用比浊法检测 24 h 尿蛋白,分析 HGF、MMP-9 与 24 h 尿蛋白的相关性。**结果:**HSP 组、HSPN 组血清、尿液 HGF,血清 MMP-9 及尿蛋白定量均高于对照组($P<0.05$);HSPN 组中 II~IV 期患者血清 HGF 及 I~IV 期尿液 HGF、III~IV 期 MMP-9 及 I~IV 期尿蛋白定量均高于 HSP 组($P<0.05$);血清及尿液 HGF 与血清 MMP-9 无明显相关性($r=0.014, 0.027, P>0.05$);血清 HGF 与尿蛋白定量无明显相关性($r=0.032, P>0.05$);尿液 HGF、血清 MMP-9 与尿蛋白定量均呈正相关($r=0.412, 0.302, P<0.05$)。**结论:**尿液 HGF、MMP-9 均参与 HSP、HSPN 病情的发展,尿液 HGF、MMP-9 水平的监测有助于评估 HSPN 的病情及预后,但 HGF 与 MMP-9 之间无明显相关性。

关键词:过敏性紫癜 / 过敏性紫癜肾炎;肝细胞生长因子;基质金属蛋白酶-9;尿蛋白定量

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Expressions of Hepatocyte Growth Factor and Matrix Metalloproteinases -9 in Henoch-Schonlein Purpura*

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ABSTRACT Objective: To investigate expressions of hepatocyte growth factor (HGF) and matrix metalloproteinases-9 (MMP-9) in henoch-schonlein purpura (HSP), and to explore the sensitive indicators of nephritis. **Methods:** 30 children with henoch schonlein purpura nephritis (HSPN) were selected as the (HSPN group, and another 30 healthy children were selected as the control group. The enzyme-linked immunosorbent assay (ELISA) was applied to detect the HGF levels in serum and urine and serum contents of MMP-9, and turbidimetric method was used to detect the 24 h urine protein. Analyze the correlation of MMP-9 and HGF with 24 h urine protein. **Results:** The levels of urine and serum HGF, serum MMP-9 and urinary protein were higher in HSP and HSPN group than in control group ($P<0.05$). The levels of serum HGF of II ~ IV stage patients, the urine levels of HGF of I ~ IV stage patients, the serum levels of MMP-9 of III~ IV stage patients and the urinary protein levels of I ~ IV stage patients were higher in HSPN group than in HSP group ($P<0.05$). Serum and urine HGF levels had no significant correlation with MMP-9 serum levels ($r=0.014, 0.027, P>0.05$). Serum HGF had no significant correlation with urine protein quantitative ($r=0.032, P>0.032$). HGF urine level and MMP-9 serum level both had a positive correlation with urine protein quantitative ($r=0.412, 0.302, P<0.05$). **Conclusion:** The urine HGF and MMP-9 participates in the development of HSP and HSPN. The monitoring on the levels of urine HGF and MMP-9 may help to assess the state and prognosis of HSPN. But there was no significant correlation between HGF and MMP-9.

Key words: Henoch-schonlein purpura/Henoch-schonlein purpura nephritis; Hepatocyte growth factor; Matrix metalloproteinases -9; Urine protein quantitative

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前言

过敏性紫癜(Henoch-schonlein purpura, HSP)是儿童继发性肾脏损害的常见疾病,常因弥漫性小血管炎和免疫球蛋白复合

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物沉积而导致肾小球改变而发展成为过敏性紫癜肾炎(Henoch-schonlein purpura nephritis, HSPN)^[1]。肾间质纤维化是HSPN的主要病理特征。近年研究显示,肝细胞生长因子(Hepatocyte growth factor, HGF)参与肾间质纤维化的进程^[2]。而基质金属蛋白酶-9(Matrix metalloproteinases-9, MMP-9)的过度表达可加强细胞外基质降解而加快肾小球硬化^[3]。但临床对于HGF与MMP-9及与HSP、HSPN的相关性尚无报道。本研究通过检测HSP与HSPN患儿血清及尿液HGF及MMP-9含量,探讨HGF与MMP-9是否可作为预测早期肾脏损害的敏感性指标的可能性。

1 资料与方法

1.1 临床资料

选取2011年7月至2012年12月在新疆医科大学第一附属医院就诊的HSP、HSPN患儿各30例。纳入标准:①符合《儿科学》中有关HSP、HSPN相关诊断标准^[4];②伴有骨关节疼痛、胃肠道疼痛、尿常规异常等症状;③HSP均为初次发病;④1个月前未使用免疫介导或激素类药物;⑤家属签署知情同意书。排除标准:①伴有严重心、肝、血液提供疾病者;②伴有糖尿病、肾病综合征、系统性红斑狼疮、川崎病者。同时选取同期在本院体检的健康儿童30名作为对照组,对照组无心、脑、肺、肝、肾、内分泌及生殖系统疾病。HSP组男19例,女11例,年龄2~13岁,平均(7.34±2.03)岁。HSPN组男17例,女13例,年龄3~14岁,平均(7.69±2.34)岁;病理分期:I期7例,II期13例,III期6例,IV期4例。对照组男17例,女13例,年龄2~14岁,平均(7.15±1.96)岁。三组临床基本资料无差异具有可比性。

1.2 检测方法

抽取3组空腹静脉血3 mL各2份,1份进行3000 r/min离心20 min,取上层血清,置于-80℃冰箱中保存,用于检测血清HGF;1份静置30 min,待其凝血后进行3000 r/min离心5 min,取上层血清,置于-80℃冰箱中保存,用于检测血清MMP-9。同时留取晨尿10 mL各2份,1份进行1500 r/min离心5 min,取上清液,置于-80℃冰箱中保存,用于检测尿液HGF。采用ELISA法血清、尿液HGF及血清MMP-9,试剂盒购自江苏晶美生物科技有限公司。1份尿液采用比浊法检测尿蛋白定量,采用酶促动力学法检测尿肌酐仪器均为Cobas MIRA plus全自动生化分析仪(瑞士生产)。为降低人为误差增加可比性,尿液HGF水平以其与尿肌酐的比值表示。

1.3 统计学方法

本研究计量资料用($\bar{x} \pm s$)表示,采用t检验;血清、尿液HGF与血清MMP-9及尿蛋白定量的相关性采用Spearman相关性检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 三组血清、尿液HGF水平比较

HSP组、HSPN组血清及尿液HGF均显著高于对照组,差异有统计学意义($P < 0.05$);HSPN组中I~II期患者血清HGF与HSP组比较差异无统计学意义($P > 0.05$),但III~IV期患者血清HGF高于HSP组,差异有统计学意义($P < 0.05$);HSPN组I~IV期尿液HGF均高于HSP组,差异有统计学意义($P < 0.05$)(表1)。

表1 三组血清、尿液HGF水平比较($\bar{x} \pm s$)

Table 1 Comparison of the serum and urine HGF levels between three groups($\bar{x} \pm s$)

Groups	By stages	Cases(n)	Serum HGF(ng/L)	Urine HGF/urine creatinine
Control group		30	251.14±83.21	0.74±0.32
HSP		30	365.38±79.35*	1.36±0.33 [#]
HSPN	I	7	388.46±85.36*	1.89±0.31 ^{#*}
	II	13	403.21±82.14*	2.64±0.42 ^{#*}
	III	6	564.12±101.24 ^{*a}	2.87±0.39 ^{#*}
	IV	4	586.53±96.53 ^{*a}	3.01±0.41 ^{#*}

Note: Compared with the serum HGF levels of control group, * $P < 0.05$; compared with the urine HGF levels of control group, [#] $P < 0.05$; comparison of serum HGF levels between HSPN and HSP group, ^a $P < 0.05$; comparison of urine HGF levels between HSPN and HSP group, * $P < 0.05$.

2.2 三组血清MMP-9水平、尿蛋白定量比较

HSP组、HSPN组血清MMP-9及尿蛋白定量均显著高于对照组,差异有统计学意义($P < 0.05$);HSPN组中I期、II期患者MMP-9与HSP组比较差异无统计学意义($P > 0.05$),但III~IV期患者MMP-9高于HSP组,差异有统计学意义($P < 0.05$);HSPN组I~IV期尿蛋白定量均高于HSP组,差异有统计学意义($P < 0.05$)(表2)。

2.3 血清、尿液与MMP-9及尿蛋白定量的相关性

Spearman相关性检验显示HSP、HSPN患者血清及尿液HGF与血清MMP-9无明显相关性($r = 0.014, 0.027, P > 0.05$);

HSP、HSPN患者血清HGF与尿蛋白定量无明显相关性($r = 0.032, P > 0.05$);尿液HGF与尿蛋白定量呈正相关($r = 0.412, P < 0.05$);MMP-9与尿蛋白定量呈正相关($r = 0.302, P < 0.05$)。

3 讨论

过敏性紫癜肾炎是多种细胞因子共同参与及促进的结果^[5]。HGF是一种强效肾脏营养因子。研究证实HGF在有害刺激后,对促小管的修复及再生起关键作用^[6];转化生长因子-β(TGF-β)的过度表达与肾脏间质纤维化密切相关,而HGF的表达降低直接导致了TGF-β的升高^[7,8]。研究证实过敏性紫癜

表 2 三组血清 MMP-9、尿蛋白定量比较 ($\bar{x} \pm s$)Table 2 Comparison of serum MMP - 9 and urine protein quantitative between three groups($\bar{x} \pm s$)

Groups	by stages	Case number	Serum MMP-9(ng/mL)	Urine protein quantitative (g/24 h)
Control group		30	51.32± 83.21	0.08± 0.17
HSP		30	242.19± 69.22*	2.16± 0.36 [†]
HSPN	I	7	268.46± 75.22*	2.81± 0.30 [‡]
	II	13	294.21± 72.67*	3.33± 0.41 [‡]
	III	6	465.13± 89.14* ^Δ	3.59± 0.36 [‡]
	IV	4	516.33± 91.06* ^Δ	4.01± 0.52 [‡]

Note: Compared with the serum MMP-9 levels of control group, *P<0.05; compared with the urine protein quantitative of control group, [†]P<0.05; comparison of serum MMP-9 levels between HSPN and HSP group, ^Δ P<0.05; comparison of urine protein quantitative between HSPN and HSP group, [‡] P<0.05.

及过敏性紫癜肾炎患者急性期血清 HGF 水平明显升高^[9,10]。本研究中,HSP 组、HSPN 组血清及尿液 HGF 均显著高于对照组,差异有统计学意义(P<0.05),与文献报道基本一致。过敏性紫癜及过敏性紫癜肾炎患者急性期 HGF 水平升高反映了内皮细胞的损伤和功能障碍。Jacobi 等^[11]研究报道,HGF 参与了过敏性紫癜肾炎的发生、发展过程,在病理分期为 I~II 期时,随着肾脏损害的加重,HGF 的浓度呈上升趋势,而在 III~IV 期时 HGF 则呈下降趋势。本研究中 HSPN 组中 I~II 期患者血清 HGF 与 HSP 组比较差异无统计学意义(P>0.05),但 III~IV 期患者血清 HGF 高于 HSP 组,差异有统计学意义(P<0.05),与 Jacobi 等^[11]研究不符。笔者认为,过敏性紫癜肾炎 I~II 期患者肾损害与过敏性紫癜患者无明显差异,血清中的 HGF 未发生较大变化,而到了 III~IV 期,随着肾损害的加重,肾脏受累,机体分泌 HGF 增多,以期实现机体自我保护作用。当肾脏受到损伤时,可诱导肾组织局部细胞因子的增加,这些细胞因子经尿液排出,因此尿液可反映肾组织局部细胞因子的状况及肾脏病变程度。HGF 分子量较大,在正常情况下不能经过肾小球滤过,70%以上的 HGF 需经肝脏代谢。而当肾脏受损时,HGF 可经肾小球滤过^[12]。因此在肾脏受损情况下,尿液 HGF 的变化较血清 HGF 变化敏感。本研究中,HSPN 组 I~IV 期尿液 HGF 均高于 HSP 组,差异有统计学意义(P<0.05)。Spearman 相关性分析显示,HSP、HSPN 患者血清 HGF 与尿蛋白定量无明显相关性(r=0.032,P>0.05);尿液 HGF 与尿蛋白定量呈正相关(r=0.412,P<0.05)。由此提示,尿液 HGF 水平比血清 HGF 更能反映肾脏损害程度。

过敏性紫癜肾炎的主要病理特征是细胞外基质在肾小球基底膜和系膜区沉积导致系膜膨胀和肾小球基底膜增厚。大量研究证实,MMP-9 可促进细胞外间质降解而参与动脉粥样硬化斑块的形成、破裂,川崎病及巨细胞动脉炎等多种血管炎性疾病的发病机制^[13,14]。研究发现小儿过敏性紫癜血清中 MMP-9 浓度显著高于健康儿童(P<0.05)^[15];过敏性紫癜与过敏性紫癜肾炎患儿血清、尿液中 MMP-9 的水平显著高于健康儿童(P<0.05)^[16]。本研究中,HSP 组、HSPN 组血清 MMP-9 显著高于对照组,差异有统计学意义(P<0.05),HSPN 组中 I 期、II 期患者 MMP-9 与 HSP 组比较差异无统计学意义(P>0.05),但 III~IV 期患者 MMP-9 高于 HSP 组,差异有统计学意义(P<0.05)。

Spearman 相关性分析显示,MMP-9 与尿蛋白定量呈正相关(r=0.302,P<0.05)。由此提示,MMP-9 与过敏性紫癜及过敏性紫癜肾炎的发生、发展密切相关。我们认为,MMP-9 作为细胞外基质降解的主要成分,其表达增加必然会对血管内皮屏障产生直接破坏作用,进而使肾脏发生损害。而血管内皮屏障的破坏可激活并释放多种细胞因子及炎性介质,进一步诱导血管内皮合成更多的 MMP-9。

综上所述,HGF 与 MMP-9 均参与过敏性紫癜与过敏性紫癜肾炎的发生及发展,二者之间是否具有相关性呢? Danilewicz 等^[17]研究发现,HGF 可显著增加诸如 MMP-9 等胶原酶的表达、降低 TIMP-1 和 TIMP-2 的表达而促进基质降解。Libetta 等^[18]研究则发现,HGF 可促进 MMP-9 的表达,而加强细胞外基质的降解而促进肾纤维化。我国学者对于过敏性紫癜、过敏性紫癜肾炎患儿 HGF 与 MMP-9 之间的关系尚无研究报道。本研究中,Spearman 相关性检验显示 HSP、HSPN 患者血清及尿液 HGF 与血清 MMP-9 无明显相关性(r=0.014,0.027,P>0.05),与 Danilewicz^[19]及 Libetta 等^[20]报道不符,其原因有待进一步研究,也许 HGF 与 MMP-9 通过不同的途径参与肾损害进程。

综上所述,尿液 HGF、MMP-9 均参与 HSP、HSPN 病情的发展,尿液 HGF、MMP-9 水平的监测有助于评估 HSPN 的病情及预后,但 HGF 与 MMP-9 之间无明显相关性。

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