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膝关节骨性关节炎患者血清和滑膜液 CC 趋化因子配体 18 浓度及与疾病严重程度的关系

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[摘　要]　**目的：**探究膝关节骨关节炎患者血清和滑膜液中CC趋化因子配体18(CC-chemokine ligand 18，CCL18)浓度及与疾病严重程度的关系。**方法：**纳入100例膝关节炎患者和70例健康对照者。采用Kellgren-Lawrence(KL)分级评估膝关节炎的严重程度；采用ELISA方法检测研究对象血清和滑膜液中CCL18浓度；用Spearman相关分析分析血清与滑膜液CCL18浓度间的相关性，血清、滑膜液CCL18 浓度与KL分级间的相关性。**结果：**膝关节炎患者血清CCL18浓度显著高于健康对照者[(54.13±8.9) ng/mL vs (38.97±6.9) ng/mL，P<0.001]，KL-4级患者血清CCL18浓度显著高于KL-3级患者[(72.26±12.90) ng/mL vs (49.56±10.3) ng/mL，P<0.05]，KL-3级患者血清CCL18浓度显著高于KL-2级患者[(49.56±10.3) ng/mL vs (45.85±8.9) ng/mL，P<0.05]。KL-4级患者滑膜液CCL18 浓度显著高于KL-3级患者[(45.43±11.09) ng/mL vs (28.49±7.98) ng/mL，P<0.05]；KL-3级患者滑膜液CCL18浓度显著高于KL-2级患者[(28.49±7.98) ng/mL vs (27.00±5.43) ng/mL，P<0.05]。血清CCL18 与滑膜液CCL18浓度显著正相关(r=0.425，P<0.001)，血清和滑膜液CCL18与KL分级显著正相关(r=0.560，P<0.001；r=0.525，P<0.001)。**结论：**血清和滑膜液中CCL18浓度与膝关节炎严重程度相关。

[关键词]　CC趋化因子配体18；骨关节炎；滑膜液；严重程度

Relationship between the concentration of CC-chemokine ligand 18 in serum and synovial fluid and the severity of knee joint in patients with osteoarthritis

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Abstract　**Objective:** To determine the relationship between the concentration of CC-chemokine ligand 18 (CCL18) in serum and synovial fluid and the severity of knee joint in patients with osteoarthritis. **Methods:** This study included 100 patients with knee joint osteoarthritis and 70 healthy controls. Disease severity was assessed using the Kellgren-Lawrence grading system and ELISA was used to assess CCL18 concentration in serum and

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synovial fluid of the study subjects. Spearman correlation analysis was used to analyze the correlation between serum and synovial fluid CCL18 concentration and correlation between serum and synovial fluid CCL18 and KL classification. **Results:** The serum concentration of CCL18 in patients with knee osteoarthritis was significantly higher than those in healthy controls [(54.13±8.9) ng/mL vs (38.97±6.9) ng/mL, *P*<0.001]. The serum concentration of CCL18 in patients with KL-4 was significantly higher than those in KL-3 [(72.26±12.90) ng/mL vs (49.56±10.3) ng/mL, *P*<0.05]. Patients with grade KL-3 were significantly higher than those with KL-2 grade [(49.56±10.3) ng/mL vs (45.85±8.9) ng/mL, *P*<0.05]. Patients with KL-4 grade synovium Liquid CCL18 concentrations were significantly higher than patients with KL-3 [(45.43±11.09) ng/mL vs (28.49±7.98) ng/mL, *P*<0.05]. Patients with KL-3 were significantly more than those with KL-2 [(28.49±7.98) ng/mL vs (27.00±5.43) ng/mL, *P*<0.05]. Serum CCL18 levels were positively correlated with synovial fluid CCL-18 levels (*r*=0.425, *P*<0.001). Serum and synovial fluid CCL18 levels were positively correlated with KL grades (*r*=0.560, *P*<0.001, and *r*=0.525, *P*<0.001). **Conclusion:** The concentration of CCL18 in serum and synovial fluid is related to the severity of knee osteoarthritis.

Keywords CC-chemokine ligand 18; osteoarthritis; synovial fluid; severity

膝关节骨性关节炎(osteoarthritis，OA)是常见的关节疾病，涉及关节软骨破坏、骨性生长和滑膜的异常[1]。膝关节OA的发病机制尚不清楚。长期慢性炎症和损伤在膝关节OA发生和进展中发挥重要作用[2]。有研究[3]表明白细胞介素-6、肿瘤坏死因子-α、C反应蛋白等一系列炎症标志物均与膝关节OA的严重程度相关。趋化因子是炎症反应过程中白细胞活化后分泌的可溶性蛋白质[4]，CC趋化因子配体18(CC-chemokine ligand 18，CCL18)是趋化因子最大的亚组之一[5]，也称为肺和激活调节趋化因子，其在单核细胞/巨噬细胞和树突细胞中高表达，诱导T和B淋巴细胞进入炎症部位[6]。膝关节OA患者关节软骨和滑膜组织中趋化因子表达量上升[7]。尽管如此，CCL18在膝关节OA患者血清和滑膜液中的浓度及与严重程度的关系尚无报道。本研究旨在调查和比较膝关节OA患者和健康对照者之间CCL18浓度的差异，并探讨其与膝关节OA疾病严重程度的相关性。

1 对象与方法

1.1 对象

本研究纳入100例膝关节骨关节炎患者，为膝关节炎组，其中男45例，女55例，年龄(61.68±8.17)岁。纳入标准：1)符合美国风湿病学会[8]对膝关节OA诊断标准的患者；2)同意参与研究者。排除标准：1)炎性关节炎患者；2)膝关节损伤患者；3)无菌性骨坏死患者。70例年龄和性别相匹配且无膝关节骨关节炎或其他关节疾病的健康志愿者作为对照组，其中男28例，女42例，年龄(62.18±7.35)岁。所有研究对象签署知情同意书，本研究通过武汉中心医院医学伦理委员会批准。

1.2 膝关节OA患者X线摄影和评估[8]

研究对象处于站立位，完全展开膝盖下行膝关节X线摄影，X射线束以关节水平为中心。Kellgren-Lawrence(KL)分级系统评估放射学严重性。膝关节OA定义为至少1个膝关节KL分级≥2。同一患者取膝关节KL分级最大者用于研究。对照组膝关节X线KL分级为0。

1.3 血清和滑膜液CCL18 浓度检测

研究对象禁食过夜采集其早上空腹上肢静脉血5 mL，1 500 r/min离心30 min获得血清，在−80 ℃冰箱保存。OA患者在第一次透明质酸治疗之前，采用无菌技术从膝关节中获得滑膜液，样本以1 000 r/min离心10 min去除细胞和杂质，获得上清液，并在−80 ℃保存。采用酶联免疫吸附试剂盒(美国R&D Systems Inc.)检测CCL18的血清和滑膜液水平，最低检测浓度为0.01 ng/mL，组间和组内的变异系数为3%和4.5%。

1.4 统计学处理

采用SPSS 19.0软件进行数据分析。结果以均数±标准差($\bar{x}\pm s$)表示。两组间比较采用非配对t检验，三组之间比较采用单向方差分析。Spearman相关分析用于检测血清、滑膜液CCL18 浓度与KL分级之间相关性。P<0.05为差异有统计学意义。

2 结果

2.1 膝关节炎组与对照组血清和滑膜液CCL18浓度比较

两组年龄和性别差异无统计学意义(P>0.05)，膝关节炎组血清CCL18浓度显著高于对照组(P<0.001)，膝关节炎组患者滑膜液中CCL18浓度为(31.24±10.1) ng/mL(表1)。

2.2 不同KL分级的膝关节OA患者血清和滑膜液CCL18浓度比较

血清和滑膜液中CCL18浓度在不同KL分级患者中差异有统计学意义(均P<0.001)，KL-4级患者血清CCL18 浓度显著高于KL-3级患者(P<0.05)，KL-3级患者血清CCL18 浓度显著高于KL-2级患者(P<0.05)。KL-4级患者滑膜液CCL18浓度显著高于KL-3级患者(P<0.05)；KL-3级患者滑膜液CCL18浓度显著高于KL-2 级患者(P<0.05，表2)。

2.3 血清和滑膜液CLL18浓度与KL-分级相关性

Spearman相关分析显示：血清CCL-18浓度与KL分级(r=0.560，P<0.001)及滑膜液CCL-18浓度与KL分级均显著正相关(r=0.525，P<0.001，图1)。

表1 膝关节OA患者与健康对照者临床特征和实验室结果比较

Table 1 Characteristics between patients with knee osteoarthritis and healthy controls

组别	n	年龄 / 岁	性别 (男 / 女)	体重指数 /(kg·cm^{-2})	血清 CCL18/(ng·mL^{-1})	滑膜液 CCL18/(ng·mL^{-1})
膝关节炎组	100	61.68 ± 8.17	45/55	28.33 ± 5.95	54.13 ± 8.9	31.24 ± 10.1
对照组	70	62.18 ± 7.35	28/42	27.42 ± 4.86	38.97 ± 6.9	—
t		3.245	4.212	5.567	9.321	—
P		0.522	0.603	0.550	<0.001	—

表2 不同KL级别膝关节炎患者血清和滑膜液CCL18浓度比较

Table 2 CCL18 levels of serum and synovium liquid in knee OA patients with different KL grades

组别	n	血清 CCL18/(ng·mL^{-1})	滑膜液 CCL18/(ng·mL^{-1})
KL-2 级	30	45.85 ± 8.9	27.00 ± 5.43$^{\#}$
KL-3 级	45	49.56 ± 10.3*	28.49 ± 7.98*
KL-4 级	25	72.26 ± 12.90*$^{\#}$	45.43 ± 11.09*$^{\#}$
F		12.234	17.345
P		<0.001	<0.001

与KL-2级比较，*P<0.01；与 KL-3级比较，$^{\#}P$<0.01。

Compare with KL-2, *P<0.01; compare with KL-3, $^{\#}P$<0.01.

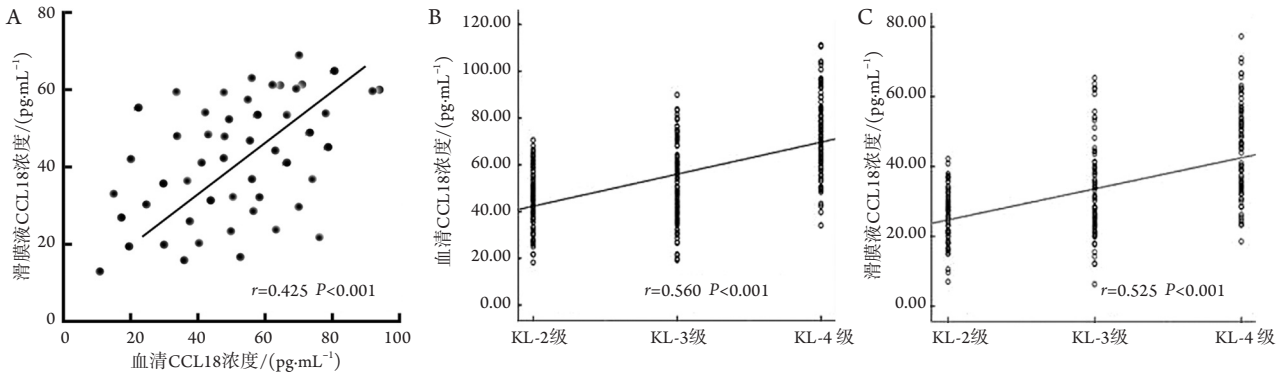

图1 血清和滑膜液CLL18浓度与KL-分级相关性分析

Figure 1 Correlation between serum and synovial fluid CCL18 and KL classification

3 讨论

膝关节OA发病率高，给患者日常生活带来巨大影响并造成沉重的医疗负担[1-2]。OA的严重程度主要通过磁共振成像和直接关节镜检查来评估，但是评估方法成本高、创伤性、缺乏客观标准[7-8]。生物标志物可用于不同疾病的诊断和评估疾病严重程度[9-10]。

膝关节OA的发生和进展与患者长期慢性炎症损伤和修复相关，CCL18是最重要的趋化因子之一，研究[11]报道巨噬细胞炎症蛋白-1α、干扰素-c-诱导蛋白-10和基质细胞衍生因子-1在滑膜液中浓度均与OA严重程度有关。本研究发现血清CCL18水平显著高于对照组，且血清和滑膜液中CCL18浓度均随着膝关节OA患者疾病严重程度增加而增加，提示CCL18浓度在评估OA的严重程度中可能发挥重要作用。有研究[7,12]报道：OA患者软骨样本中CCL18 mRNA的表达水平显著增加，血清CCL18浓度显著增加。本研究与上述结果一致。

本研究发现血清和滑膜液CCL18随着KL分级增加而增加，KL分级是常规评估OA患者严重程度的放射学标准[8]，提示CCL18是膝关节OA的发病机制中重要参与者。CCL18 参与膝关节炎的发病机制尚不清楚，可能与软骨基质的重塑有关。基质金属蛋白酶是软骨基质重塑过程中的关键因子，其产生与OA过程中的软骨破坏相关[13]。Takayasu等[13]报道用CCL18刺激成纤维细胞样滑膜细胞可显著促进基质金属蛋白酶-3的表达。此外，CCL18在小鼠骨关节中的过表达增强了基质金属蛋白酶-2和基质金属蛋白酶-9的释放[13]。上述结果显示CCL18刺激的不同金属蛋白酶的过量产生对OA软骨产生有害作用，导致其降解和损害。

体外研究[14]显示CCL18促进OA患者滑膜组织中培养的成纤维细胞样滑膜细胞中白细胞介素6的表达。CCL18可能通过促进炎性信号途径促成软骨组织破坏和恶化。炎性因子如肿瘤坏死因子-α、白细胞介素-4和白细胞介素-13可促进膝关节OA患者血液和关节液中多形核中性粒细胞或滑膜组织中CCL18的分泌[15]。本研究还发现血清与滑膜液中CCL18浓度与KL-分级呈正相关，提示血清与滑膜液中CCL18浓度与膝关节骨关节炎病情严重程度呈正相关。CCL18可促进炎症反应，促进软骨损伤和OA的进展，即KL分级越高，血清和滑膜液中CCL18显著增加。鉴于此，检测滑膜液和血清中CCL18浓度可能有助于临床医师对骨关节炎患者疾病严重程度进行评估。

本研究存在如下不足：本研究是个横断面研究，纳入的样本较少，需要加大样本进行前瞻性研究以进一步验证；其次，本研究并未评估滑膜组织中CCL18的免疫组织化学表达水平，值得进一步研究。

综上，本研究发现血清和滑膜液中CCL18水平与膝关节OA的放射学分级密切相关，CCL18介导的炎症反应可能参与OA的发病机制；血清和滑膜液中CCL18是评估OA严重程度潜在标志物；CCL18水平的检测具有一定的临床价值。

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