

血清脂联素水平与膝骨关节炎严重程度相关性研究

郑 双,徐建华,丁长海,黄淑婷,何 凡

摘要 目的 探讨膝骨关节炎(KOA)患者血清脂联素水平与KOA严重程度的相关性。方法 选取KOA患者179例,用骨关节炎指数(WOMAC)评分方法系统评估其关节症状严重程度;用Kellgren-Lawrence(KL)分级方法对KOA患者双膝关节放射学严重程度进行评估,选取较严重一侧膝关节纳入研究;使用酶联免疫吸附法(ELISA)测定患者血清脂联素水平。结果 ①男女血清脂联素水平差异无统计学意义($Z = -1.417, P = 0.157$)。②血清中脂联素水平与病程($r_s = -0.156, P < 0.05$)、KL分级($r_s = -0.235, P < 0.01$)及载脂蛋白A($r_s = -0.323, P = 0.001$)存在负相关性;与WOMAC总分、WOMAC疼痛、WOMAC僵硬、WOMAC功能障碍、体重指数、血糖等均无相关性($P > 0.05$)。③血清脂联素水平与X线KL分级呈负相关性($r_s = -0.235, P < 0.01$)血清脂联素水平在各KL分级中分布差异有统计学意义($\chi^2 = 10.656, P < 0.01$)。④以脂联素作为应变量进行多元线性回归分析显示:KL分级与脂联素及腰臀比之间差异有统

计学意义($\beta = -6259.967, P = 0.008; \beta = 79165.563, P = 0.047$)。结论 血清脂联素水平与KOA患者关节症状严重程度无关,与关节放射学严重程度相关,提示脂联素可能对关节软骨起到保护作用。

关键词 膝关节;骨关节炎;脂联素

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骨关节炎(osteoarthritis, OA)是一组异质性疾病,以关节软骨病变为主要特征,同时伴有肌肉、韧带、滑液的病变。OA的危险因素主要包括年龄、性别、肥胖、外伤、关节承重、遗传等,但具体发病机制尚不清楚。在这些危险因素中,肥胖是OA发病的一个重要的可调控因素。肥胖所导致关节破坏,不仅有生物力学因素的作用,而且通过各种脂肪组织分泌的脂肪细胞因子^[1]所导致的代谢性炎症在OA的发病中可能发挥重要的作用。有研究^[2-3]证明,许多细胞因子与膝骨关节炎(knee osteoarthritis, KOA)的疾病活动度相关,并在OA的发病中扮演着重要的角色。在许多情况下,脂联素可作为一种抗炎介质,但其与OA的关系还存在着争议。该研究主要探讨血清脂联素水平与KOA严重程度的相关性。

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作者单位: 安徽医科大学第一附属医院风湿免疫科,合肥 230022

作者简介: 郑 双,女,硕士研究生;

徐建华,女,教授,主任医师,博士生导师,责任作者, E-mail: xujianhua86@aliyun.com;

丁长海,男,教授,博士生导师,责任作者, E-mail: chang-hai.ding@utas.edu.au

lung adenocarcinoma and 37 benign pleural effusions were collected. Specimens were analyzed for the expression of claudin-18 using immunocytochemistry and Western blot. Furthermore, claudin-18 expression was compared with exfoliated cytology. The significance of claudin-18 expression in malignant effusion and its association with clinicopathological features and therapeutic effect were also analyzed. **Results** With the immunocytochemistry method, the sensitivity, specificity, accuracy were 38%, 100%, 64.4%, respectively. There was no statistically significant difference when claudin-18 expression was compared with exfoliated cytology (38% vs 54%). However, with the combination of both methods to detect malignant pleural effusion, the sensitivity increased to 68%. The expression rate of claudin-18 protein was 50.0% in lung adenocarcinoma of nonsmokers, significantly higher than that in lung adenocarcinoma of smokers (12.5%, $P = 0.011$), while no association was found between claudin-18 and sex, age, tumor size, nodal metastases, distant metastases or therapeutic effect. **Conclusion** Claudin-18 protein may be involved in the carcinogenesis of lung adenocarcinoma, especially in nonsmokers. The combining examination of claudin-18 expression and exfoliated cytology can enhance the positive diagnostic rate of malignant pleural effusion and has differential diagnosis value.

Key words lung neoplasms; malignant pleural effusion; Claudin-18; immunohistochemistry

1 材料与方法

1.1 研究对象 选择2012年1月~2013年11月就诊于安徽医科大学第一附属医院风湿免疫科并确诊的症状性OA患者179例,其中女154例,男25例,男女比例1:6.16,年龄34~74(55.53 ± 8.26)岁。入选标准:患者诊断符合:1986年美国风湿病学会(American College of Rheumatology, ACR)修订的KOA诊断标准^[4]。排除标准:①伴有类风湿关节炎及其他严重疾病,如:牛皮癣性关节炎、狼疮、活动性癌症、严重心血管和肾脏疾病等;②严重KOA近期拟进行关节置换手术的患者;③MRI的禁忌症如装有心脏起搏器、人工金属瓣膜和角膜、动脉瘤夹闭术后、动脉夹存留、眼球内金属异物、幽闭恐惧症等。本研究得到安徽医科大学第一附属医院伦理委员会的批准,患者均已签署书面知情同意书。

1.2 标本采集 抽取5 ml空腹静脉血置入抗凝管中,分离血清后置入-80℃冰箱中保存,待测脂联素浓度。同时收集患者的一般检验资料结果包括:空腹血糖(fasting blood-glucose, FBG)、总胆固醇(total cholesterol, TC)、三酰甘油(total glycerin, TG)、高密度脂蛋白(high density lipoprotein, HDL)、低密度脂蛋白(low density lipoprotein, LDL)、极低密度脂蛋白(very low density lipoprotein, VLDL)、载脂蛋白A(apolipoprotein A, ApoA)、载脂蛋白B(apolipoprotein B, ApoB)。采用酶联免疫吸附法(ELISA)检测血清脂联素水平。脂联素试剂盒购自美国eBio-science莱兹生物技术公司,所有步骤按照说明书操作。

1.3 调查内容 问卷内容包括3部分:①患者的一般情况,包括性别、年龄、身高、体重、腰围、臀围、职业、学历、病程等,计算体质指数(body mass index, BMI) = $\frac{\text{体重(kg)}}{\text{身高(m)}^2}$ 、腰臀比(waist-to-hip ratio, WHR) = $\frac{\text{腰围(cm)}}{\text{臀围(cm)}}$;②根据骨关节炎指数(the western ontario and mcmaster universities osteoarthritis index, WOMAC),对其膝关节的疼痛、僵硬、关节功能进行自我评估,评估其关节炎症状严重程度,分值越高代表疼痛、僵硬越明显或关节功能越差;③其他资料包括疾病史、吸烟史、生育史、日照情况、近1周服药情况等。

1.4 关节放射学严重程度评估 受试者均行负重站立位膝关节前后位、外侧位和轴位X线片检查。由两名经过专业培训的影像学医师,采用Kellgren-

Lawrence(KL)分级系统评估方法^[5]对膝关节的严重程度进行评估。根据KL分级系统对其严重程度进行评估,共分5级:0级,完全正常,无X线片改变;1级,可疑的关节腔狭窄(joint space narrowing, JSN)和可能的唇样性增生;2级(轻度),明确的骨赘(osteophyte, OP)形成及可能的JSN;3级(中度),中等OP及明确的JSN,骨质硬化及可能的骨形态改变;4级(重度)为较大的OP,明确的JSN,严重骨质硬化,骨畸形。KOA患者KL分级至少一侧膝关节 ≥ 2 级,可诊断为放射学KOA,以严重一侧膝关节纳入统计。

1.5 统计学处理 采用SPSS 13.0软件进行分析,数据以 $\bar{x} \pm s$ 表示,两组间比较采用 χ^2 检验;血清脂联素水平不符合正态分布,采用中位数(四分位间距)表示;组间比较采用Mann-Whitney U检验或Kruskal-Wallis检验;相关性分析采用Spearman相关性分析。多分类有序资料比较采用Ordinal Logistic回归。

2 结果

2.1 血清中脂联素水平 179例受试者脂联素水平为18.60(52.26) $\mu\text{g/ml}$,女性为20.45(47.34) $\mu\text{g/ml}$,男性为8.68(49.58) $\mu\text{g/ml}$,男女之间差异无统计学意义。

2.2 血清中脂联素水平与各临床指标的相关性 血清中脂联素水平与病程($r_s = -0.156, P < 0.05$)、ApoA($r_s = -0.323, P < 0.01$)呈负相关性;与WHR、臀围、腰围、身高、体重、TC、LDL、WOMAC总分、WOMAC功能障碍等均无相关性,见表1。

2.3 血清脂联素在各放射学级别中浓度的分布及相关性研究 血清脂联素水平与X线片KL分级呈负相关性($r_s = -0.235, P < 0.01$)。对脂联素浓度在各KL分级水平中分布使用Kruskal-Wallis检验进行组间比较,发现KL分级与脂联素水平差异有统计学意义($\chi^2 = 10.656, P < 0.01$),见表2。

2.4 血清脂联素水平的变化及其影响因素 分别以KL分级作为应变量,以年龄、性别、病程、WHR、BMI、WOMAC总分、脂联素水平作为自变量进行Ordinal Logistic回归分析。结果显示:KL分级均与年龄和病程有关,且随年龄、病程的增加,KL分级越来越重。KL分级与其他项目差异无统计学意义。见表3。以脂联素作为应变量,以年龄、性别、

表1 血清脂联素水平与各临床指标的相关性

项目	r_s 值	P 值
年龄	-0.042	0.579
病程	-0.156	0.037
腰围	0.039	0.607
臀围	-0.078	0.299
WHR	0.111	0.137
身高	-0.080	0.284
体重	-0.057	0.445
BMI	0.014	0.852
FBG	0.060	0.421
TG	-0.016	0.834
TC	0.082	0.276
HDL	-0.028	0.715
LDL	0.101	0.180
VLDL	0.029	0.698
ApoA	-0.323	0.001
ApoB	0.008	0.920
WOMAC 总分	0.055	0.464
WOMAC 疼痛	0.046	0.545
WOMAC 僵硬	-0.033	0.661
WOMAC 功能障碍	0.066	0.381

表2 KL 分级中脂联素浓度分布

KL 分级	n	脂联素浓度 ($\mu\text{g/ml}$) 中位数(四分位间距)	χ^2 值	P 值
1	50	6.03 (36.04)	10.656	0.005
2	75	26.27 (47.29)		
3~4	54	6.97 (29.40)		

病程、WHR、BMI、WOMAC 总分、KL 分级作为自变量进行回归分析。结果显示: KL 分级、WHR 与脂联素差异有统计学意义($\beta = -6\ 259.967$, $P = 0.008$; $\beta = 79\ 165.563$, $P = 0.047$), 见表4。其他项目与脂联素差异均无统计学意义。

表3 KL 分级影响因素的 Ordinal Logistic 回归分析

项目	SE	Wald	P 值	OR (95% CI)
年龄	0.021	15.580	0.001	1.086 (1.042 ~ 1.131)
病程	0.002	6.516	0.011	1.006 (1.001 ~ 1.011)

表4 脂联素影响因素的线性回归分析

项目	β 值	SE	t 值	P 值
KL	-6 259.967	2 346.392	-2.668	0.008
WHR	79 165.563	39 521.390	2.003	0.047

3 讨论

脂联素是脂肪组织分泌的一种内源性生物活性多肽化合物,脂联素在代谢性疾病^[5],如糖尿病、冠心病等中扮演着重要的角色。有研究^[6]表明脂联

素与冠心病之间的相关性,在调整过脂质(尤其是HDL)后则无相关性。研究^[7-8]显示血清脂联素的水平与HDL有显著相关性,而ApoA是HDL主要的载脂蛋白,本研究显示,血清脂联素水平与ApoA呈负相关,与文献^[9]报道一致。

关于脂联素在关节炎发病机制的抗炎作用还存在着争议。近期研究^[10]表明,脂联素可以诱导中性粒细胞和单核细胞在炎症关节内沉积,使关节软骨降解,对关节软骨存在破坏作用。然而,Chen et al^[11]研究认为脂联素能下调软骨细胞白细胞介素-1诱导的基质金属蛋白酶-13的表达,上调基质金属蛋白酶抑制剂-2的表达,提示脂联素作为抗炎介质对关节软骨可能有保护作用。

本研究显示,血清脂联素水平与X线片KL分级呈负相关性,即血清中脂联素水平越低,KOA的严重程度越高。研究^[12]表明,血清脂联素水平的降低可增KOA的严重程度,这与本研究结果一致。这些相关性可以解释,在KOA中脂联素作为抗炎介质起到保护作用。因此,低水平的脂联素可能是发生严重的KOA的危险因素之一。然而,Filkova et al^[13]研究发现,侵蚀性OA患者的脂联素水平高于非侵蚀性OA患者,可能是由于身体在对抗OA破坏的过程中增加了脂联素的产生。近期研究^[14]结果表明脂联素与KOA的严重程度无关。这些结果存在差异,可能是由疾病进程的差异、群体的差异、混杂变量不完全控制所造成的。

综上所述,血清脂联素水平与KOA放射学严重程度相关,提示脂联素可能对关节软骨起到保护作用。未来还需要更多队列研究来观察脂联素在KOA的进展和预后方面的作用。然而,不同亚型的脂联素在KOA的发病中起到不同的作用,需要更多前瞻性研究各亚型脂联素在OA发病中具体的作用。

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Correlation of plasma adiponectin with knee osteoarthritis severity

Zheng Shuang, Xu Jianhua, Ding Changhai, et al

(Dept of Rheumatology and Immunology, The First Affiliated Hospital of Anhui Medical University, Hefei 230022)

Abstract Objective To measure adiponectin concentrations in plasma of patients with knee osteoarthritis(KOA) and to investigate the correlation of adiponectin levels with disease severity. **Methods** 179 OA patients were enrolled in this study. The severity of symptoms was assessed with The Western Ontario and McMaster Universities osteoarthritis index(WOMAC) scoring system; the Kellgren-Lawrence(KL) criteria were adopted to evaluate X-ray changes observed in anteroposterior knee radiography; adiponectin levels in plasma and synovial fluid were determined by enzyme-linked immunosorbent assay(ELISA). **Results** ① There was no significant difference($Z = -1.417$, $P = 0.157$) between male and female of the serum adiponectin. ② Adiponectin concentrations in plasma showed significant inverse correlation with disease duration($r_s = -0.156$, $P < 0.05$), KL grade($r_s = -0.235$, $P < 0.01$) and apolipoprotein A($r_s = -0.323$, $P = 0.001$). There was no correlation between adiponectin and total WOMAC scores, pain scores, stiffness scores, dysfunction scores, body mass index and fasting blood-glucose($P > 0.05$). ③ The differences of serum adiponectin level in the KL grades distribution had statistical significance($\chi^2 = 10.656$, $P < 0.01$). ④ Additionally, linear regression analysis showed that adiponectin and KL classification and WHR had statistical significance($\beta = -6\,259.967$, $P = 0.008$; $\beta = 79\,165.563$, $P = 0.047$, respectively). **Conclusion** In this pilot study, we find that adiponectin serum concentrations are not associated with knee osteoarthritis symptom severity. However, adiponectin serum concentrations are associated with knee osteoarthritis radiographic severity. These findings suggest that adiponectin may protect the articular cartilage and play a protective role in OA.

Key words knee; osteoarthritis; adiponectin