

[1]刘晓飞,王志中,王勇,等.早期类风湿关节炎患者肠道微生物群落分析[J].第三军医大学学报,2012,34(23):2411-2415.

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## 早期类风湿关节炎患者肠道微生物群落分析(PDF)

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Title: Analysis of intestinal microbial community structure in patients with early rheumatoid arthritis

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关键词: 肠道菌群; DGGE; 早期类风湿关节炎; 多样性指数

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摘要: 目的 研究类风湿关节炎(rheumatoid arthritis, RA)患者肠道微生物群落分布特点,探讨类风湿关节炎与肠道菌群的关系。 方法 收集30例早期RA患者(RA组)与21例健康受试者(对照组)粪便样本,培养与测定肠道菌群计数水平;提取粪便样本基因组DNA,PCR扩增细菌V6-V8区16SrDNA,产物经变性梯度凝胶电泳分离,获得肠道菌群DGGE图谱,分析肠道细菌丰富度、Shannon-Wiener指数、均匀度指标,用非加权类平均法对图像进行相似性聚类分析,并绘出聚类树形图,研究RA患者肠道菌群结构与健康受试者的差异。 结果 双歧杆菌含量:RA组为(6.21±1.48)CFU/ml,显著低于对照组(8.55±0.90)CFU/ml( $P<0.01$ ),脆弱类杆菌的含量:RA组为(6.54±1.28)CFU/ml,显著低于对照组(8.73±0.90)CFU/ml( $P<0.01$ ),乳杆菌、梭菌、肠杆菌、肠球菌数量差异无统计学意义( $P>0.05$ )。肠道菌群DGGE图谱示:与对照组[丰富度:(22.8±3.12);Shannon-Wiener指数:(2.98±0.26);均匀度:(0.96±0.05)]相比,RA组丰富度[(16.0±1.05), $P<0.01$ ]、Shannon-Wiener指数[(2.48±0.14), $P<0.01$ ]和均匀度[(0.90±0.05), $P<0.01$ ]显著降低,分别表明RA患者肠道细菌的种类(丰富度)、菌群结构复杂程度、细菌的相对密度(均匀度)显著降低,在DGGE图谱下方区域RA患者肠道菌群条带数量减少,消失明显。图谱相似性聚类分析显示:20例样本聚为两类,一类

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样本中包含10例患者与1例健康人，二类样本中包含9例健康人。表明RA患者肠道菌群有较高的相似性，RA与健康人肠道菌群相似性低。 结论 早期RA患者肠道存在菌群失调，肠道细菌数量及结构与健康人有显著差异，其差异变化可能是类风湿关节炎的发病因素之一。

**Abstract:** **Objective** To analyze the features of intestinal microbial community structure in patients with rheumatoid arthritis (RA), and to investigate the relationship between RA and intestinal flora. **Methods** Fecal samples were collected from 30 patients with RA (RA group) and 21 healthy individuals (control group). The intestinal flora was cultured and the intestinal flora counts were determined. Bacterial DNA was extracted, and the amplicons of the V6-V8 variable regions of bacterial 16S rDNA were analyzed by denaturing gradient gel electrophoresis (DGGE). The richness, Shannon-Wiener index and evenness of the intestinal flora were analyzed after the DGGE profiles were obtained. A distance tree was constructed using clustering analysis with the unweighted pair-group method with arithmetic mean (UPGMA). The structures of the intestinal flora between the RA patients and healthy subjects were compared. **Results** The fecal microbiota of the RA group contained significantly less *Bacillus bifidus* and *Bacteroides fragilis* ( $6.21 \pm 1.48$  and  $6.54 \pm 1.28$  CFU/ml) than that of the control group did ( $8.55 \pm 0.90$  CFU/ml and  $8.73 \pm 0.90$  CFU/ml,  $P < 0.01$ ), while the counts of Lactobacillus, Clostridium, Enterobacter and Enterococci were not significantly altered ( $P > 0.05$ ). As compared with the control group (richness:  $22.8 \pm 3.12$ , Shannon-Wiener:  $2.98 \pm 0.26$  and evenness:  $0.96 \pm 0.05$ ), the richness ( $16.0 \pm 1.05$ ,  $P < 0.01$ ), Shannon-Wiener ( $2.48 \pm 0.14$ ,  $P < 0.01$ ) and evenness ( $0.90 \pm 0.05$ ,  $P < 0.01$ ) of the RA group significantly reduced, suggesting the number of bacterial species (richness), the diversity of total bacteria and their relative abundance (evenness) in the RA patients decreased remarkably. Moreover, some bands were not detected in the DGGE profile of the RA group. In the cluster analysis of the DGGE profiles, two clusters were found in 20 samples. One cluster included 10 RA samples and 1 control samples, and another cluster contained 9 control samples. It represented high similarity in the same sample group, but low similarity between different groups. **Conclusion** Enteric dysbacteriosis is detected in patients with early RA, and remarkable differences are observed in intestinal microflora between RA patients and healthy individuals. The changes of intestinal flora may be related to the pathogenesis of RA.

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备注/Memo: -

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