不孕女性生殖道UU,CT,NG和MG感染状况分析及不同检测方法结果比较*

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摘 要:目的 分析不孕症女性生殖道常见微生物的分布状况,比较核酸恒温扩增检测技术(SAT)与培养法或乳胶法解脲支原体(UU)、沙眼衣原体(CT)、淋病奈瑟菌(NG)和生殖支原体(MG)检出的差异。**方法** 2016年6月~9月于南京医科大学附属苏州医院生殖中心就诊的467例女性不孕患者,年龄20~48(31.52±6.83)岁,分析UU,CT,NG和MG的感染分布状况。选择其中352例行辅助生殖技术的女性患者,年龄21~46(30.67±6.67)岁,取生殖道拭子标本,一份以培养法或乳胶法检测UU或CT。另一份用SAT法进行检测,根据实验结果评估两种方法检测相应微生物的敏感度和特异度差异。**结果** 467例不孕女性生殖道拭子检测结果中UU阳性率最高62.53%(292/467),CT阳性率1.93%(9/467),NG阳性率0.21%(1/467),MG阳性率1.71%(8/467)。与正常对照组[23.81%(25/105)]相比不孕女性组UU感染率更高($\chi^2=52.01$,$P<0.01$)。对352例行辅助生殖技术的不孕女性患者用不同检测方法检测UU,CT,UU拭子培养的阳性率为48.9%,UU-SAT检测阳性率为63.9%,SAT法阳性率高于培养法。拭子培养和SAT检测结果经配对四格表χ^2检验,差异具有统计学意义($\chi^2=41.93$,$P<0.01$),显示SAT法检测UU具有更高的敏感度。CT-SAT检测阳性率为1.71%,CT乳胶法检测阳性率为0.28%。乳胶法检测结果与SAT结果相比差异具有统计学意义(Fisher确切概率法统计分析,$P<0.05$),SAT阳性率和敏感度更高。**结论** 不孕女性生殖道常见病原体以UU最为多见,其次为CT和MG。SAT法与培养法或乳胶法检测UU和CT相比较,前者具有更高的敏感度。

关键词:支原体;衣原体;非淋菌性尿道炎;核酸恒温扩增检测法
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Analysis of UU, CT, NG and MG in Infertile Women and Comparison of Different Detection Methods

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Abstract: Objective To analyze the distribution of pathogens in the genital tract of infertile female, and comparing traditional methods with simultaneous amplification and testing (SAT) in the detection of UU, CT, NG and MG. **Methods** 467 female infertility patients were selected from the reproductive center of Suzhou Hospital Affiliated to Nanjing Medical University between June and September 2016 to analyze the distribution of UU, CT, MG and NG. The age was between 20 to 48 years old (mean 31.52±6.83 years old). 352 cases of female patients with assisted reproductive technology were selected, aged from 21 to 46 years old (mean 30.67±6.67 years old). The swabs were tested by traditional methods or SAT. The sensitivity and specificity of the methods in detecting the pathogens were evaluated according to the experimental results. **Results** Among the 467 infertile women, the number of UU positive cases was the highest, the positive rate was 62.53% (292/467), the positive rate of CT was 1.93% (9/467) and the positive rate of NG was 0.21% (1/467), and the positive rate of MG was 1.71% (8/467). UU infection rate was higher in infertile women than normal control group 23.81% (25/105) ($\chi^2=52.01$, $P<0.01$). 352 cases of female patients with assisted reproductive technology were selected for further analysis. For UU detection, the positive rate of swab samples detected by liquid culture was 48.9%, while the positive rate detected by SAT was 63.9%. Obviously the positive rate of SAT was higher than that of liquid culture. Swab culture and SAT results were analyzed by paired χ^2 test ($\chi^2=41.93$, $P<0.01$). The positive rate of CT-SAT was 1.71%, and the positive rate of CT-latex method was 0.28%. There was significant difference between CT latex method and SAT (Fisher exact probabilistic method statistical analysis, $P<0.005$), which indicated that SAT method had a higher sensitivity. The positive rate (1.7%) and sensitivity (100%) of SAT were also higher than that of traditional method. **Conclusion** UU was the most common

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pathogen in female reproductive tract pathogens, followed by CT and MG. The SAT method has higher sensitivity than the conventional method in detecting of UU and CT.

Keywords: mycoplasma; chlamydia trachomatis; non-gonococcal urethritis; simultaneous amplification and testing

生殖道感染对女性生育的影响一直受到大家的重视，但个别生殖道微生物对生育的影响还存在着争议[1]，如解脲支原体（ureaplasma urealyticum，UU）。随着分子生物学技术的飞速发展，检测方法也越来越精准。新技术的出现让我们能更快速和准确地检出各种生殖道病原体。流行病学研究表明，解脲支原体（UU）、沙眼衣原体（CT）、生殖支原体（MG）是非淋菌性尿道炎（NGU）最常见的病原体。其中UU的感染率为11%～26%，CT为11%～50%，MG为6%～50%[2]。UU是泌尿生殖道的常见微生物之一，因其可以分解尿素而得名[3]。目前，我国人群中UU的感染率日趋上升，由于反复感染以及抗生素滥用等原因，使之成为发病率较高的性传播疾病之一。UU感染后，患者多无明显症状，也易造成医生漏诊[4]。本文研究对象为不孕女性群体，旨在阐明该群体的生殖道微生物分布特征。随着分子生物学诊断技术的发展，生殖道病原体的检测经历由培养法、免疫法到核酸检测法多个阶段[5～7]。病原体分离培养法被认为是“金标准”方法[8]。然而分离培养操作复杂，所需时间较长和敏感度易受标本采集方式等影响，对临床常规检测和流行病学筛查带来了一定的困难。加之培养法取样采用拭子样本，该取样方式给病患带来一定的痛苦，尤其是男性患者，一些检测方法结果判定具有一定的主观性，容易引起假阳性和假阴性，给临床诊断带来一些困难。免疫学检测技术也得到了广泛的应用[9]，但由于质控困难，并且只能定性不能定量，加之有交叉抗原的存在[10]，故其应用得到了很大的限制。DNA检测技术有效地弥补上述不足，敏感度较高，其缺点在于不能进行药敏试验以及无法区分死亡和活动的病原体，故对于在治愈初期的病患，检测结果也可能是阳性[2]。故临床上急需建立一种灵敏、可靠的检测方法，以求能够准确检测出病原体。本研究运用核酸恒温扩增技术（Simultaneous amplification and testing，SAT）检测女性阴道分泌物中的UU，CT，MG和NG，并将其与培养法及乳胶法进行比较分析，现将结果报告如下。

1 材料与方法

1.1 研究对象 2016年6～9月于本院生殖中心门诊就诊的不孕女性患者467例，年龄20～48岁，平均年龄31.52±6.83岁。其中352例行辅助生殖技术（assisted reproductive technology，ART）治疗。对照组选取105例已生育妇女体检拭子标本进行检测。采集标本之前1月内所有受试对象均未采取阴道灌洗、抗生素治疗等措施，并处于非月经期。本研究经医院伦理委员会允许，患者知情同意。

1.2 试剂与仪器 UU，CT，NG和MG核酸检测试剂盒（YZB/国3011-2014）；MagX全自动核酸提取仪（上海仁度生物科技有限公司）；定量PCR仪（西安天隆科技有限公司）；UU培养鉴定药敏试剂盒（珠海丽珠生物科技有限公司）；CT，NG乳胶免疫层析法试剂盒（南京黎明生物制品有限公司）。

1.3 标本采集 用无菌棉拭子伸入女性宫颈口1～2 cm，旋转几周并停留数秒后取出，行ART的患者同时取两份拭子标本，分别用不同的方法进行检测。

1.4 样本检测

1.4.1 培养法（用于UU检测）：将采集的标本插入培养液中，挤压旋转三次，将培养液置于37℃温箱中培养48 h后观察结果，若培养液变为红色并清亮透明，即为阳性。不变色即为阴性。若培养液变红并伴有浑浊，则将培养液接种于10 mg/dl血平皿中，按照临床检验操作规程对微生物进行分离培养和鉴定。

1.4.2 乳胶法（用于CT，NG检测）：将采集的标本插入生理盐水中，挤压旋转三次，即为待测样本。测试时，将待测样本滴入检测卡加样孔内，阳性标本在测试区（T）内会出现一条红色条带。阴性样本由于不含检测目的抗原，在测试区（T）内不能形成夹心复合物，故没有红色条带出现。

1.4.3 SAT法：拭子标本取出后放入1 ml生理盐水充分浸泡后贴壁挤干，取0.5 ml与等体积的样本保存液混合，即为待测样本。使用MagX全自动核酸提取仪进行实验。首先，病原体被裂解释放出核酸，与核酸提取液中的相应磁性颗粒特异度结合，利用磁珠吸附纯化UU，CT，NG和MG的靶标RNA。加入42℃预热的SAT酶液，于PCR仪上扩增40个循环，荧光标记的优化探针与RNA拷贝特异性结合，产生的荧光由仪器进行检测。结果判断：dt表示样本曲线与阈值线交点的循环数，dt≤35的标本判为阳性。

1.5 统计学分析 应用SPSS 17.0软件对实验结果进行统计学分析，计数资料采用χ^2检验，两方法对比使用配对四格表χ^2检验，当$T<1$时使用Fisher确切概率法分析，$P<0.05$为差异具有统计学意义。

2 结果

2.1 不孕女性生殖道UU,CT,NG和MG分布状况 467例不孕女性生殖道拭子UU,CT,NG和MG经SAT法检测结果如下。其中UU阳性例数最多,阳性率62.53%(292/467)。CT阳性9例,阳性率1.93%(9/467)。NG阳性1例,阳性率0.21%(1/467)。MG阳性8例,阳性率1.71%(8/467)。与正常生育女性组23.81%(25/105)对比,不孕女性组UU感染率明显增高,差异具有统计学意义($\chi^2=52.01$,$P<0.01$)。

2.2 UU培养法和SAT检测结果阳性率比较 选取其中352例受检女性进行UU拭子培养和SAT检测结果比较。SAT法检测UU阳性率为63.9%(225/352),敏感度95.9%,特异度66.7%。培养法检测阳性率为48.9%(172/352),敏感度73.3%,特异度94.5%,两样本经配对四格表χ^2检验差异具有统计学意义($\chi^2=41.93$,$P<0.01$)。其中60例样本拭子培养阴性而SAT法检测阳性,有7例样本培养法检测阳性SAT法检测阴性。

2.3 CT乳胶法和SAT检测结果阳性率比较 352例受检女性CT乳胶法检测结果。SAT法检测CT阳性例数为6例,阳性率1.7%(6/352),敏感度100%,特异度98.6%。乳胶法检测阳性1例,阳性率0.3%(1/352),敏感度16.7%,特异度100%,两样本经Fisher确切概率法统计分析,差异具有统计学意义($P<0.05$)。NG两种检测法均无阳性检出,MG仅用SAT法检测未作比较。

3 讨论 UU是泌尿生殖道常见的一种共生微生物,其感染与多种疾病相关,包括非淋菌性尿道炎、宫颈炎、慢性前列腺炎、附睾炎以及不孕不育等[11,12]。CT是一类胞内寄生的微生物,能够感染男、女性泌尿生殖道[13]。在女性中能引起盆腔炎、宫颈炎、宫内感染等多种疾病[14]。然而在CT感染者中,约70%~80%的女性常常无明显的临床症状[15],故可靠的实验室检测结果对疾病的诊断显得尤为重要。NG感染可导致一系列疾病,以宫颈炎和尿道炎最常见,并可进一步上行入侵引起睾丸炎、盆腔炎、输卵管炎,甚至扩散至全身引起广泛的炎症反应。淋病还可导致女性不孕、胎膜早破、异位妊娠和男性不育等病症,使临床检测和治疗面临着严峻的挑战[16]。MG不仅可以引起尿道炎和宫颈炎,并且近60%子宫内膜炎患者的宫颈黏液中能够检测出MG[17]。MG感染与子宫内膜炎和复发性盆腔炎有相关性[18]。值得一提的是,MG的检测仅限于核酸扩增试验。培养法所需时间过长,需要数月,并且敏感度不佳[19]。至今为止,血清学测定法和抗原检测等方法均不能应用于MG的检测。核酸扩增试验是MG唯一可行的诊断方法[20]。本研究结果显示,不孕症女性中UU感染率为62.53%,CT感染率为1.93%,NG感染率为0.21%,MG感染率为1.71%。UU是不孕女性生殖道检出率最高的微生物。显示UU和女性不孕症存在着密切的关系。值得注意的是,本研究中CT或MG阳性的患者中仅一例为UU阴性,其余均为并发感染,提示我们不同病原体感染之间可能存在着一定的关联性。

随着分子诊断技术的发展,病原体检测方法需要满足准确、高效和灵敏等要求。RNA检测技术因其特异、灵敏的优势广泛的用于生殖道病原体的检测。RNA检测技术包括SAT和转录介导扩增(transcription-mediated amplification,TMA),这两种检测方法的扩增原理完全相同。由于病原体细胞中的RNA存在多个拷贝,故RNA检测技术相较于以DNA有更高的准确度,更重要的是只有活的病原体中存在完整RNA片段,故能排除治愈后已死亡病原体对检测结果的影响[2]。RNA具有易降解、不稳定的特性,使得该法交叉污染可能性更小,可以有效减少假阳性的产生,大大提高了检测结果的可靠性,有助于临床诊断的准确度和对疗效的监测[21,22]。此外,RNA检测结果还可以用于临床疗效监测,符合精准医疗的要求,是目前生殖道病原体检测的首选方法。本研究中泌尿生殖道感染患者的拭子样本,SAT法结果和培养法检测UU相比较,差异具有统计学意义,SAT法的阳性率更高,敏感度更强。这与郑渠等[23]报道的培养法与SAT法检测效能相当不同。SAT法与乳胶法检测CT结果相比,前者阳性率以及敏感度亦明显更高。基于SAT法可以以尿液检测的优势,兼具检测时间短、假阳性率低等优势,尤其当受检者正处于感染初期,病原体数量较少,DNA含量少,而RNA较多,SAT法对该类病人的检测具有独特的优势[24],故该技术更适合作为体检检测方法进行人群筛选。然而由于SAT法敏感度强,容易被环境中的病原体或异物污染,故对实验室条件的要求更多,成本更高。此外,培养法可直接进行药敏试验,有助于指导临床用药,该法更适合作为筛选后的确诊方法。综上所述,SAT法和常规法联合检测UU,CT,NG和MG将为临床提供既准确又快捷的诊断依据。

综上所述,与其他方法比较,SAT法的优势更明显,如无创、操作简便、重复性好、敏感度高,并且一份标本可以进行多个项目的检测,避免多次取样之间的差异。更重要的是运用SAT法对病原体进行定量检测还可以应用到分子生物学研究、流行病

学的统计与调查、疾病的早期诊断、分型与预后等多个方面为临床提供强有力的理论依据。

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