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腺苷脱氨酶活性对自身免疫性肝病的临床意义研究*

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摘要 目的:探讨腺苷脱氨酶(ADA)在自身免疫性肝病患者血清中的变化及其临床意义。**方法:**利用酶法试剂盒检测自身免疫性肝病患者血清中的总 ADA(tADA)及其同工酶 ADA1 和 ADA2 的活性变化,利用受试者工作曲线(ROC)分析总 ADA、ADA1 及 ADA2 活性的诊断价值。利用 Spearman 相关性分析自身免疫性肝病患者各指标之间的相关性。**结果:**与对照组相比,tADA 和 ADA2 活性在自身免疫性肝病患者血清中均极显著升高($P<0.001$),ADA1 活性有一定程度升高($P=0.035$)。不同自身免疫性肝病亚型患者之间的血清 tADA、ADA1 及 ADA2 活性均无显著差异。ROC 分析显示,血清 tADA 和 ADA2 活性具有诊断价值,ADA1 活性无诊断价值。tADA 活性截断值取 13.5 U/L 时,诊断特异性为 93.3%,敏感性为 81.2%。血清 ADA2 活性截断值取 9.5 U/L 时,诊断特异性为 85.0%,敏感性为 83.3%。而 ADA1 无显著的诊断价值,ADA 活性与白蛋白球蛋白比值呈显著负相关($r=-0.41, P=0.004$),与球蛋白水平具有一程度的正相关($r=0.34, P=0.018$),与 ALT 呈弱正相关($r=0.29, P=0.042$),与其他指标的相关性均无统计学显著性。**结论:**血清 tADA 及 ADA2 活性在自身免疫性肝病患者血清中显著升高,并且具有一定的诊断价值。

关键词:自身免疫性肝病;腺苷脱氨酶;诊断

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Clinical Significance of Adenosine Deaminase Activity in Autoimmune Liver Disease*

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ABSTRACT Objective: To investigate the changes and clinical significance of serum adenosine deaminase activity (ADA) in patients with autoimmune liver disease (AILD). **Methods:** The activities of total ADA (tADA) and its isoenzymes ADA1 and ADA2 in serum of AILD patients were detected by enzyme assay. The diagnostic value of serum tADA, ADA1 and ADA2 activities were analyzed by using receiver operating curve (ROC). Spearman correlation was used to analyze the correlation among indexes in AILD patients. **Results:** Compared with the control group, the activities of tADA and ADA2 in serum of AILD patients were increased significantly ($P<0.001$), and the activity of ADA1 was increased to a certain extent ($P=0.035$). There were no significant differences in serum tADA, ADA1 and ADA2 activities among patients with different AILD subtypes. ROC analysis showed that serum tADA and ADA2 activities had diagnostic value. When the tADA activity cut-off value was 13.5 U/L, the diagnostic specificity and sensitivity were 93.3% and 81.2% respectively. When the cut-off value of serum ADA2 activity was 9.5 U/L, the diagnostic specificity and sensitivity were 85.0% and 83.3% respectively. ADA1 has no significant diagnostic value. ADA activity was correlated negatively with albumin globulin ratio ($r=-0.41, P=0.004$), correlated positively with globulin level to a certain extent ($r=0.34, P=0.018$), correlated weakly with ALT ($r=0.29, P=0.042$), and had no statistical significance with other hepatic function indexes. **Conclusion:** Serum tADA and ADA2 activities were increased significantly in AILD patients, and it could be a diagnostic biomarker for AILD.

Key words: Autoimmune liver disease; Adenosine deaminase; Diagnosis

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前言

自身免疫性肝病(Autoimmune liver disease, AILD)是体内免疫功能紊乱引起的一组特殊类型的慢性肝病,属于自身免疫性疾病。原发性胆汁性胆管炎(Primary biliary cholangitis,

PBC)、自身免疫性肝炎(AIH)和原发性硬化性胆管炎(Primary sclerosing cholangitis, PSC)是 AILD 的典型类型。AILD 的发病机制尚不清楚,机体免疫调节的紊乱既是患者的重要临床表现,同时也在 AILD 发生发展过程中发挥重要作用^[1]。

腺苷是机体循环和微环境中的免疫抑制信号,在体内发挥

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"Alarm signal"的作用,可以防止机体出现过激的免疫反应,在机体免疫稳态调控中作用关键^[2,3]。腺苷脱氨酶(Adenosine deaminase, ADA)可以催化腺苷的降解,在免疫系统发育和免疫反应调节中发挥关键作用^[4]。ADA包含两种同工酶:ADA1(基因ID:100)和ADA2(基因ID:51816),ADA1主要表达于胞内,血浆或血清中可以检测少量ADA1活性,ADA2主要由单核-巨噬细胞分泌表达至胞外,因此血清总ADA(Total ADA, tADA)活性主要由ADA2组成^[5,6]。由于ADA1和ADA2在机体免疫调节中的关键作用,因此其可能参与自身免疫性疾病的发生发展过程,已有研究显示ADA活性在多种自身免疫性疾病患者血清中升高,包括系统性红斑狼疮,类风湿性关节炎等^[7-9],然而有关ADA1及ADA2活性改变的研究相对较少。本文目的是分析ADA活性,包括tADA、ADA1和ADA2活性在AILD患者血清中的改变及其临床意义。

1 材料与方法

1.1 材料与仪器

材料:收集2016年至2018年在空军军医大学唐都医院收治的48例AILD患者为观察组,其中包含18例自身免疫性肝炎(AIH)和30例原发性胆汁性胆管炎(PBC)。选择同期体检健康者60名作为正常对照组。本研究经空军军医大学唐都医院伦理委员会审核批准,符合知情同意豁免的要求。收集AILD患者和健康对照血清,并分装冻存于-80℃冰箱备用。

仪器:全自动生化分析仪(BECKMAN, COULTER-AU5800)。

1.2 指标检测

血清tADA活性检测:采用腺苷脱氨酶检测试剂盒(上海

科华生物工程公司,中国),利用全自动生化分析仪(BECKMAN, COULTER-AU5800)进行测定。ADA2活性检测:向100 μL血清中加入1 μL ADA1抑制剂-ENHA(终浓度为100 μM),然后利用全自动生化分析仪进行酶活性测定。ADA1活性=tADA-ADA2,活性单位为U/L。肝功能指标AST、ALT、TP、ALB、GLO、TBA、TBIL、IBIL、TBA和DBIL检测:利用全自动生化分析仪进行分析。

1.3 受试者工作特征(ROC)曲线分析

对不同水平的血清ADA活性绘制ROC曲线图,当约登指数最大时,为最佳截断点,此时,曲线下面积(Area under the curve, AUC)最大,指标的诊断特异性(将患者正确判断为阳性的百分率)和敏感性(将健康对照正确地判断为阴性的百分率)最优。ROC曲线分析及绘图利用R软件Version 4.0.2执行。

1.4 统计分析

本文中定量数据不符合正态分布,采用中位数和四分位区间(Interquartile range, IQR)表示。采用Wilcoxon检验比较患者与对照组ADA水平的差异。性别差异采用卡方检验。利用Spearman相关性检验分析各指标之间的相关性。 $P<0.05$ 表示差异有统计学显著性。统计分析利用R软件Version 4.0.2执行。

2 结果

2.1 AILD患者和健康对照基本信息

如表1所示,纳入AILD患者与健康对照性别年龄无显著差异。AILD患者谷草转氨酶(AST)、谷丙转氨酶(ALT)、碱性磷酸酶(ALP)、总胆红素(TBIL)、直接胆红素(DBIL)、间接胆红素(IBIL)及总胆汁酸(TBA)水平升高,总蛋白(TP)及白蛋白(ALB)水平下降。

表1 AILD患者和健康对照基本信息
Table 1 The detail information of AILD patients and healthy controls

Index	AILD	Healthy control	P value
Age	59.50 (36.25 - 98.00)	55.50 (51.00 - 60.25)	0.369
Female/Male	37 / 11	44 / 16	0.571
AST(U/L)	59.50 (36.25-98.00)	24.00 (20.00-26.00)	0.000
ALT(U/L)	52.00 (22.75-93.50)	21.00 (15.00-28.00)	0.000
ALP(U/L)	220.00 (137.75-349.50)	81.00 (93.00 - 101.00)	0.000
TP(g/L)	63.20 (58.80-69.50)	70.50 (67.65-73.30)	0.000
ALB(g/L)	32.90 (30.58-35.40)	42.60 (41.35-43.65)	0.000
GLO(g/L)	30.55 (27.45-34.22)	28.10 (25.65-29.90)	0.004
TBIL(μmol/L)	33.05 (19.13-49.75)	13.40 (11.30-16.85)	0.000
DBIL(μmol/L)	18.15 (7.80-30.40)	4.90 (3.70-5.50)	0.000
IBIL(μmol/L)	13.25 (9.88-22.50)	8.90 (7.25-11.45)	0.000
TBA(μmol/L)	49.30 (16.60-135.60)	3.50 (2.20-5.10)	0.000

2.2 AILD患者血清ADA活性升高

与对照组相比,AILD患者血清中的tADA活性极显著升高($P<0.001$,图1A);AILD患者血清ADA1活性有一定程度的升高($P=0.035$,图1B);AILD患者血清ADA2活性极显著升

高($P<0.001$,图1C)。此外,两种AILD亚型AIH和PBC患者血清tADA,ADA1及ADA2活性均无统计学差异($P>0.05$,图1D-F)。

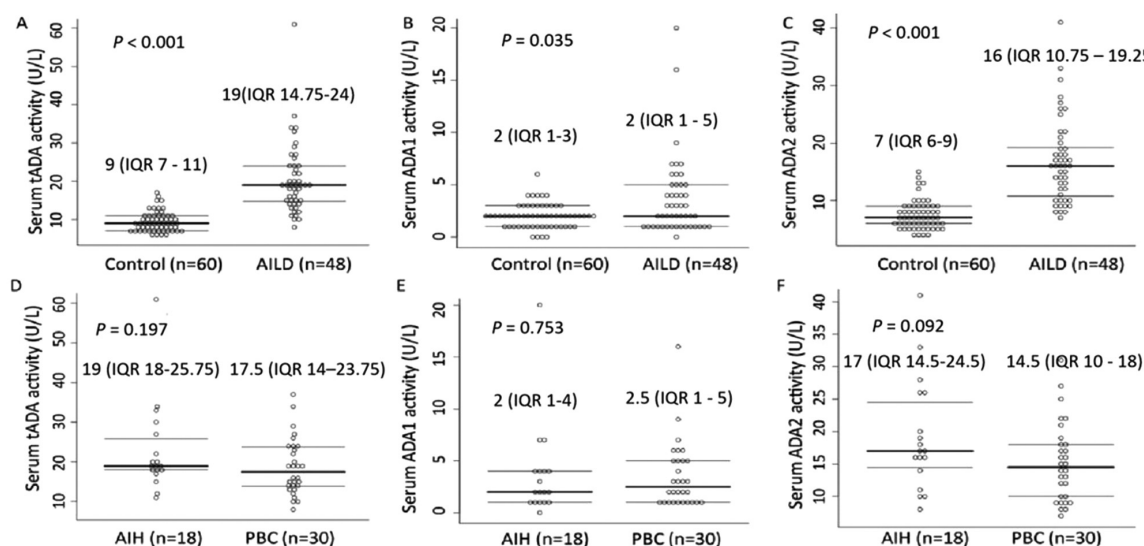


图1 AILD患者血清ADA活性

(A-C) AILD患者及对照血清中的tADA、ADA1和ADA2活性。(D-F) AIH和PBC患者血清中的tADA、ADA1和ADA2活性。

Fig. 1 Serum ADA activity of AILD patients

(A-C) Serum tADA, ADA1 and ADA2 activity of AILD patients and healthy controls. (D-F) Serum tADA, ADA1 and ADA2 activity of AIH and PBC patients.

2.3 AILD患者血清ADA活性之间的相关性

利用Spearman相关性分析,结果显示,AILD患者血清中的tADA与ADA1活性无显著相关性(图2A),而tADA与

ADA2活性显著正相关(图2B),ADA1与ADA2活性无相关性(图2C)。

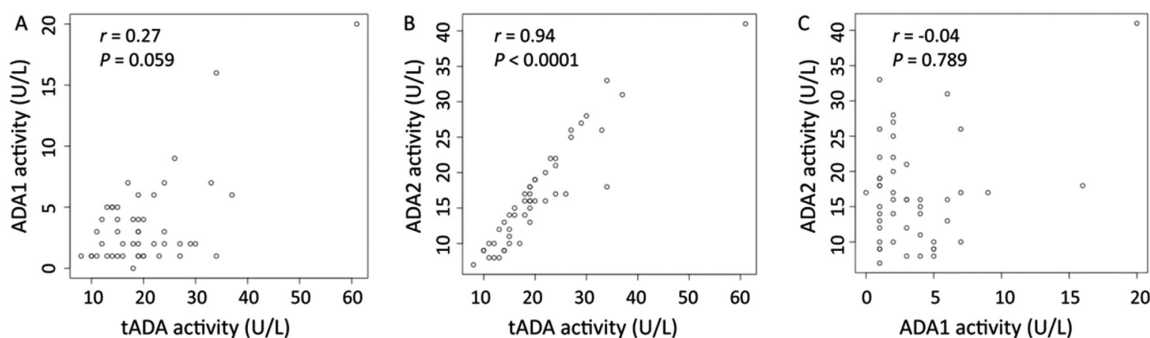


图2 AILD患者血清中的tADA、ADA1、ADA2活性相关性(r:Spearman相关系数)

(A) 血清中tADA与ADA1活性相关性。(B)血清中tADA与ADA2活性相关性。(C)血清中ADA1与ADA2活性相关性。

Fig.2 The correlation among serum tADA, ADA1 and ADA2 of AILD patients (r: correlation coefficient).

(A) The correlation between serum tADA and ADA1. (B) The correlation between serum tADA and ADA2. (C) The correlation between serum ADA1 and ADA2.

2.4 AILD患者血清ADA活性诊断价值

ROC曲线分析显示(图3),当tADA活性截断值取13.5 U/L时,tADA活性检测对AILD的诊断价值最优:特异性为93.3%,敏感性为81.2%。ADA1无显著的诊断价值。血清ADA2活性取9.5 U/L时,诊断效能最优:特异性为85.0%,敏感性为83.3%。tADA与ADA2的诊断效能无统计学差异($P=0.93$)。

2.5 血清tADA活性与肝功指标的相关性分析

利用Spearman相关性检验,分析了AILD患者ADA活性与其他肝功指标之间的相关性,如图4结果显示:ADA活性与白蛋白/球蛋白比值显著负相关($r=-0.41$, $P=0.004$),与球蛋白水平具有一定程度的正相关($r=0.34$, $P=0.018$),与ALT呈弱正相

关($r=0.29$, $P=0.042$),与其他指标的相关性均无统计学显著性。其他肝功指标之间的相关性如下:白球比与TBA之间有一定的负相关性($r=-0.29$);ALB与胆红素呈负相关性,ALB与TBA呈显著负相关($r=-0.62$);ALP与胆红素呈正相关,ALP与GGT显著正相关($r=0.81$);ALT与胆红素及TBA水平显著正相关;AST与胆红素及TBA水平显著正相关。

3 讨论

自身免疫性肝病发病率相对较低,发病机制尚不明确。AIH发病率在十万分之0.67~2.00之间,PBC的发病率在十万分之0.33~5.80之间^[10-12]。AILD作为一种自身免疫性疾病,机体免疫失调导致免疫系统攻击自身是其发生发展的重要因素。

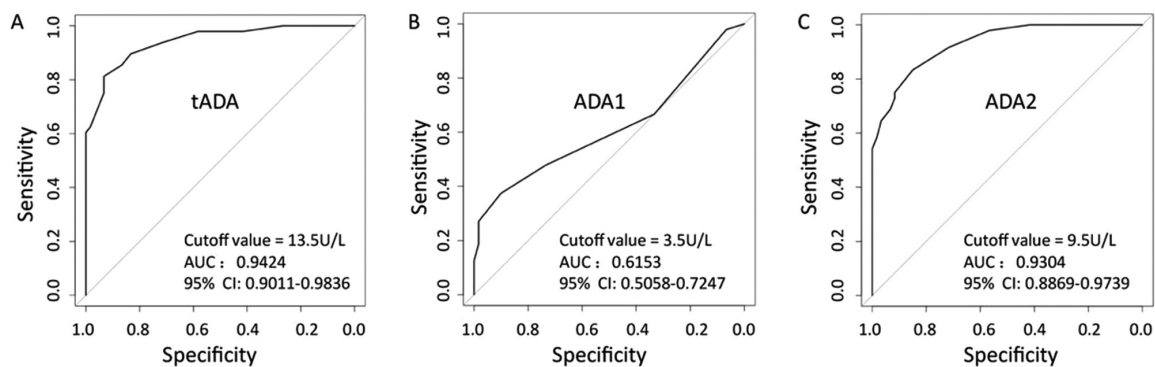


图3 血清 ADA 活性对 AILD 患者的诊断价值
(A-C)tADA、ADA1、ADA2 的诊断效能

Fig. 3 Diagnostic value of serum ADA for AILD patients.

(A-C) Diagnostic value of serum tADA, ADA1 and ADA2 for AILD patients.

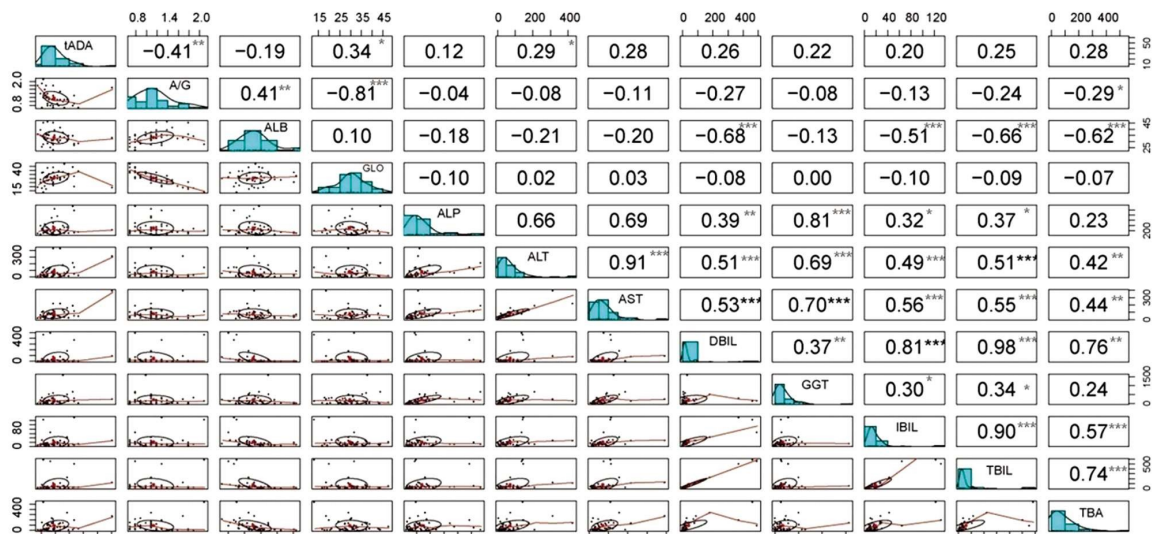


图4 AILD 患者 ADA 活性与其他肝功指标之间的相关性矩阵(* $P<0.05$; ** $P<0.01$; *** $P<0.001$)

Fig.4 Correlation matrix between ADA activity and other liver function indexes in AILD patients (* $P<0.05$; ** $P<0.01$; *** $P<0.001$)

素^[13]。ADA 可以催化腺苷的降解,腺苷是一种公认的免疫抑制因子^[14,15]。腺苷信号通路包括一系列酶促反应过程,首先,三磷酸腺苷(ATP)由 CD39 催化降解为单磷酸腺苷(AMP);然后,AMP 由 CD73 水解生成腺苷,腺苷由 ADA 水解生成肌苷^[16]。腺苷存在于机体循环及微环境中,可以通过激活免疫细胞表面的不同类型的腺苷受体,执行防止机体免疫过激的功能^[17-19]。目前针对腺苷信号通路相关分子的研究主要集中于 CD39, CD73 以及腺苷受体,而对 ADA 的研究相对较少^[20,21]。ADA 在机体免疫系统发育、免疫应答、免疫调节过程中具有重要作用^[22,23]。ADA1 和 ADA2 表达方式不同,ADA1 主要在胞内表达,而 ADA2 主要为分泌型表达,研究显示,ADA1 和 ADA2 对免疫细胞的调节作用具有显著差异^[24,25]。目前针对 ADA1 和 ADA2 的研究大都关注其基因突变所致功能缺陷,进而引起的相关疾病,如 ADA1 基因缺陷可导致儿童罹患重症联合免疫缺陷(即 ADA-SCID),ADA1 基因或蛋白替代疗法也已经进入临床应用^[26,27]。ADA2 缺陷可导致儿童患中风及血管炎症^[28,29]。ADA1 和 ADA2 在自身免疫性疾病的中变化及临床意义研究相对较少。

本文研究了 AILD 患者和健康对照的血清 ADA 活性,包括 tADA、ADA1 和 ADA2 活性。结果显示,与对照组相比,tADA

及 ADA2 活性在 AILD 患者血清中显著升高($P<0.001$),血清 ADA1 活性有一定程度的升高($P=0.035$)。AIH 与 PBC 患者血清 tADA、ADA1 及 ADA2 活性均无显著差异($P>0.05$)。此外,tADA 与 ADA2 活性高度正相关,而与 ADA1 活性无相关性,这种现象与 ADA2 是分泌表达蛋白有关。ROC 曲线分析表明,血清 tADA 和 ADA2 活性检测有助于区分 AILD 与健康体检者,并且两者的诊断效能相似,无统计学差异,而血清 ADA1 活性无诊断价值。Torgutalp 等曾对 AIH 患者 tADA 活性进行了研究^[30],结果显示,当 tADA 的截断值为 25.25 U/L 时,其敏感性和特异性分别为 84.0%和 88.9%,与本文结论相似。然而,诊断截断值的差异可能是由于 ADA 测定方法和检测对象种族的不同所致。因此,临床实验室在检测血清 ADA 活性时,应根据实际情况制定相应的参考区间。与其他肝功指标的相关性分析结果显示,tADA 与白球比呈负相关,与球蛋白和 ALT 呈一定程度的正相关性。

综上所述,AILD 患者血清 tADA 和 ADA2 活性极显著升高,血清 tADA 和 ADA2 活性检测可用于临床诊断 AILD,且成本低、灵敏度高。然而,ADA 活性的变化仅仅是 AILD 伴随性改变,还是本身发挥功能,参与 AILD 发生发展,尚需进一步深

入研究。

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