

HBeAg 特异免疫复合物检测及临床意义

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摘要 采用单克隆抗-HBe 固相 ELISA 法检测 118 例 HBV 感染者血清中 HBeAg 特异免疫复合物(HBeAg/IC), 结果表明: HBeAg/IC 与 HBV 复制相关, 可作为 HBV 复制的一种血清学标志, HBeAg 阴性、HBVDNA 阳性者中, 62.2% 患者 HBeAg/IC 阳性, 提示 HBeAg/IC 的形成致使夹心法不能检出 HBeAg, 这类患者仍可以是 HBV 野毒株感染, 并非均为 HBeAg 缺陷变异株感染; HBeAg/IC 阳性血清 ALT 异常率明显高于 HBeAg/IC 阴性血清($P < 0.01$), 提示 HBeAg/IC 存在与肝损伤有关。

关键词 乙肝病毒; e 抗原特异免疫复合物; 乙肝病毒脱氧核糖核酸; 套式多聚酶链反应

HBeAg 为临床判断乙肝病毒(HBV)复制、传染性的重要血清标志, 然而血清 HBeAg 阴性时仍可检出 HBVDNA, 故应重新评价 HBeAg 检测的临床意义。业已证实 HBV 感染血清中存在 HBeAg 特异免疫复合物(HBeAg/IC), 而现行双抗体夹心法不能检出免疫复合物中的 HBeAg。本文建立单克隆抗-HBe 固相 ELISA 法检测 HBeAg/IC, 结合 HBVDNA、HBeAg、抗-HBe 检测评价 HBeAg/IC 的临床意义。

对象与方法

1 研究对象 118 例 HBV 感染者系 1995 年 8 月~1996 年 5 月我院传染科住院和门诊随访的 HBsAg 阳性肝炎患者。诊断依据 1995 年北京第五届全国传染病寄生虫病学术会议《病毒性肝炎防治方案》。正常对照 40 例系本校医学生和体检者, 无肝炎病史及症状和体征, 肝功正常, HBsAg 阴性。

以上病例 IgM 抗-HAV、抗-HCV、抗-HDV、抗-HBV 均阴性。

2 研究方法 采静脉血分离血清, -20℃ 保存, 统一检测, 采血同时查肝功。

2.1 HBeAg/IC 检测 采用自建单克隆抗-HBe(3G8 细胞株)固相 ELISA 法^[1]。抗-HBe 包被, 与待检血清作用后, 加 HRP 标记抗人 IgG 抗体, 显色, 测 OD₄₅₀, S/N 2.1 为阳性。

2.2 血清 HBV DNA 检测 采用套式多聚酶链反应(n-PCR), 依 HBV 基因组 S 区设计套式引物, 上海细胞生物学研究所合成^[2], 第二次扩增产物电泳后出现 206bp 条带为阳性。

2.3 HBsAg、HBeAg、抗-HBe 采用 ELISA 法, IgM 抗-HAV、抗-HCV、抗-HDV、抗-HEV 采用固相免疫法, 严格按试剂盒说明书操作。

4 统计学处理 卡方检验和四格表确切概率法。

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结 果

1 40 例正常对照血清均未检出 HBeAg/IC 和 HBV DNA。118 例 HBV 感染者中 64 例(54.2%) 检出 HBeAg/IC, 其血清 HBV DNA 均阳性, 54 例 HBeAg/IC 阴性血

清中 28 例(51.9%), HBV DNA 阳性, 两组比较差异显著($P < 0.01$), 见表 1。

2 血清 HBeAg 阴性、HBV DNA 阳性的 37 例中 23 例(62.2%) HBeAg/IC 阳性。HBeAg 阴性、HBeAg/IC 阴性而 HBV DNA 阳性仅 14 例(见表 1。)

表 1 HBeAg/IC 与 HBV DNA 检测结果比较

HBeAg/IC	抗-HBe ⁺ /HBeAg ⁻ HBV DNA		抗-HBe ⁺ /HBeAg ⁺ HBV DNA		抗-HBe ⁻ /HBeAg ⁻ HBV DNA		抗-HBe ⁻ /HBeAg ⁺ HBV DNA	
	+	-	+	-	+	-	+	-
+	13	0	5	0	10	0	36	0
-	10	10	3	5	4	11	11	0

3 HBeAg/IC 阳性者 ALT 异常率(90.63%) 明显高于 HBeAg/IC 阴性者(51.85%), 见表 2。

表 2 HBeAg/IC 与血清 ALT 水平的关系

ALT (U/L)	HBeAg/IC		P
	+	-	
40	6	26	< 0.01
> 40	58	28	

HBeAg/IC 形成而未能检出 HBeAg, 并非均为 HBeAg 缺陷变异株感染^[4]。

有学者认为 HBeAg/IC 形成与肝损伤相关^[6], 本研究发 现 HBeAg/IC 阳性血清中 ALT 异常率(90.63%) 明显高于 HBeAg/IC 阴性血清(51.85%), HBeAg/IC 存在意味抗-HBe 应答出现, HBeAg 特异 Th 活化, HBeAg 和 HBcAg 在 Th 水平上存在交叉反应, 因而也意味着 HBcAg/HBeAg 特异 Th 活化。活化的 Th 细胞产生 Th₂ 类细胞因子促进 B 细胞活化产生抗-HBe, Th₁ 类细胞因子促进 CTL 活化, 识别感染肝细胞表面靶抗原(HBeAg/HBcAg) 导致肝细胞破坏^[7], 并非 HBeAg/IC 直接致肝损伤。

讨 论

本文结果显示, HBeAg/IC 阳性的 64 份血清 HBV DNA 均为阳性, HBeAg/IC 阴性血清中 HBV DNA 检出率为 51.9%(28/54), 两组比较差异显著($P < 0.01$)。HBeAg/IC 形成致使现行双抗体夹心法不能检出复合物中 HBeAg^[1,3,4]。本文建立的单克隆抗-HBe 固相 ELISA 法检测 HBeAg/IC, 简单、敏感、特异。可作为临床判断 HBV 复制的一种血清标志, 克服和补充双抗体夹心法查 HBeAg 的局限性, 尤其适用于 HBeAg 阴性而又无条件查 HBV DNA 的患者。

HBV 前 C 区变异可阻断 HBeAg 合成和分泌(HBeAg 缺陷变异), 为血清 HBeAg 阴性、HBV DNA 阳性的重要原因^[5]。然而, 本文 HBeAg 阴性、HBV DNA 阳性的 37 例中 23 例(62.2%) 检出 HBeAg/IC, 提示这类患者中半数以上仍存在野毒株 HBV 感染, 因

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DETECTION OF HBeAg-SPECIFIC IMMUNE COMPLEX AND ITS CLINICAL SIGNIFICANCE

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Abstract A method (ELISA) for detection of HBeAg-specific immune complex (HBeAg/IC) using monoclonal antibody was developed and 118 patients with HBV infection were investigated. The results showed that HBeAg/IC was related to HBV active replication, could serve as a marker of HBV replication; Of 37 patients with HBeAg-negative and HBV DNA γ -positive, 23 (62.2%) had HBeAg/IC in their sera, suggesting that HBeAg/IC formation prevents

the detection of HBeAg by "Sandwich" ELISA and this kind of patients could be still infected with wild type HBV. It is not true that all patients with HBeAg-negative and HBV DNA-positive are infected with HBeAg-defect mutant; The rate of abnormal ALT level is much higher in patients with HBeAg/IC in serum than that in patients without HBeAg/IC, $P < 0.01$ (90.63% vs 51.85%), indicating that the existence of HBeAg/IC is related to liver damage.

Key words HBV; HBeAg-specific immune complex; HBV DNA; nested polymerase chain reaction

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CLINICAL CURATIVE EFFECT ANALYSIS OF THE FORTIFIED TREATMENT OF NOVOLIN TO DIABETIC NEPHROPATHY

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Abstract 35 cases of diabetic nephropathy in chronic renal dysfunction of azotemia stage were treated by fortified treatment of novolin in a short period and compared with routine therapy group of novolin which was set as control. The results showed that the blood glucose of the patients who received

fortified treatment was controlled ideally after the course of treatment. Meanwhile, the endogenous creatinine clearance rate (CCR) rise up significantly. It suggests that fortified treatment to diabetic nephropathy can defer the deterioration process of renal dysfunction.

Key words novolin; fortified therapy; diabetic nephropathy; endogenous