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青海地区献血者 HBV 感染隐匿风险与基因型分析 *

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摘要 目的:探讨青海地区献血者乙型肝炎病毒(hepatitis b virus,HBV)感染隐匿风险与基因型的相关性。**方法:**采用回顾性研究方法，选择 2014 年 2 月 -2018 年 1 月在我院进行无偿献血的青海地区人群 750 例，采用聚合酶链式反应 - 限制性内切酶片段法(PCR-RFLP)检测 HBV DNA 基因的多态性,并进行 HBV 感染隐匿风险分析。**结果:**在 750 例人群中,检出 HBV 隐匿性感染 8 例,检出率为 1.1%,其中窗口期感染 3 例,一过性感染 5 例;基因 C 型 6 例,基因 B 型 2 例,基因 B 型患者的都为窗口期感染,核酸定量都≤ 20 IU/mL,与基因 C 型患者对比差异有统计学意义(P<0.05)。多因素 Logistic 回归分析显示基因 C 型、核酸定量、家属病史、吸烟为导致 HBV 隐匿性感染的独立危险因素(P<0.05)。**结论:**青海地区献血者 HBV 感染隐匿风险相对比较低,多为基因 C 型,基因 C 型为导致 HBV 隐匿性感染的独立危险因素。

关键词:青海地区;献血者;HBV 隐匿性感染;基因型

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Risk Factors and Genotype Analysis of HBV Occult Infection in the Blood Donors in Qinghai*

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ABSTRACT Objective: To explore the correlation between the genotype of HBV and occult infection in the blood donors in Qinghai. **Methods:** A retrospective study was performed, February 2014 to January 2018, the polymorphism of HBV DNA gene in 750 blood donors from Qinghai were detected by the polymerase chain reaction restriction endonuclease fragment (PCR-RFLP), and the risk of HBV occult infection were analyzed. **Results:** 8 cases of HBV occult infection were detected in the 750 donors, the detection rate was 1.1%, including 3 cases of window stage infection and 5 cases of transient infection. And there were 6 cases of gene C, 2 cases of gene B type, and genotype B patients were all window stage infection, and the nucleic acid quantity were ≤ 20 IU/mL, and the difference were statistically significantly compared with genotype C patients (P<0.05). Multivariate Logistic regression analysis showed that genotype C type, nucleic acid quantification, family history and smoking were independent risk factors for HBV occult infection (P<0.05). **Conclusion:** The risk of HBV occult infection in blood donors in Qinghai is relatively low, and most of them are genotype C, gene C is an independent risk factor for HBV occult infection.

Key words: Qinghai area; Blood donors; HBV occult infection; Genotype

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前言

乙型肝炎病毒(hepatitis b virus,HBV)感染是当前重大传染疾病之一,可引起急 / 慢性肝炎、重症肝炎、肝硬化、原发性肝癌,具有较高的致残率与死亡率[1-3]。我国曾经是 HBV 感染的高流行区,随着免疫规划的顺利进行,人群中乙肝表面抗原(HBsAg) 携带率下降，但一般人群的 HBsAg 阳性率依然在 5%左右,特别是 HBV 变异株显著增加[4,5]。隐匿性 HBV 感染是目前 HBsAg 无法检测的，但血清或肝脏中依然存在 HBV DNA 的感染，其主要与 HBV 病毒株的变异和病毒极低复制水平有关[6,7]。HBV 隐匿感染者的病毒载量大多低于 10^4 IU/mL,只有高度敏感的方法才能够检测出[8]。现代研究显示 HBV 感染是一种多基因遗传病，是遗传因素和环境因素等共同作用的结果,其发生与遗传因素有关[9,10]。本研究主要探讨了青海地区献血者 HBV 感染隐匿风险与基因型的相关性。现总结报道如下。

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1 资料与方法

1.1 研究对象

选择 2014 年 2 月 -2018 年 1 月在我院进行无偿献血的青海地区人群 750 例。其中,男 400 例,女 350 例;年龄最小 25 岁，最大 44 岁，平均年龄 36.33± 2.19 岁；平均体重指数为 22.92± 1.29 kg/m^2;平均献血次数 1.62± 0.45 次。纳入标准:献血资料与一般资料完整;青海地区户籍、民族不限;献血人群知情同意本研究,自愿进行无偿献血;年龄 25-45 岁;梅毒螺旋体抗体阴性;抗 -HCV 阴性、抗 -HIV 阴性;医院伦理委员会批准了此次研究;经 ELISA 法初筛的 HBsAg 阴性的合格献血人;精神系统疾病或长期心理压力过大患者。排除标准:献血资料与一般资料缺失;妊娠与哺乳期妇女;合并其他自身免疫性疾病。

1.2 HBV DNA 检测

提取外周血基因组 DNA，采用聚合酶链式反应 - 限制性内切酶片段法(PCR-RFLP)检测 HBV DNA 基因的多态性,严格按照操作说明书进行。根据 GenBank 中 HBV DNA S 基因核苷酸序列资料及软件 Primer Premier 5.0 设计引物。HBV DNA 以不同区域的两套巢式 PCR 进行检测,A 序列如下:上游:5'-AACATCGGCTGTCTATTATAATC-3';下游:5'-AAATAACACGTACGATACTGAAG-3'。B 序列如下：上游:5'-ATCCACTCATGCATATTGACCA-3';下游:5'-AGC CAT GTC TCA GCT ACCACA-3'。PCR 扩增条件:94℃ 3 min,然后 94℃ 30 s,48℃ 55 S,72℃ 45 s,共 30 个循环,最后 72℃ 延伸 10 min。PCR 反应体系均为 25 μL,含 2× Tag PCR Masterix 12.5 μL,上下游引物各 1 μL,模板 DNA 1.5 μL,不同 Taq DNA 聚合酶在反应体系液中的浓度按说明书加入。PCR 产物进行 2%凝胶电泳鉴定,以紫外凝胶成像系统记录结果,基因扩增后由上海英骏生物技术有限公司用 ABI 3100 DNA 全自动测序仪进行测序。

1.3 资料调查

记录与调查所有献血人员的一般人口学指标,内容包括性别、年龄、献血次数、体重指数、生活习惯、疾病史等。

1.4 统计学分析

使用 SPSS23.00 软件进行数据分析,计数资料采用例数表示,正态分布的计量资料以均数± 标准差($\bar{x}$± s)表示,非正态分布计量资料采用中位数表示，对比方法为 t 检验与卡方分析,危险因素分析采用多因素 logistic 回归分析,均为双侧检验,以 P<0.05 为差异具有统计学意义。

2 结果

2.1 HBV 的感染隐匿情况

在 750 例人群中，检出 HBV 隐匿性感染 8 例，检出率为 1.1%,其中窗口期感染 3 例,一过性感染 5 例。

2.2 HBV 隐匿性感染的基因型

在 HBV 隐匿性感染的 8 例患者中,基因 C 型 6 例(75%),基因 B 型 2 例(25%),基因 B 型患者的性别、年龄、体重指数、献血次数等与基因 C 型患者对比差异无统计学意义(P>0.05)。见表 1 与图 1。

表 1 不同 HBV 隐匿性感染患者的一般资料对比

Table 1 Comparison of the general information of patients with different HBV occult infections

Type	n	Sex (male/female)	Age(year)	Body Mass Index (kg/m^2)	Frequency of blood donation (times)
Genotype C	6	3/3	38.22± 2.49	22.45± 1.44	1.45± 0.22
Genotype B	2	1/1	37.98± 3.19	22.19± 1.84	1.39± 0.41
t or x^2		0.000	0.342	0.311	0.233
P		1.000	0.589	0.603	0.687

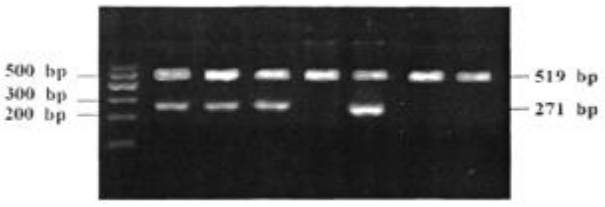

图 1 HBV 隐匿性感染患者的基因型 PCR

Fig.1 Genotype PCR of patients with HBV occult infection

2.3 HBV 基因型的核酸定量与感染类型对比

基因 B 型患者的都为窗口期感染,核酸定量都≤ 20 IU/mL,与基因 C 型患者对比差异有统计学意义(P<0.05),见表 2。

2.4 相关性分析

以献血人群的 HBV 隐匿性感染作为因变量，以调查的内容、核酸定量、基因型作为自变量,多因素 Logistic 回归分析显示基因 C 型、核酸定量、家属病史、吸烟为导致 HBV 隐匿性感染的独立危险因素(P<0.05),见表 3。

表 2 不同 HBV 基因型的核酸定量与感染类型对比

Table 2 Comparison of the nucleic acid quantification and infection types between different HBV genotypes

Type	n	Nucleic acid quantificatio		Type of infection	
		n≤ 20 IU/mL	>20 IU/mL	Window phase	Transient
Genotype C	6	1	5	0	6
Genotype B	2	2	0	2	0
x^2		4.444		8.000	
P		0.035		0.005	

表 3 青海地区献血者 HBV 感染隐匿风险的多因素分析

Table 3 Multivariate analysis of HBV infection occult risk among blood donors in Qinghai

Influence factor	β	SE	Wald	P	OR(95%CI)
Genotype C	0.323	0.178	34.119	<0.05	1.893(1.541-2.386)
Nucleic acid quantitation	0.627	0.208	34.396	<0.05	2.154(1.82-2.737)
Family medical history	0.525	0.338	23.132	<0.05	2.162(1.554-2.771)
Smoke	0.451	0.196	28.175	<0.05	2.041(1.885-2.786)

3 讨论

HBV 感染是一个世界性公共卫生问题,HBV 感染所致的肝硬化、肝功能衰竭和肝癌当前也比较常见,每年因肝功能衰竭和 HBV 相关肝癌死亡的病例超过 100 万，也严重影响了青海地区居民的身心健康[11-13]。相关研究表明 HBV 感染可造成肝细胞损伤,引起病程慢性化,最后导致肝组织纤维化、肝硬化,诱发肝癌的形成[14,15]。HBV 感染在不同地区间流行程度的差异,既与人群的感染年龄、医疗卫生状况、感染途径、免疫遗传特征、生活习俗等因素有关,也与病毒基因型等病毒自身的生物学特征有关[16]。传统研究认为血清中的病毒血症、HBsAg 阳性是 HBV 感染的主要依据，患者血清 HBsAg 转阴及出现抗-HBs 表现出现 HBV 的清除。但随着 HBV-DNA 检测技术灵敏度及特异性的进步,隐匿性的 HBV 感染现象已经被广泛关注。最新研究表述将现有的检测方法检测为 HBsAg 阴性，但血清或肝脏中存在 HBV DNA 的称为隐匿性 HBV 感染[17]。

本研究显示在 750 例人群中,HBV 隐匿性感检出率为 1.1%,低于相关报道,这可能是与本地区的人口流动性比较低有关。相关研究显示我国 HBV 感染在要为基因型 B 和 C,北方主要为 C 型,南方主要为 B 型[18,19]。而随着疾病从慢性乙型肝炎到肝硬化、肝癌的进展,基因型 C 在 HBV 感染中所占比例显著上升[20,21]。本研究中 HBV 隐匿性感染 8 例患者中,基因 C 型 6 例,基因 B 型 2 例。基因 B 型患者的都为窗口期感染,核酸定量都≤ 20 IU/mL，与基因 C 型患者对比差异有统计学意义。当前研究也显示 HBV 隐匿性感染的核酸定量值多小于最低检出量,基本无法定量,说明 HBV 隐匿性感染的核酸检出存在一定的机会性[22-24]。同时,当前 HBV 核酸检测也会缩短血清学窗口期,特别是变异株不仅影响血清学检测,也会造成核酸漏检[26,27]。随着病毒载量的增多,基因型 C 的病毒载量比其他基因型显著增加,其发生肝癌的风险也相应增加,但也有研究显示 B 基因型与 C 基因型 HBV-DNA 水平差异无统计学意义。

HBV 感染是遗传因素和环境因素共同作用的结果，某一种或多种因子或酶类基因表达异常是 HBV 隐匿性感染那发生的重要环节[28]。本研究结果显示基因 C 型、核酸定量、家属病史、吸烟为导致 HBV 隐匿性感染的独立危险因素。其中,吸烟与病毒感染密切相关,可使患病毒感染性血清标志物阳性患者患原发性肝癌的危险性明显增高[29]。相关研究显示 HBsAg 阳性标本以 B 型为主，而 HBsAg 阴性隐匿性 HBV 感染标本 HBV DNA 分型以 C 型为主,隐匿性感染 HBV DNA 分型 C 型显著高于 HBsAg 阳性携带者,表明 C 型更易出现隐匿性感染[30]。

总之,青海地区献血者 HBV 感染隐匿风险相对比较低,多为基因 C 型,基因 C 型、核酸定量、家属病史、吸烟为导致 HBV 隐匿性感染的独立危险因素。

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