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Prevalence of different alleles of apolipoprotein E (ApoE) gene with respect to the genetic risk for Hepatitis B virus infection in healthy north Indian population

Vikas Pahal[1], Rohit Randhawa[2], Gurdyal Singh[3], Ankita Chandel[4] and Krishan Swaroop Dadhich[5]

[1,2,4]Department of Medical Biotechnology, Dolphin (PG) College of Science and Agriculture, Chunni Kallan, F.G. Sahib, Punjab, India

[3]Department of Biotechnology, Panjab University, Chandigarh, India

[5]Department of Microbiology, Dolphin (PG) College of Science and Agriculture, Chunni Kallan, F.G. Sahib, Punjab, India

Abstract: **-Several single nucleotide polymorphisms (SNPs) in various human immunogenes have been shown to be responsible for resistant or susceptibility to hepatitis B virus infection (HBV). It has been shown by many studies that various polymorphism in apolipoprotein E (ApoE) gene have direct effect on various disease manifesto including HBV, HIV, HCV and several neurological disease. The allele ε2 has been observed as a disease susceptible allele for HBV infection, whereas it confirms resistance to HCV infection. The aim of this study was to find out the different polymorphic variant of apolipoprotein E (ApoE) gene in our healthy population. Seventy-four healthy volunteers were included in this study. Genomic DNA was extracted and PCR was performed with two different systems and the result was analyzed for different banding pattern of the gene to find out the respective alleles. Allele ε2 was found to be the dominant allele in both male and female of our healthy population, which is not a good sign regarding HBV-infection**.

Keywords: Apolipoprotein E gene, HCC, Hepatitis B virus

Abbreviations: BQW: best quality water; HDL: high density lipoprotein; VLDL: very low density lipoprotein; LDLR: low density lipoprotein receptor; HBV: hepatitis B virus; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; HIV: Human immunodeficiency virus; Nt: Nucleotide; SD: Standard deviation

I. INTRODUCTION

Hepatitis B virus (HBV), a non-cytopathichepatotropic virus, belongs to *hepadnaviridae* family of viruses causing various spectrum of liver disease worldwide [1,2]. In India, chronic HBV infection comprises 15% of total pool of the world HBV infection. Infection with HBV may cause different clinical outcomes from asymptomatic carriers to the HCC- the gravest finale [1-5]. In India 80-90% HCC cases are only due to HBV infection [6]. Progression of HBV-mediated liver disease dependsupon many factors including the virus strain and host genetic factors [1-3]. Host genetic polymorphism is responsible for differential immune response towards HBV infection [7,8]. So, different polymorphic forms of various immunogenes plays an important role in determining susceptibility or resistant to HBV infection.

It has been demonstrated by many studies that cellular lipids plays an important role in the replication of HBV. *ApoE* is a plasma protein which is found in HDL (high density lipoprotein) and VLDL (very low-density lipoprotein) and produced by hepatocytes [9-12]. It has several functions like transport of lipids, regulation of immune response and as a modulator of cell growth and differentiation [10-16]. Out of 30 kinds of polymorphisms, only three have been found to have an effect on *ApoE* protein function [12]. These 3 common alleles (ε2, ε3 & ε4) resulted in six different genotypes (ε2/2, ε3/3, ε4/4, ε3/2, ε4/3, ε4/2). Some studies demonstrated the influence of different genotypes of *ApoE* in the progression of several neurological and cardiovascular diseases [9-14]. Further confirming the effect of these host genotypes on disease progression, some other studies relate the genotypes with outcomes of various viral diseases including hepatitis B virus (HBV), hepatits C virus (HCV), herpex simplex virus (HSV) and human immunodeficiency virus (HIV) infection [10-19].

So, keeping in mind the importance of *ApoE* gene alleles with respect to their influence on HBV-directed disease, we try to

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find out dominating allele of *ApoE* gene in our healthy population. This will help us to find out the population group which is at the risk of HBV infection.

II. MATERIAL AND METHODS

Samples collection

We included 74 healthy volunteers (male-41, female-33) for this study. All the isolates were negative to HCV/HBV/HIV infections with a median age 20.80±1.14 for male & 19.12±1.13 for female volunteers. Before inclusion to the study a written consent was taken from all the volunteers. All of the volunteers in the present study were from our college Dolphin P.G College of Science and Agriculture. The study was approved by the Institutional Ethics Committee (I.E.C).

Chemicals

Analytical grade chemicals were used in the present study. PCR reagents, DNA ladders and MilliQ water were purchased from Chromous Biotech (INDIA). Tris buffer, EDTA, glacial acid, ethidium bromide and agarose were purchased from Himedia (INDIA) and plastic wares were purchased from AXYGEN (AXYGEN, Scientific Pvt. Ltd., New Delhi).

DNA extraction & amplification of different alleles of *ApoE* genes

DNA was extracted from the peripheral blood of our isolates using standard chloroform-phenol method [20]. Primers were designed on the basis of available literature and with the use of Primer 3 software [12]. The primers were synthesized by Chromous Biotech on payment basis. To validate the amplification results a negative control using MilliQ water in place of DNA, and a positive control comprises the amplified 228-bp fragment of LDLR gene were used. Various primers used in the present study are shown in Table 1.

Table 1. Details of primers used for amplification of *ApoE* gene from human DNA. Sense and antisense primers are donated by (+) and (-) signs respectively.

Primers	Sequence (5' - 3')
P1(+)	ATGCCGATGACCTGCAGAATT
P2(+)	ATGCCGATGACCTGCAGAATC
P3(+)	CGCGGACATGGACGTTTT
P4(+)	CGCGGACATGGACGTTTT
P5 (-)	GTTCAGTGATTGTCGCTGGGCA
P7(+)	AACAACTGACCCCGCTGGCG
P8(-)	ATGGCGCTGAGGCCGCGCTC

To study the polymorphism in *ApoE* gene in healthy population, we used two PCR systems (System A & System B) for each DNA sample [12]. Final master mixture for both the systems had 25 µl volumes and contained 0.1 µl (5U/µl) DNA *Taq* polymerase enzyme (New England BioLabs), 200 µM each of dNTPs, 1 µM of each primer (for system A primers were P1, P3 and P5 and for system B the primers were P2, P4 and P5. Internal primers were P7 & P8 in both the system), 3.0 mM $MgCl_2$, 2.5µl of 10x Buffer (NH_4^+) and 2.5 µl target DNA. The PCR amplification profile was as follows: initial denaturation at 95°C for 5 min, then 35 cycles with denaturation at 96°C for 45 s, annealing at 65°C for 45 s and extension at 72°C for 1 min, followed by the last cycle of extension at 72°C for 5 min. *ApoE* gene is classified into six genotypes. Different genotypes shows different banding pattern (which depends on the size of amplified products) on agarose gel in different system (System A& System B) as shown in Table 2.

Table 2. Amplification products of the six common ***ApoE*** genotypes in different PCR system

Genotype	PCR production (bp)	
	A system	**B system**
ε2/2	588 &451	No band
ε 3/3	588	451
ε 4/4	No band	588 & 451
ε 3/2	588 &451	451
ε 4/3	588	588 &451
ε 4/2	588 &451	588 &451

Detection of DNA on agarose gel for identification of genotypes

The 2.0 % ethidium bromide stained agarose gel was prepared for detection of different banding patterns of different alleles of *ApoE* gene.

Statistical analysis

Different alleles and genotyped frequency were extracted by direct counting and using Hardy-Weinberg equilibrium method. For comparison of two independent proportions, the chi square (χ^2) or Fischer exact test was used and statistical differences were calculated using SigmaPlot software (version 11.00). Differences were considered significant if P values obtained was found to be less than 0.05 (P<0.05).

III. RESULTS

Sixty-nine isolates were genotypes accurately for *ApoE* gene polymorphism (Figure 1). Out of these 69 isolates, 40 were male and 29 were female (Table 3). Genotype **ε**2/2 was found to be more prevalent in our population followed by **ε**4/2, **ε**4/4and **ε**3/2genotypes (Figure 2).

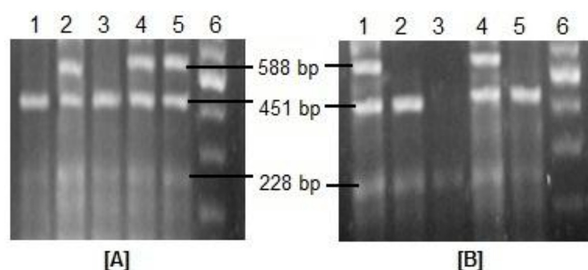

Figure 1. Amplified products of '***ApoE***' gene of various human isolates on 2.0% ethidium bromide stained agarose gel for PCR system A& B. **[A]** Lanes 1 & 3 show 451 bp amplified products from different isolates with PCR system A, Lanes 2,

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4 & 5 show 588 & 451 bp bands and Lane 6 shows molecular weight marker (100 bp). **[B]** Lanes 1 & 4 show 588 & 451 bp amplified products from different isolates with PCR system B, Lanes 2 and 5 show 451 bp amplified band and Lane 6 shows molecular weight marker (100 bp). For both system A & B, a 228 bp band was amplified as an internal control system (positive control).

Table 3. Presence of different genotypes and alleles of ***ApoE*** gene in our healthy volunteers

Genotype	Total	Male	Female
ε2/2	34	20	14
ε3/2	10	5	5
ε4/2	13	8	5
ε4/4	12	7	5
Total	**69**	**40**	**29**
Measurement of different Allele (frequency)			
ε2	91.0 (65.94%)	53.0 (66.25%)	38.0 (65.51%)
ε3	10.0 (7.24%)	5.0 (6.25%)	5.0 (8.62%)
ε4	37.0 (26.81%)	22.0 (27.50%)	15.0 (25.86%)
	ε2	**ε3**	**ε4**
Allele frequency Male *vs.* Female	(53/91 vs. 38/91) 58.25% vs. 41.75% [P>0.05]	(5/10 vs. 5/10) 50.0% vs. 50.0% [P>0.05]	(22/37 vs. 15/37) 59.45% vs. 40.54% [P>0.05]

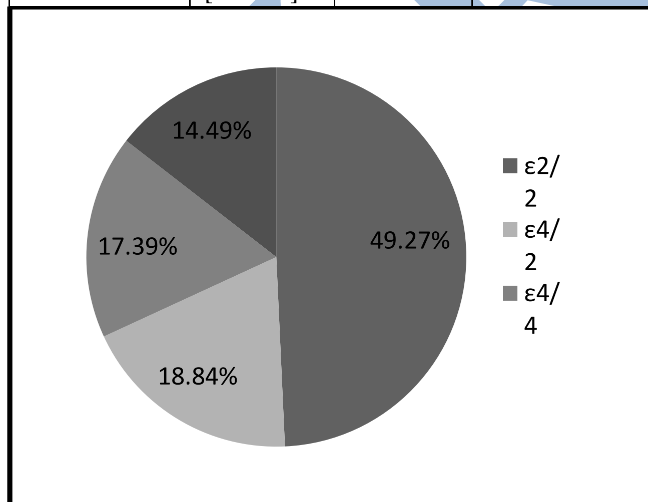

Figure 2. Percentage prevalence of different genotype of *ApoE* gene in our population

In male population, genotype ε2/2 was found to be more prevalent followed by ε4/2, ε4/4 and ε3/2 as shown in the table 3 & Figure 3. Similarly, in female population ε2/2 was found again the dominant genotype. Other genotypes were found to be equally distributed as shown in the table 3 and Figure 3.

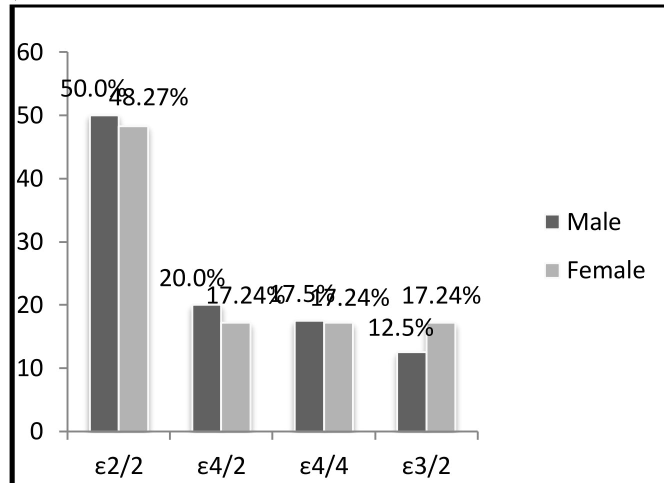

Figure 3. Percentage prevalence of different genotypes of *ApoE* gene in male and female population

Allele **ε**2 (65.94%) was found to be the dominant allele (P<0.05) in our population followed by allele **ε**4 (26.81%) and **ε**3 (7.24%). In both male and female population **ε**2 remains the dominant allele (Figure 4).

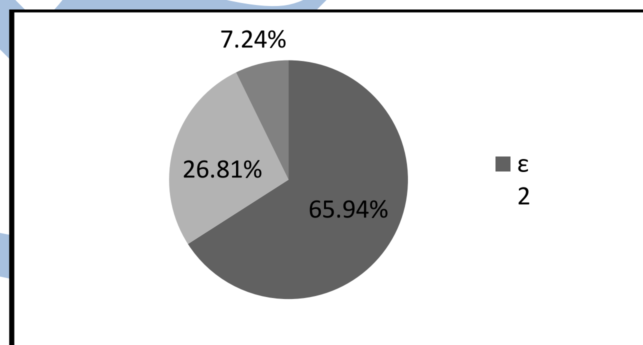

Figure 4. Percentage prevalence of different alleles of *ApoE* gene in our healthy population

Allele **ε**2 (58.25% vs. 41.75%) &**ε**4 (59.45% vs. 40.54%) were observed more with male in comparison to female of our group, whereas allele **ε**3 prevalence was equally distributed in both male and female population as shown in Figure 5.

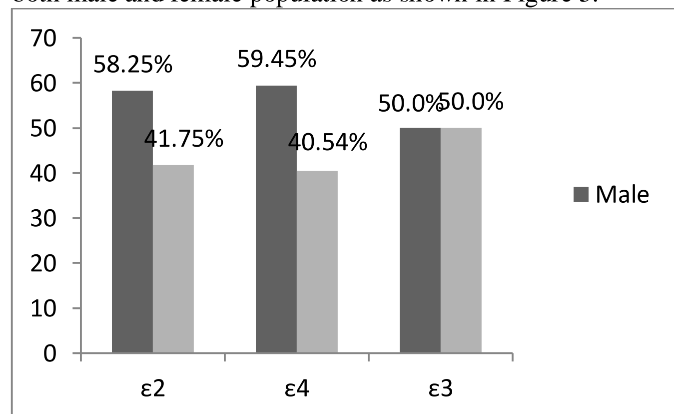

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Figure 5. Percentage prevalence of different alleles of *ApoE* gene in male and female population

IV. DISCUSSION

A complex combination of environmental, pathogen and host genetic factors plays an important role in determining both susceptibility and resistant to HBV infection and its pathogenesis. Many researchers throughout the world found the individual or ethnic differences in infection, severity and outcomes of the disease caused by the HBV [21]. Human genome has nearly 3.5 million SNPs [22] and presences of these SNPs are responsible for the observed genetic diversity between different ethnic groups and also in between different people in the same ethnic population. The virological and immunological factors of HBV infection have been extensively studied, but the literature has very scanty information about the relationship between polymorphisms of *ApoE* gene and HBV infection.

It has been observed by many studies that some alleles of *ApoE* gene have protective effect against some pathogen, but make susceptible to another one. For example, ε4 allele hasbeen found to have protective effect against HCV infection, but a risk factor for herpes labialis infection [18]. It is speculated that *ApoE*-ε2 allele product binds poorly to the LDLR and up-regulate its synthesis and because LDLR facilitate the endocytosis of HBV entry into the hepatocytes this can be the reason why this allele is susceptible to HBV infection [12]. Additionally, same allele product give resistance to HCV infection due to LDLR mediated defective uptake of HCV lipoviral particles [11, 18]. In many studies it has been confirmed that genetic polymorphisms of *ApoE* gene is responsible for differential progression of several pathogenic infection including HCV and HIV [10, 11, 13, 18, 19]. In both male (58.25 %) and female (41.75%) healthy volunteers, we have found ε2 as the dominant allele followed by ε4 and ε3 allele.

If we consider only ***ApoE*** gene influence on HBV pathogenesis then we are at the risk of susceptibility to HBV infection, but safe from the side of HCV infection. This could be the reason why HBV is responsible for 80-90% cases of HCC in our population.Since genetic interactions are very complex and it is unlikely that a single allelic variant is responsible for HBV resistance or susceptibility. So, it was concluded that, the collective influence of several SNPs or haplotype(s) may exert the synergistic protection against HBV.Identification of HBV resistant alleles (alleles which are associated with favorable outcome and low risk of progression of HBV infection) and HBV susceptible alleles (alleles that are responsible for severe HBV infection and high risk of progression of HBV infection) will be helpful in diagnosis and therapeutic strategies in future.

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