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Genotype in Hepatitis B Patients in Tertiary Care Hospital in North Zone of India

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A B S T R A C T

This study evaluated the distribution of HBV genotypes among serological positive patients of HBV infected patients in North zone of India. Samples were selected from HBsAg positive patient and tested for HBeAg. HBeAg positive patient were selected for HBV genotype. Genotype-D was the most prevalent. The present study did not find other genotype. Genotype D is the most prevalent in North India.

Introduction

Hepatitis B virus (HBV) induces a range of liver diseases, ranging from acute or fulminant Hepatitis to liver cirrhosis (LC) and hepatocellular carcinoma (HCC)[(1)]. Hepatitis is self-limiting in most adult patients with acute infection but approximate 1% - 2% of patients progress to fulminant hepatic failure, while some progress to chronic infection[(2)]. The rate of progression from acute to chronic HBV infection is reported to be 90% in newborns and 5% -10% in adults[(3)].HBV is largely transmitted through exposure to body fluids containing the virus through unprotected sexual contact, blood transfusions, re-use of contaminated needles, vertical transmission from mother to child during birth[(4)].

Other risk factors for developing HBV infection are occupational linked like working in a health care setting related to transfusion, dialysis, acupuncture, tattooing, and residence in an institution[(5)]. HBV was discovered by Blumberg in 1965[(6)]. HBV was initially known as serum hepatitis[(7)]. HBV is a small DNA virus with unusual features similar to

retroviruses[8]. The genome of HBV is a partially double-stranded circular DNA of about 3.2 kilobase (kb) pairs[9]. HBV sequence is characterized by > 8% nucleotide differences for genotype and on this bases HBV is characterized into ten genotypes that is A to J[10]. Relationship between different genotypes and geographical distribution with relation to disease progression, prognosis and response to antiviral treatment is also studied and documented[11].

Geographic distribution of HBV genotypes may be correlated to route of exposure for example; genotypes B and C are more common in high-endemic regions and are associated with perinatal or vertical route of viral transmission[12]. For A, D, E route of exposure is pivotal in predicting disease progression and determine appropriate antiviral therapy e.g. acute infection with genotypes A and D leads to higher rate of chronicity[13]. Genotype C generally is considered as a risk factor for perinatal infection[14] and related to severe liver disease, including cirrhosis and HCC[15,16,17]. Patients infected with genotype B or C have a lower opportunity to gain serological response to tenofovir [18,19]

All genotypes A, B, C and D except for E, G and H are subdivided into several geographically localized subgenotype[20]. Subgenotype A1 in central and Southern Africa, subgenotype A2 predominates in northern Europe, subgenotypes B1-B7, C_1 and C2 predominate in Asia[21] while C3, C4 and D4 are the most prevalent on the Australian continent.

Materials and Methods

It was research and observational study done at Govt. Medical college hospital after having permission from Institutional Ethical Committee (IEC) of Government Medical College (GMC) Jammu. Sample received for testing of HBV infection GMCH to OPD (collection centre) of Microbiology GMC Jammu taken in duration of one year (November 2017 to November 2018). Samples were collected from patients of chronic hepatitis B, positive for HBsAg and above 15 years of age with exclusion criteria of patient of acute hepatitis, Cirrhosis, HCC and patient positive for HBsAg, but at same time positive for anti–HDV, anti–HCV or anti –HIV and pregnant women.

5 ml of venous blood was collected from chronic HBV patients attending to the Medicine OPD of Government Medical College (GMC) Jammu. Samples were sent to Department of Microbiology GMC Jammu for further processing. Serum was separated by centrifugation, aliquoted and stored at -20°C. Serum was tested for HBeAg Enzyme linked immunosorbent assay (ELISA) for HBeAg (Make: Bioneovan). Test was performed as per manufactures instructions. All the samples positive for HBeAg were subjected to DNA extraction by Viral DNA extraction kit (Make: Machery Nagel). Primer sequence was designed[22].

P1b -: TCACCATATTCTTGGGAACAAGA (number of bases 23)
S1-2-: CGAACCACTGAACAAATGGC (number of bases 20)
B2-: GGCTCMAGTTCMGGAACAGT (number of bases 20)
BA1R -: CTCGCGGAGATTGACGAGATGT (number of bases 22)
BB1R -: CAGGTTGGTGAGTGACTGGAGA (number of bases 22)
BC1R -: GGTCCTAGGAATCCTGATGTTG (number of bases 22)
BD1-: GCCAACAAGGTAGGAGCT (number of bases 18)
BE1 -:

CACCAGAAATCCAGATTGGGACCA
(number of bases24)

The extracted DNA is subjected to multiplex PCR with PCR primers (outer primer pairs and inner primer pairs) designed on the basis of the conserved nature of nucleotide sequences in regions of the pre-S1 through S genes, irrespective of the six HBV genotypes. P1 (sense) and S1-2 (antisense) with universal outer primers & inner primer (sense)[22]. Extracted DNA & PCR primers will be subjected to PCR cycles.

As PCR completed the product will be subjected into electrophoresis on 1% agarose gel. Sample was loaded in respected well and run at 75 volts for 2 hrs. The sizes of PCR products are estimated according to the migration pattern of a 100 bp DNA ladder**.** Gel was visualized under UV light in E gel imager (Applied biosystem). The gel will then stained by ethidium bromide, the results adjacent to DNA ladder 100 bp will be viewed under UV light[23].

Results and Discussion

Among 50 patients, 29 were positive for HBeAg from 29 HBeAg positive samples, 26 samples showed genotype whereas in 3 samples no genotype was detected. In our study, out of 50 chronic hepatitis B infected patients, 29 (58%) samples were positive for HBeAg. Out of which 26 were positive for Genotype D and in 3 samples no genotype was detected.

Non detection of genotype in three patients would, be due to low viral load, technical error or patients were on treatment since longer duration. Chattopadhyay *et al.,* 2006[24])detected most common genotype D (62.2%) from New Delhi and Tuteja *et al.,* 2014[25] reported most common genotype D (62.2%) from Punjab, Himachal Pradesh, Delhi and other regions of North India. Study done in Uttar Pradesh on 105 samples by Kumar *et al.,* 2011[26] and all HBeAg. Positive and HBeAg negative sample were subjected to PCR genotyping and Genotype D was detected. 78% genotype D was reported by Rizvi, (2018)[27] in Northern region of India. Kumar *et al.,* (2011)[26] reported most common genotype A (53%) in Bihar, Eastern India. Mukherjee *et al., (*2013)[28] detected most common genotype D (84%) in Hyderbad.

Biswas *et al.,* (2013)[29] detected most common genotype A from Eastern India. Borkakotry *et al.,* (2008)[30] reported genotype A (41.6%) in Arunachal Pradesh, Assam, eastern state of India. Banerjee *et al.,* 2006[31] and Chandra *et al.,* (2007)[32] reported genotype D (49.6%) from Kolkata. Ismail *et al.,* (2014)[33] reported Genotype D (77%) in Andhra Pradesh, Chennai and south part on India.

Gopal Krishnan *et al.,* 2013[34] reported Genotype A(72%) in Kerala, South part on India. Kumar *et al.,* (2011)[26] reported that Western India and the Andaman and Nicobr Islands originally both genotype A and genotype D were in equal proportion but latter genotype D was most predominant over genotype A. Shrestha *et al.,* 2012[35] reported genotype D from Nepal.

Mahmood *et al.,* 2016 [36] conducted study at Pakistan reported that most common genotyped D (71.2 %) followed by genotypes A. Ansari *et al.,* 2015,[10] Imam Khomeini Hospital of Ahvaz and reported most common genotype D. Ayaz *et al.,* 2013 [37]conducted study at Kohat region of Khyber Pakhtunkhwa in Pakistan and concluded that genotype A, C, D and F were the most common genotypes. Rahman *et al.,* (2016)[38]detected genotype A in Bangladesh. Ying Ma *et al.,* 2011[39] identified genotype C dominant genotype in patients from Shenyang, China (Fig.1a-d).

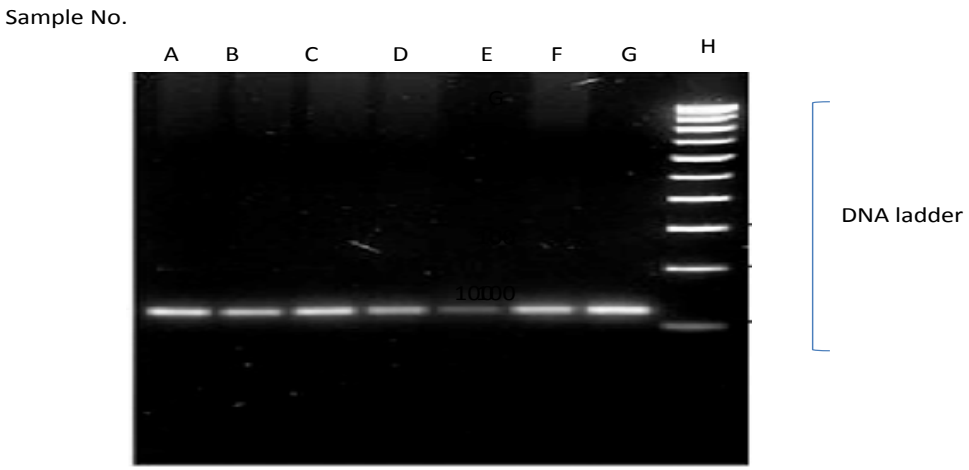

Fig.1a I indicate DNA ladder 100bp (Make:Qiagen) and A,B,C,D,E,F and G are sample number 1,2,3,5.6.13 and 14

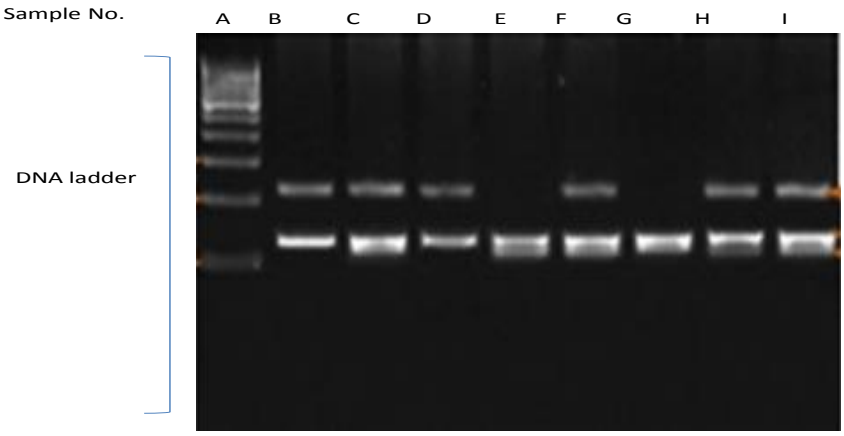

Fig.1b A indicate DNA ladder 100bp (Make:Qiagen) and B,C,D,E,F,G and H are sample number 16,17,18,22,24,25,27 and 28

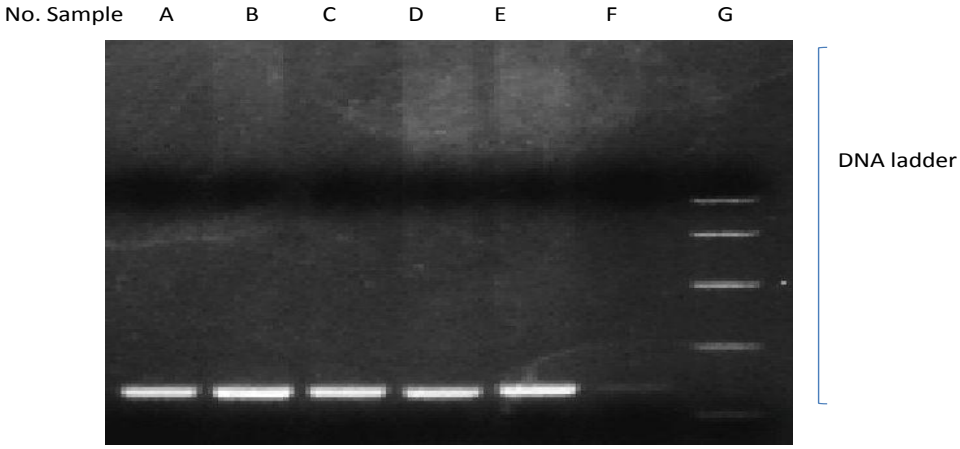

Fig.1c G indicate DNA ladder 100bp (Make:Qiagen) and A,B,C,D,E,F and G are sample number 32,38,39,40,41, and 42

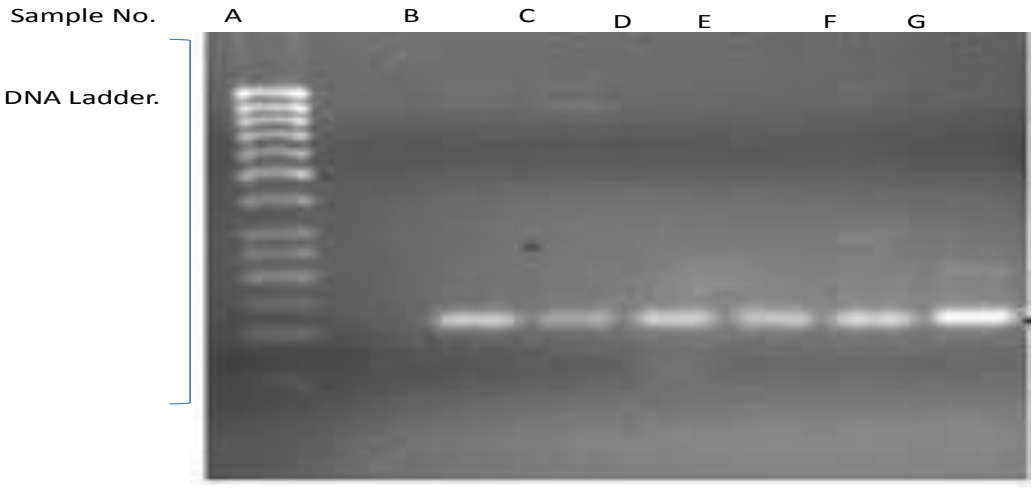

Fig.1d A indicate DNA ladder 100bp(Make:Qiagen) and B,C,D,E, and G are sample number 44,45,46,48,49, and 50.

In the study genotype D was reported whereas in similar study from neighboring state of our region, Himachal Pradesh, Punjab, Delhi most common genotype reported was genotype D. In neighboring state of our region Pakistan, Nepal, Pakistan occupied Kashmir genotype D was reported. Therefore because of geographical niches and due to marriages and duty posting in neighboring states and due travelling in neighboring countries could be the reason for detection of genotype D.

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