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CLINICAL STUDY

Investigation of Hepatitis B Virus DNA among HBsAg Positive Patients in Kabul, Afghanistan

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ABSTRACT
Background: Hepatitis B virus (HBV) infections are one of the world's health problems that annually kill about 500,000 to 1,200,000 people. Investigation of HBV DNA in the person infected with HBV is a definitive indicator of activation and replication of HBV. **Objectives**: The aim of this study is to investigate the DNA of HBV in HBsAg positive patients and to study the risk factors for virus activation. **Methods**: This study was conducted on 106 HBsAg positive patients from January 2020 to Jully 2020 in Kabul. After informed consent, 3 to 5 milliliters of blood was collected for the HBV-DNA testing using the Real-time PCR method. **Result**: Out of 106 HbsAg positive patients, 74 (69.8%) were males and 32 (30.2%) females. The patients were aged between 11 and 65 years. Hepatitis B virus DNA was positive in 58 (54.7%) of the samples, 41 (70.7%) were male and 17 (29.3%) were female. The viral DNA load was in the range of 9.85 x 102 to 9.3 x 108 copies/ ml. Most of the patients were aged between 20 and 30 years. **Conclusion**: From 106 HbsAg positive patient, 23(39.7%) were in age group of 20 – 30 years, and males were more infected than females. Majority of the patients were married and had informal job with education below grade 12. No specific differences were found in the availability of HBV DNA between patients who received hepatitis B treatment before and those who did not.

KEYWORDS: HBV; ELISA; PCR; Kabul; Replication

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INTRODUCTION

Hepatitis B virus (HBV) is still one of the major public health problem in the world. This virus belongs to the family Hepadnaviridae and the genome contains double-stranded DNA. The virus has seven genotypes (A - G)[1]. The genotypes in Afghanistan are D (35.67%), C (32.16%), A (19.30%), B (7.02%) and the rest (6.07%)[2]. Infection due to the HBV is one of the major liver diseases in the world. The virus causes a wide range of liver diseases including acute, chronic inflammation and non-communicable infections. It is also a major cause of liver cirrhosis and hepatocellular carcinoma. More than 350 million people worldwide have chronic HBV[3]. And annually about 500,000 - 1200,000 people die from various HBV-related illnesses, and on average, 5% of people infected with acute hepatitis B become chronic[4]. The risk of developing chronic infections in children under the age of one year reaches 90% and about 25% of them die before adolescence due to cancer and liver cirrhosis[5]. From Untreated chronic hepatitis B patients 15% - 40% may develop cirrhosis and hepatocellular carcinoma[6]. However safe vaccine for prevention is available, still 50 million new cases are diagnosed annually. Various factors such as the country's health policies, health practices, knowledge level, and economic status change the incidence of this infection[7]. HBV can be transmitted through blood products, drug abuse, unprotected intercourse, during childbirth and vertically from the mother to the infant[8].

In the last two decades, serological, virological, biochemical and histological methods have been used to identify HBV infection status. One of the first serologic features to appear after the HBV infection in the patient is the hepatitis B surface antigen (HbsAg). If the antigen is present for more than 6 months, it will be called chronic Hepatitis B infection[9]. HBsAg screening tests have not always been sufficiently sensitive to receive HBV infections. Serologic tests of HBcAg, Anti-HBc IgM, Anti-HBc IgG, and Anti-HBe are also used with HBsAg for the detection of HBV infection[10].

Investigation of HBV DNA in infected patient serum is a good and definitive indicator of HBV activation and replication. It is well known that HBV activation is a key factor in the progression of the disease[11]. For example, high viral load plays a major role in causing chronic form

and low viral load reduces the risk of liver cancer[3]. However, the prediction and relationship between DNA availability indexes in HBs Ag positive sera are still unclear[11]. In vitro Propagation of nucleic acid (NA) sequence is one of the most important steps in molecular biology. This method helps in the determination of drug resistance, treatment follow-up and infection diagnosis. Real time PCR is a simple and fast that allows the quantification of viral DNA[4].

The aim of this study is to investigate the presence of hepatitis B virus DNA by Real-time PCR in HBsAg positive patients and the risk factor for HBV activation among HbsAg positive patients.

MATERIALS AND METHODS

The study was conducted on 106 seropositive patients who referred to City Medical Complex Hospital for PCR examinations. After informing of consent, each individual was interviewed for demographic characteristics. Information form including sex, age, marital status, education, place of birth, history of operation, prison, blood transfusion and occupation, and their consent was completed. 3-5 ml of blood was taken from each patient and labeled with a specific number. DNA amplification done using QIAGEN One-step RT-PCR Kit. The data obtained were analyzed by SPSS version 21.

RESULTS

Hepatitis B virus DNA was detected in a total of 58 out of 106 (54.7%) patients serum samples. HBV DNA measured by Real-Time PCR and the copy number of DNA was 9.85 x 102 to 9.3 x 108 copies/ml (mean±SD, 30716411.83 ± 137956102.2). Of these DNA positive patients, 39 (67.2%) were males and 19 (32.8%) were females and 38 (65.5%) were married. The majority of patients 23 (39.7%) were located at age group 3 (20-30 years) figure 1. From 58 DNA positive patients, 37 (63.8%) patients had education below grade 12. Of these DNA positive patients, 37 (63.8%) of patints had informal jobes. Risk factors and other demographic data are presented in table 1.

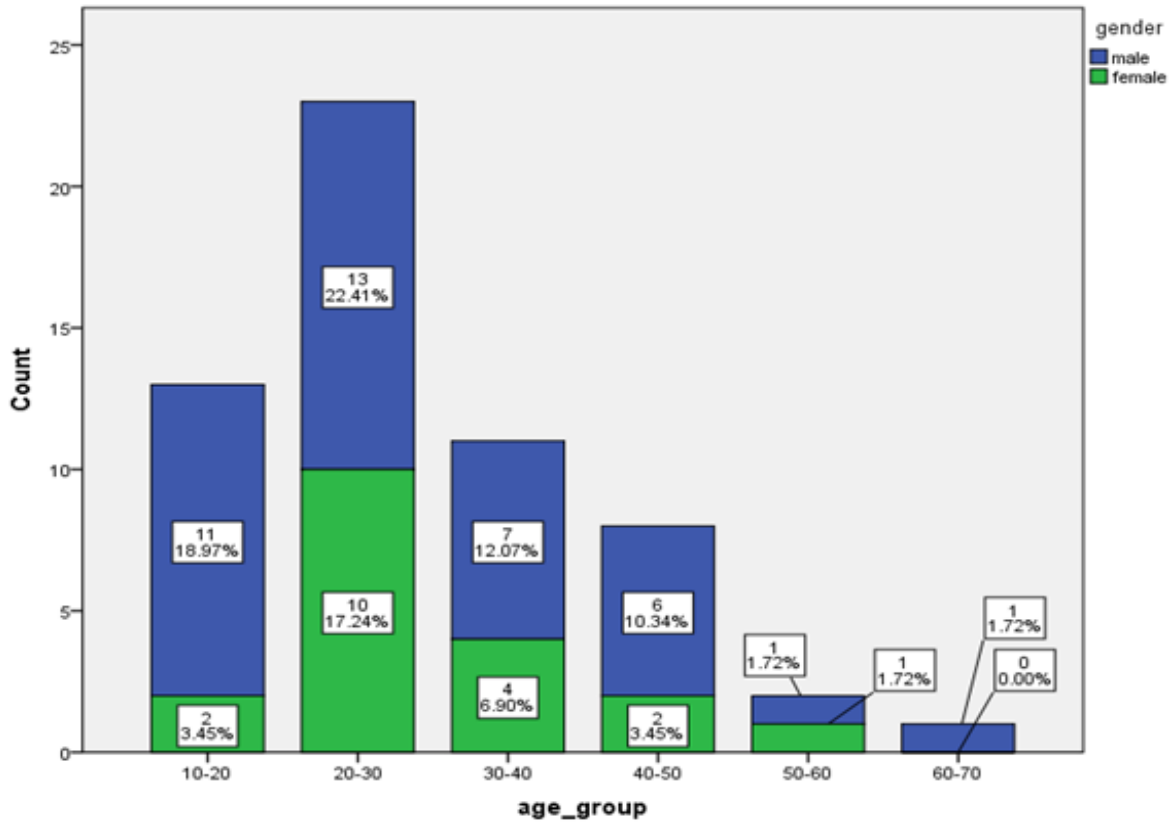

Figure 1: Prevalence of HBV DNA positive patients according to the gender and age-group (years).

DISCUSSION

Although there have been several research done on HBV infection in Afghanistan, there is limited information on HBV DNA level among HbsAg positive Afghan patients. This is the first study of the HBV viral load in Hepatitis B seropositive patients in Kabul-Afghanistan.

This study was conducted on 106 HBsAg positive patients. Hepatitis B virus DNA detected in a total of 58 out of 106 (54.7%) patients serum samples. Despite the study of risk factors in the population under study, the route of transmission was not clear. Exposure to contaminated needle and Blood has been shown to be one of the major transmission routes of the virus in the US and Europe[12].

The results have shown that 12.1% had a history of surgery and 6.9% had a blood transfusion and none of the patients studied had a history of imprisonment and addiction, where drug addicts could play a role in transmitting HBV through partnering with infected needles. According to a new study in Afghanistan, the prevalence of HBV among drug addicts is 3.7%[13], while in another study the prevalence of HBV among drug addicts in Kabul shown 6.5%[14]. The prevalence of HBV among addicts in Iran is 3.9%, and among prisoners, it is 2% [8]. And among injecting drug addicts it is 6%[15].

Table 1: Frequency of Hepatitis B risks factors and Demographic Varibles among HBV DNA Positive patients in Kabul Afghanistan (n=58).

Characteristics		Count	Column N %
Gender	Male	39	67.2%
	Female	19	32.8%
Marital_status	Single	20	34.5%
	Married	38	65.5%
Original_residence	Kabul	6	10.3%
	Other provinces	52	89.7%
Current_residence	Kabul	11	19.0%
	Herat	19	32.8%
	Other provinces	28	48.3%
Education	<12	37	63.8%
	>12	21	36.2%
Addiction	yes	0	0.0%
	no	58	100.0%
Prison	yes	0	0.0%
	no	58	100.0%
Surgery	yes	7	12.1%
	no	51	87.9%
Blood transfution	yes	4	6.9%
	no	54	93.1%
Occupation	formal	11	19.0%
	informal	37	63.8%
	student	10	17.2%
	jobless	0	0.0%
Treatment	treated	26	44.8%
	untreated	32	55.2%
Age group	3 (20-30)	23	39.7%
	2 (10-20)	13	22.4%
	4 (30-40)	11	19.0%
	5 (40-50)	8	13.8%
	6 (50-60)	2	3.4%
	7 (60-70)	1	1.7%

Studies have shown that literacy plays a major role in the control and treatment of HBV infection. This study shows significant correlation between viral load and literacy. From 58 HBV DNA positive patients, 63.8% had education below grade 12 and the majority of patients (63.8%) had informal jobs. Moreover, there is significant correlation between age and presence of viral DNA at the 0.05 level ($p < 0.05$). The result of our study revealed that 67.2% of HBV DNA positive patients were adults' males' which is in agreement with the report in turkey[16]. Male gender is a risk factor for virus activation (presence of virual DNA in blood) since 67.2% of HBV DNA positive cases are males. Horizontal transmission is the most important route of infection to adults during unprotected sex with hepatitis B infected parteners. Having multiple sex partners is unusual in female in Islamic countries. Positive HBsAg may indicate chronic infection[8]. All patients in the study were positive for HBsAg, which may have a chronic infection. 89.7% of HBV DNA positive patients were born in other provinces and 10.3% of patients were born in Kabul, indicating that the majority of the patients were infected with the virus before settling in Kabul.

The limitations of this study include the lack of information about the history of infection, family history, and sex partnership. On the other hand, the majority of patients were not ready to cooperate in the data collection. Also, in this study, we were not able to find HBV genotypes in patients due to lack of access to the necessary equipment and chemicals. Earlier studies have shown that D genotype is the most common genotype in Afghanistan[2].

CONCLUSION

The prevalence of hepatitis B is high in the age group of 20 – 30 years and males are more infected than females. The majority of the patients were married with education under grade 12 and an informal job. Determination of viral load and treatment follow-up can help to reduce the cost of diagnosis and treatment in such patients, as well as, reducing the risk of transmission. No specific differences were found in the availability of HBV DNA between patients who received the hepatitis B treatment before and those who did not.

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AUTHORS' CONTRIBUTIONS

The participation of each author corresponds to the criteria of authorship and contributorship emphasized in the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly work in Medical Journals of the International Committee of Medical Journal Editors. Indeed, all the authors have actively participated in the redaction, the revision of the manuscript, and provided approval for this final revised version.

COMPETING INTERESTS

The authors declare no competing interests with this case.

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