International Journal Dental and Medical Sciences Research
Volume 3, Issue 6, Nov-Dec 2021 pp 450-454www.ijdmsrjournal.com ISSN: 2582-6018

Study of Screening ofCamp BloodDonorsfor Occult Hepatitis B Infection

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Submitted: 05-12-2021 Revised: 17-12-2021 Accepted: 20-12-2021

ABSTRACT: -

Background: Hepatitis B virus (HBV) infection is one of the major global public health problemsinfecting nearly two billion subjects worldwide, of which 250 million livewith HBV infection. Transfusion-associated HBV (TAHBV) is a major problem in India, especially in patients receiving repeated transfusions, despite mandatory screening for HBsAg by ELISA for over 20 years.

Aims and Objectives:

To determine the frequency of Occult Hepatitis B infection in the camp blooddonors.

To determine the number of blood samples with negative ELISA result andpositive PCR amplification of HBV DNA results.

Materials and methods: The present study was carried out over a period of 22 months (January2017 to November 2018), in the department of pathology at a tertiary care center and NationalInstitute of Virology. Total 1200 participants were included in the study whose blood samplescollected were subjected to HBsAg detection by ELISA and for HBV DNA by PCR amplification.

Results: Out of 1200 blood donors 10 were HBsAg reactive donors, 11 donors were PCRreactive and 1 was occult Hepatitis B positive donor. Rate of HBsAg seropositivity was 0.83%.Prevalence of HBV PCR reactive blood donor was 0.91% and occult Hepatitis B reactive donorwas 0.08%.

Conclusion: This study suggests that therisk of HBV transmission from blood donors can be minimized by subjecting the donor blood to PCR assays. The introductionofHBVDNA assays in pre-transfusion blood analysis will be auseful tool in achieving near zero risk of HBV transmission.

Key Words: Occult Hepatitis B infection, ELISA, HBsAg, PCR amplification

I. INTRODUCTION:-

Hepatitis B virus (HBV) remains a major public health problem worldwide[(1)]. Aapproximately two billion of the world population have been infected with hepatitis B virus, out of which about 250 million livewith HBV infection [(2)]. Infection with HBV is highest among developing countries [(3,4)]

Transfusion-associated HBV (TAHBV) is a major problem in India, especially in patients receiving repeated transfusions, despite mandatory screening for HBsAg by ELISA for over 20 years [(5,6)]. It has been demonstrated in studies that transmission of HBV can still occur through blood component which have been tested negative for HBsAg [(7)]. Persistence of HBV DNA in low levels have been demonstrated in serum and liver tissues, both in acute self-limiting or chronic HBV infection after clearance of HBsAg has brought about the concept of occult HBV infection indicating the presence HBV DNA with undetectable HBsAg [(8,9).] The occult hepatitis B infection(OBI) represents a form of hepatitis B with positive/negative antibodies to hepatitis B core antigen in serum but absence of detectable levels of HBsAg, as a result of mutations occurring naturally in thesurface antigen gene of hepatitis B virus (HBV) [(10,11)]. Donors with occult HBV infection are a potential source of HBV infection, who lack detectable HBsAg and might have exposure to HBV infection [(11)]. Hence the aim of this study is to determine the presence/ absence of HBV DNA to assess the magnitude of occult HBV infection in these subjects.

II. MATERIALS AND METHODS: -

The present study, a cross sectional analysis of occult hepatitis B infection in camp blooddonors was carried out over a period of 22 months (January 2017 to NNovember 2018), inthe

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department of pathology at a tertiary care centera. Thestudy was performed with respect to parameters such as age, sex, education, occupation,residence, marital status and the number of times the person has donated the blood. Total1200 participants were included in the study whose blood samples collected in BD vacutainerwith serum clot activator were subjected to HBsAg detection by ELISA and for HBV DNA byPCRamplification.

Inclusion Criteria: All donor samples. (Findings were interpreted in respect to history given by donors in the blooddonation requisition form.)

Data Analysis: Data was analyzed by using Microsoft excel sheet and EPI Info7 software. As the data was qualitative, chi square test was used to assess the associationbetween parameters included in the study. A value of p <0.05 was taken as significant and <0.01as highly significant. Whereas p>0.05 was taken as non-significant.

III. RESULTS: -

In the present study 1200 voluntary donors were included over a period of 22months(January 2017 to November 2018). Out of the 1200 blood donors 1090(91%) were malesand 110(9%) were females. Out of total donors 556(46%) were married and 554(54%) wereunmarried. According to residential area 659(55%) were from urban area while 541(45%)were from rural area. 53% donors were first time donors while 47% were repeat donors. Byoccupation 47%were students, 36% were laborer/workers and 17% were engineers/ officers.Most of the donors were literate. Illiterate donors were 277(23%), primary/secondary schoollevel donors were 150(13%) while 773(64%) were having qualification up to the level ofcollege/university. Most of the donors belonged to the age group of 18-40 years constituting91%(1093); further 13%donors fell in the age group of 18-20 years, 48% were in the age groupof 21-30 years, 30% of the donors fell in the age group of 31-40 years while 8% fell in the agegroup of 41-50 years. Only 1% of the donors fell in the age group of 51-60 years.Out of 1200 blood donors 10 were HBsAg reactive donors, 11 donors were PCR reactive and 1was occult Hepatitis B positive donor. Rate of HBsAg seropositivity was 0.83%. Prevalence ofHBV PCR reactive blood donor was 0.91% and occult Hepatitis B reactive donor was 0.08%.

1) HBsAg Reactive, PCR Reactive and Occult Hepatitis B Donors co-relation with –Marital Status, Area of Residence, Occupation and Education (Table-1)

Parameters	Total donors (1200)		HBsAgReactive Donors n=10		PCR Reactive Donors n=11		Occult Hepatitis B Donors(n=1)	
	No.	%	No.	%	No.	%	No.	%
Marital Status								
Married	556	46%	9	90%	10	91%	1	100%
Unmarried	644	54%	1	10%	1	09%	0	00%
Area of residence								
Rural	541	45%	7	70%	3	27%	0	00%
Urban	659	55%	3	30%	8	73%	1	100%
Occupation								
Labour/Worker	427	36%	8	80%	9	82%	1	100%
Student	565	47%	1	10%	1	9%	0	00%
Engineer/Officer	208	17%	1	10%	1	0%	0	00%

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Education								
Illiterate	277	23%	6	60%	6	55%	0	00%
Primary/Secondary	150	13%	2	30%	3	27%	1	100%
college/University	773	64%	2	20%	2	18%	0	00%

2) Values of HBsAg test compared to the PCR method. (Table-2)

		PCR		Total
		Positive	Negative	
HBsAg ELISA	Positive	10	0	10
	Negative	1	1189	1190
Total		11	1189	1200

3) **Comparison between ELISA and PCR Test with respect to PPV, NPV, Sensitivity and Specificity(Table-3)**

	ELISA	PCR
Positive predictive value (PPV)	100%	100%
Negative predictive value (NPV)	99.92%	100%
Sensitivity	90.91%	100%
Specificity	100%	100%

Prevalence of HBsAg seropositivity was 0.83% and of HBV PCR reactive blood donor was 0.91%.

There was significant association between occurrence of Positivity of HBsAg and HBV PCRpositivity with Marital Status, Type of Area, ooccupation, Education Level.

There was no significant association between occurrence of Positivity of HBsAg and HBVPCR positivity with Male – Female Distribution, Type of Donors, Age wise Distribution.

Prevalence ofOccult Hepatitis B reactive blood donor is 0.08%. There issignificant association between occurrence of Occult Hepatitis B with male-female distribution (p-0.009), Area of residence(p-0.45), Level of education(p-0.03) and Age wise distribution(p-0.019)

There isno significant association between occurrence of Occult Hepatitis B with Marital Status (p –0.463), Type of donor(p-0.472) and Occupation (p-0.404).

The current study helped in determining the prevalence of hepatitis B in camp blood donors using the ELISA and PCR techniques for the determination HBV virus. The overall prevalence of hepatitis B surface antigen (HBsAg) was (9.02%), and hepatitis B DNA (HBV DNA) was the highest one (9.29%).

In comparison between the positive results of PCR technique with those of the ELISA test, the PCR technique is found to be more sensitive and reliable than the ELISA test.

As PCR technique showed 100% sensitivity and specificity, it is assumed as the gold standard method for the detection of HBV infection. Detection rate sensitivity of HBsAg test is 90.91% and its specificity is 100%. Also for HBsAg detection test, positive predictive value (PPV) is 100% and its negative predictive value (NPV) is 99.92%.

IV. DISCUSSION: -

The Prevalence of HBsAg(Hep B infection), varies from place to place. In this study,among the 1200 blood donors screened, the seroprevalence of HBsAg is observedto be 0.83%. According to the WHO classification, this region qualifies as a lowprevalence area (<2%). The lower prevalence may be a result of several factors. One of the important factors being the implementation of strict pre-donation counselling and donor selection criteria help in excluding the possibly infected

donors. The hepatitis B surface antigen (HBsAg) is most frequently used to screen for the presence of this infection [12,13]. It is the first detectable viral antigen to appear during infection [14]. However, early in an infection, this antigen may not be present and it may be undetectable later in the infection as it is being cleared by the host [15]. The obtained results confirm the great importance of the PCR technique in accuracy and reliability of detection and diagnosis of hepatitis viral infection. According to our obtained result, we can strongly recommend the use of PCR as the reliable most accurate test for detecting(HBV) rather than used as confirmatory test to promote the health of the community.

Table no 4)

Study	Year	Place	Prevalence (%) of HBsAg positivity
Nanu et al	1997	Delhi	2.23
Srikant et al	1999	Bangalore	1.86
Chandrasekaran et al	2000	Madurai	4.0
Methai et al	2002	Kerala	3.1
Son wane Bret al	2003	Rural India	2.78(v);4.84(r)
	2007	Maharashtra	2.15
Chattoraj et al	2008	Pune	0.99
Karandeepsingh et al	2009	Costal karnataka	0.62
Gagandeepkaur et al	2010	Chandigarh	0.65(v);1.07(r)

If we compare the HBsAg positivity in other developing countries of the world the rate is quite high as compared to India.

From the findings of this study, it can be inferred that in most of the HBV infection cases, the HBsAg detection method value for the diagnosis of HBV infection is acceptable as compared to the PCR method, except in positive cases with low index that need further investigation with the PCR technique. Similar results were found in the study by Ly et al[16], O‘ Brien et al[17] and Chen et al[18].

V. CONCLUSION:-

This study suggests the residual risk of HBV transmission from blood donors can be minimized by employing PCR assays. Hence, the introduction of HBVDNA assays in pre-transfusion blood analysis willbe a useful tool in the quest to approach nearzero risk of HBV transmission. We therefore recommend that to minimize the risk of acquiring HBV burden in transfusedindividuals, blood donor samples in blood bank should be screened for OBI status by PCR technique prior to transfusion.

Currently, the challenging task in safe transfusion of blood is to identify blooddonorswith OHB, and then to avoid this route of transmission that too by implementing sensitive HBV PCR blood screening technologycost-effectively .

"Compliance with Ethical Standards" –

1) Disclosure of potential conflicts of interest - None . Author 1) Kalpana Baliram Rathod declares that she has no conflict of interest. Author 2) Ujwal R Devakate. Declares that he has no conflict of interest. Author 3) Sudeshna Gajbhiye Declares that she has no conflict of interest.

2) Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution..

3) Informed consent: Informed consent was obtained from all individual participants included in the study.

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