1
2
3
4
5 Screening for occult hepatitis B infection among Chronic Liver Disease patients attending
6 tertiary care hospital
7
8 ABSTRACT

9 **Background and objectives**- Occult Hepatitis B Virus infection (OBI)is defined as a condition
10 when HBsAg virus infection is undetectable in serum, despite the presence of HBV DNA in liver
11 or blood. This study was done to screen for occult hepatitis B infection among patients with
12 CLD (chronic liver disease) attending gastroenterology OPD of tertiary care hospital. **Materials**
13 **and methods**- Observational Cross-sectional study conducted for period of one year.All patients
14 with history of chronic liver disease were tested for HBsAg by ELISA. All HBsAg negative
15 patients were tested for total antiHBc antibody by ELISA and HBV DNA by real time PCR.
16 **Results**- Prevalence of OBI(confirmed OBI) is 2.96% with isolated anti HBc positivity (probable
17 OBI)being 7.24% in this study. Prevalence of seropositive OBI was 1.93% and prevalence of
18 seronegative OBI was 0.96%. Fair agreement was observed between Total antibody against core
19 antigen by ELISA and RTPCR for the diagnosis of OBI(k value=0.354). **Conclusion** It is
20 recommended that Total Anti HBc by ELISA may be used as a surrogate marker for diagnosis of
21 OBI in HBsAg negative patients with CLD and for confirmation HBV DNA PCR should be
22 done if molecular facility is available.

23 **INTRODUCTION**

24 *Hepatitis B Virus* (HBV) infection is a major public health problem which affects
25 approximately 250 million people globally.[1] Occult Hepatitis B Virus Infection (OBI) is a
26 condition in which the HBsAg virus is undetectable in serum despite the presence of HBV
27 DNA in the liver or blood.[2] Occult hepatitis B infection is basically the persistence of HBV in
28 the liver which appears to be a heterogenous entity to the presence of key parameters - Past
29 HBsAg positive chronic Hepatitis B infection , detectable anti-HBc (+/- anti-HBs) antibodies,
30 mutations in specific regions of the HBs Ag coding gene, other conditions such as liver
31 disease, HIV, hepatitis C virus coinfection or immunosuppressive therapies. Based on the

UNDER PEER REVIEW IN IJAR

sensitivity of HBV DNA detection assays, sample size, and real-time PCR detection of HBV DNA in liver tissue and serum, OBI prevalence differs by location. HBV carriers who are negative for HBsAg but have low amounts of HBV-DNA in serum or liver tissue can now be identified thanks to the development of highly selective polymerase chain reaction techniques. In order to avoid the spread of OBI, it is necessary to evaluate occult HBV infection. HBV DNA testing should be implemented in blood donors, chronic liver disease patients, and immunosuppressive patients, as HBV DNA has a lot of clinical significance in particular clinical circumstances.[3]

In 1985, HBV DNA was discovered in patients with chronic liver disease (CLD) who were negative for hepatitis B surface antigen (HBsAg). Until the mid-1990s, this finding was divisive, with approximately equal numbers of research confirming or refuting the original finding. Such discrepancies were because of the differences in epidemiology and performance of the methods utilized for HBV DNA detection. Occult HBV infection (OBI) was emphasised in a significant number of research, confirming the existence and scientific significance of this new aspect of this "old" viral infection.[4] In 2008, an international workshop in Taormina, Italy, where a group led by G Raimondo defined OBI as the presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) of persons who tested HBsAg negative using currently available detection methods tests.[5] Prevalence of OBI is generally underestimated because prevalence in different areas and individuals seems to be higher among patients at high risk for HBV/HCV infection and with liver disease than among individuals at low risk of infection and those without liver disease .[6]

HBV nucleic acid amplification testing (NAT),which is a PCR technique is highly sensitive and specific and it has detection limits of 10 copies HBV DNA per response, became the gold standard for diagnosing OBI. Anti-HBc should only be used as a less-than-ideal surrogate marker for probable detection of seropositive OBI people in cases of blood, tissue, or organ donation, and when immunosuppressive medication is required, if testing by highly sensitive HBV DNA is not possible.[5] The presence of anti-HBc antibody in serum is a crucial factor in OBI detection. There is a requirement for evaluation of Occult HBV infection in order to prevent the spread of Occult Hepatitis B.

This study was done with the aim to assess the prevalence of OBI in a tertiary care hospital, its clinico epidemiological profile and role of anti HBc as surrogate marker of OBI and diagnosis of OBI by PCR.

UNDER PEER REVIEW IN IJAR

64 **MATERIALS AND METHODS**

65 The Observational Cross-sectional study was conducted at Microbiology and Gastroenterology
66 Department of Govind Ballabh Pant Institute of Postgraduate Medical Education and Research
67 New Delhi over one year amongst CLD ,cirrhosis patients attending Gastroenterology OPD .
68 The study was conducted after approval from the Institutional Ethical Committee.

69 **INCLUSION CRITERIA:** All HBsAg negative adult patients (18 -70years) with CLD and cirrhosis.

70 **EXCLUSION CRITERIA**

71 1. All patients less than 18 yrs age.

72 2. All those patients who were not willing to take part in the study.

73 All patients were subjected to the following:

74 1) Informed consent was obtained from all patients before participation in the study.

75 2) History taking was done with special emphasis on history of alcohol intake , history of
76 hepatitis, family history, previous treatment and use of immunosuppressive drugs.

77 3) Thorough clinical examination with special emphasis on local examination of liver and
78 spleen and detection of ascites was done.The clinical history of the patients was recorded
79 by filling a clinical proforma.

80 4)Laboratory investigations: Qualitative screening of serum sample from all study
81 participants for HBsAg was done by ELISA (BIO-RAD MONOLISA HBsAg ULTRA
82 kit) . For all HBsAg negative samples, Total anti HBc antibody ,Anti HBs by ELISA
83 (DIA PRO DIAGNOSTICS BIOPROBE kit) was done and HBV DNA was done by real
84 time PCR. DNA extraction (FAVORGEN BIOTECH CORP kit) from plasma was done
85 and real time PCR for amplification using Geneproof kit for quantitative detection of
86 HBV DNA. Liver function tests (LFT) was performed for all study participants.

87 Probable OBI was diagnosed as a condition when HBsAg is undetectable in serum and only
88 Total antiHBc antibodies are positive by ELISA with no HBV DNA detected by real time PCR..

UNDER PEER REVIEW IN IJAR

Confirmed OBI was diagnosed as a condition when HBsAg is undetectable in serum , total anti HBc(+/-) and anti HBs (+/-) by ELISA and HBV DNA by real time PCR is present in serum.

Seropositive OBI : Presence of HBV antibodies (+anti HBc / +_anti HB s) and HBV DNA .

Seronegative OBI : Total HBV antibodies (antiHBc/antiHBs) are negative and HBV DNA is present.

STATISTICAL ANALYSIS

The collected data was entered in Microsoft Excel and then was analysed and statistically evaluated using SPSS version 25. Qualitative data was expressed in percentage and difference between the proportions was tested by chi square test or fischer's exact test.P value less than 0.05 would be considered statisticaly significant.

RESULT

Total of 207 participants were included in the study. Out of total cases (n=207), patients were mostly in age group of 36-45years of age(46.4%) and only (1%) case was <65 years of age. Mean age was 42.53+-8.80 years. Out of total 207 patients , 182 male patients(87.9%) and 25 female patients(12.1%) were there in the study. LFT was normal (55.6%) in 115 patients and deranged in 92 patients(44.4%). Total antibody against core antigen (Anti HBc by ELISA) was positive in 15 patients (7.24%) (Table 1) out of total number of cases indicating probable OBI. HBV DNA PCR was positive in 6 cases (2.96%) indicating confirmed OBI. Out of 6, 4(1.93%) was prevalence of seropositive OBI and 2 (0.96%) was prevalence of seronegative OBI. Out of total OBI cases(n-6),5 patients were male and of age group 36-45 years(83.3%),1 patient was female in age group 26-35years(16.7%). Table 2 shows the OBI markers in all cases. Out of total 207, 15 samples were positive for Total antiHBc. Out of 15, only 4 were positive for HBV DNA . Out of the 4 (both Total antiHBc and HBV DNA positive cases), only one sample was positive for antiHBs also. Out of 192 samples that were negative for Total antiHBc, only 2 samples were positive for HBV DNA only. There was no statistically significant correlation (table 3) of demographic profile among OBI positive and negative cases . LFT mean values (table 4) of OBI seropositive cases were also comparatively more as compared to OBI seronegative cases with no statistical significance. P value was >0.05 which

UNDER PEER REVIEW IN IJAR

was statistically not significant,hence there was no correlation (table 5) between risk factors and OBI positive and negative cases. Kappa value of 0.354 (table 6) depicts fair agreement between Total antibody against core antigen by ELISA and RTPCR for the diagnosis of OBI. Among confirmed OBI cases by RT PCR, highest HBV DNA level was LOG 5.15IU/ml while lowest HBV DNA level was LOG2.9IU/ml. The correlation between DNA level with total antibody to HBV core antigen (anti HBc) is not statistically significant.

Table 1: OBI prevalence by ELISA and RT-PCR (n=207)

Test		No	%
Anti HBc+ive by ELISA (probable OBI)		15	7.24%
HBV DNA by RTPCR (confirmed OBI)		6	2.96%
Seropositive OBI(N=4) PREVALENCE- 1.93%	Seronegative OBI(N=2) PREVALENCE- 0.96%		

Table 2 : OBI biomarkers in all cases

HBsAg	AntiHBs	Total AntiHBc	HBV DNA	Interpretation	TOTAL CASES
-	-	+	+	Seropositive OBI	3
-	+	+	+	Seropositive OBI	1
-	-	-	+	Seronegative OBI	2
-	-	+	-	‘HBc alone’	11
-	-	-	-	No OBI	190
Total					207

Table 3 : Comparison of demographic profile among OBI positive and OBI negative cases

Gender	**OBI +ve**	**OBI -ve**	**p value**

UNDER PEER REVIEW IN IJAR

Male	5 (83.3%)	177 (88.1%)	0.54
Female	1 (16.7%)	24 (11.9%)	
Mean age in years	40.0±6.03 years	42.6±8.87 years	0.50

135
136
137
138
139
140 **TABLE 4 : COMPARISON OF LFT (MEAN) (SEROPOSITIVE OBI AND SSERONEGATIVE OBI**

141

	OBI (seropositive)	OBI (seronegative)	P value
AST(MEAN)	52IU/l	48IU/l	0.46
ALT	52IU/l	51IU/l	0.45
ALP	88IU/l	90IU/l	0.66
S.BILURUBIN	2.2mg/dl	1.mg/dl	0.56
S.ALBUMIN	3.4mg/l	3.2mg/dl	0.67

142
143
144 **TABLE 5 : Risk factor analysis among OBI cases with OBI negative cases**

	OBI +	OBI-	P Value
H/O alcohol intake	3 (50.0%)	32 (15.9%)	0.06
H/O past CLD	0	17 (8.5%)	1.0
H/O previous treatment	1 (16.7%)	8 (4.0%)	0.23
H/O Malignancy	0 (0%)	0 (0%)	-
H/O family history of CLD	1 (16.7%)	5 (2.5%)	0.16

UNDER PEER REVIEW IN IJAR

H/O previous HBV exposure	0 (0%)	1 (0.5%)	1.0
H/O blood transfusion	0 (0%)	1 (0.5%)	1.0

145
146
147
148
149 **TABLE 6 : Correlation of Total anti HBc by ELISA with RTPCR for OBI diagnosis**

	TOTAL ANTI HBc ELISA POSITIVE	**TOTAL ANTI HBc ELISA NEGATIVE**
RTPCR HBV DNA+ (N=6)	4 (66.66%)	2 (33.33%)
RTPCR HBV DNA- (N=201)	11 (5.47%)	190 (94.52%)

150 **Kappa value = 0.354; p value <0.001**
151
152
153
154
155
156
157 **DISCUSSION**

158 OBI is a clinical condition which is difficult to diagnose and it mainly has two important
159 characteristics: Absence of HBsAg, and low viral replication. Researchers have suggested that
160 the lack of HBsAg in OBI may be due to rearrangements in the HBV genome that interfere
161 with gene expression or lead to the production of an antigenically modified S protein.[7] This
162 study was done with the aim to assess the prevalence of OBI in a tertiary care hospital and the
163 role of Total antiHBc as surrogate marker of OBI and diagnosis of OBI by PCR.
164 In India, majority of research on OBI has been done on blood donors. A total of 207
165 individuals were enrolled in this OBI screening study, mean age being 42.53±8.80 years (table
166 1). Majority of the patients were male 182(87.9%) and 25 were female (12.1%). The

UNDER PEER REVIEW IN IJAR

167 prevalence of OBI in this study(table 2,3) was found to be 7.24% using total antibody against
168 the core antigen(Anti HBc) as surrogate marker of OBI which is lower than other studies in
169 India (10.2–58.8%).[8,9] . However, HBV DNA PCR is recommended for accurate diagnosis of
170 OBI. Overall OBI prevalence using HBV DNA PCR was 2.96 percent in this study, with 1.93
171 percent having seropositive OBI and 0.96 percent having seronegative OBI. Rizvi et al in their
172 study [10] reported a higher OBI prevalence of 25.6% using antiHBc as surrogate marker and
173 7.6% OBI prevalence using HBV PCR . The prevalence of seropositive OBI was higher than
174 that of seronegative OBI in the present study . OBI was found to range from 3.9 percent in
175 Kolkata (eastern India) and 0.78 percent in New Delhi (northern India) to 0.05 percent in
176 Chandigarh (north-western India) and 0.15 percent in Vellore in studies conducted across India
177 (southern India) using the HBV DNA PCR.[8,11,12] According to past studies which were
178 conducted,based on the endemicity of HBV infection, countries were divided into three groups:
179 high, intermediate, and low endemic areas. Hepatitis B virus (HBV) has been discovered in
180 approximately 200 percent of the world's population, with 350 million individuals living with
181 the infection long-term. With a disease burden of around 50 million people (prevalence of 2–
182 7%, with an average of 4%), India is in the intermediate endemicity zone. In tribal regions,
183 pockets of increased endemicity can be identified, where the high burden is sustained by
184 intracaste marriages, tribal customs, illiteracy, and a lack of access to health-care resources. [13]
185 OBI is found in a wide range of people. It is estimated that 1% to 95% of people worldwide are
186 affected, depending on several factors that influence prevalence rates, including: (1)
187 geographical variations (endemicity), (2) patient characteristics, such as the presence of
188 concomitant disorders like diabetes, and (3) the various diagnostic procedures used, each with
189 a different sensitivity.[14,15]

190 In a study published in 2019,Raimando G et al discovered that serum was negative for all
191 HBV infection serological indicators in roughly 20% of OBI, 50% were positive for hepatitis
192 B core antibody (anti-HBs), and 35% were positive for hepatitis B surface antibody (anti-
193 HBc). Based on these HBV antibody profiles, OBI can be categorised as seropositive or
194 seronegative, with seronegative patients testing negative for both anti-HBc and anti-HBs
195 people have lowest HBV levels .[16]

196 In this study, the prevalence of OBI among anti-HBc seropositives was found to be 1.96
197 percent. The variability in OBI prevalence is most likely attributable to the use of HBsAg
198 assays of varied sensitivity, the prevalence of HBV, and the type of population studied. [17]

UNDER PEER REVIEW IN IJAR

Regarding the demographic profile of OBI cases (table 4), 83.3 percent were male with age group 36-45 years while 1(16.7 percent) patient was female in the age group 26-35 years. There was no statistically significant difference regarding age and gender among OBI cases and patients with no OBI . There is very less data on age and gender of individuals with OBI available in previous studies. Most of the studies show generally higher prevalence of HBV infection in men. In the current study, out of 207 total number of patients 182 were male (87.9%)and 25 were female(12.1%) because of which the OBI prevalence might be more in males in this study as the male participants were already more in number. One study on OBI showed 100% males in Western Europe (on a small number of cases), 62% in South East Asia (genotype B/C) and 88% in Italy, Spain, Poland (genotype D). The authors recommended that the difference might be related to the generally more efficient anti-viral activity of the female immune system but this hypothesis would require further studies to be supported by firm evidence. [10,11,12,18] There was a study conducted by Diarra B et al in 2018 and it was found that majority of OBI carriers mainly constituted of men (9/16) in the age group 31–50.[91] In a study condusted in areas of dominance of genotype A2 (Europe), B/C (South East Asia) and D (Mediterranean basin), median age, irrespective of gender,OBI transmission ranges between 45 and 55 years [10,11,12].

Out of total 207 patients , in 115 (55.6%) patients LFT was normal and deranged in 92(44.4%) patients. All cases of OBI had deranged LFT , with mean AST,ALT , bilirubin levels on the higher side in OBI cases when compared to Non OBI cases.. And among OBI cases (table 5), it was found that LFT mean values of OBI seropositive cases were comparatively more as compared to OBI seronegative cases. AST range in OBI positive cases was 48-58U/l, ALT range 40-62U/l, ALP range 24-146U/l, total bilirubin range was 1.3-4mg/dl, Total albumin mean was in a range of 2.4-4g/dl. This is similar to the findings of Rizvi et al [10] where total bilirubin levels were significantly deranged in OBI patients. Although occult hepatitis B infection has been documented in a variety of clinical circumstances, it is unknown if occult HBV causes liver damage. Persistent HBV infections might frequently experience flare-ups in viral replication and liver damage with elevated liver enzymes. A study done previously showed that during the enzyme flares, HBV DNA was detected in 7/7 study patients versus 3/8 controls, p = 0.026 suggesting a relationship between occult hepatitis B and liver damage. It was found that HBV infection can cause flare-ups in viral replication, which were linked to

UNDER PEER REVIEW IN IJAR

elevated liver enzymes.[19] Anti-HBc reactive samples were tested for LFTs in a study conducted by Dhawan HK etal. The serum bilirubin levels were all normal, and two (18%) of the samples had elevated liver enzymes. Increased liver enzyme levels in blood donors indicate underlying hepatitis or liver injury, which can only be ruled out by additional tests like hepatic ultrasonography, liver biopsy, and genetic testing.[20]

Risk factor analysis (table 6) in present study showed that history of alcohol intake was the most common risk factor seen in 50% (3/6) patients out of total 6 OBI cases however it was found to be statistically not significant (P value was >0.05) . In fact there was no statisitical significance between any risk factors among OBI positive and negative cases indicating that none of the risk factors were associated with OBI. In India, alcohol is currently the most common cause of CLD, and this representation has arisen over the previous few years. This is attributed to growing alcohol consumption rates rather than a true drop in HBV infection prevalence (which has remained steady over time) or the influence of vaccination (as the vaccine programme in India on mass basis started only in 2011).[13] Very few studies have analyzed risk factor among OBI cases. One study conducted by Azarkar et al [19] showed that OBI was significantly associated with some risk factors like diabetes, tattooing , tooth surgery, imprisonment, and multisexual behaviors were associated although these have not been seen or reported anywhere else in the literature.

The most reliable OBI biomarker is low-level serum HBV DNA detected using a sensitive, quantitative HBV PCR assay . In the absence of HBsAg and antiHBs antibody, when HBV DNA test is unavailable or intermittent viremia is suspected, detection of anti-HBc antibody, a surrogate marker of OBI, is beneficial.[13] When OBI is characterised only by the presence of anti-HBc antibody, erroneous anti-HBc positivity and negativity in OBI detection should be taken into account. Not everyone who tests positive for anti-HBc also tests positive for HBV DNA. Furthermore, the lack of antiHBc antibody does not rule out seronegative OBI.[6] Although anti-HBc is not an ideal OBI biomarker still it indicates the risk .

OBI can occur after infection, with antibodies to the hepatitis B surface antigen (anti-HBs) and persistent low-level viraemia, escape mutants undetected by HBsAg testing, or healthy carriage with antibodies to the hepatitis B e antigen (anti-HBe) and the hepatitis B core antigen (anti-HBc) (anti-HBc). Anti-HBe and, later, anti-HBc may become undetectable in the latter circumstance over time.

UNDER PEER REVIEW IJAR

263 In the present study, it was found that there is a fair level of agreement (table 7) between total
264 antibody against core antigen by ELISA and RTPCR (Kappa value = 0.354) .This corelation
265 between total antibody against core antigen ELISA and RTPCR was found to be statistically
266 significant as p value <0.001 is less than 0.05.
267 Some researchers believe that the HBsAg immunoassay s inferior sensitivity for detecting
268 HBV compared to polymerase chain reaction (PCR) is to blame for the emergence of OBI.
269 However, the reduced replication rate of HBV seen in OBI cannot be explained by this
270 variation in assay sensitivity. The precise underlying mechanisms of OBI, which could be
271 complex, remain unknown. Both host and viral variables appear to play a role in viral
272 replication inhibition and infection control.[21]
273
274 In earlier studies, evolution of mutations in gene of HBV surface antigen resulted in virus
275 escaping which is undetectable in HBsAg-negative patients by common serological tests
276 hence making it difficult to diagnose Occult HBV infection (OBI) as stated earlier too. It can
277 be diagnosed by highly sensitive and specific molecular biology techniques like the real time
278 PCR.[22] Sensitive detection and quantification of HBV-DNA is essential for assessment of
279 occult HBV infection and therefore Real time PCR assays with remarkable analytical and
280 clinical sensitivity, calibrated against the WHO 1st International standard (HBV-DNA values
281 are reported in the international format (IU/mL)) are needed .[23]
282 The gold standard for OBI diagnosis is the detection of HBV DNA from the liver, as cccDNA
283 persists in the hepatocytes and HBV DNA is many a times detected in the liver when HBV
284 DNA is absent in serum.[6] As obtaining liver tissue is an invasive procedure ,hence obtaining
285 hepatic HBV DNA is difficult in clinical practice. In addition, real-time PCR based assays for
286 serum (or plasma) HBV DNA detection have been used with better sensitivity to detect OBI in
287 majority of cases. To improve the sensitivity of the test, at least 1 mL of serum should be
288 collected when a blood sample is used. Using oligonucleotide primers specific for distinct
289 HBV genomic areas and complementary to highly conserved nucleotide sequences, DNA
290 extracts should be amplified by highly sensitive nested PCR or a real-time PCR approach that
291 can detect fewer than 10 copies of HBV DNA. Each PCR experiment should contain
292 appropriate negative and positive controls. In addition, periodic testing for HBV DNA will
293 improve diagnosis of OBI especially in high-risk patients, as intermittent viremia can occur in
294 occult HBV infection.[24] When highly sensitive HBV DNA testing cannot be performed, anti-

UNDER PEER REVIEW IN IJAR

HBc could be used as a possible surrogate marker for identifying potential seropositive OBI cases. [24]

In this study, among the 6 OBI cases confirmed by quantitative real time PCR for HBV DNA , highest HBV DNA level was 141550IU/ml(LOG 5.15IU/ml) and lowest was 912IU/ml (LOG2.9IU/ml). A study conducted by Bajpai et al in New delhi [24]observed that HBV‑DNA levels in OBI cases ranged from log 2.19 to 4.93 IU/ml with median log value of 3.16 IU/ml .It was observed in the present study that on correlating LFT with viral load , mean AST , ALT ,S BIL,S ALB was found to be comparatively lower in OBI cases with HBV DNA <2000IU/ml as compared to those with HBV DNA >2000IU/ml although it was not statistically significant. A study conducted by Dhawan et al, suggested a relationship between occult hepatitis B and liver damage. HBV infection was seen to be showing flare-ups in viral replication, which were linked to elevated liver enzymes.[5,20]

CONCLUSION

OBI has high prevalence among patient of CLD. All HBsAg negative with total anti HBc positive individuals are probable OBI and it is recommended that all such cases should be screened with PCR for HBV DNA.

For confirmed OBI, HBsAg negative patients should be tested for Total anti HBc by ELISA and HBV DNA by PCR. It is recommended that real time HBV DNA PCR should be performed in all HBsAg negative patients with chronic hepatic inflammation (deranged LFT).

Total Anti HBc by ELISA may be used as a surrogate marker for diagnosis of OBI if molecular facility is not available.

References

1. Makvandi M. Update on occult hepatitis B virus infection. World J of gastroenterol.2016; 22(39):8720.

UNDER PEER REVIEW IN IJAR

324 2. Sood AK, Pangotra C , Manrai M. Prevalence of occult hepatitis B infection in patients
325 visiting tertiary care hospital. MJAFI 2016;72(2):140-4.

326 3. Akram A. Occult Hepatitis B Virus Infection: A Review Update. Bangladesh J Infect Dis.
327 2018 ;5(1):32-8.

328 4. Allain JP. Global epidemiology of occult HBV infection. Ann Blood. 2017;2(7):1-3.

329 5. Raimondo G, Allain JP, Brunetto MR. Statements from the Taormina expert meeting on
330 occult hepatitis B virus infection. J Hepatol 2008;49:652-7.

331 6. Shire NJ, Rouster SD,Blackard JT,Martin CM, Fichtenbaum CJ et al. The prevalence and
332 significance of occult hepatitis B virus in a prospective cohort of HIV- infected patients.J
333 Acquir.Immune Defic.2007;44:309-314.

334 7. Levast M, Larrat S, Thelu MA. Prevalence and impact of occult hepatitis B infection in
335 chronic hepatitis C patients treated with pegylated interferon and ribavirin. J Med Virol2010;
336 82(5): 747–754.

337 8. Asim M,Ali R, Khan LA, Husain SA, Singla R and Kar P. Significance of anti-HBc
338 screening of blood donors and its association with occult hepatitis B virus infection:
339 Implications for blood transfusion. Indian J Med Research 2010;132:312-7.

340 9. Chaudhuri V, Nanu A, Panda SK, Chand P. Evaluation of serological screening of blood
341 donors in India reveals a lack of correlation between antiHBc titre and PCR amplification
342 HBV DNA Transfusion 2003;43:1442-8.

343 10. Rizvi M, Azam M, Sultan A, Shukla I, Malik A, Ajmal MR et al. Prevalence of genotype D
344 in chronic liver disease patient with occult HBV infection in northern region of India. Indian
345 J Pathol Microbiol. 2014;57:537-41.

346 11. Bhattacharya P etal: Significant increase in HIV, HBV, HCV and syphilis infections
347 among blood donors in West Bengal, Eastern India 2004-2005: exploratory screening
348 reveals high frequency of occult HBV infection. World J Gastroenterol 2007,13:3730-
349 3733.

350

351 12. Makroo RN,Chowdhary M, Bhatia A, Arora B, Rosamma NL. Hepatits B core antibody
352 testing in Indian Blood donors: A double-edged sword! Asian J Transfus Sci.2012;10-3.

353

UNDER PEER REVIEW IN IJAR

13. Puri P. Tackling the hepatitis B disease burden in India. *J Clin Exp Hepatol.* 2014;4:312–319.
14. Schmeltzer P, Sherman KE. Occult hepatitis B: clinical implications and treatment decisions. Dig Dis Sci 2010; 55: 3328-3335 [PMID: 20927592 DOI: 10.1007/s10620-010-1413-0]
15. Samal J, Kandpal M, Vivekanandan P. Molecular mechanisms underlying occult hepatitis B virus infection. Clin Microbiol Rev 2012; 25: 142-163 [PMID: 22232374 DOI: 10.1128/CMR.00018-11]
16. Raimondo G etal; Update of the statements on biology and clinical impact of occult hepatitis B virus infection. J Hepatol. 2019 Aug;71(2):397-408.
17. Keechilot CS, Shenoy V, Kumar A, et al. Detection of occult hepatitis B and window period infection among blood donors by individual donation nucleic acid testing in a tertiary care center in South India. Pathog Glob Health 2016;110(7-8):287–291
18. Zahn A, Li C, Danso K, et al. Molecular characterization of occult hepatitis B virus in genotype E-infected subjects. J Gen Virol 2008;89:409-18.
19. Azarkar Z, Ziaee M, Ebrahimzadeh A, Sharifzadeh G, Javanmard D. Epidemiology, risk factors, and molecular characterization of occult hepatitis B infection among anti-hepatitis B core antigen alone subjects. J Med Virol. 2019;91:615–622. https://doi.org/10.1002/jmv.25343
20. Dhawan, Hari-Krishan et al. "Anti-HBc screening in Indian blood donors: still an unresolved issue." *World journal of gastroenterology* vol. 14,34 (2008): 5327-30. doi:10.3748/wjg.14.5327.
21. Liu CJ etal. Transmission of occult hepatitis B virus by transfusion to adult and pediatric recipients in Taiwan.*J Hepatol* 2006; 44: 39-46
22. Kannangai R etal. Liver enzyme flares and occult hepatitis B in persons with chronic hepatitis C infection. J Clin Virol. 2007 Jun;39(2):101-5.
23. Raimondo G, Pollicino T, Squadrito G. What is the clinical impact of occult hepatitis B virus infection? Lancet 2005; 365: 638-640 [PMID: 15721459 DOI: 10.1016/S0140-6736(05)17961

UNDER PEER REVIEW IN IJAR

24. Bajpai V, Gupta E, Chaudhary A, Bajpai M. Clinical relevance of hepatitis B core total antibody in the detection of occult hepatitis B infection in patients with liver disease and blood donors. Asian J Transfus Sci. 2021 Jul-Dec;15(2):256-257.

UNDER PEER REVIEW IN IJAR

416
417
418
419
420
421
422
423
424
425
426

UNDER PEER REVIEW IN IJAR