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Prevalence of Occult Hepatitis B Virus infection in HBsAg negative adult patients with Acute Leukemia in a tertiary care cancer centre in South India

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Abstract

Background: Though typically being a hepatotrophic virus (1), HBV also binds to peripheral blood mononuclear cells (PBMC) and infects hematopoietic cells. HBV infection has been associated with an increased risk for hematologic malignancies. (2,3) Occult HBV DNA persists in the lymphoid cells of the individual (4) and it is difficult to detect persistence of occult hepatitis B virus infection by routine HBV surface antigen testing. Anti-HBc may be used as a surrogate marker for detection of OBI. Although it may not be as sensitive as molecular methods, it has been shown to be an useful screening tool for detection of occult hepatitis B virus infection (5). We enrolled 70 leukemia patients for the study and tested them for anti HBc. We later analysed only 69 of these patients for OBI as 1 patient was HBsAg positive (overt HBV infection) and hence excluded from the study. We found 4.35% of the patients to be anti HBc positive indicating occult HBV infection.

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Introduction

Hepatitis B virus (HBV) is a DNA virus with Pro-oncogenic properties which infects nearly 250 million people worldwide and is a leading risk factor for the development of hepatocellular carcinoma causing nearly 340,000 liver cancer cases annually (1). HBV has been classified as a group 1 human carcinogen and apart from HCC, studies have shown an increase in the incidence of haematological malignancies in persons with hepatitis B virus. (2,6) While increased prevalence of overt HBV infection (Hepatitis B surface antigen (HBsAg) positive) has been already reported in the serum of patients with leukemia, (7) occult HBV infection may also have a role to play in development of leukemia. HBV binds to peripheral blood mononuclear cells (PBMC) and infects hematopoietic cells and their progenitor cells. (8) HBV DNA replicative intermediates, HBV mRNAs, and expression of HBV-specific proteins - HBsAg and HBV-DNA have been detected in PBMCs.(4)

HBV-associated carcinogenesis is a multi-factorial process that includes both direct and indirect mechanisms that might act synergistically. HBV DNA integration into the host

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genome occurs at early steps of clonal tumor expansion and induces both genomic instability and direct insertional mutagenesis of diverse cancer-related genes.
HBV can promote carcinogenesis by three different mechanisms. (9)
a) Integration of viral DNA into host pro-oncogenic genes like TERT, CCNE1, and MLL4.
b) Promotion of genomic instability as the result of both the integration of viral DNA into the host genome and the activity of viral proteins
c) Ability of wild-type and mutated/truncated viral proteins (HBx, HBc and preS) to affect cell functions, activate oncogenic pathways.

HBV and hematopoietic malignancies
HBV infection has been associated with an increased risk of hematologic malignancies like malignant lymphoma, Acute myeloid leukemia and non-Hodgkin's lymphoma (NHL). (2,3) Serological studies have demonstrated an increased prevalence of NHL in patients infected with Hepatitis B Virus (HBV) infection. Risk of B-cell subtypes of NHL, especially DLBCL has been documented to be significantly elevated in HBV infected patients. (2)

Occult HBV infection
Occult hepatitis B infection (OBI) is HBV persisting latently in hepatocytes and lymphocytes despite patient being HBsAg negative. (10) The molecular basis of persistent OBI is related to the long-lasting persistence of HBV cccDNA in the nuclei of hepatocytes and/or lymphocytes with very low levels of viral replication. Where molecular methods are not available, anti HBc is commonly used as a surrogate marker for detecting OBI.
Based on anti HBC profile, OBI may be distinguished as Seropositive and Seronegative occult HBV infection.
There have been studies (including studies from our Institute/Department) in acute leukemia patients, showing evidence of OBI that may partly explain the Hepatitis B virus reactivation in patients in whom anticancer chemotherapy has been started. (11)

Methodology
This was a prospective study and samples were collected for a period of 6 months from January 2021 to June 2021. A total of 70 samples were collected from Acute leukemia patients attending a tertiary care cancer centre in Southern India. The study subjects were newly diagnosed adult patients (over 18 years of age) with clinically and haematologically confirmed acute leukemia. Institute Ethical clearance was obtained prior to the study and informed consent was taken from all patients. Only HBsAg negative patients were enrolled as we wanted to study OBI.
Five ml of peripheral blood was obtained from patients with acute leukemias. Blood was collected in tubes with Ethylenediamine tetra acetic acid (EDTA) as anticoagulant and stored at 4°C till plasma was separated the same day for testing later. EDTA blood was centrifuged at 4000 rpm for 10 mins and the plasma was separated in a sterile cryovial which was used to perform HBV serological markers. All samples were handled following standard precautions. (12)
Serological markers.
Hepatitis B surface antigen (HBsAg) and HIV were tested in all patients by standard chemiluminescent immunoassay. All assays were carried out and results interpreted according to the manufacturer’s instructions.

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Serological analysis of OBI

Total Anti-HBc antibodies was tested using a competitive ELISA kit (MBS NEW S.R.L: C1221-70). It is a one-step competitive Immunoassay for the detection of total antibodies to Hepatitis B Virus (HBV) core antigen in human plasma. The assay was carried out according to manufacturer's instructions. Briefly, after addition of conjugate, microplate was sealed and incubated. After washing, chromogen/substrate was added and incubated. After blocking the Enzymatic reaction with stop solution, the plate was read at 450 nm using a Microplate Spectrophotometer.

Results

A total of 70 patients diagnosed with acute leukemia were enrolled in the study. All patients were HIV non-reactive. One patient was HBsAg positive and hence excluded from the final analysis. Of the remaining 69 patients, 50 were male and 19 were female patients. The median age of the patients ranged from 18 to 46 years of age. Past HBsAg vaccination status could not be elicited and was unlikely in our patients as most of them were from the lower socio-economic status. Hence, as per our Institute policy, in order to prevent reactivation hepatitis post anticancer chemotherapy induced immunosuppression, all patients under medical Oncology are administered HBsAg vaccination course before starting chemotherapy. Fifty-one patients had received this pre-chemotherapy hepatitis B vaccination on diagnosis of acute leukemia, while 18 had not yet been vaccinated at the time of sample collection. Sixty-three Patients had already been started on chemotherapy while 6 patients were treatment naive at the time of sample collection. One patient had type 2 diabetes and was on anti-diabetic medication, but other patients did not have any associated co-morbidities.

Leukemia subtype	**Number**	**Anti-HBc positive**
Pre-B-ALL	10	1
Pre-T-ALL	1	-
T-ALL	3	-
B-ALL	3	-
ALL (not subtyped)	18*	-
AML-M2	11	1
AML-M4	6	-
AML-M7	1	-
AML (not subtyped)	17	1
TOTAL	70	3

* One of the patients was an 18-year-old female HBsAg positive patient and therefore excluded from diagnosis of OBI.

Footnote: ALL: Acute Lymphoid Leukemia, AML: Acute Myeloid Leukemia

Anti-HBc total antibody detection using ELISA was positive in 3 (2 female and 1 male) out of 69 patients tested. Out of the 3 patients, 2 patients with AML and 1 with Pre-B-ALL tested positive for Anti-HBc.

Discussion:

According to Global Cancer Observatory (GLOBOCAN), the number of newly diagnosed leukemia cases have increased globally from 354.5 thousand in 1990 to 474.5 thousand cases in 2020, constituting 2.6 % of all cancers worldwide. (13) In India, there were 48,419 new cases of leukemia (3.7% of all cancers) in 2020 with 35,392 deaths.

Considering this huge burden of leukemias, it becomes imperative to find out newer mechanisms of patho-oncogenesis with a future aim to reduce the incidence of these

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apparently ‘non communicable’ cancers. HBV is not typically considered causal in the development of leukemias. However, keeping in mind the ability of this potential oncogenic DNA virus to infect not only hepatocytes but also haematopoietic cells both overtly and occultly, it would be useful to study the association of HBV with haematological malignancies also. Prevalence rates of OBI especially in cancer patients gave a wide range of prevalence rates ranging from 1–87% though there is limited information of studies linking the association of OBI in patients with leukemia. (14)

Not only overt but occult HBV infection also plays a major role in oncopathogenesis of haematological malignancies. In resource limited settings where it may not be feasible to perform molecular tests routinely, the anti-HBc assay can also help in identifying majority of these occult infections. Anti-HBc persists for years following acute HBV infection, therefore it is often used as a surrogate marker for detecting past infection with HBV and OBI. In the present study, 4.35 % of HBsAg negative acute leukemia patients were anti-HBc positive. Although a small percentage, occult HBV infection could possibly play an etiopathological role in the oncogenesis. An OBI prevalence rate of around 5% indicates that there is a small risk of immunosuppression induced HBV reactivation hepatitis in acute leukemia patients, and hence it is a good practice to vaccinate the patients before initiating chemotherapy. Moreover, as a part of serological surveillance, in addition to screening for HBsAg, cancer patients should also be screened for OBI as HBV is known to have pro-oncogenic properties. Some cases of seronegative OBI maybe missed by serological tests alone and hence OBI should ideally be tested my molecular methods wherever possible.

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