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ASSOCIATION OF HHV-6 WITH HUMAN CANCERS

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Abstract : The seroprevalence and titre of IgG antibodies to human herpesvirus-6 (HHV-6) were studied in normal subjects and in patients with Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), acute lymphoblastic leukaemia (ALL) and carcinoma of uterine cervix (CC), breast (BC) and oral cavity (OC) using immunofluorescence and immunoperoxidase techniques. All the HL and ALL patients were positive for the antibody and in these groups the titres were highly elevated. NHL, CC and BC pateints showed no significant elevation in titre, though a slight increase in the prevalence (95%) was noted in NHL only. Seroprevalence and titre were found highly elevated in OC patients.

1. Introduction

Human herpesvirus-6 (HHV-6), initially described as human B-lymphotropic virus (HBLV), was first isolated by Salahuddin et al. (1986) from the peripheral blood lymphocytes of 4 patients with various lymphoproliferative disorders and 2 patients with AIDS. Since then, the virus has been isolated from patients with various other diseases and normal individuals, and seroepidemiological studies have shown it to be ubiquitous in human populations. Studies conducted in America, Europe, Africa, Japan and Malaysia showed the presence of serum antibodies to HHV-6 in 60 to 87% of normal adults, with titres of 10 to 160 (Ablashi et al., 1988; Yadav et al., 1991). Elevated levels of anti-HHV-6 antibodies have beeen found in patients with Exanthum subitum, chronic fatigue Syndrome, Kikuchi's lymphadenitis, a type of mononucleosis, AIDS, some lymphoid malignancies and in organ transplant patients on immunosuppressive drug therapy (Ablashi et al., 1991).

Studies have been made on the seroprevalence and titre of the antibodies to HHV-6 in normal subjects and in patients with some types of malignancies (Clark et al., 1990; Yadav et al., 1990 a, b; Yadav, 1991). Yadav (1991) reported not only a high prevalence of the antibody in lymphomas but also high titres of 160 to 5120. Investigations have not yet been made in India either on the seroprevalence or on the levels of anti-HHV-6 antibodies in normal population or in cancer patients. Hence this preliminary study.

2. Materials and Methods

Twenty-eight patients with Hodgkin's lymphoma (HL), 59 non-Hodgkin's lymphoma (NHL), 51 acute lymphoblastic leukaemia (ALL) 127 oral cancer (OC), 50 breast cancer (BC) and 50 patients with carcinoma of uterine cervix (CC) were included in the study. One-hundred and fifty age-matched, healthy subjects served as control. None of the patients had undergone any type of treatment for cancer prior to the blood collection for the study. Venous blood was collected, serum separated, inactivated at 56°C for 30 min, cleared by centrifugation, and as far as possible, the tests were carried out on the day of collection itself, or else the serum was stored at -70°C for maximum period of 2 weeks.

The presence of IgG antibodies to the viral capsid antigen of the Z29 strain of HHV-6 and the antibody titre in serum were evaluated using indirect immunoflouresecence assay and im-

Table 1. Antibody prevalence and titre to human herpesvirus-6 (HHV-6) in the sera of normal subjects and of cancer patients

Subjects	Prevalence	Antibody titre Range	Mean
Normal			
Control (n=150)	78%	10-160	48
NHL (n=59)	95%	10-320	88
HL (n=28)	100%	320-5120	1109
ALL (n=51)	100%	160-5120	1208
CC (n=50)	74%	10-160	53
BC (n=50)	78%	10-160	62
OC (n=127)	96%	80-10240	2042

NHL: Non Hodgkin's Lymphoma; HL: Hodgkin's Lymphoma; ALL: Acute Lymphoblastic Leukaemia; CC: Uterine Cervix Cancer; BC: Breast Cancer; OC: Oral Cancer.

munoperoxidase techniques. Molt-3 cells, infected with HHV-6 in culture, were used as the source of HHV-6 viral capsid antigen (Yadav et al 1990a). The cells were maintained in RPMI-1640 medium with 10% fetal calf serum (5% CO2, at 37°C) and were infected with the Z29 strain of HHV-6 in the presence of polybrene (2ug/ml). Post-infectionally, the Molt-3 cells were grown in the medium supplemented with 5% fetal calf serum until 50% or more atypical blast cells were present in the culture. The Molt-3 cells were harvested, washed 3 times with phosphate buffered saline (PBS), smeared on teflon-coated slides with circular windows, fixed in cold acetone for 10 min and stored for a maximum period of 3 weeks at -20°C.

Indirect immunofluorescence assay was done following Yadav and Ablashi (1990). Starting from 1/10, a series of 2-fold dilutions of the sera were prepared. The slides with the smears of HHV-6 infected Molt-3 cells were incubated with the diluted sera at 37°C for 30 min in a humidity chamber, washed with PBS, then incubated with flourescein-conjugated anti-human IgG antibody (Behringwerke, Germany) at 37°C for 30 min, washed thoroughly in PBS, dried, mounted with fluoremout (Pan-data Systems, USA) and covered with coverslips. The slides were observed under the 100x objective of a fluorescence microscope. Positive and negative control sera were routinely incorporated in every batch.

Immunoperoxidase staining was carried out following Jones and Gregory (1988). Endogenoeus peroxidase activity was blocked by treating the smears with 0.3% H202 in methanol for 10 minutes. Incubation with the test sera was done as detailed above. The smearss were washed, and then incubated with horse radish peroxidase conjugated anti-human IgG antibody (Dakopat) at 37°C for 45 min and washed thoroughly in PBS. Subsequently, PBS containing 0.05% diaminobendidine and 0.01% hydrogen peroxide was added as the substrate, incubated at room temperature for 5 min and washed thoroughly in PBS. The slides were mounted with aqua- mount (BDH), covered with coverslips and studied.

3. Results and Discussion

The results of the study are presented in Table 1 and 2, The data obtained from immunofluorescence and immunoperoxidase techniques were identical in the initial standardisation studies, and hence the results are not given separately. The prevalence of anti-HHV-6 antibodies in normal control was 78%, and the titre ranged from 10 to 160 (mean, 48). In HL and ALL, titres of 320 to 5120 (mean, 1109) and 160 to 5120 (mean, 1208) respectively. The prevalence of the antibody in the sera of NHL patients was elevated to 95%, and the titre was slightly elevated, 10 to 320. CC and BC patients showed no elevation either in the seroprevalence or titre of the antibodies. In OC prevalence was elevated to 96% and the titre was very high, 80 to 10250 (mean, 2042).

Table 2. Anti-HHV-6 antibody titres in the sera of normal subjects and of patients

Subjects	Total Numbers	No. of persons having the antibody titre of <10	10-80	160-640	1280-5120	10240
Normal	150	33	102	15	-	-
NHL	59	3	36	20	-	-
HL	28	-	-	12	16	-
ALL	51	-	-	35	16	-
OC	127	4	7	51	54	11
CC	50	13	35	2	-	-
BC	50	11	33	6	-	-

Yadav (1991) reported a high prevalence (80%) of anti HHV-6 antibodies in the sera of HL and ALL patients. In the present study, we observed the presence of the anit-viral antibody in all patients with HL and ALL. The titre of the antibody observed in the present series of patients was same in HL and ALL, but was considerably higher than the titres reported by Yadav (1991). It is known that in HL and ALL, the blast cells include those with T-cell markers (Kadin, 1985). HHV-6 has been reported to infect T-lymphocytes at various stages of differentiation in vivo (Ablashi et al., 1990; Yadav et al., 1991). The high prevalence and titre of anti-HHV-6 antibody in the sera of HL and ALL patients may be due to an increase in the number of cells with HHV-6 receptors in these diseases. Another possible explanation might be that the impairment of cellular immune response caused by HL and ALL permits the reactivation of a latent infection leading to increased levels of antibody.

Oral cancer is the most prevalent cancer in India, and forms about 27% of the total cancer incidence in this part of the country. There are no previous reports on the association of HHV-6 with oral cancer. In the present study, anti-HHV-6 antibody was observed in 96% of the serum samples from OC patients. The titre of the antibody was highly elevated in the vast majority of the patients. Vijayakumar and Vasudevan (1985) have observed derailment of cell-mediated immunity in OC patients. This may lead to the reactivation of the virus, which in turn could result in the elevated levels of the antibody. HHV-6 has been reported to be cytopathic to the host T-cells, which are important in cell-mediated immunity. A reactivated HHV-6 infection could possibly increase the severity of the disease by further weakening the already impaired immune response. Alternatively, the derangement in immunity in OC may be due to the newly acquired HHV-6 infection. Further work is underway to understand the role of HHV-6 in the pathogenesis of OC, and also to see whether the viral sequences become integrated into the genetic material of oral cancer cells.

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