JOURNAL OF EXPERIMENTAL PATHOLOGY
Volume 6, Number 2, 1992
Mary Ann Liebert, Inc., Publishers

Anti-HHV-6 Antibodies in Normal Population and in Cancer Patients in India

K.R. SHANAVAS,[1] V. KALA,[1] D.M. VASUDEVAN,[1] T. VIJAYAKUMAR,[2] and M. YADAV[3]

[1]Department of Biochemistry[1], Medical College, Trichur, Kerala, India
[2]Department of Science, Technology and Environment, Trivandrum, Kerala, India
[3]Department of Genetics and Cellular Biology, University of Malaya, 59100 Kuala Lumpur, Malaysia

ABSTRACT - The prevalence and titre of IgG antibodies to human herpesvirus type 6 (HHV-6) were assayed in the serum samples from normal subjects and patients with Hodgkin's lymphona (HL), non-Hodgkin's lymphoma(NHL), acute lympho-blastic leukaemia (ALL) and oral cancer (OC) using immunfluorence and immunoperoxidase techniques. This forms the first study on the sero-prevalance and titre of anti-bodies to HHV-6 in India. There was no considerable difference in the prevalance (76%) and titre (10-160) of the antibodies in normal population from those reported for normal adults in other parts of the world. All the HL and ALL patients studied showed no significant elevation in the antibody titre, though a slight increase in the prevalence (95%) was noted. Antibody titre and prevalence were found highly elevated in OC. OC remained totally unstudied for the presence of anti-HHV-6 antibodies, and this is the first report of elevated levels of the antibody in this cancer. The role of HHV-6, if any, in the pathogenesis of OC is worth investigating.

INTRODUCTION

SALAHUDDIN et al. (1986) isolated a new virus f peripheral blood lymphocytes of 4 patients with lymphoroliferative disorders and 2 patients with Al virus was named Human B-Lymphotropic Virus (HBLV) some of the patients from whom the virus was deri B-cell lesions. Subsequent molecular biological and structural studies, however, revealed that this vi typical features of a Herpes virus which predominantly the T-lymphocytes. The virus was, th renamed as human herpesvirus type 6 Seroepidemiological studies have shown HHV-6 ubiquitous in human populations, and it has been from the blood and saliva of normal adults (Harnett 1990; Levy et al. 1990; Yadav, 1991).

The prevalence of the antibodies to HHV-6 in norma populations worldwide ranges from 60 - 85% (Yadav The antibody titre ranges from 10 - 360, and may d in the individuals above 40 years of age.Elevated of anti-HHV-6 antibodies have been found in patient Exanthum Subitum, chronic fatigue syndrome, Ki lymphednitis, a type of infectious mononucleosis,AIDS lymphoid malignancies and in organ transplant patie immunosuppressive drug therapy (Ablashi et al. 1988).

Studies have been made on the seroprevalence and ti the antibody to HHV-6 in normal subjects as well cancer patients (Saxinger et al. 1988; Okino et al. Clark et al. 1990, b; Yadav, 1991). Yadav (1991) re not only a high prevalence rate of anti-HHV-6 anti in lymphomas but also high titres of 160 to >512

against a low titre of 10 to 320 in normal adults. Studies have not yet been made in India either on the seroprevalence or on the titre of anti-HHv-6 antibodies in mormal population or in cancer patients. Hence this preliminary study.

MATERIALS AND METHODS

Twenty-eight patients with Hodgkin's lymphoma (HL), 59 non-Hodgkin's lymphoma (NHL), 51 acute lymphoblastic leukaemia (ALL) and 127 patients with oral cancer (OC) were included in the study. Seventy five age-matched healthy subjects were also included in the study as contol. 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, 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 a maximum period of 2 weeks.

The presence of IgG antibodies to the viral capsid antigen of the Z29 strain of HHV-6 and its titre in serum were evaluated using indirect immunofluorescence assay and immuno-peroxidase 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 Molt-3 cells, maintained in RPMI-1640 medium with 10% fetal calf serum (5% CO2 at 37°C), were infected with Z29 strain OF HVV-6 in the presence of polybrene (2 g/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 in phosphate buffered saline (PBS), on teflon-coated multi-well slides, fixed in cold for 10 min and ~~stored~~ stored at -20°C until used.

The indirect immunofluorescence assay was done fo Yadav and Ablashi (1990). Starting from 1/10, a ser 2-fold dilutions of the sera was prepared. The slide the smears of HHV-6 infected Molt-3 cells were in with the diluted sera at 37°C for 30 min in a h chamber, washed with PBS, then incubated with fluor conjugated anti-human IgG antibodies at 37°C for 3 washed thoroughly in PBS, dried, mounted with fluo (Pan-data Systems, USA) and covered with coverslip slides were observed under the 100x objective fluorescence microscope. Anti -HHV-6 antibody positi negative control sera were incorporated in every batch.

Immunoperoxidase staining was carried out following and Gregory (1988). Endogenous peroxidase activit blocked by treating the smears with 0.03% H202 in me for 10 minutes. Incubation with the test sera was d detailed above. The smears were washed, and then inc with horse radish perioxidase conjugated anti- huma antibodies at 37°C for 45 min and washed thoroughly i Subsequently, PBS containing 0.05% diaminobenzidine 0.01% hydrogen peroxide, was added as the subs incubated at room temperature for 5 min and thoroughly in PBS. The slides were mounted with aqua (BDH), covered with coverslips and studied under the objective of a microscope.

RESULTS

The results of the study are presented in Table 1 a

Subjects	Prevalance	Antibody titre	
		Range	Mean
Normal Control (n = 75)	76%	10-160	47
NHL (n = 59)	95%	10-320	88
HL (n = 51)	100%	320-5120	1109
ALL (n = 51)	100%	160-5120	1208
OC (n = 127)	96%	80-10240	2042

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

NHL : Non Hodgkin's lymphoma; HL: Hodgkin's lymphoma; ALL: acute lymphoblastic leukaemia; OC: oral cancer

Subjects	Total Numbers	No. of persons having the antibody titre of				
		<10	10-80	160-640	1280-5120	10240
Normal	75	18	37	10	-	-
NHL	59	3	36	20	-	-
HL	28	-	-	12	16	-
ALL	51	-	-	35	16	-
OC	127	4	7	51	54	11

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

HHL : Non-Hodgkin's lymphoma; HL: Hodgkin's lymphoma; ALL:Acute lymphoblastic leukaemia;

OC : Oral cancer

The results obtained by 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 76%, and the titre ranged from 10 to 160 (mean, 47). In HL and ALL, the antibodies could be detected in all the serum samples, with titres of 320 to 5120 (mean,1109) and 160 to 5120 (mean, 1208) respectively. The prevalence of the antibody in the sera of NHL and OC patients was 95% and 96% respectively. While the antibody titre was relatively low in NHL (10 to 320; mean, 88); it was seen to be very high in OC (80 to 10250; mean, 2042).

DISCUSSION

Yadav (1991) has 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 anti-viral antibody in all the 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 (Devita, 1973; Kadin, 1985; Nadkarni et al. 1978). HHV-6 has been reported to infect T-lymphocytes at various stages of differentiation in vivo (Yadav, 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.

Oral cancer is the most prevalent carcinoma in India, and forms about 27% of the total cancer incidence in this part of the country (Padmanabhan and Vasudevan, 1981). There are

no previous reports on the association of HHV-6 wit cancer. In the present study, anti-HHV-6 antibo observed in 96% of the serum samples from OC patient titre of the antibody was highly elevated in the majority of the patients. Saranath et al (1985 Vijayakumar and Vasudevan (1985) have observed dera of cell-mediated immunity in OC patients. This may l the reactivation of the virus which in turn could in the elevated levels of the antibody. HHV-6 ha: reported to be cytopathic to the host T-cells, whi important in cell-mediated immunity. A reactivated infection, hence, could possibly increase the sever the disease by further weakening the already ir immune response. Or the derangement in immunity in be due to the newly acquired HHV-6 infection. Howeve exact role of HHV-6 in OC remains to be determined t further investigations.

ACKNOWLEDGEMENT- Our grateful thanks to Dr. D.V.Ab National Cancer Institute, USA, for advice and to Mr Anil Kumar, Department of Biochemistry, Medical Co Trichur, for technical assistance.

REFERENCES

1. Ablashi DV, Joseph SF, Buchbinder A, Hellm Nakamura S, Llana T, Lusso P, Kaplan M, Dahlb Memon S, Imam E, Ablashi KL, Markham PD,Kramar Krueger GRE, Biberfeld P, Wong-stall F, Sala SSZ, Gallo RC. Human B-lymphotrophic virus (huma pesvirus-6). J Virol Methods 21, 29-48, 1988.

2. Clark DA, Alexander FE, Mckenney PA, Robert

O'Brien C, Jarret RF, Cartwright RA, Onions DE. The seropidemiology of human herpesvirus-6 (HHV-6) from a case-control study of leukaemia and lymphoma. Int J Cancer 45, 829-833, 1990.

3. De Vita VT. Lymphocyte reactivity in Hodgkin's disease: a lymphocyte civil war. New Engl J Med 289, 801-802, 1973.

4. Harnett GB, Farr J, Pietroboni GR, Bucens MR. Frequent shedding of human herpesvirus-6 in saliva. J Med Virol 30,128-130, 1990.

5. Jones FL, Gregory J. Immunoperoxidase methods. In: Catty D, Ed-'Antibodies, a practical approach, Vol.II', New York IRL Press, 155-178, 1988.

6. Kadin ME. Common activated helper T-cell origin for lymphomatoid papulosis, mycosis fungoides and some types of Hodgkin's disease. Lancet ii, 804-865, 1985.

7. Levy JA, Ferro F, Greenspan D, Lennette ET. Frequent isolation of HHV-6 from saliva and high seroprevalence of the virus in population. Lancet i, 1047-1050,1990.

8. Nadkarni JJ, Nadkarni JS, Advani SH. Surface markers in human lymphoid leukaemias. Indian J Cancer 15, 43-47,1978.

9. Okuno T, Takahashi K, Balachandra K, Shiraki K, Yamanishi K, Takahashi M, Baba K. Seroepidemiology of human herpesvirus-6 in normal children and adults.J Clin Microbiol 27, 651-653, 1989.

10. Padmanabhan TK, Vasudevan DM. A statistical analysis

of cancers registered at the Regional Cancer Ce
Trivandrum. Indian J cancer 18, 189-196, 1982.

11. Salahuddin ZS, Ablashi DV, Markham PD, Josephs
Sturzenegger S, Kaplan M, Halligan G, Biberfel
Wong-Staal F, Kramarsky B, Gallo RC. Isolation
new virus HBLV in patients with lymphoprolifer
disorders. Science 234, 596-601, 1986.

12. Saranath D, Mukhopadhyaya R, Rao RS, Fakih AR,
SL, Gangal SG. Cell-mediated immune status in pati
with squamous cell carcinoma of the oral cav
Cancer 56, 1062-1070, 1985.

13. Saxinger C, Polesky H, Eby N, Grufferman S, Mu
R, Tegtmeir G, Parekh V, Memon S, Hung G. Anti
reactivity with HBLV (HHV-6) in U.S. population
Virol Methods 21, 199-208, 1988.

14. Vijayakumar T, Vasudevan DM. High affinity ros
forming cells in carcinoma of the oral cavity, ute
cervix and breast. Cancer letters 27, 339-345, 1985.

15. Yadav M. Active human herpesvirus-6 infections
associated diseases. Mal J Child Health 3, (in pre
1991.

16. Yadav M, Ablashi DV. Nasopharyngeal carcinoa:
of elevated antibody to human herpesvirus-6 in t
patients.Asean J Clin sci 10, 81-84, 1990.

17. Yadav M, Ponniah K, Umamaheswari S. IgG antib
to human herpesvirus-6 in Malasians. J. Biosc
1, 59-63, 1990a.

18. Yadav M, Umamaheswari S, Ablashi DV.Low prevalence of antibody tc human herpesvirus-6 (HHV-6) in Kadazans. Southeast Asian J Trop Med Hlth 21, 259-263, 1990b.

Correspondence and reprint requests to :

Dr. D.M. Vasudevan

Professor and Head

Department of Biochemistry

Medical College

Trichur - 680 596

Kerala, India