

Other Viruses and Miscellaneous Studies

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Studies on Human papillomavirus, Epstein-Barr virus, herpes simplex virus infections in Oral cancers

V Gopalkrishna
gopalvk58@hotmail.com

Human papillomaviruses (HPV) have been associated with cervical, oral and some oesophageal cancers. HPV DNA type 16 and 18 are considered as “high risk” genotypes in the development of such cancers, whereas HPV 6 and 11 are the “low risk” ones. Herpes viruses such as Epstein Barr viruses, cytomegalo viruses and herpes simplex viruses have also been found to be associated with human malignancies. Synergistic role of these viruses is incompletely understood.

Objectives

- Detection, characterization of “high risk” and “low risk” human papillomavirus types associated in oral malignancies.
- To study the prevalence of Epstein Barr Virus (EBV) and HSV infections in same study subjects.
- Studies on the nature of integration of HPV genome into the host cell DNA in these tumours.
- Clinical correlation of these viral infections with the aggressiveness/or stage of the disease

Achievements

During the study period, a total of fifty-five surgically operated oral cancer tissue specimens were obtained from local collaborating hospitals of NIV, Pune, for the detection and characterization of HPV, EBV DNA by polymerase chain reaction (PCR). Cancer cell lines such as SiHa, Hela and Raji were used as positive controls. All the DNA samples isolated from the tumor tissues were initially screened for α -globin gene that served as internal control. A total of 42.2% of HPV DNA positivity was established in oral cancer patients. When type specific HPV primers were used, HPV type 16 was the most predominant “high risk” type found in 31.2% of the patients. HPV 18 was detected only in 6.6% and HPV 6, 11 in 2.2% of the cases respectively. A high prevalence of EBV DNA positivity i.e. 44.5% was also observed by PCR when primers of BamHI-W repeated region of EBV genome used. However, primers of BNLF-1 transforming region of EBV were used, only 13.3% of the cases showed positivity. Interestingly, co-infections of HPV and EBV were also observed in 11.2% of males (n=27) and 22.2% of females respectively. EBV DNA (BNLF-1) positivity could also be observed in HPV negative oral cases. Normal control samples showed only 10-13% positivity of either of the viruses studied. All the patients were having tobacco

chewing/ brushing habits for more than 15 years duration. The present study so far been done suggests, HPV 16 could contribute to some extent in oral carcinogenesis. High prevalence of EBV (BamHI-W) by PCR indicates increased viral shedding of oral mucosa due to immunodepression. However, detection of BNLf-1 single copy gene of EBV in certain proportion of oral cases suggests the ability of EBV to transform oral epithelial cells and the level depends upon the severity of the immunosuppression which can lead to the progression to malignancy.

A total of fifteen surgically operated oral cancer tissues tested by PCR for CMV and HSV for found to be negative.

Future plan

Study will be continuing. Individual genotyping of high risk HPV's will be carried out by using type specific viral primers. Viral integration studies are being conducted in HPV positive cases in order to rule out the transforming ability of the viral genes in to the host.

Human papillomavirus infection in HIV infected women attending STD clinics and its correlation with cervical abnormalities

V Gopalkrishna
SN Joshi, S Mehendale (NARI)*
gopalvk58@hotmail.com

* National Aids Research Institute

Human Papillomaviruses have been implicated as sexually transmitted viral agents in the development of cancer of the cervix. “High risk” HPV type 16 and 18 are considered as potential risk factors during progression of the disease. Recently, HIV infection and related immunosuppression are found to be associated with excess risk for cervical neoplasia with persistence HPV infections. It is important to rule out whether HIV infection and related immunosuppression can play an important role in modulating the natural history of HPV associated diseases.

Objectives

- To detect the prevalence of HPV infections in HIV infected women with cervical abnormalities.
- To characterize “high risk” and “low risk” HPV genotypes associated in HIV infected women with cervical abnormalities.
- To correlate the viral infections with clinical and other epidemiological risk factors.

Achievements

During the present study period, a total of 120 cervical swabs collected from the HIV infected women were subjected to DNA extraction and HPV study. Cytological diagnosis of the cervical abnormalities was carried out by using 'PAP' test. Polymerase chain reaction (PCR) method was used to detect HPV DNA and also genotyping of “high risk” and “low risk” HPV's. 'PAP' smear abnormalities were more commonly observed among women aged 30 yrs and above (P=0.035, OR 2.8, 95% CI 1.04-7.8). Of the 120 randomly selected cervical swabs screened, HPV 16/18 DNA was detected in 33% (95% CI 23.9-43.1) of the cases. HPV 16 was the predominant type found and dual infection of both 16 and 18 were also observed in one case. HPV 16/18 was present in 28.3% of women below 30 years of age and 40.5% women aged above 30 years.

Future plan

High risk” HPV patient group will be constantly monitored and regularly followed up for any progression of higher lesions. HPV positive cases will be characterized further for viral integration studies, in order to rule out the transforming ability of the HPV DNA in to the host cells. Antiretroviral therapy patients will be constantly monitored for HIV viral load , CD4 counts, also PAP smear abnormalities and HPV genotyping and its influence on cervical lesions.

Surveillance of Acute Flaccid Paralysis (AFP) cases from Karnataka State as a part of WHO-SEAR Polio Lab Network.

PN Yergolkar
nivbng@bgl.vsnl.net.in

Laboratory Surveillance work for Global Eradication of Poliomyelitis programme was initiated by the Govt. of India in collaboration with the WHO/WHO-SEARO/WHO-NPSP in India since June 1997. NIV Field Station, Bangalore is responsible for processing all the stool specimens from Karnataka State.

Objectives

- To isolate and identify wild poliovirus from AFP cases until detection of last case of wild poliovirus in South East Asian Region and declaration of Polio free SEAR and Global Eradication of Poliomyelitis.
- To participate as one of the 8 National Polio Laboratories in India in the WHO-SEAR Polio Lab Network as per guidelines of WHO's Global Polio Eradication Programme.
- To co-ordinate with State EPI Officers and National Programme Managers National Polio Surveillance Unit (Dept. of Family Welfare) & National Polio Surveillance Project (WHO) at New Delhi with weekly and monthly reports and to send all polio virus isolates to ERC, Mumbai for intratypic differentiation tests.
- To fulfil 7 criteria of WHO for annual laboratory accreditation by the WHO to be part of the WHO-SEAR Polio Lab Network.

Achievements

During the period from April 2004 to March 2005, a total of 1215 stool specimens were received from 610 AFP cases (data as on 12-04-05), of these 589 cases were from Karnataka, 8 from Andhra Pradesh, 6 from Kerala, 5 from Tamil Nadu and 2 from Maharashtra. All cases were investigated in Karnataka State.

Virological results of AFP cases are, Polio vaccine virus-32, NPEV only-189, Negative-350 and results pending for 39 cases which are <28 days of receipt. Break up of 32 polio vaccine virus are P1-9, P2-3, P3-7, P1+P2-3, P1+P3-6, P2+P3-1 and P1+P2+P3-3.

No wild poliovirus was isolated during the period and last wild polio virus (P1) was isolated in February, 2004 in Raichur district of Karnataka and one year has completed without wild poliovirus isolation.

Results are presented in Tables, District-wise Table-10, Other States- Table-11, Month wise Table-12, Age & Sex of AFP cases Table-13 and Age & sex wise results in table 14.

During the period 20 stool specimens from healthy contacts of 4 index AFP cases @ 5

contacts below 5 years per index case were also tested from 4 districts of Belgaum, Bijapur, Gulbarga and Mysore in the months of April, August, October and December respectively. No wild poliovirus was isolated from any these contact specimens and the virological results are, P3 vaccine virus-1, NPEV only-13 and 6 Negative.

AFP surveillance indicator targets were fully achieved for the year.

WHO Accreditation for NIV Field Station, Bangalore in the WHO-SEAR Polio Laboratory Network

Polio Laboratory was fully accredited by the WHO for the year 2004 during April 2004 Onsite review visit. Criteria and targets achieved during 2004 for the Accreditation for the year 2005 are fully achieved and are as following for the period 1-1-2004 to 31-12-2004 except for pending on onsite review visit (planned for 28 & 29 April 2005) and pending of announcement of Proficiency Test Panel Results of February 2005.

Criteria for accreditation and targets achieved are as follows for the review period from 1-1-2004 to 31-12-2004

Target	Achievement
Tests are performed on at least 150 Stool specimens annually	1104 stool specimens tested - 552 from AFP cases and 20 specimens from Healthy Contacts (Total = 1104 + 20 = 1124 specimens)
Score on annual onsite review is at least 80%	Onsite review Score is 99% in April 2004 visit (Pending for 2005)
Test results on 80% of all AFP specimens are reported within 28 days	100% - 1124/1124 reported < 28 days.
Accuracy of poliovirus typing is at least 90%	100% - 93/93 from AFP cases & 1 from Contact Total 94/94
Atleast 80% of poliovirus isolates from AFP cases are forwarded for intratypic differentiation within 7 days	100% - 94/94 Polio isolates sent within < 7 days
Results on most recent Proficiency Test Panel is atleast 80%	100% for Feb 2004 (Result Pending for 2005)
Internal Quality Control procedures are implemented atleast Quarterly	Implemented internal quality control procedures quarterly

Conclusion

No wild poliovirus was isolated during the period with high quality surveillance standards achieved in Karnataka state and it is more than a year without wild poliovirus isolation and the last wild P1 was isolated in February 2004. No P2 wild virus was isolated since June 1997 and last P3 wild virus in Karnataka was in Dec 2000.

Future plan

There is continued need for high quality AFP surveillance both field and laboratory until Global Eradication & Certification Containment of Laboratory Wild Polio virus and cessation policy on OPV-immunization and contingency plan for any future cases of poliomyelitis either due to wild or vaccine derived polioviruses are implemented globally as per WHO and Govt. of India decisions.

Virological and Immunological studies of Lymphadenopathy in HIV Infected patients

SS Tikute, AC Mishra
acm1750@rediffmail.com

In HIV infected patients, lymphadenopathy is a common manifestation. According to recent report, from Memorial Sloan Kettering Cancer Center, USA, the incidence of Non Hodgkin's lymphoma (NHL) is over hundred times increased and Hodgkin's disease (HD) over ten times increased in HIV infected population. Majority of HIV related HDs are linked to Epstein Barr virus, which ranges from 80 to 100%. A study on clinical profile of AIDS in India suggests 22.5 to 28% HIV patients had generalized lymphadenopathy. In other studies of malignancies associated with HIV infection, lymphoma has been reported from India.

Objectives

- To investigate association of different viruses like HTLV, EBV, HSV and HRV-5 in AIDS associated lymphadenopathy

Achievement

Serological Detection for Epstein Bar virus antibodies (IgG) has been done where 80% reactivity is found with ELISA (Trinity Biotech kit).

PCR with HHV-8 primers has done. Till date 43 DNA samples are tested from these four samples are showing results, these will be studied further.

Studies on Ganjam virus activity around Pune

MV Joshi
mvjoshi46@rediffmail.com

Ganjam virus has been isolated earlier from *Haemaphysalis intermedia* (*H. intermedia*) ticks collected off domestic animals from Orissa, Andhra Pradesh and Karnataka state. Recently, we have reported isolation of Ganjam virus from ticks collected off domestic animals from certain localities around Pune. Six of the ten virus isolates obtained were identified and confirmed as Ganjam virus. Four isolates remained as unidentified.

Objectives

- Identification and molecular characterization of unidentified tick virus isolates.

Achievement

Four unidentified virus isolates obtained from ticks collected from certain villages around Pune were reinoculated in 2-3 old infant Swiss albino mice for further adaptation. The brains of sick mice were harvested and stored individually for identification of these isolates and molecular characterization studies.

In order to determine the prevalence of antibodies to Ganjam virus among humans, a total of 153 human sera were collected from Mulshi, Paud and surrounding localities of Pune (from where the Ganjam virus was isolated), during October 2001. These sera were tested for the presence of CF antibodies to Ganjam (G619) virus. Eight (5.2%) of the 153 sera had CF antibodies to Ganjam virus.

Biotechnological approach for defining Epizootic Ulcerative syndrome in freshwater fishes

A Roy

Epizootic Ulcerative Syndrome (EUS) is today a semi-global problem among the freshwater fishes of the tropics. It was first reported in Australia in 1972 and thereafter appeared in India in the Barak Valley region during 1988. Now it is found all over India and the different indigenous fish species affected by EUS disease. The large-scale mortality caused by EUS in fishes has caused immense economic crisis to the fishing industry as well as to the nutritional balance of fish eating population of Eastern and North-eastern India. During recent times the intensity and fatality rate of EUS has decreased considerably, however the risk for the fresh wave of such disease remains till we understand its etiology and epidemiological features to full extent.

Objectives

- Isolation and characterization of the etiological agent(s) from the specimens collected from the fresh water fishes affected with EUS.

Achievements

The details on isolation of the viral agent from the freshwater fish samples, its susceptibility to various fish cell lines; physical and chemical properties of the viral agent were mentioned in the last report. Work on physical and chemical properties, Electron Microscopy and other studies of the viral agent were continued.

Effect of Chloroform

The infected virus suspension (1 ml) grown in BF2 cells was mixed with 0.5 ml Chloroform (Reagent Grade) and incubated at room temperature for 10 Min. The infected virus suspension without Chloroform treatment kept as control. After incubation the tubes were centrifuged at 3000 RPM for 10 Min and the supernatant was used for titration. Complete inactivation of the virus was observed with the virus suspension treated with Chloroform.

Effect of pH

One part of the virus grown in L-15 Medium was mixed with one part of the L-15 Medium (pH 3) and similarly virus grown in L-15 Medium was mixed with L-15 Medium (pH 7) as control. These mixtures were incubated at room temperature for 30 Min and then were titrated for the activity. Coxsackie A 24 virus grown in MEM (E) Medium mixed with L-15 Medium (pH 3) was kept as positive control. The virus treated with L-15 Medium (pH 3) was neutralized by 0.1 N NaOH before virus titration.

The viral agent was found completely inactivated at pH 3.

Susceptibility with Mammalian cell lines

Four mammalian cell lines viz: HeLa, PS, RD and Vero were used for the infection with the above viral agent. All the cell lines were grown in 25cm² bottles and at 60 to 70% confluency; these were infected with 200 μ l of the virus suspension (Titer 10⁸ /TCID₅₀). These were kept at 37°C and observed for 10 days. No CPE was noticed in any cell lines. Further, the infected fluid from these cells were collected and inoculated on BF2 cells, which were kept at 28°C for the incubation. These were observed for one week with no CPE. The results suggest that, the agent is very host specific. Therefore, further experimentation is needed with the cell line obtained from the skin of the fish as lesions were observed in deep muscle of the fish infected with EUS.

Heamagglutination assay

Heamagglutination assay was performed to further characterize the viral agent. The RBC's from three different species (Fowl, Goose, Guinea pig) were used to perform the assay. The experiments were carried out in microtitre plates and 2-fold dilution of the virus was used in duplicates for the assay. Then 0.5% RBCs suspension from three species prepared in PBS; (pH 7.2) were added in each well with controls. The plates were kept at room temperature for 1 hr and checked for heamagglutination. Further, the plates were kept at 4C for overnight and observed on next day. No haemagglutination was observed with any species of the RBCs.

Future Plan

Characterization and identification of the virus by immunological and molecular tools. Experimental infection of healthy fishes with this isolate.

Human Parvovirus B19 infection in Tribal Populations with Homozygous Sickle Cell Disease

Atanu Basu
basua@icmr.org.in

Objectives

Testing for the prevalence of human parvovirus B19 infections in different tribal populations from India with sickle cell disease on samples referred from various sickle cell centers of India (Table : 1)

Achievements

Using a commercial EIA ELISA kit (Biotrim, Ireland), antibodies to B19, both IgM and IgG were screened. A nested PCR based on NS1 sequence to detect the B19 virus in the pooled sera of SCD cases was also used. Antibodies to human parvovirus B19 in tribal populations with SCD disease

Table 1 : Distribution of Parvovirus antibodies in Tribal Population.

S.No	Tribe/Region	B19 IgM	B19 IgG	Sera pools tested for B19 DNA by PCR
1.	Pawra (Western India)	01/109	86/128	0/45 The IgM positive sera was tested B19 positive and not included in the pools
2.	Gond (Central India)	ND	03/06	0/02
3.	Halba (South-central India)	01/27	05/27	0/09
4.	Kuruma (South India)	0/03	10/21	0/07
5.	Paniya (South India)	ND	05/11	0/04
6.	Chetty (South India)	ND	04/10	0/04

Other **Viruses**

Isolation and characterization of Herpes viruses in organ transplant patients

V Gopalkrishna
gopalvk58@hotmail.com

Both primary and reactivated herpesvirus infections can cause severe disease in organ transplant recipients. Therefore the need to identify, genotype and study the drug resistance profile of herepsviruses in such settings is important.

Objectives

- Detection, isolation and characterization of CMV, EBV, HSV, and BK viruses associated in organ transplant patients.

Achievements

The 84335 strain of cytomegalovirus (NIV repository isolate) was grown in vero and MRC 5 cells and a PCR assay for detection of the virus standardized. Clinical specimens from renal transplant cases were inoculated in the MRC5 cells and four virus isolations confirmed by PCR.

Miscellaneous Studies

Screening of some naturally occurring and synthetic compounds with respect to antiviral activity on selected cell-virus system

V Gopalkrishna
gopalvk58@hotmail.com

Objectives

To test the antiviral properties of the herbs identified in the Western Ghats and presently being used as Folk Medicine against different ailments.

Achievements

Antiviral activity of Clematis and Swertia plant extracts

Initially the cytotoxicity levels of Clematis rosemoor, Family-Gentian and Swertia chirata, Family-Renunculaceae extracts were assayed in HeLa, Vero, Hep-2 and RD cell lines. The toxicity range for Clematis was 1\50 dilution of 10% extract and Swertia was 1\100 dilution of 10% extract to the above cell lines. The antiviral activity of both the extracts were checked against Coxsackie A-24(CA-24), RNA viruses and Herpes Simplex Virus type 1 and 2(HSV 1 and 2), DNA viruses in above cell lines at subtoxic dose of the above plant extracts. Studies are currently ongoing.

Miscellaneous Studies

Arboviral studies on a newly developed cell line (*Culex tritaeniorhynchus*)

A new cell line was developed from *Culex tritaeniorhynchus* mosquitoes and its susceptibility to JE, WN, CHIK, CHP, VSV, KSO, GAN, REO and Chittoor was studied. Fluorescence antibody technique (IFA) was used for the first 4 viruses to study the susceptibility while growth of the cell line grown virus in vero cells was used for the rest of the viruses. The cell line supported the growth of all the viruses except the tick-borne viruses Kaisodi and Ganjam. Attempts are being made to determine the virus yield in the cell line on different post infection days. Cells were infected with respective viruses, harvested on fixed post infection days and assayed on vero cells (plaque assay) to determine the virus content.

Establishment and characterization of a new *Aedes aegypti* cell line

A new cell line from the mosquito was established and characterized in the laboratory. The Susceptibility of the cell line to 10 arboviruses was studied using either IFA or passage in vero cells. The cell line supported the growth of JEV, WNV, DEN-2, CHIK, CHP, VSV, Chittoor and Reo viruses. However, the cell line did not support the growth of Ganjam and Kaisodi viruses, the two tick borne viruses used in the study.

Studies on Zoonoses

A total of 97 blood samples were collected from the veterinarians working at different Government Veterinary Clinics in Pune district under Department of Animal Husbandry (Maharashtra State). These samples were sent to the Microbiology Department of B.J. Medical College for testing against leptospira antibodies. 6 out of 40 samples tested were found positive. The samples were also sent to ICAR Research Complex, Goa for testing against Listeria & Brucella antibodies. 7 and 15 out of 97 samples found positive for Listeria & Brucella respectively. Two samples were found positive for both the pathogens. 37% Seroprevalence was noticed for HEV.

Suspected Cowpox zoonosis at Lohgaon, Pune

Clinical specimens (blood & scabs) from human, cattle and dog were collected to investigate suspected cowpox zoonosis. Virus isolation attempts were made by using cell culture, egg embryo inoculation & animal (rabbit) inoculation (Figure 1). No virus isolate was obtained (In the collaboration with the Clinical Pathology division).



Fig.1: Pock lesions on hand of suspected cowpox case

Screening of Mycoplasma infections in stock cell cultures and virus stocks by using highly sensitive assay, PCR-ELISA-RFLPS

Mycoplasmas are one of the most frequent contaminant agents found in cell culture systems. The sources of their infections are often due to bovine serum, reagents, Mycoplasma infected cultures/ laboratory personnel. Mycoplasma screening is routinely being done in the laboratory by using PCR-ELISA-. PCR/RFLP method is being used in order to identify the species specific mycoplasmas present in cell cultures. Further, Mycoplasma infected cell lines were treated with BM-cycline continuously for three passages. When results were compared before treatment and after treatment with BM-cycline indicated that Mycoplasma infections were completely eliminated from the cell cultures as compared to the original cell cultures. Study is routinely being carried out.