IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Global Outbreaks Of Chandipura Viral Encephalitis And Pathophysiology

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Abstract - CHPV causes acute encephalitis in pediatrics population under the age of 15 years. The critical features of CHPV is sudden onset of the clinical symptoms including neurological complications and high fatality rate. Due to the short duration between the onset of clinical features and neurological illness, serological diagnosis is not useful. However, CHPV re- emerged during 2003 in the form of an encephalitis outbreaks affecting 11 districts of Andhra Pradesh with a high fatality ratio of about 56%. CHP encephalitis (CHPE) outbreaks was simultaneously documented in 15 districts of Maharashtra a during the same time. During the subsequent years, CHPV outbreaks with 70% case fatality rate in the pediatrics population of Vadodara district of Gujarat was documented. It has been associated indifferent states of India viz.

Key words - CHPV, Encephalitis and pathophysiology.

Introduction: Chandipura virus (CHPV), an arbovirus belonging to genus Vesiculovirus in the family Rhabdoviridae has gained global attention as an encephalitis causing virus after the 2003-2004 outbreaks in central India.

A total of 322 child deaths:- 183 in Andhra Pradesh (AP) , 15 in Maharashtra and 24 in Gujarat were reported during the outbreaks.

Case fatality rate (CFR) in AP and Gujarat were 56 to 75% respectively.

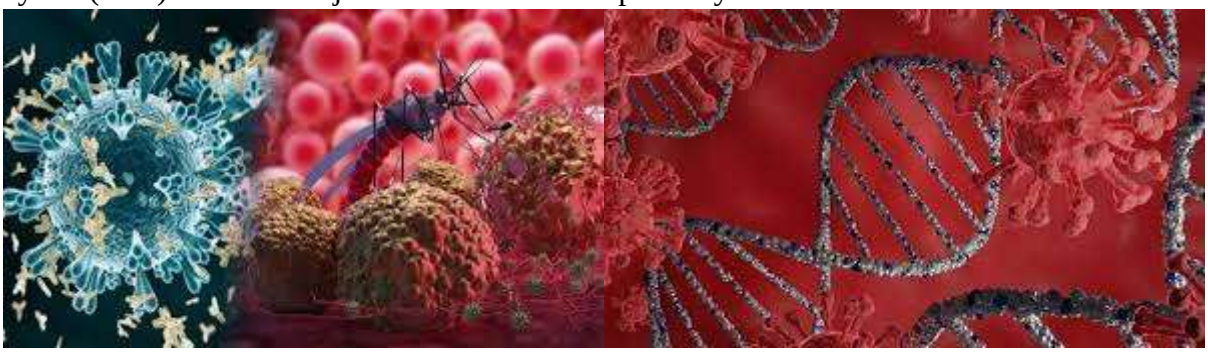

Figure-1: Chandipura arbovirus

Distribution:- Till date, the presence of this virus is recorded from India subcontinent, Sri Lanka and Africa . Hence, it is speculated that CHPV may be present in other parts of the country. Although, this was first time identified in India during 1965 but the retrospective serological studies indicate exposure of the human population as early as during 1957-58. Earlier reports suggest that the association of CHPV with a few undiagnosed outbreaks occurred in 1954 in Bihar. However, CHPV was isolated from sera collected from clinically confirmed encephalitis cases in 1983 from Raipur and the Warangal district of Andhra Pradesh (1997 and 2002) suggesting its wide circulation in the country.[1-4]

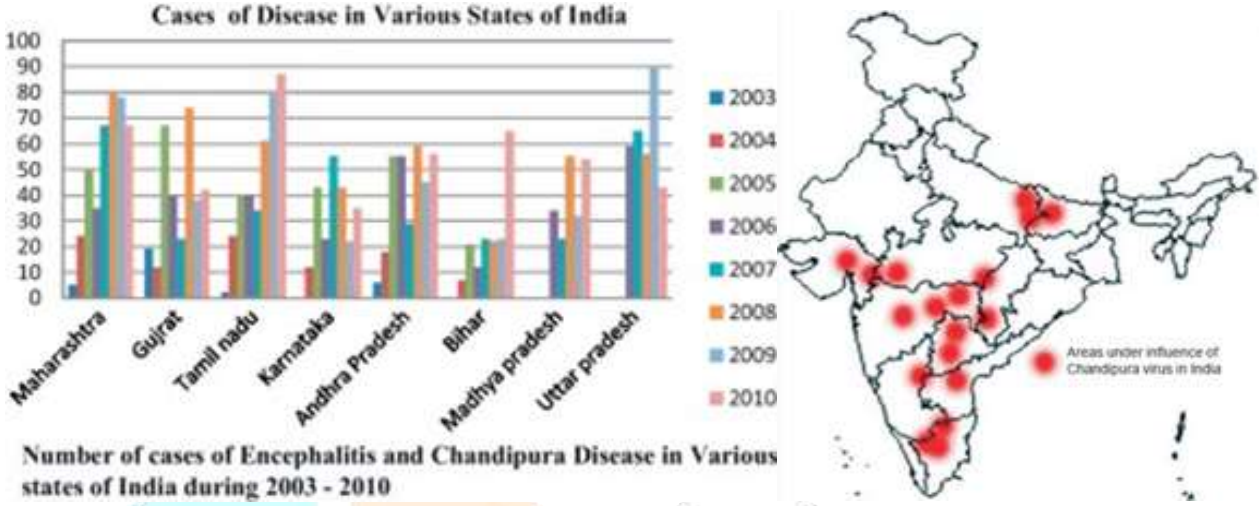

Figure-2: Outbreak of Chandipura virus

However, CHPV re- emerged during 2003 in the form of an encephalitis outbreaks affecting 11 districts of Andhra Pradesh with a high fatality ratio of about 56%. CHP encephalitis (CHPE) outbreaks was simultaneously documented in 15 districts of Maharashtra a during the same time. During the subsequent years, CHPV outbreaks with 70% case fatality rate in the pediatrics population of Vadodara district of Gujarat was documented. It has been associated indifferent states of India viz. Andhra Pradesh in 2003 and 2007, Gujarat in 2004, Maharashtra in 2007 and 2009, and Odisha in 2015. The CHPV was also been isolated on Nigeria from hedgehog and in Sri Lanka from macaques.

Incubation period: symptoms may be appearing within in 24hrs of reported mortality.

Clinical manifestations/symptoms:

- High fever followed by seizures
- Altered sensorium
- Diarrhoea
- Vomiting, followed by death in majority of the cases.

Classification:

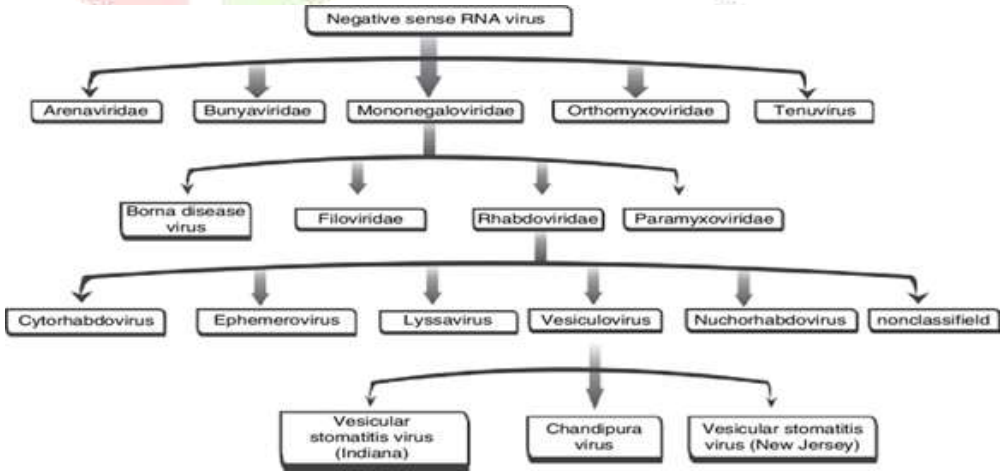

Figure-3: Classification of Chandipura virus

Symptoms of Chandipura virus on skin:-

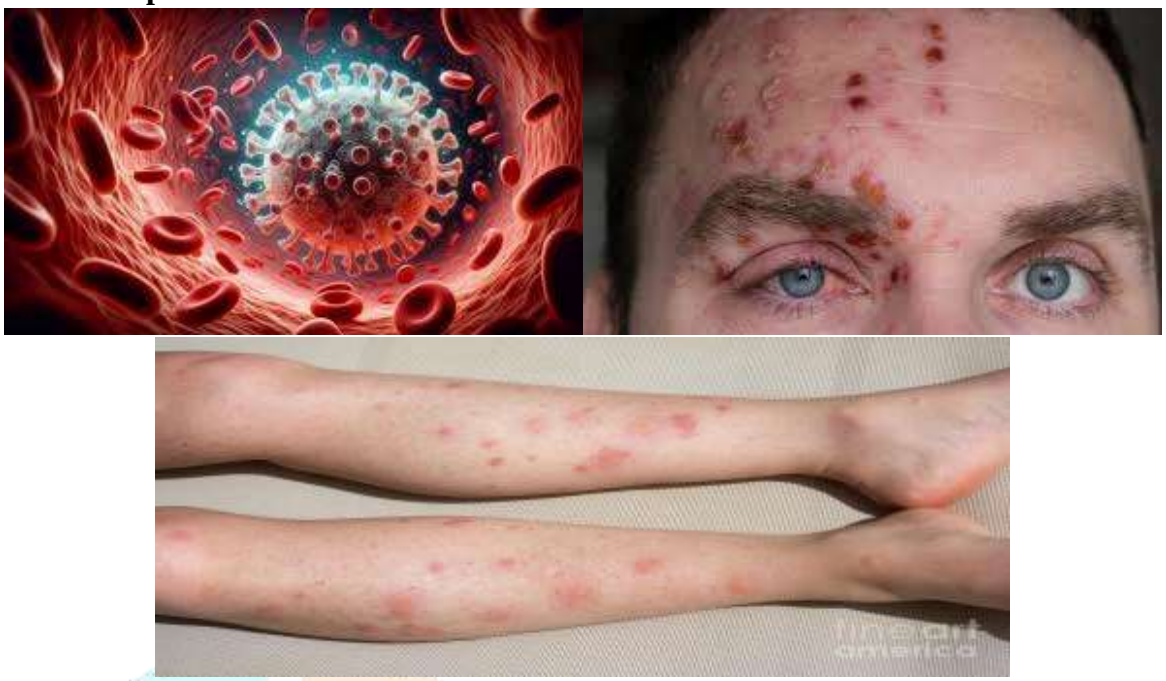

Figure-4: Chandipura symptoms

Case definition: Fever (100%), Convulsions (76.3%), Sensorium (34.2%), Headache (23.7%), Vomiting (44.7%) and Diarrhea (23.7)

Diagnosis of Chandipura virus:

1. *Molecular diagnostics assays for CHPV:* CHPV causes acute encephalitis in pediatrics population under the age of 15 years. The critical features of CHPV is sudden onset of the clinical symptoms including neurological complications and high fatality rate. Due to the short duration between the onset of clinical features and neurological illness, serological diagnosis is not useful. The virus has been detected in CSF as well as sera collected from the patient in the acute phase of illness using CHPV specific one step RT-PCR assay that detects 10-100 pFu /ml of the virus in human clinical specimens . Real time one step RT-PCR indicates linear relationship for a wide range of viral RNA 10^2-10^{10}. When RNA from other viruses or healthy individual was used, specificity was found to be 100%.[5-7]

2. *Serological diagnosis assay for CHPV:*- CHPV specific IgM capture ELISA with specific polyclonal antibodies shows polyclonal antibodies masking the specificity of the assay to be used for the detection of anti CHPV IgM antibodies in the patient's CSF and sera. Monoclonal antibodies were generated and replaced in anti CHPV IgM ELISA to increase the sensitivity, specificity and rapidity of the assay. Plaque reduction neutralization test is considered as 'gold standard' to detect neutralizing antibodies against CHPV. However, the test is cumbersome to perform, time intensive and reading is subjective. Recently developed micro neutralization ELISA detects neutralizing antibodies against CHPV with readouts in the form of optical Density and shorter turnaround time this test may sera as an alternative to conventional assay in sero surveillance and vaccine studies.[8-10]

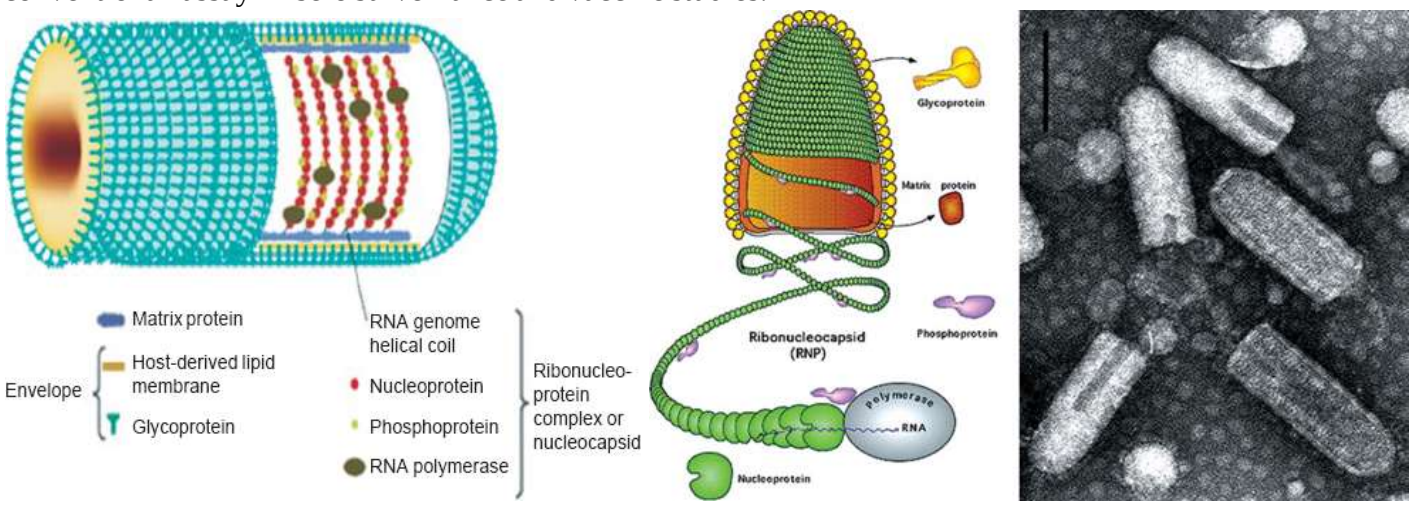

Figure-5: Chandipura viral cascade structure

3. *Future direction of CHPV research in India:-* CHPV activity was detected in India in a few selected geographic region of the country. However, further investigation in the country and in the other geographic region are needed. The natural cycle of CHPV is still not clear but the demonstration of anti CHPV antibodies in rodents, cattles , sheep, goat, pigs, frog , hedgehog, lizard and rodents etc. indicates the wider range of the maintenance hosts explored by the virus in nature. Further studies are necessary to unravel the animal's species that are infected by the transmission vector in nature is resolved but frequent isolation of CHPV from sandflies may relate them as the principal vector of transmission in nature.

Evolutionary studies on CHPV indicate substantial genomic and antigenic stability of the virus during the last 47 years. Hence, it is possible to obtain protection against all the circulating strains with a vaccine developed against any one of the strain. The efforts are needed for CHPV vaccine candida. Alternative approaches should also be considered while planning for complete care of the problem by adopting novel technologies boosting production and safeguarding health of humans and animals, through the use of immunomodulation and immunomodulatory agent on health with some bioactive principles, modes of action and potent biomedical application and the innate immune receptors with ingenious anti-viral roles. However, further efforts are necessary to used it for the susceptible population. Hence, the development of ELISA for serological investigation is needed.[11-13]

Figure-6: Serology of Chandipura virus

Mode of transmission:- Increased expression of CHPV phosphoprotein has been demonstrated upto 6h post infection (PI) showing the replication of CHPV in neuronal cells. The investigation reported rapid apoptosis of infected neurons though FAS associated death domain via an extrinsic pathway following the activation of caspases-8 and caspases-3 as well as prominent cleavage of ADP ribose polymerase. They also demonstrated reduction in apoptosis when the pathway was blocked using interfering small RNA.

Range:- the disease was predominating the lower income of the population and the affected age group ranged from 2.5 months to 15 year old.

Disease pathogenesis:-

1. *CHPV infection in laboratory rodents (mice and rats):-* studies have shown that 16 years old mice when inoculated subcutaneous with CHPV, 5th day post infection hind limb weakness was observed that continues to 7-8 PID and then mice recovered . However histological investigation showed no grass changes in any of the organs with CHPV infection in the infant mice, Frank sickness was observed with ataxia, hyperesthesia, convulsions, quadriplegics and death. Interestingly marked histological changes were observed only in the brain and spinal cord in gradation of the post infection period. Earlier studies have shown that rats of two weeks age could be suitable anime for studying the pathogenesis, host virus interaction, and drug development etc. for CHPV. Degeneration of neurons, antigen detection in cytoplasm of neurons and chromatoysis of neurons as well as localization of antigen in purkinje cells and choroid have been needed. Neuropathogenesis of virus has been established but its route of entry to the CNS and mechanism of neuronal death is unknown. One way to enter the nervous system is by retrograde movement from peripheral nerves or factory nerves and the other one is through damaged blood brain barrier by cytokinase and chemo kinase produced in response to peripheral infection. After entering neurons, it triggers cellular stress factor and release of reactive oxygen species while initiate neuronal death. Recent evidence shows that the virus induces death by triggering death domain or microglial activation.[14-19]

Figure-7: Pathogenesis of Chandipura virus

2. *Generic characterization and phylogenetic analysis of CHPV*:- till date, a total of 8 complete genomes of CHPV isolated in India are available and 2CHPV isolates have been documented in African continent. The phylogenetic analyses of the whole genome sequences indicates stability of the virus isolated obtained during the last 47 years divergence of the CHPV whole genomes isolated in India varied from 3.54-3.71 with respect to the prototype isolate of 1965. The comparison with full genome sequences of African continent indicates higher genetic divergence of 5-6% from Indian isolates indicating that these independently emerged different continents and are independently evolving. These observations indicate that CHPV is independently evolving with time.

3. *Bioinformatics approach to identify the markers for pathogenesis*:- search of possible hotspot in complete genomic sequences of CHPV was compared with others members of Rhabdoviruses that may be responsible for pathogenesis. This virus is in the cluster with the Isfahan virus, however, maintain several functional motifs of others the Rhabdoviruses. There is different with the prototype vesiculovirues in flanking sequence of the M protein. Several mutations in G protein have been mapped on to probable antigenic sites. Mutations in N protein mapped have been shown crucial for N-N interaction and a putative T cell epitope. A mutation in the case in kinase (‖) phosphorylation site in P protein may attributes to increased rates of phosphorylation. Further protein – protein interactions between host and virus protein is in progress to reveal ways by which the virus manipulates the biological pathways of hosts in its favor and evades immune system.[20-24]

4. *Immunological marker:*- The susceptibility of mice and humans to CHPV infection is age dependent. Experimental information in mice secrets significant amount of pro-inflammatory cytokinase. Monocytes and B cells support active replication of CHPV. An elevated levels of cytokinase and chemokinase observed in monocytes may help in predicting the pathogenicity of CHPV and possible entry into the CNS Children who recover from natural infection with the virus shows significant amount of TNF-alpha production. Suggestion that innate immunity plays a major role in response to CHPV. TLR are key host molecules involved in innate immune response in infection. CHPV infection activates TLR4, which leads to the secretion of pro inflammatory cytokinase and nitric oxide.

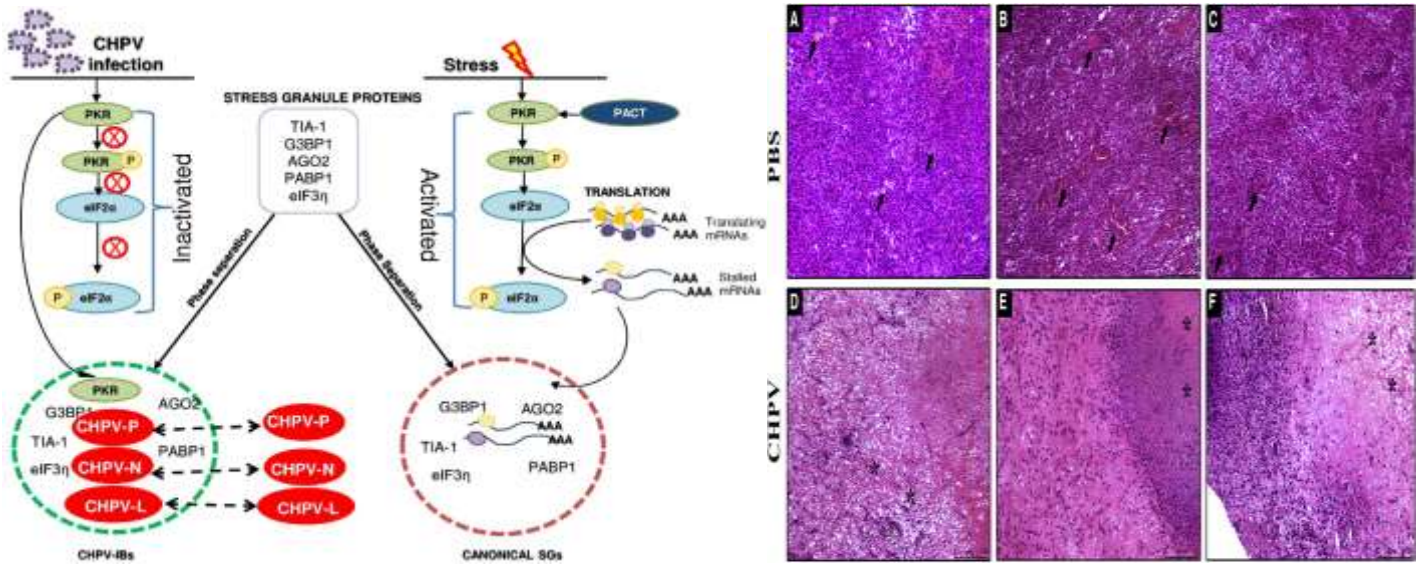

Figure-8: Immunology of Chandipura virus

Despite activation of the innate immune system, mortality was observed in young mice. Particle protection in TLR4 mutant mice and NO inhibitors treated wild type mice indicated that TLR4 and NO contributes to disease pathogenesis, IL-2 was detected in most of the early acute cases , this probably is associated with recovery. During CHPV infection in mice, drastic reduction in CD4, CD 8 + and CD19 + cell was reported. Depletion of lymphocytes in spleen suggested that the reduction may be due to regulatory mechanisms of immune system to prevent the by stander host tissue injury. The role of regulatory cells in homeostasis with regards to CD 4+ T regulatory cells from the infected mice suggest induced of CD 4+ T regulatory cues and expression of PD -1 in infection mice may be one of the mechanism by which the immune system control the activated lymphocytes and maintains homeostasis. It was also reported that microglial activation might be one of the triggering factors for the neuronal apoptosis in CHPV infection.

Figure-9: Vaccination for Chandipura virus

5. *Approaches to vaccine developed*: veracess based inactivated vaccine candidate against CHPV elicited efficient protection after two doses upon challenge with live virus in mice. Antibody titer after the third dose ranged between 1:80 and 1:320.

Mice, which demonstrated neutralizing antibody titer above 1:20, survived live virus challenge through even intracranial route. In another approach, a candidate vaccine employing recombinant CHPV glycoprotein gene using Baculo virus expression system showed intracerebral challenges to the immunized mice with 100 LD 50 of the homologous strain with 90% protection.

6. *Laboratory transmission experiment*:- several studies demonstrated the central transmission of arbovirus by its arthropod vectors that might serve as one of the mechanism for horizontal transmission. Lab experiment documented vertical and venereal transmission of CHPV in *Aedes aegypti*. The minimum infection rate among the progeny of infected females was documented to be 1.2%. The venereal infection rate of CHPV among inseminated females was 32.7 %. The study indicated the possible occurrence of vertical and venereal transmission of CHPV in insect vector. Experiment conducted on phlebotomus papatasi to determine the possible role of males in maintaining or sustaining the CHPV activity in nature indicated that infection males are capable of passing on the virus to female sandflies while mating. The infection rate was found to be 12.5% in uninfected females when mates with infected males. The occurrence of venereal transmission of CHPV may contribute to the epidemiology and in the natural cycle of CHPV. In India, *P. argentipes* is one of the predominant sand fly species found in many CHPV endemic areas and 65% of the lab- grown *P. argentipes* were susceptible to CHPV infection by the oral route. Transmission experiment were also carried of refeeding problems with this species. After incubation for 24 hours efficient transmission of CHPV to mice was observed. The estimated minimum transmission rate among the inoculated flies was 32%. CHPV in sandflies as well as in mice, was detected and confirmed by immunofluorescence antibody assay and RT-PCR assays. The potential to transmit the virus by bite might contribute towards the natural transmission of CHPV.

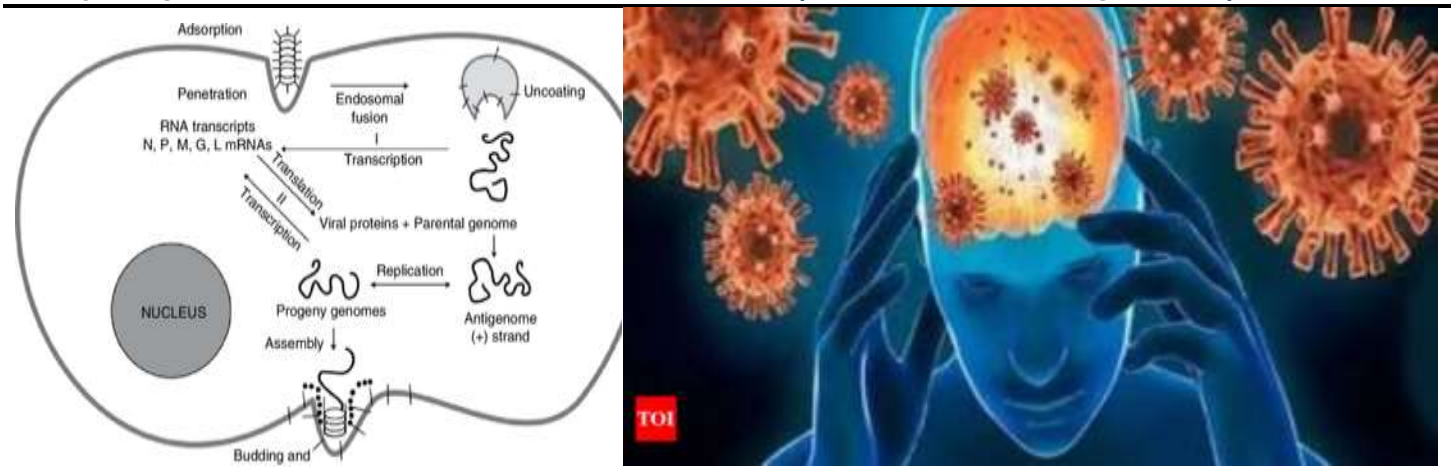

Figure-10: Chandipura in transmission

Natural cycle:- Earlier studies relevant that CHPV is predominantly circulation in the central part of India. The presence of anti – CHPV neutralizing antibodies in the blood collected from pigs , buffalos , cattle, goats and sheep. Suggests continuous circulation of the virus in this region. This demonstrates exposure of the domestic animals to CHPV. It is interesting to note that earlier serological investigation of CHPV activity suspected in the area of Andhra Pradesh, Maharashtra (Nagpur and beed district) and Karnataka (Bangalore) did not show anti- CHPV IgM antibodies in the 191 human sera. This is suggestive of sporadic nature of this virus. The anti- CHPV neutralizing antibodies have also been detected in the sera collected from frog (2/33), lizard (2/14) and rodents (26/32). This indicates a probable role of these of these or such animals in maintaining the virus in nature. However, this virus has capabilities to affect larger population and appear in outbreaks form. Serological investigation of CHPV in Andhra Pradesh state demonstrated high level exposure of the pediatrics population as anti- CHPV neutralizing antibodies detected in about 81% (237/291) human sera. The anti- CHPV sero prevalence was higher in the age group of > 15 years in 33(16 affected and 17 unaffected) localities of 6 district in Maharashtra state. Anti- CHPV IgM antibodies were detected in 5.5% (30) sera and anti-CHPV neutralizing antibodies were detected in 15.1% (82) sera collected from pediatrics population. As the laboratory data in mice indicates that infants are highly susceptible thus CFR is high in this pediatrics age group, however, adults develop IgM antibodies. Besides studies have been conducted for understanding the role of bats in a natural cycle maintenance cycle.[25-29]

Figure-11: Prevention of viral attack

Prevention:

1. Hygiene practice: washing hands regularly with soap and water, especially after handling animals or being in potentially contaminated environment.
2. Avoidance of wildlife: minimizing contact with wild animals and their habits particularly in coastal areas where the virus is prevalent.
3. Personal protective equipment: when handling potentially infected animals or their tissue, wear appropriate protective gear such as gloves and masks to reduce the risk of transmission.
4. Vector control: given the potential role of insects in transmitting the virus, using insect repellent and mosquito nets can helps mitigate exposure.

Management: Early recognition and prompt Medical intervention are crucial in managing chandipura virus infection. If symptoms suggestive of respiratory illness develop, individual must seek medical attention immediately. Doctors may recommend supportive care, including rest, hydration, and a symptomatic treatment to alleviate fever and respiratory symptoms.

As we navigate the complexities of the CHPV, efforts to understand its epidemiology transmission dynamics, and potential for spread are ongoing, understanding the collaborative efforts needed across scientific disciplines and international borders. By staying informed and adopting preventing measures, we can collectively mitigate that impact of emergency, infectious diseases like the CHPV, safe guarding both individual and community health.

Treatment:- There is currently no specific antiviral treatment or vaccine for the CHPV. Early diagnosis and supportive treatment are crucial. According to a 2014 document by the Gujarat government on 'Epidemiology and management of chandipura encephalitis' management includes airways, breathing and circulation support through oxygen therapy and ventilation if necessary. It also involves managing fluid and electrolyte balance, hyperpyrexia, raised intracranial pressure, and seizures as well as preventing secondary bacterial infections. In response to the current outbreaks union health ministry, along with experts, reviewed the chandipura virus cases and acute encephalitis syndrome cases in Gujarat, Rajasthan and Madhya Pradesh. This review aims to enhance understanding and improve measures combat to spread and impact of the virus.[30-34]

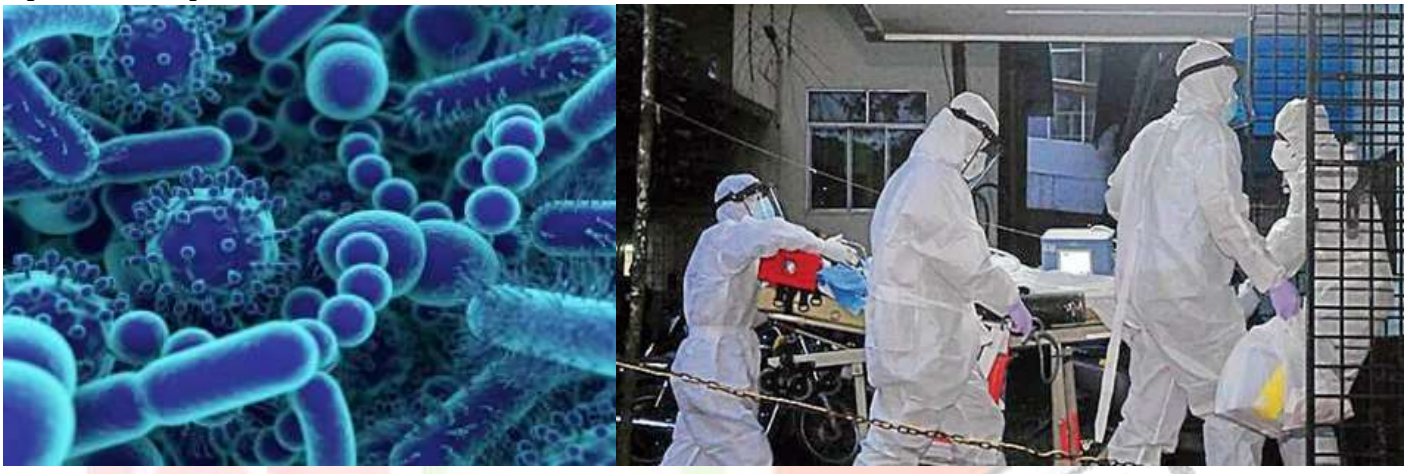

Figure-12: Management of viral attack

Result:- CHPV infection leads to neuronal cell death phase contrast microscopic image showed significant cell shrinkage and membrane bleeding in CHPV infected cells at 12 hrs post infection compared to their respective time matched mock infected controls. The morphological change was validated with MTS assays and TUNEL staining in neuro 2A cells with the MTS assays. An approximate 2 fold reduction in cell viability after CHPV infection at 12 hpi was observed. Concomitantly, approximately a 4 fold higher number of TUNEL positive cells at the same time point was also observed. A similar result was obtained in the case of our in vivo experiment. It was observed that upon infecting 10 days pups through the intraperitoneal route, the infected animals died within 96 hrs, an observation that corroborates earlier finding. The CHPV matrix protein, apart from having importance roles to play as a structural protein, interferes with the host transcriptional and translational machinery, thus accounting for cytopathic effects within the host upon infection. Double immunostaining with TUNEL and CHPV M protein was performed, and the representative graphs and images substantial the fact that neurons are dying post CHPV infection in both *in-vivo* and *in-vitro* models. To negate the fact that neuronal death in Vitro is due to inflammatory response, cytokine bead array analyses of the culture supernatant were performed from various incubation time point, which showed that there was no significant changes in the infected samples compared to those of their time matched controls. Cell death after CHPV infection is characterized to be apoptosis:- cell shrinkage and membrane blebbing are the two hallmark of apoptosis. In order to confirm CHPV mediated neuronal death to be apoptosis, annexin V and PI staining were done along with cell death detection by ELISA using a commercial CDDE kit.

The CDDE kit is based on the principle of identifying mono or oligonucleosomes in a " sandwich enzyme immunoassay " from a fraction of cell lyastes of apoptosis cells where fragmentation of DNA happens before membrane blebbing. Thus, it can specifically distinguish between apoptosis and necrosis, since in necrosis fragmentation DNA is released in the growth medium due to premature rupture of the plasma membrane. Annexin

V-PI staining of mock infected control and CHPV infected neuro 2A cells through various time points is shown in. At 12 hpi , it can be seen that CHPV infected neuro 2A cells enter into a late apoptosis phase, with a higher number of annexin V and PI positive stained cells than its time matched control. CDDE marked a significant amount of apoptosis in 12 hpi, similarly suggesting that there is an enhanced apoptosis due to CHPV after the particular incubation period in neuro 2A and SHSY – 5Y cells. CHPV P protein expression kinetic:- CHPV phosphoprotein is a component, which plays a dual role. RdRp act as a transcriptional activator within in the host and switched it's function to genome replication in the replicative stages. Immunoblotting showed that CHPV P protein expression in infected neuro 2A cells significantly increased at 6 hpi, which depicts the replicative phase of CHPV. A similar result was observed in an vivo experiment, with a rise in CHPV P protein expression at 4 dpi.

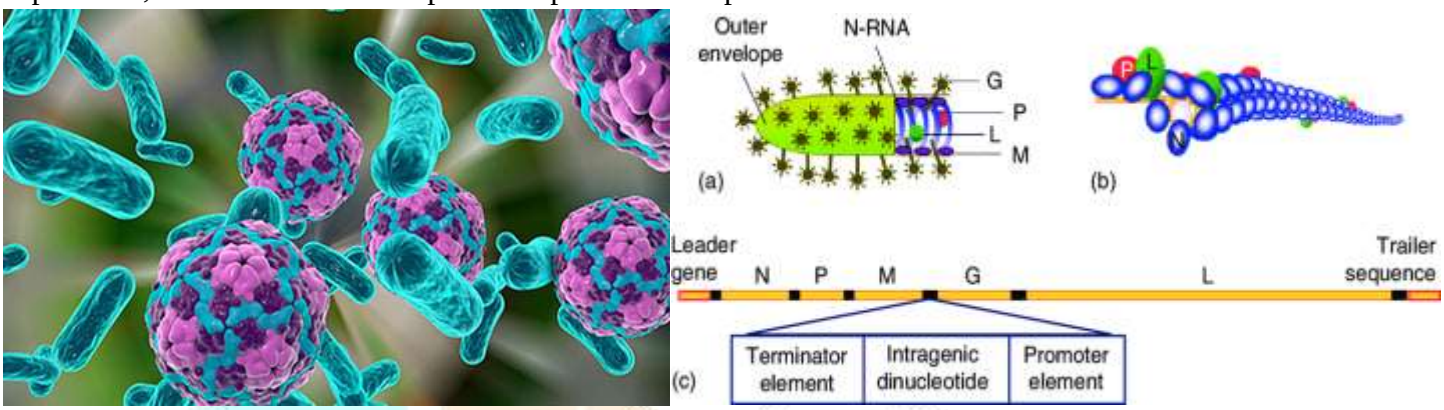

Figure-13: CDDE kit for identification oligonucleosomes of Chandipura virus

We performed some further experiments to identify whether CHPV infection in neuronal is productive or abortive. CHPV infected neuro 2A supernatants from various time period were collected and used for plague assay analysis. The result sgiwa significant rise in viral load in culture media collected from later time points. To strengthen our assertion that the viral progeny which was released in culture medium was equally effective in causing neuronal death, the culture supernatants were used as per the calculated PFU and to infect fresh batches of neuro2A cells. Which after 12 hpi were subjected to annexin V- PI analysis that showed a significant amount of cell death. The above described results help us to conclude that CHPV infection in neuronal cells is productive. CHPV infected neurons undergo apoptosis through the FADD mediation pathway:- significant foldb changes of fas ligand and its receptor were observed in qPCR arrays analysis, which was also reinforced by surface staining analysis using flow cytometry of CHPV infected neuro 2A cells at 3 hpi with fast antibody. Immunoblotting of FADD showed enhanced expression in infected neuro 2A cells and mouth brain at early time points, marketing the onset of apoptosis. We pretreated neuro 2A cells with Kp7-6 prior to CHPV infection. Immunoblotting of FADD expression in Kp7-6 pretreated neuro 2A cells compared to the CHPV infected ones showed significant inhibition at 3 hpi. In order to establish that FADD is the only death domain initiator of the apoptotic pathway is CHPV infection, apoptosis assay way perfomed in Kp7-6 pretreated neuro 2A cells by the CADD kit . Kp7-6 pretreatment was found to reduce apoptotic death by about 75% compared to their untreated counterparts. Kp7-6 did not have any cytotoxic effect at the mentioned concentration on pretreatment in neuro 2A cells.[35-40]

Figure-14: Chandipura outbreak through mosquito

Neuronal death after CHPV infection is caspase mediated:- Referring to the qPCR array data we observed that caspase -3 expression yielded a significant fold change in both infected neuro 2A and primary neurons. Immunoblot results showed that caspase-8 expression was enhanced in early time points but diminished at 12 hpi, while cleaved caspase -3 and cleaved PAPR expression increased at 12 hpi in CHPV infected controls. XIAP expression was suppressed through the time point in the neuro 2A cells after CHPV infection. Similar results were obtained in mouse whole brain lysate. The striking observation was that there was no significant changes in expression levels of caspases-9 in CHPV infected samples. This bolsters our assertion that the host cell follows the extrinsic apoptotic pathway after CHPV infection. In addition to these analyses, we also performed double immunostainin of cleaved. Caspases-3 / CHPV M protein in neuro 2A cells at 12 hpi, which showed clear colocalization of CHPV affirms the claim that the cells are undergoing apoptosis through a caspase mediated pathway upon being infected by CHPV directly and not through any antiviral response. QVDoph is a pan – caspase inhibitor which we used in our study to validation our proposition that the death is caspase mediated. Pretreating cells with QVDoph prior to CHPV infection attenuated the expression levels of caspases-8 and cleaved caspase-3 significantly at 6 hpi and 12 hpi. With the help of a CDDE kit, we confirmed that use of QVDoph significantly reduced apoptosis compared to the untreated cells. This finding seems to support our hypothesis that apoptosis is not happening through any of the caspase independent pathways. At the mentioned concentration, QVDoph did not have any cytotoxic effect on pretreatment in neuro 2A cells. We also verified the consistency of our result at a higher MOI by infecting neuro 2A cells with an MOI of 5, compared to our working MOI of 0.1. We obtained similar results in annexin V-PI and immunoblot analysis. Caspases-3 is a potential target to restrain CHPV infected neurons to undergo apoptosis:- specific activity levels of caspases-3 was measured and verified in two model cell lines, neuro 2A and SHSY – 5Y . In both cases, we found a highly significant rise in caspases-3 activity at 12 hpi . I order to prove that caspase-3 plays of pivotal role in CHPV mediated neurodegeneration, it was knocked done by using esiCaspase -3 in neuro 2A cells. It was observed that there is a significant downregulation in cleaved caspase-3 and its downstream molecule cleaved PARP in immunoblot analysis at 12 hpi. That potential of the esiCaspase-3 was analyzed using a CDDE kit and annexin V-PI assay. Significant suppression of apoptosis was obtained was observed in our treated samples compared to that of the untreated ones at 12 hpi.[41-45]

Conclusion:- However, CHPV has also been isolated from human cases, during 1971-72 in Nigeria, and hedgehog during entomology surveillance in Senegal, Africa (1990-96) and recently referred samples from Bhutan and Nepal and from wild toque macaques at polonnaruwa, Sri Lanka during 1993 suggest its circulation in many tropical countries. Based on the limited study on vector related report it appears that sandflies may be the principal vector.

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