MEDICAL VIROLOGY

History of Virology

Virology, it refer study of characteristics of viruses,

Medical virology, it refer study of medically important viruses

Father of virology – Martinus Beijerinck is often called the father of virology, In 1892 , **Dimitry Ivanovsky** used one of the filter to show that sap from a diseased tobacco plant remained infectious to healthy tobacco plants despite having been filtered . **Martinus Beijrinck** called the filtered infectious substance a virus(contagium vivum fluidum – contagious living fluid) and this discovery is considered to be the beginning of virology.

Bacteriophages are viruses that infect and replicate in bacteria they were discovered by English bacteriologist **Frederick Twort(1877-1950)** and **Felix d, Herelle(1873 – 1949)**

Thomas Milton Rivers defined **viruses as obligate parasites** ,

In 1885 **Louis Pasteur** developed **rabies vaccine**

It is to be **particles and crystal form** example Tobacco Mosaic virus , rather than a fluid by Wendell Meredith **Stanley**(1904 – 1071)

In **1931** the **electron microscope** was invented by **Ernst Ruska,** used for **study of viruses**.

GENERAL PROPERTIES

VIRUSES are smallest infectious agents. Animals , plants and bacteria are susceptible to infection caused by viruses.

Three properties are differentiate viruses are from other microbes, that includes

1, **Size** : viruses are smaller than other microbe , they are vary in size from 10nm to 300nm.

2, **Genome :** it contain either DNA or RNA, Viruses contain any one type nucleic acid as their genetic material

3, **Metabolically inert** : Viruses have no metabolic activity outside susceptible host cells, they do not possess active ribosome or protein synthesizing apparatus, viruses can multiply only inside living cells, not on inanimate media. Inside a susceptible cell, the virus redirects the cells synthesizing machineries to prepare new virus components

Virus structure – It consists of a core of nucleic acid (genome) surrounded by a protein coat or protein shell, this coat protect the viral genome from inactivation by adverse environmental factors e.g, nucleases in the bloodstream, both protein and genome are antigenic and responsible for antibody production.

Virion: a complete virus particle or intact virus particle

Capsid : the protein coat or protein shell

Capsomeres: protein structural units which form capsid

Nucleicacid : it contain either DNA or RNA

Nucleocapsid : consist of genome and protein coat

Envelope: some viruses the nucleocapsid is covered by lipoprotein envelope, contains viral antigen, this envelope is partially derived from nuclear membrane or cytoplasmic membrane of host cell

Symmetry - Viruses show three types of symmetry

1, Cubic – it is polyhedron , having icosahedral protein shell, the icosahedrons has 20 faces, 12 vertices and 5 fold, 3 fold, 2 fold , it covers the genome e.g, *Adenovirus*

2, Helical - nucleocapsids are arranged in helical form or spiral form e.g, *Influenza virus*.

3, Complcx : it is neither cubic nor helical, Brick shaped with ridges on the external surface and a core and lateral bodies inside e.g, *Pox virus.*

Cultivation of viruses : Viruses can only replicate within living cells, three methods are used for cultivation of viruses

1, Tissue culture : cells obtained from humans or animals grown in artificial culture media in a glass vessels in the laboratory

2, Chick embryo : some viruses are cultivated in chick embryo for production of vaccine

Laboratory animals : some viruses are isolated from Rabbit or Mice after inoculated with clinical specimens.

Effects of viruses on host cells

Viruses may affect host cells in four ways

1.Death : it causes death of host cell or cytopathic effect (CPE)

2, Transformation : it causes transformation normal cell into malignant cell or cancerous cell

3, Latent infection , here, virus remain within a the cell in a potentially active state,

4, Haemadsorption- envelope of this virus contain haemagglutinin antigen it will adhere to erythrocyte causes haemagglutination.

5, Inclusion body formation – during course of virus multiplication within host cell, virus specific structures are abundantly seen this structure is called as inclusion body, it have high affinity for acid dye (eg - eosin) , Inclusion body can be seen host cell nucleus eg- *Herpes virus*, host cell cytoplasm eg – *Pox virus.*, intracytoplasmic inclusion in nerve cell(Negri body) by *Rhabdo viruses*. I.body helps in diagnosis of viral infection

6, Chromosome damage , during course of virus multiplication within host cell, virus can damage the host cell chromosome , damage includes fragmentation, rearrangement, changes chromosome number, deletion, translocation seen in cancer cell (leukemia)

Physical and chemical properties of viruses or effect of physical and chemical agents on viruses

1, Heat : most viruses are inactivated at 56^0C for 30 minutes or at 100^0C for few seconds

2, Cold : most viruses are stable at low temperature, can be stored at – 70^0C

3, Drying :most viruses are rapidly inactivated at high temperature

4, Ultraviolet radiation : it can inactivate viruses

5, Organic solvents (chloroform, ether) it can inactivates enveloped viruses and resistant to non-enveloped viruses

6,Oxidizing and reducing agents(formaldehyde, iodine, chlorine and hydrogen peroxide) can inactivate viruses

7, Beta – propiolactone and formaldehyde are used for inactivation of viruses

8, Phenols : most viruses are resistant

9, Disinfectants - hypochlorite solution and glutaraldehyde are best to control the viruses

Virus diseases.

Most viruses diseases in humans are mild , few viral diseases are severe

Entry

Viruses enter the human body in four ways :

1**, Inhalation** – via respiratory tract e.g, Covid -19

2, **Ingestion** - via gastrointestinal tract e.g. Rota viruses, picor na viruses.(enteroviruses)

3, **Inoculation** – sexual transmission HIV(AIDS) , mosquito bite (Yellow fever virus) and sharing of infected injection needle (Hepatitis virus B),

4**, Congenital** – from mother to foetus HIV(AIDS)

Invasiveness

Spreading or transmission of virus from cell to tissues to organs

Chemical composition of Viruses

Viral protein

The major role is to transfer viral nucleic acid from one host cell to another and also protect the viral genome from nucleases, participate in the attachment

of virus particle to a susceptible cell, the protein determine the antigenic properties of the virus, the influenza virus have a surface protein haemagglutinnin it will agglutinate red blood cell and provide the structural symmetry of the virus particles

Some viruses carry enzymes (which are protein) inside the virions and may involve replicative processes., for example influenza virus(orthomyxoviruses) and rhabdoviruses carry RNA polymerase, this enzyme involve first m RNA synthesis, retrovirus carry reverse transcriptase , this enzyme involve in synthesis of DNA from RNA., many enzymes are present in core region of Pox virus and this enzyme involve in transcriptional processes.

Viral Nucleic Acid

Viruses contain a single kind of nucleic acid , either DNA or RNA, that encodes the genetic information necessary for replication of the virus, the genome may be single or double stranded, circular or linear and segmented or non-segmented,. based on nucleic acid viruses can be classified into various groups for example DNA viruses contain DNA as their genetic materials,

The size of genome is ranges from 3.2kbp (Hepadna viruses) to 375kbp (Pox viruses) , single linear positive sense+RNA (+ RNA is act itself as m RNA)as their genetic material for Picorna viruses, segmented form of negative sense -RNA (-RNA is itself act as template for synthesis of m RNA)in Orthomyxoviruses and Rhabdoviruses.

Viral lipid envelopes

A number of viruses contain lipid envelope as part of their structure, the lipid is acquired when the viral nucleocapsid buds through a cellular membrane in course of maturation, lipid – containing viruses are sensitive to ether and other organic solvents, non- lipid containing viruses are resistant to ether.

Viral glycoprotein

Some viruses the envelope contain glycoprotein, which are derived from the host cell, the envelope glycoprotein are virus encoded. The surface glycoprotein of an enveloped virus helps the virus particles to attaches the host cell receptor and also involved in fusion step of infection, the glycoprotein are also important viral antigens, they are frequently involved in the interaction of the of the virus particle with neutralizing antibody.

Viral Assay

The number of virus particles present in a specimen can be assayed in two ways :

1, total virus particles count

2, infectious virus assay

Total virus particles count

,Electron microscopy – In this method the virus particles are mixed with known concentrated latex particles and is stained by negative stain and counted directly under the electron microscope

Haemagglutination – In this method, clinical specimen is serially diluted and added into known concentrated RBC cell, if specimen contain haemagglutinin producing viruses are present , it will agglutinate with RBC to produce reddish agglutination reaction, highest titre is significant , minimum 10^7 influenza virus required to produce haemagglutination reaction. Both infective and non-infective particles give this reaction, this method used to measure total quantity of virus present in the specimen

Infectious Virions assay

Plaque assay

Here, viral suspension is inoculated into monolayer of cultured cells in a bottle or Petri dish, after adsorption the medium is replaced with solid agar gel, each infectious particles give rise to localized focus on infected cell known as plaques that be seen naked eye, the number of plaques indicates the number of infectious virus particles,

Pock aasay

Here, viral suspension is inoculated into chorioallantoic membrane of chick embryo, the virus particle produce the visible pocks, the number of pocks produced on CAM corresponds to the number of virus particles.

Purification of viruses

The first step is concentration of virus particles using ammonium sulphate or ethanol or polyethylene glycol or ultracentrifugation. Haemagglutination and elution can be used to concentrate Orthomyxoviruses. Once concentrated viruses can be separated from host materials by differential centrifugation , density gradient centrifugation, column chromatography and electrophoresis

In purification , first to remove the non - virus materials, in density gradient centrifugation in the presence of cesium chloride, potassium tartarate, potassium citrate or sucrose, the virus particles migrate to an equilibrium position where density of the solution is equal to virus buoyant density and form a visible band.

Column chromatography used for separation of virus particles from contaminants, here viruses are bound to phosphocellulose and then eluted by changes in p H.

Icosahedral virus particles are easy to purify than enveloped viruses, it is very difficult to achieve complete purity of viruses.

Replication of Viruses

Viruses multiply only in living cells, The host cell must provide the energy and synthetic machinery and low molecular weight precursors for synthesis of viral proteins and nucleic acids. The viral nucleic acid carries the genetic code for all virus –specific macromolecules.

After synthesis of viral nucleic acid and viral proteins, the components are assembled to form new infectious virus.

The duration for single virus replication is range from 6-8 hours for *Picorna virus*, more than 40 hours for some *Herpes viruses.*

Eclipse period – the time taken for virus for single replication processes is known as eclipse period

Permissive cell – cell that support the virus replication , (productive infection)

Non-permissive cell- cell not support the virus replication (defective or abortive infection)

Defective virus – virus that lack of some functional genes, this virus require helper virus for multiplication, for example *Hepatitis virus D* require helper virus *Adenovirus* or *Hepatitis virus B.*

Latent infection – viral genome persists in the host cell chromosome, no sign of any symptoms

General steps in viral replication(multiplication)

1, Attachement

Here , viruses attaches to host cell with the help of host cell specific receptor, chemical composition of receptors are varied , for example protein sequences for *Picorna viruses*, oligosaccharides for *Orthomyxoviruses* and *Paramyxoviruses,* glycoproteins for most viruses. CD4 receptor on cell of the immune system for HIV virus, CD21 receptor for Epstein – Barr virus, receptor number is more than one 100,000 in single susceptible cell. Two type of receptor for polio virus one is intestinal tract and another one is in nervous system.

2, Penetration or engulfment

Most viruses penetrate into the host cell through the plasma membrane, in other cases fusion of virus envelope with the plasma membrane of the host cell.

3, Un-coating

After penetration, physical separation of viral nucleic acid from protein coat is occurred., in some cases acidic p H is required.

4, Expression of viral genome and synthesis of viral components

After un-coating , m RNA synthesis occurred , two type of genes are present in viral genome, early gene, late gene, early gene produces proteins that are enzymes involved in viral genome replication, late gene produces proteins that are structural proteins assembled into new virus particle.

Pathway for transcription for various types of viruses

Type of nucleic acid	_	Type of m RNA	Example

ds DNA (+ -)	None	m RNA (+)	Adenoviruses
ss DNA(+)	ds DNA	m RNA(+)	ØX bacteriophage
ds RNA(+-)	none	m RNA(+)	Reoviruses
ss RNA(+)	ss RNA +	m RNA(+)	Picorna virus , Corona virus (covid-19)
ss RNA(-)	None	m RNA(+)	Rhabdovirus Orthomyxovirus, Paramyxoviruses
ss RNA((+)	DNA (-), DNA (+ -)	m RNA(+)	Retroviruses

Positive (+) (positive - sense)RNA strand means, this RNA itself act as m RNA.

Negative (-) RNA means this RNA not itself act as m RNA require RNA polymerase for synthesis of m RNA.

For example *Rhabdovirus ,Ortho, Paramxyovirus carry RNA plolymerase for synthesis of m RNA.*

Viruses replicate in host cell nucleus is Herpes viruses, in host cell cytoplasm is *Pox viruses,*

Splicing(processesing of mRNA) mechanism can be seen in replicative processes of *Adenovirus* .

Larger viruses are more susceptible to antiviral chemotheraphy

Many viral protein undergoes glycosylation seen in *Ortho, Paramyxovirus* (adding of sugar molecule into protein), acylation(adding of acyl group into protein for example adding of acetyl chloride) , cleavage.

5, **Morphogenesis and Release**

It refer assembling of newly synthesized genome and proteins to form progeny or new viruses. Sometimes icosahedral capsids assembled to form new virus without genome, but helical capsids not assembled to form new virus without genome.

Non- enveloped viruses are released from host cell after lysis of host cell, Enveloped viruses mature and release by a budding process, virus specific glycoproteins are inserted into host cell membrane.

Genetics of Viruses

Viruses that have stable antigens on their surface, such viruses can be controlled by vaccination for example - *Polioviruses, Measles viruses.*

Viruses that have not stable antigens on their surface such viruses cannot be controlled by vaccination for example – COVID-19, Influenza virus.

Wild virus or field virus – natural strain without changing genetic character.

Mutant strain – strain with changing genetic character.

Genotype – genetic system of the virus

Phenotype - observable character of the virus

Ecology

It refer interaction between viruses and their environment,

Transmission

Viruses transmitted from one man to another via faecal- oral route example Polioviruses, via sexual contact example *HIV, Hepatitis B virus, Herpes simplex* type 2 virus, via aerosol or droplet example COVID-19, *Influenza virus, Measles, Small pox*, via bite of dog example *Rhabdo virus* or rabies virus, via hand- mouth, hand-eye , mouth-mouth example *Herpes virus* type 1, *Rhino virus.* Via contaminated blood and needle example *Hepatitis B virus.,* via bite of mosquito example *Yellow fever virus.*

Viroids – these are smaller than viruses and cause diseases in plants, these are protein free nucleic acid molecule and contain single stranded RNA

Prions- these are infectious particles made up of protein with no nucleic acid, resistant to heat, formaldehyde and UV rays, they cause disease s such as scrapie of sheep and goat, spongiform encephalopathy, Kuru and Creutzfelt-Jacob disease , these are not true viruses.

Treatment of Viral infections

Antiviral chemotherapy

Viruses are obligate intracellular parasites, antiviral agents must be capable of selectively inhibiting viral infection without damaging host, Furthermore, an ideal drug would reduce disease symptoms without affecting immune system of the host.

Nucleoside analogs – nucleoside means nitrogenous base(Purines – adenine and guanine, Pyrimidines – cytosine and thymine) and ribose(RNA) or deoxribosugar(DNA). Or nitrogenous base and sugar

Analogs means – compounds structurally similar functionally differ,

1, **Acyclovir** – is an analog of guanosine and deoxyguanosine that strongly inhibit *Herpes viruses* especially virus encoded DNA polymerase and thymidine kinase (enzyme add phosphate group into DNA)

2, **Didanosine** – is a analog of dideoxynucleoside that inhibits HIV reverse transcriptase and blocks the proviral DNA formation.This drug was approved for treatment of HIV infections in 1991.

3, **Ganciclovir** – is a guanine anlogs, inhibit the DNA polymerase of *Cytomegalovirus*.

4, **Idoxuridine** – is a pyrimidine anolog ,inhibite thymidine kinase of *Herpes simplex virus*, topical agent(applied directly to affected part of the body) for eye infection caused by *Herpes virus*

5, **Lamivudine** – nucleoside analog , inhibits reverse transcriptase of HIV. This enzyme responsible for conversion of viral RNA into DNA, this DNA form provirus and integrated into host cell chromosome. Blockage of DNA formation this virus is failed to cause diseases,

6, **Ribavirin** – guanosine analog, used for treatment of infection caused by *Influenza* and *Respiratory syncytial virus*, Lassa fever.caused by *Lassa mammarinavirus*.

7, **Stavudine** – thymidine nucleoside analog, inhibits the reverse transcriptase of HIV.

8, **Trifluridine** – pyrimidine nucleoside analog , it interfere the DNA synthesis of *Herpes.*

9, **Vidarabine** – a purine analog , it blocks the viral DNA synthesis of *Herpes virus, Varicella* –zoster , Cytomegalovirus, *Hepatitis virus B* and *Herpes simplex virus*.

10, **Zalcitabine** – nucleoside analog, inhibits reverse transcriptase enzyme of HIV

11, **Zidovudine** – thymidine analog , it blocks or inhibits the proviral DNA formation by HIV.

12, **Remdesiver** – adenosine analog used for treatment of Covid-19, it will (bind) inhibit RNA dependent RNA polymerase, so virus failed to replicates, it is approved by Food and Drug Administration(FDA).

13, **2-deoxy-2-D- glucose(2DG**) developed by DRDO – Defence Research and Development Organisation , anti – COVID drug , it will reduce supplemental oxygen dependence, oral drug.

Nucleotide analog- nucleotide means nucleoside plus phosphate compound or nitrogenous base , sugar plus phosphate group.

Compounds similar to nucleotide in structurally and functionally differ is nucleotide analog

1, **Cidofovi**r – it inhibite DNA polymerase of *Herpes virus* and *Cytomegalo virus*.

Non nucleoside inhibitor

Nevirapine – inhibits or blocks the synthesis of reverse transcriptase of HIV.

Protease inhibitors

This enzymes are synthesised by viral genome for cleavage of structural protein during assembly

Or maturation of virus particles. Without this enzymes the virus produces non-infectious virus particles.

1, **Indinavir**

2, **Ritonavir**

3, **Saquinavir** – the three drugs used for treatment of HIV-I, HIV-II.

Other types of antiviral drugs

1, **Amantadine** - a synthetic amine compound, this compound will block the un-coating processes of Influenza viruses.

2, **Foscarnet** – pyrophosphate analog, it will block the pyrophosphate binding site by pyrophosphate during DNA polymerase by DNA viruses and reverse transcriptase by retroviruses used for treating *Cytomegalo virus, Epstein – Barr virus, Hepatitis virus B, Varicella –zoster virus, Retroviruses, Herpes simplex virus*,

3, **Methisazone** – it will block the pox viral replication at final stage that result in production of immature or non-infectious virus particles.

Cytokines – are small protein molecule released by virally infected cell, including lymphocytes, macrophages example for cytokines are interferon, interleukin and tumour necrosis factor

Interferon - are cytokine , three types

1, **Alpha interferon** – produced by leucocytes

2, **Beta** – produced by fibroblast

3, **Gamma** – by T- lymphocytes

Characteristics of interferon

Host –specific – human interferon act only human cell, not in animal cell

Broad spectrum – it will block wide range of viruses

Function – it will inhibits virus replication (blocks the transcription and protein synthesis)

Chemotherapeutic agent

It is an ideal chemotherapeutic agents against viruses, useful for treatment of chronic viral hepatitis.

Prevention of viral diseases

Viral infection can be prevented by vaccination

Vaccine – is a biological preparation that provide active acquired immunity to a particular disease A vaccine typically contains an agent that resembles a disease causing microorganisms and is often made from weakened or killed form of microbe, its toxin or one of its surface proteins. The agent stimulate immune system to produce antibodies against the viruses. Vaccines are prophylactic agent.

Vaccination- administration (injection)of vaccine into the body.

Based on preparation vaccine can be **classified into various types**:

1, **Killed vaccine** – it contain biological material that are killed form

For example polio vaccine prepared from human diploid cell line or monkey kidney, route of administration is subcutaneous

Rabies vaccine prepared from human diploid cell line, route of administration is intramuscular

2, **Live attenuated vaccine** – it contain less virulent strain , but good antigenic properties

For example , vaccine for *Varicella, Measles, Mumps, Rubella*, vaccine prepared from tissue culture(chick embryo) route of administration is subcutaneous.

3, **Sub-unit vaccine** - it contain microbial antigens only

For example vaccine for hepatitis B prepared from recombinant Yeast, route of administration is intramuscular, haemagglutinin antigen from Influenza virus.

4, **Live vaccine** – it contain live strain , pathogenic character is absent, but antigenic character is similar to causative agent.

For example live Vaccinia strain used for small pox caused by *Variola virus.*

5,**Oral polio vaccine** - contain attenuated culture of *poliovirus* prepared from tissue culture (human diploid cell line or monkey kidney) route of administration is oral.

6, **Vaccine for Covid-19**

1,**Covishield** :it contain non replicating viral- vector based vaccine, , two doses, given intramuscularly, manufactured by Oxford University , Serum Institute of India, Maharashtra , Pune

2, **Covaxin** : it contain inactivated Covid -19 virus two doses, given intramuscularly, manufactured by Bharat Biotech Ltd, Hyderabad,

3, **Sputnik V vaccine** : :it contain non replicating viral- vector based vaccine, single dose, manufactured by Gamaleya Research Institute of Epidemiology and Microbiology Moscow,(Russia) .In India, it has collaborated with Dr. Reddy s Apollo Hospitals, Hyderabad.

Preservation of viruses

Viruses can be preserved by sub freezing temperatures, and some withstand lyophilisation and can be preserved at 4^0C or even at room temperature,

Enveloped viruses are heat labile, icosahedral or non-enveloped viruses are heat stable

Stabilization of viruses

Viruses are stabilized by Mg SO_4, $MgCl_2$, and Na_2SO_4

The stability of viruses are important in vaccine preparation

Radiation – Ultraviolet , X-ray can inactivate viruses

P H – most viruses are stable at p H 5-9.0.

Laboratory safety in Virology Lab

Many viruses are human pathogens and laboratory acquired infection can occur. Laboratory procedures are often potentially hazardous, if proper techniques are not followed

While working in virology lab, use of protective coats and gloves,

Wear mask to avoid entry of airborne pathogens and inhalation of aerosols or droplets or droplet nuclei.

Aerosols are generated by homogenization of infected tissues, centrifugation, broken glassware

No drinking, eating and smoking in the laboratory

Immunize relevant vaccines

Avoid mouth pipetting,

Wash the hands frequently

Laboratory diagnosis of Viral infection

Viral diseases are diagnosed mainly by immunological techniques that is demonstration of antigen – antibody reaction to reveal the virus infection

Electron microscopy – used for detection of viruses from patient stool , viruses include rota virus and other enteroviruses

Serology – typically a virus infection stimulate or elicits immune responses against one or more viral antigens, both cellular and humoral immune responses usually develop, measurement of either may be used to diagnose the viral infection.

Cellular immunity may be assessed by dermal hypersensitivity, lymphocyte transformation and cytotoxicity test,

Dermal hypersensitivity –it refer finding any itchy scattered red papules in skin caused by virus

Lymphocyte transformation - finding proliferative T cells caused by virus infection

Cytotoxicity test - it refer finding abnormality of cells caused by viruses

Humoral immunity may be assessed by Neutralization test(Nt), Complement fixation test(CF), Haemagglutination inhibition test(HI) and immunofluorescence test (IF)

During viral infection , antibodies Ig M appear initially followed by Ig G antibodies, The IgM antibodies disappear in several weeks, where as the IgG persists for many years

Collection of blood specimen

Detection of antibodies

Blood Serum are essential for diagnosis of virus specific antibodies, blood specimen should be withdrawn without anticoagulants and the serum separated and stored at 4^0C or – 20 ^{0}C, Before performing serological test, it may be necessary to heat the serum 56^0C for 30 minutes

Neutralization test(Nt) – in this test serum is collected from infected patient and inoculated into cell culture, if virus specific antibodies are present, culture cell

failed to produce cytopathic effect(CPE), while control cell culture which have received virus plus a serum free of antibody develop cytopathic effects.

Complement fixation test(CF) – in this test the patient serum contain virus specific antibodies it will fix the complement in the presence of virus antigen extracted from cell culture that result in no lysis of sheep RBC, if serum contain no virus specific antibodies that result in lysis of sheep RBC.

Haemagglutination inhibition test(HI) - in this test the patient serum contain virus specific antibodies it will fix the haemagglutinin antigen extracted from tissue cell culture and free from erythrocytes, so erythrocytes are settled at bottom of test tube. if serum contain no virus specific antibodies that result in haemagglutination – formation of reddish color. This test used for identification of Influenza virus.

immunofluorescence test (IF) - in this test the patient serum contain virus specific antibodies it will fix the fluorescent labelled antigen extracted from tissue cell culture and examined under fluorescent microscope , presence of fluorescence indicate positive,

Antigen detection

ELISA – Enzyme Linked Immunosorbent Assay – used for detection of virus specific antigens(Direct ELISA) as well as antibodies.(Indirect ELISA) used for quantitative estimation of either antigen or antibodies.

Western blot – used for detection of virus specific protein (antigen) from clinical specimen

Inclusion body – virus induced masses(Viral protein plus viral genome) can be seen in host cell cytoplasm or nucleus for example Rabies (Negri bodies).

Virus isolation – it require living cells, since virus can not grow on inanimate media

Three system used for this

1, tissue culture

2, chick embryo

3, laboratory animals

In tissue culture the cells are propagated in the presence of balanced and buffered salt solution with added amino acids, vitamins and serum, antibiotics are added to prevent the bacterial contamination.

It is grown in stoppered test tubes or screw capped bottle or Petri plate with 5-10% CO2, temperature for cell is 37^0C for some viruses, for some required 33^0C., primary cell line is short lived example monkey kidney cell, continuous cell line is He La cells (derived from human cervical cancer cell), it will be subcultured indefenitly. Clinical specimen is inoculated in tissue culture.

Virus growth is recognized by CPE(killing of cells), Haemadsorption(adhering of erythrocytres in cells), immunofluorescet test,,

Chick embryo – rarely used for virus diagnosis, useful for preparation of bulk virus for vaccine production.

Laboratory animals – here clinical specimen is injected into lab animal like Rabbit or Mice, , useful for study viral pathogenesis

PCR – polymerase chain reaction used for diagnosis of viral genome from clinical specimens,

RT- PCR is used for diagnosis of COVID-19 from clinical specimen like respiratory or mouth swab of patient.

Classification of viruses

Classification of DNA viruses and their diseases

Family	**Viruses**	**Diseases**
Poxviruses	Variola molluscum	Smallpox Molluscum contagious
Herpesviruses	Herpes simplex Varicella-zoster Cytomegalovirus EB(Epstein Barr) virus HHV6	Herpes chickenpox,shingles infection in the immunocompromised infectious mononucleosis exanthema subitum
Adenovirus	adenoviruses	Sore throat , conjunctivitis

Hepadnaviruses	hepatitisB	hepatitis
Papovaviruses	Papilloma JC virus	Warts progressive multifocal leuconcephalopathy
Parvoviruses	B19	Erythema infectiosum,aplastic crises

Classification of RNA viruses and their diseases

Family	**Viruses**	**Diseases**
Orthomyxoviruses	influenza	influenza
Paramyxoviruses	Parainfluenza ,respiratory syncytial ,measles,	respiratory infection Measles mumps

	mumps	
Coronaviruses	coronavirus	respiratory infection
Rhabdoviruses	rabies	rabies
Picornaviruses	Enteroviruses Rhinoviruses hepatitisA	meningitits, paralysiscolds hepatitis
caliciviruses	SRSVs(small round structured viruses)	gastroenteritis
Togaviruses	Alphaviruses(GroupA arboviruses)rubivirus	encephalitits, haemorrhagic fevers rubella
Flaviviruses	Flaviviruses(GroupB arboviruses) hepatitis C	Encephalitis,haemorrhagic fevers hepatitis
Bunyaviruses	Some arboviruses	encephalitits,haemorrhagic fevers fever,renal involvement
Reoviruses	rotavirus	gastroenteritis
Arenaviruses	Lymphocytic Choriomeningitis Machupo virus Junin virus	haemorrhagic fevers
Retroviruses	HTLV- II Hiv -1,2	T cell leukaemia, lymphoma,paresis AIDS
Filoviruses	Marburg virus Ebola virus	marburg disease Ebola haemorrhagic fever

Baltimore classification

The viruses are classified into six groups on the basis of their nucleic acid and m RNA production.

Family	**Viruses**	**Genomes**
Poxviruses	Variola Molluscum, Vaccinia	DS DNA
Herpesviruses	Herpes simplex Varicella-zoster Cytomegalovirus EB(Epstein Barr) virus HHV6	DS DNA
Adenovirus	adenoviruses	DS DNA
Hepadnaviruses	hepatitisB	DS DNA
Papovaviruses	Papilloma JC virus	DS DNA
Parvoviruses	B19	SS DNA
Orthomyxoviruses	Influenza A	SS - RNA
Rhabdoviruses	rabies	SS - RNA
Paramyxoviruses	Parainfluenza, respiratory syncytial measles, mumps	SS - RNA
Arena	Lassa fever,	SS - RNA
Bunya	Hemorrhagic fever	SS - RNA
Picorna	Polio virus	SS+ RNA
Flavi	Yellow fever virus	SS+ RNA
Filo	Marburg	SS+ RNA
Corona	Corona virus	SS+ RNA
Calici	Small round structured viruses SRSV	SS+ RNA
Retroviruses	Retrovirus	SS+ RNA
Reovirus	Rota virus	DS RNA

Host response to Virus infection or immunity to virus infection

The body defence mechanisms to virus infection are of two types:

1. Non-specific
2. Specific

Non-specific defence mechanisms

The body has defence which are not specifically directed at particular infectious agent, but which serve as non-immunological barriers to infection;

1. Skin: an effective and impermeable barrier unless breached by injury ,disease, etc

2.**Respiratory tract:**

Upward flow of mucus by ciliated epithelium removes virus particles to prevent invasion of the lower respiratory tract.

3.**Gastrointestinal tract:** stomach acid inactivates acid –labile viruses .Bile (which lyses enveloped viruses)movement of intestinal contents and uptake of virus by lymphoid tissue all aid elimination of ingested viruses.

4.**Urinary tract**: flow of urine remove viruses.

5**.Conjunctiva**; tears flush viruses from the eye

6.**Phagocytosis**; an important defence mechanism in bacterial infection and in virus infections also : invading viruses – like bacteria – are ingested by two types of scavenger cell;

(a)**neutrophil** polymorphonuclear leucocytes

(b)**macrophages** (or mononuclear cells of the reticuloendothelial system)- of two types;

(i)free macrophages in lung alveoli, peritoneum

(ii)fixed macrophages in lymph nodes , spleen, liver(Kupffer cells), connective tissue (histiocytes)and CNS (microglia).

Phagocyosis is enhanced by antibody (a specific immune mechanism) and complement : this effect is known as **opsonisation**.

Macrophages are `activated` by cytokines released by T lymphocytes

Specific (immunological) defence mechanisms

Humoral response - is mediated by immunoglobulins IgM, IgG, IgA are responsible for neutralization of viruses.

Cell mediated immunity

CD4 helper T- cells require MHC – Class II antigens for their activation, liberate cytokine, it also interact with B- lymphocytes for antibody production.

CD8 cytotoxic T cells – they lyse virus infected cell and tumour cells

Natural killer(NK) cells and killer(K) cells are responsible for destruction of virus infected cell and tumour cells.

Interferon - are cytokine , three types

1, Alpha interferon – produced by leucocytes

2, Beta – produced by fibroplast

3, Gamma – by T- lymphocytes

Characteristics of interferon

Host –specific – human interferon act only human cell, not in animal cell

Broad spectrum – it will block wide range of viruses

Function – it will inhibits virus replication(blocks the transcription and protein synthesis)

Bacteriophages or Viruses of Bacteria or Bacteria eater

Bacteriophages are viruses that infect bacteria, were discovered independently by **Frederick Twort** in 1915 and **Felix Herelle** in 1917. The word bacteriophage means bacteria eater, filterable agent , invisible, smallest, self - replicating microbe that was parasitic for bacteria, , it is widely used for genetic research.

General Characteristics of bacteriophage.

Bacteriophages are widely distributed in nature, it is made up of nucleic acid surrounded by protein coat, it occur in different shapes, they have tail through which they inoculate their nucleic acid into the host cell.

There are two types of bacteriophage 1, lytic or virulent 2, temperate or avirulent

When lytic phage infect cells, the cells respond and produce many number of phage particles at the end of the incubation period the host cell burst or lyses , releasing new phage particles, this is called as lytic cycle.

In temperate phage infection , the viral nucleic acid is integrated into host cell chromosomal DNA and replicated in the host bacterial cells from generation to generation without any cell lysis, However ,temperate phage spontaneously become virulent, generate new phage particle that are released after burst of host cells,

Morphology of Bacteriophge

Most phages shows cubic or helical symmetry, cubic phages regular solids or polyhedra, helical phages are rod- shaped, polyhrdral phage are icosahedral in shape – 20 triangular faces, 12 vertices.

Some bacteriophages such as T- even coliphage(T2,T4 and T6) have complex structure(binal symmetry) because head is icosahedral, tail is helical tail,

Typically, bacteriophages are made up of head (icosahedral) tail (helical) the tail have tail fiber used for attaches to host cell

Based on morphology the phages are classified into seven groups

1, phage have binal symmetry- hexagonal head, rigid tail with contracted sheath and tail fibers .Examples : T2,T4,T6 – they have ds DNA.

2, Similar to one but lack of contractile sheath , Examples : T1, T5 they have dsDNA.

3,This phage is characterized by a icosahedral head with a shortest tail, Examples: T3,T7 they have ds DNA.

4, This phage head(cubic) is made up of large capsomeres, but has no tail Examples: Ø x 174, S13.

5, This phage head(cubic) is made up of small capsomeres, but has no tail Examples: MS2, f2.

6, Filamentous type Example: fd, f1

7. Enveloped spherical Example: MV-12 (Plasmoviridae)

Bacteriophages of E.coli

T1 ,T2,T3,T4,T5,T6,T7 Phages having dsDNA as their genetic material

Ø x 174, fd, M13 Phages having ssDNA as their genetic materials

f2,R17,QB Phages having ss RNA as their genetic material

Replication of Bacteriophages

Bacteriophage show two types of life cycle

1, Lytic cycle.or virulent

2, Temperate or lysogenic cycle

It consists of following steps - **Lytic cycle**

Adsorption

This is first step, phage attaches to bacterial cell with the help of tail fiber, , in some cases the specific receptor of the bacterium is lipopolysaccharides, flagella, pili, carbohydrates, protein in the membrane or cell wall., some bacterial mutants have lost the ability to synthesize specific receptor, become resistant to phage infection,

Penetration

The virus injects its genetic material with the help of contractile sheath of tail

Transcription

Phage genome have two types of genes, one is early gene it will produces early proteins act as enzyme for synthesis of new viral genome, late gene it will produces structural protein, both structural protein , new viral genome are assembled to form new phages,

Assembly and Release

The new phage particles are range from 150-200 , formed within 25 minutes, new phages are released after bursting of bacterial cell,

This kind of replication can be seen in virulent phage or lytic cycle.(for example T4 phage)

Temperate or lysogenic cycle (lambda phage)

Here, phage fail to lyse the host cell, The phage DNA either gets integrated with the bacterial chromosome or exists as a free plasmid in the bacterial cell, when

the phage nucleic acid integrated into bacterial chromosome it is known as **prophage**

The prophage replicates along with the bacterial chromosome,This is called as **lysogeny,** a bacterium that carries a prophage is called as **lysogenic bacterium.**

Prophage confers certain new character or properties on the **lysogenic bacterium,** This phenomenon is called as **lysogenic conversion** or **phage conversion**

Occasionally , the integrated prophage may become excised from bacterial DNA, This excised prophage can initiate lytic cycle. This is known as spontaneous induction of prophage.

Factor which induces the lytic cycle in temperate phage is physical factors like hydrogen peroxide, UV rays.

The lysogenic bacterium is not re-infected by the same or related phages. This is known as **super infection immunity**.

Uses of bacteriophages

Medical aspect of lysogeny

Diphtheria is a human diseases caused by bacterium ***Corynebacterium diphtheria***, this bacteria produce a toxin , it can produce toxin when it carries a temperate phage. In the same way only those **streptococci** which carry a temperate phage can produce the **erythrogenic** rash producing **toxin** of scarlet fever, **botulism** toxin are produced by ***Clostridium botulinum*** as a result of lysogeny This phenomenon in which prophage is able to make changes in the properties of a host bacterium in lysogen is termed lysogenic conversion.

Bacterial lysogeny is a good model for the study of oncogenic or cancer producing viriuses.

Since the virulent or lytic phages can destroy their host bacterial cells, it was logical that inoculation of such phages into a bacteria –infected individuals would result in elimination of the pathoges, However, after numerous studies, there is no evidence to show phages can be used therapeutically to destroy bacterial pathogens in the human body.

In **recombinant DNA technology**, M13 phage genome , lambda genome are act as gene cloning vectors.

Isolation and cultivation of bacterial viruses – bacterial viruses are easily isolated and cultivated in young actively growing culture of bacteria in broth or on agar plates. In liquid cultures, lysing of the bacteria may cause a **cloudy culture to become clear,** whereas in agar plate culture , clear zone or **plaques** become visible to the unaided eye.

Orthomyxoviruses (Influenza virus)

These are RNA viruses, belong to family orthomyxoviridae, infects vertebrates, this virus causes influenza , myxa means mucus of respiratory tract , this virus has highly affinity with mucin (protein of R.tract), R means respiratory.

Morphology

Size : is medium 80-120 nm in diameter

Shape: is spherical, pleomorphic and filamentous form.

Genome is single stranded negative sense RNA in eight pieces, it also contains RNA dependent RNA polymerase.

Envelope: is present having two kinds of glycoprotein projection or spike one is haemagglutinin (peplomeres) and another is neurominidase (mushroom shaped- spikes), the envelope chemically lipid derived from host cell membrane during process of budding or maturation.

The envelope is made up of two layer inner protein layer , it is called as matrix or M protein ,it is virus coded and another layer is outer lipid layer derived from host cell membrane.

Cultivation

Egg inoculation – influenza virus is cultivated in the amniotic cavity of 11-13 days old chick embryo, influenza virus type A and B can grow in both allantoic and amniotic cavities. Type C can grow only in amniotic cavity,

Virus growth can be detected by haemagglutination test.

Tissue culture

Virus can grow on primary monkey kidney or human embryo kidney cells.

Virus growth can be detected by haemadsorption or haemagglutination test.

Animal inoculation

Virus is inoculated through the nasal into mice for study of pathogenesis of virus

Physical and chemical properties

Inactivate by heat at 56^0C for 30 minutes

Virus is viable for one week at 4^0C, at -20 ^{0}C for one year.

Sensitive to ether, phenol and formaldehyde

Antigens

Four types of antigens present.

1, RNP- ribonuleoprotein antigen(not show antigenic variation)

2, M protein or matrix antigen (two components M1 and M2)

3, Haemagglutinin(HA) – glycoprotein, two polypeptides HA1 and HA2, show antigenic variation , antibodies are formed following infection, HA helps the virus to fuse with the cell membrane.

4, Neuraminidase (NA) – glycoprotein enzyme destroy the host cell receptor , show antigenic variation , antibodies are formed following infection, helps the new virus particles to release form host cell at the end of the life cycle, it also prevent self-aggregation of viruses by removing sialic acid residues from viral glycoproteins

Based on nature of RNP and M proteins, the virus is classified into three types

A,B and C,

Type A, B and C only infect humans, type A also infect horse, chicken, pigs, ducks.

Antigenic variation

Two types of antigenic variation can be seen in HA,NA, due to mutation in gene code for HA, NA.

Antigenic variation is two types

1, **Antigenic shift**

It is an abrupt(sudden and unexpected), major antigenic change in HA and NA resulting in the emergence of new strain unrelated to predecessor(old) strain

Antigenic shift is probably due to gene assortment or gene recombination,

The new variant are not neutralized by antibodies of the predecessor, antigenic shift is associated with major epidemics and pandemics of influenza.

2, **Antigenic drift**

It is a minor change in gene code for HA and NA, this is due to mutation. Antiserum to the predecessor strain can react with the mutants to some extent

Haemagglutination

It is an important property of influenza virus, used for detection of virus in tissue culture

Replication

It has single stranded negative sense RNA, (SS - RNA)

Pathogenesis

Virus enter the lungs via R.tract or inhalation of aerosols or droplets or droplet nuclei from infected person, after entry the virus neuraminidase destroy the mucus lining of R. Tract, that is ciliated epithelial cells, death of ciliated epithelial cells render the R. tract susceptible to secondary bacterial infections,

Incubation period is 1- 4 days

Clinical symptoms include chills, fever, sore throat, cough, headache and myalgia

Laboratory diagnosis

Fluorescent microscopy

Specimen – nasopharyngeal secretion, nasal swab,

Specimen is treated with fluorescent labelled antibodies and examined under fluorescent microscope, the cells containing viral antigen will fluoresce.

Isolation of viruses

Clinical specimen can be inoculated into amniotic cavity of 11-13 days chick embryo or monkey or human kidney cells

Virus growth is identified by haemadsorption or haemagglutination

Serology

Complement fixation test

Haemagglutination inhibition test

Direct or indirect ELISA for demonstration of viral antigen and antibody.

Epidemiology

Influenza virus A causes epidemic and pandemics, Influenza virus B causes sporadic and epidemics

The pandemic and epidemics of Influenza virus is due to antigenic variation in the virus

Both A and B strain may evolved from avian or animal reservoir either by mutation or due to recombination of human strain,

Immunity

Neutralising antibody responsible for controlling re-infection caused by virus

Prevention or prophylaxis

1, Inactivated vaccine (subunit vaccine)

Influenza virus is cultivated in allantoic cavity of chick embryo is inactivated with formalin or betaprobiolactone, the purified vaccine contain only haemaggulitinins and neuraminidase and is known as **subunit vaccine.**

It induces the formation of neutralising antibodies after immunization

It gives protection for one year

2, Live attenuated vaccine

Temperature sensitive mutant strain of Influenza virus is cultivated at 33^0C ,used for preparation of live vaccine, administered intra-nasally or aerosol spray

It stimulate Ig A antibodies production

Treatment

Amantadine or rimantadine is effective for treatment of Influenza virus infection .

Paramyxoviruses

It includes parainfluenza virus, mumps virus, measles, and respiratory syncytial virus, these are human pathogens , all are airborne pathogens.

All members of paramyxoviridae family initiate infection via respiratory tract, replication of respiratory pathogens is limited to the respiratory epithelia, where as measles and mumps become disseminated throughout the body and produce generalized disease.

Rubella virus is a toga virus member because in physic-chemical properties, on the basis of epidemiologic this virus is included in paramyxovirus.

Morphology

Size : 100 -300 nm in diameter

Shape : spherical

Nucleic acid : negative sense single stranded RNA as a single piece, nucleocapsid is **helical.**

Envelope: present, it contains two types of projections or spike(HANA - haemagglutinin neuraminidase) responsible host cell attachment and fusion protein(F) is a key factor for pathogenesis , it mediate fusion of viral envelope with the plasma membrane of the host cell, also responsible for cell- to –cell fusion which permits the viral spread. It also causes large cell formation (giant cell) Fusion protein also glycoprotein.

Parainfluenza viruses

About 10-15% of R.infection in children are caused by parainfluenza viruses, four types of influenza viruses 1,2,3 and 4.

Type 1 is haemagglutinating virus (sendai virus) ,type 2 is known as Croup associated virus or CA virus , it grow in monkey kidney or human cells producing syncytial cytopathic effect., type 3 is haemadsorption virus

They causes lower R. infection, such as pneumonia, bronchitis and bronchilities

Type 4 is associated with mild R. illness in children

The infection is transmitted by droplets and by contact with the R. secretion

Incubation period : is 2-6 days

Lab diagnosis

Demonstration of viral antigens in the R.secretion by immunofluoresce or ELISA

Isolation of virus from throat and nasal swabs in monkey kidney cells, virus growth is identified by haemadsorption , type of virus is confirmed by haemagglutination inhibition test

Complement fixation test, demonstration of neutralization, ELISA.

Newcastle disease virus or Raniket virus

It causes pneumoencephalities or influenza in ploultry

Mumps virus

It causes epidemic **parotitis** in children characterized by enlargement of the parotid glands

This virus agglutinate the RBC of guinea pigs and humans

Cultivation

It can grow in amniotic and allantoic cavities of chick embryo or primary kidney cells.

Transmitted by saliva or aerosols from infected persons and the portal of entry is R.tract

Incubation period is 15-18 days

The virus multiplies in the upper R.tract and in the local lymph nodes, it then spreads to other part of the body via blood (primary viremia) like in parotid

gland and causes swelling of one or both P.gland (**parotitis),** in brain causes encephalitis, arthritis, pancreatitis, myocardities and renal failure.

primary viremia

It refer to the initial spread of virus in the blood from the first site of infection,

Secondary viremia - it occurs when primary viremia has resulted in infection of additional tissues via bloodstream, in which virus has replicated and once more entered the circulation

Laboratory diagnosis

Demonsration of virus in R.secretion or saliva by immunofluoresce test

Isolation of virus from saliva or cerebrospinal fluid (CSF) or urine, grow on monkey kidney cell culture, virus growth is identified by haemadsorption.

Detection mumps specific IgM by ELISA

Prevention

MMR(Mumps-Measles- Rubella) **vaccine**, it contain live attenuated mumps virus used as preventive medicine.

Measles virus (Rubeola virus infections)

It is highly infectious virus causes diseases in children, transmitted by respiratory secretions

Incubation period is 10-12 days

Measles virus enter the body via R. tract and multiply locally, it then invades the blood stream and infect macrophages or reticuloendothelial system, after multiplying there, it is transported to epithelial surfaces of skin, mouth, R.tract and conjunctiva.

The disease is characterised by high fever, cough, conjunctivitis and maculopapular rash in skin(characterized by a flat , red area on the skin that is covered with small confluent bumps), -Koplik,s Spot(small white spot with reddish background) can be seen in buccal mucosa.

It also causes otitits media(ear infection), bronchopneumonia and Croup(inflammation of larynx and trachea in children), a single infection confer life long immunity

Laboratory diagnosis

Demonstration of virus in nasal.secretion by immunofluorescence test

Demonstration of giant cells from nasal secretion by Giemsa stain

Isolation of measles virus from blood, conjunctiva, nasopharyngeal swab or throat washing using human or monkey kidney cell culture or amniotic cavities of chick embryo, virus growth is identified by immunofluorescence test

Demonstration of measles specific IgM antibody in the serum by ELISA

Haemagglutination inhibition test, neutralization test, complement fixation test

Prevention

MMR(live atteneuated) vaccine injected subcutaneously.

Respiratory Syncytial Virus (RSV)

It causes lower R.infection in children

Medium **size** 150-300 nm in diameter

It **lacks** both haemagglutinin and neuraminidase

But envelope contain two **glycoproteins G and F**. The G protein helps the virus to attach the cell surface, The F protein induces fusion of viral and host cell membrane. It also brings about cell to cell fusion resulting in the formation of multinucleated syncytia.

RSV does not grow in chick embryo, can grow in He La , or monkey kidney cells. It is antigenically stable and contains two subtypes A and B.

It causes bronchilities and pneumonitis in infants , in older children and adults it causes rhinitis and common cold

RSV is transmitted by contaminated hands, fomites, etc.

Incubation period is 4-5 days

This viru multiplies in the nose and throat and spread into lower R.tract causes bronchilitis and pneumonia.

The virus shed in R.secretions

Laboratory diagnosis

Detection of viral antigens in the nasopharyngeal secretion by immunofluorescece and by ELISA.

Isolation of viruses in He La cell culture , virus growth is identified by immunofluorescence test.

Rubella (German measles) virus infection

Rubella is a mild disease , but if it affect the early pregnancy the virus cause severe **congenital** abnormalities and diseases in the **foetus.**

Clinical features

A mild fever with a macular rash which spreads from face to behind ears, pharyngitis enlargement of the cervical lymph glands

Incubation period is 14 – 23 days (average 18 days)

Virus is present in both blood and pharyngeal secretions and is shed during incubation period for 7 days before the appearance of rash., 2 weeks after rash appears.

Immunity after rubella good after both naturally and vaccine- acquired rubella

Congenital infection

Congenital defects occur only if mother has rubella in the first 16 weeks of pregnancy

Defects include

Cataract

Nerve deafness

Cardiac abnormalities

Hepatosplenomegaly, thrombocytopenic purpura , low birth weight, mental retardation, jaundice, anaemia eytc.

It cause (teratogenic properties) early abortions.

Epidemiology

Mainly seen in children under 15 years old, before introduction of vaccination programme, rubella has shown decline in incidence since the introduction of MMR vaccine

Virology

A non –arthropod borne togavirus, one serological type

RNA virus single stranded negative sense RNA.

Pleomorphic, enveloped particles, medium size 50-75 nm, helical symmetry.

Haemagglutinate avian erythrocytes.

Grow in rabbit kidney cell line with the production of CPE

Diagnosis

Serology

Detection of IgM antibody by ELISA or immunofluorescence

Molecular level diagnosis

PCR is used for detection of viral genome

Isolation

Rabbit kidney cell line or rabbit cornea cell line , observe CPE

Prophylaxis

MMR vaccination , one dose subcutaneously or intramuscularly. It contain live attenuated measles, mumps and rubella virus strains

Protection

Excellent, immunity to be long lasting.

Rabies virus

Morphology

Rabies virus belong to family Rhabdoviridae,

Shape : bullet shaped with one end round and other end plane or concave

Size : 75-180nm

Envelope is present , contains a lipoprotein envelope carrying peplomers, below the envelope is the membrane or matrix (M) protein layer

Nucleic acid : nucleocapsid shows **helical symmetry,** genome is unsegmented single stranded negative sense **RNA genome** and RNA dependent RNA polymerase

Host range- all mammals are susceptible to rabies infection, cattle, cats and fox are highly susceptible, while humans and dogs have intermediate susceptibility.

Natural strain is **street virus**, it produces intracytoplasmic inclusion bodies known as **Negri bodies** are seen in brain of mice,

When the street virus is subjected several passages in rabbits, it undergoes certain changes. Such virus is known as **fixed virus**, it is more neurotropic and used for vaccine production.

Chick embryos – rabies virus can be grown in the yolk sac of chick embryo, live attenuated vaccine strains are developed by this method.

Strains adapted to grow in duck eggs have been used for preparation of inactivated vaccine

Tissue culture

Rabies virus can be grown in hamster kidney , chick embryo fibroblast, **human diploid cells** and Vero culture, growth is identified by immunofluorescence test

Rabies virus is susceptible to ethanol, ether, chloroform, acetone, iodine, soaps and detergents, quaternary ammonium compounds

It can be inacitvated by phenol, formalin, **beta-propiolactone,** UV irradiation, sunlight and heat at $50^{O}C$ for one hour

The virus can remain viable for weeks at 4^0C and for years at -70^0C or when lyophilized.

Antigenic properties

The glycoprotein G present in the surface spikes is strongly antigenic, it possess haemagglutinating activity.

Pathogenicity

Rabies virus naturally infects dogs ,cats and bats and is excreted in the saliva of such animals, man get the infection by the bite of rabid dog or other animals

During the bite the virus present in the saliva is deposited in the wound. The virus multiplies in the muscles, connective tissues or nerves and travel towards the spinal cord and brain, it multiplies in the brain and spreads along the peripheral nerves to various part of the body including salivary glands, the virus multiplies in the salivary glands and excreted in the saliva.

In human the incubation period varies from seven days to three years depending upon the site of bite, if bite near the neck or face that result in shorter incubation period

The disease undergoes **four stages** :

Prodormal phase : lasts for 2-4 days, symptoms include malaise, head ache, fever,, fatigue, anxiety , irritability and nervousness.

Encephalitic phase: patient develops hyperactivity, aggression, convulsion and difficulty in drinking water, fear at the site or sound of water (hydrophobia)

Coma : encephalitic stage is followed by coma which may last for a few hours or days

Death : death occurs due to respiratory arrest during convulsion

Laboratory diagnosis

Demonstration of rabies antigen by direct immunofluorescence test.

Demonstration of **Negri bodies** in salivary or conjunctival smears.

Isolation of virus from the brain , CSF, saliva and urine by intracerebral inoculation in mice or in tissue culture cell line

Detection of rabies antibodies in the serum and CSF of patients by ELISA

Detection of rabies virus RNA by **RT-PCR**

Prophylaxis

Rabies the only human disease that can be prevented by vaccination after infection, since the incubation period is long, active immunity develops before onset of disease

Two types of vaccines are available neural vaccine and non-neural vaccines

Neural vaccine – here rabies virus is cultivated in nerve tissues of animals

Semple vaccine- it consist of 5% suspension of infected sheep brain inactivated by 5% phenol, it is widely used vaccine

Betapropiolactone (BBL) vaccine - it is a Semple vaccine inactivated by beta propiolactone instead of phenol, more antigenic, prepared in India.

However, neural vaccines are not satisfactory because:

Poor immunogens

Many contain few infectious particles causes neurological complication

Non- neural vaccines

These are not prepared from nerve tissues,

There are two types of non neural vaccine

1, egg vaccine

2, **tissue culture vaccine**

Egg vaccine – live attenuated chick embryo vaccine used for the vaccination of animals

Tissue culture vaccine

Here fixed virus is cultivated in human diploid cell culture, the virus particles are inactivated with beta-propiolactone, highly antigenic, no side effects, highly expensive

Vero cell culture vaccine are equally effective, more economical and widely used.

The vaccines are given subcutaneously,

Adenoviruses

Adenoviruses were first detected in human adenoid tissue,, mastadenovirus infect both humans and animals, Human adenovirus are divided into 6 groups A-F,

Morphology

Size: medium sized 70 nm to 90 nm

Shape: spherical

Nucleic acid : ds DNA

Cubic symmetry ,,it is an icosahedron has 20 triangular facets and 12 vertices and five fold, three fold, two fold , each vertices have projects an apical fibre with a knob at the free end giving the virus appearance of a space vehicle

Cultivation

Adenoviruses are highly host specific and can grow in tissue culture of human origin only, example Human embryonic kidney cell, HeLa cell.

Cytopathic changes consists of rounding and aggregation of swollen cells into grape like clusters.

Intranuclear inclusion are seen in stained preparation

Physico-chemical properties

The viruses are heat - labile, destroyed at 56^0C , resistant to ether and bile salts

Pathogenesis

Adenoviruses are transmitted by direct contact, faecal – oral route.

It cause infection of the eye, respiratory tract, urinary tract and gastrointestinal tract and then spread to lymph nodes, incubation period is 6-7 days.

Clinical syndromes includes :

Pharyngitis and tonsilities - due to serotypes 1-7

Pneumonia – by serotypes 3 and 7

Acute respiratory diseases – serotypes 4,7, 21

Pharyngoconjucnctival fever - by serotypes 3,7 and 14

Epidemic keratoconjunctivitis - due to serotype 8

Follicular conjunctivitis – due to serotypes 3,4 and 11

Acute hemorrhagic cystitis – due to serotype 11 and 21

Infantile gastroenteritis – due to serotypes 40-41

Laboratory diagnosis

Microscopy – demonstration of virus particles in faecal specimen by electron microscope

Immunofluorescence test - demonstration of virus antigen in urine , cells from eye, respiratory tract, autopsy or biopsy materials using monoclonal antibodies

Isolation- viruses are isolated and identified by the cytopathic effects,

Serology – includes demonstration of antibodies and antigens using ELISA.

CFT is used for detection of antibodies

PCR is used for detection of virus specific genome

Polio virus

It is a causative agent of infantile paralysis or polio or poiliomyelitis, this virus belong to family Picornaviridae (pico means small , RNA virus), poilo was eradicated disease from India. WHO(World Health Organization) had declared India polio - free in 2014.

Size : small 20-30nm in diameter.

Shape : spherical

Nucleic acid : single stranded positive sense RNA.

Capsid : icosahedrons, cubic symmetry, it contains virus specific four antigens VPI, VP2, VP3 and VP4,

Non –enveloped

Cultivation

Grown in monkey kidney tissue culture, vero, HeLa

Cytopathegenic effect is characterized by cellular swelling

Physico-chemical properties

They are stable and viable in water and sewage for many months

Viable at 4^0C for months at -20 ^{0}C for years.

Susceptible to UV light, formaldehyde and chlorine

Resistant to chloroform, ether, bile

Poliovirus is three types

1, type1 – common epidemic type,

2, type 2 – causes endemic infection

3, type 3 – occasional epidemic

Pathogenesis

Poliomyelitis is a human disease, enters the human body via digestive tract , multiplies in lymphatic tissues of oral-pharynx and intestine, the virus enter the blood stream from where it is carried to the spinal cord and brain

It destroys the anterior horn of spinal cord causing flaccid paralysis

Clinical features

Incubation period is about 10 days

Symptoms include fever, malaise, head ache and sore throat

Poliovirus infection occur in four forms

1, Inapparant infection – 90-95 % of infection, do not have symptoms, but virus can be isolated from stool and throat swab

2, Minor illness -4-8% of infection, here patients suffer mild influenza like illness.

3, Non-paralytic poliomyelitis- -seen in 1-2% of infection, symptoms include headache, stiffness of the neck, back pain

4, Paralytic poliomyelitis – seen in 0.1-2% of infection, symptoms include flaccid paralysis

Laboratory diagnosis

1, demonstration of virus in stool by electron microscope

2, Isolation of the virus in tissue culture

3, Demonstration of antigen or antibody by ELISA

4, PCR – for detection of viral genome

Prophylaxis

Killed polio vaccine (salk vaccine)

It was developed by Salk in 1953

It contain formalin inactivated vaccine contain 3 types of strain grown in monkey kidney cell line

It is given as an injection

It provides 80-90 5% protection

Live attenuated vaccine (Sabin vaccine)

It was developed by Sabin in 1962

It contains live attenuated stains of poliovirus grown in monkey kidney cell line or human diploid cell culture.

It is administered orally known as oral polio vaccine (OPV)

The vaccine is stabilized by magnesium chloride.

It gives life long immunity.

Rhinoviruses

It causes common cold , rhino means nose, non-enveloped virus, cubic symmetry, capsid contain four viral protein VP1, VP2, VP3 and VP4.

Size, shape, nucleic acid are similar to polio virus, heat stable,

On the basis of neutralization test it has 113 serotypes,

Based on their growth in tissue culture rhinoviruses are divided into three groups H ,M and O .

H strain grow only on human cells

M strains grow both in human and monkey cells

O strain grow in nasal cells

Pathogenesis

The virus is transmitted by droplet via the R. tract, after entry the virus multiply in upper R. tract , the infected cell release chemokine and cytokines it will cause inflammation of R.tract.,

Incubation period is 2-4 days

Clinical feature includes

Profuse watery discharge, nasal obstruction, sneezing, sore throat, cough and head ache

Laboratory diagnosis

Rhinovirus can be isolated from nasal swab or throat swab or nasopharyngeal secretion in tissue culture

Demonstration of viral antigen and antibody by ELSA

PCR – used for detection of viral genome

Prophylaxis

There is no effective vaccine available

Small pox

Small pox is an acute , highly infectious, often fatal disease, it is characterized by high fever and aches with widespread eruption of papules that blister produce pus and leave a permanent pockmarks, it is caused by ***Variola virus ,***

It is now eradicated , it is the first vial disease to be completely eradicated by worldwide. On May 8, 1980 the WHO (World Health Organization) announced the global eradication of smallpox.

The eradication of small pox was possible because of the following factor: no animal reservoir, availability of an effective vaccine

Though the smallpox virus now exists only in lab, it is the major use in bioterrorism.

Variola virus

It is large size 300nm in diameter, it can be seen under ordinary light microscope,

Shape: brick shaped

Nucleic acid : ds DNA

Structure : biconcave, dumb – bell shaped, ds DNA surrounded by double layered membrane. On either of the concave there is a lens shaped structure called lateral body. The envelope which is covered by a irregular shaped outer membrane.

Cultivation

It can grow in chorioallantoic membrane of 10-14 days old chick embryo and tissue culture.

Pocks are produced within 48-72 hours, pocks are white , small, shiny, convex

Tissue culture

Monkey kidney, He La and chick embryo cells, this virus produces inclusion bodies(Guarneri bodies) in host cell cytoplasm , I.B consists of aggregation of virus particles

Physical-chemical properties

Variola virus is susceptible to sunlight, UV rays, formalin

Resistant to 50% glycerol 1% phenol and ether, remain viable for months at room temperature and for years at low temperature.

Antigens

More than 20 different antigens present, some are heat stable , others heat labile, some agglutinogen, some haemagglutinin

Vaccines

Live Vaccinia virus is used as vaccine for smallpox.

Herpes viruses

These are ds DNA viruses that infect both humans and animals, the most important viruses are *Herpes simplex virus, Varicella –Zoster virus, Cytomegaloviruses* and *Epstein –Barr virus*

Size : medium 100 – 200nm in size

Shape : icosahedral

Nucleic acid : ds DNA

Envelope – is present, lipid envelope containing peplomers, tegument between capsid and envelope

Cultivation

The virus can be cultivated on chorioallantoic membrane, rabbit kidney , HeLA cell .

Multiply in the nucleus of the host cell producing **Cowdry type A** intracellular inclusion bodies

Physical -chemical properties

Remains viable for years at low temperature

Susceptible to alcohol, ether, chloroform and bile salts

Herpes simplex virus -1(HSV-1)

It causes infection of mouth, eyes, CNS, etc

Examples : Gingivostomatitis, herbes labialis, kerotoconjuunctivities,dedrtic keratitis, eczema herbaticum and meningoencephalities

Transmission is by direct contact or droplets spread from cases or carriers

Herpes simplex virus -2 (HSV-2)

It causes genital infections

Examples genital herpes, neonatal herpes

It can be grown on CAM of chick embryo, monkey or rabbit kidney, HeLa cell culture

On CAM it produces white, shining, non-necrotic pocks

In tissue culture, typical intranuclear inclusion bodies are produced

Differentiation of HSV-1 and HSV-2

On chick embryo CAM type -2 produces large pocks than type -1

Type-2 replicate much better than type-1

Antiviral agents cytarabine less effective on type-2

Type-1 show less neurovirulence than type-2

Both are differ in antigenically and restriction endonuclease analysis

Pathogenesis

Human are natural host for HSV. The primary infection occur through the skin and mucous membrane of mouth and eyes.

After entering through the skin or mucous membrane the virus multiplies locally and spread to the neighbouring cells, once inside the body, HSV-2 travel to nerve roots of spinal cord and settle there.

Sometimes the virus migrates from the nerve to the skin and mucosa and causes cutaneous and mucosal lesion, HSV infection is a self-limiting and lifelong latency and periodic reactivation .

Common cold, fever, pneumonia, stress, exposure to sun light etc, can provoke reactivation

Antibodies can not prevent reoccurrence ,

Reoccurrence is more frequent and severe in immunodeficient individuals

Laboratory diagnosis

Specimens : skin scrapings , vesicle fluid, corneal scrapings, CSF and saliva

Direct microscopy

Smear is prepared from the base of vesicles and stained with 1% toluidine blue and examined under microscope,

Geimsa stained smears are examined for the presence of **Cowdry type A intranuclear inclusion bodies.**

Smears is prepared and examined under electron microscope for the presence of virus particles

Immunofluorecencent stained antibodies are used for detection of viral antigen in skin scrapings and tissue preparation

Tissue culture

HSV grow on human embryonic kidney, human fibroblasts and cytopathic changes appear in 24-48 hours

Serology

ELISA, neutralization test complement fixation test used for detection of virus specific antibodies

Molecular level diagnosis

PCR is used for detection of viral genome.

Chemotherapy

Acyclovir and vidarabine

Varicella-Zoster virus

VZV is single virus that causes two type of infections in humans varicella and zoster

Varicella is other name is chickenpox seen in children and zoster is other name is shingles seen in adults above the fifty, morphologically VZV is similar to HSV.

VZV is not able to grow on chick embryo, grow in human fibroblasts, HeLa cells producing intranuclear inclusion bodies, VZV is susceptible to disinfectants. It can be treated by acyclovir and vidarabine.

Varicella is a childhood viral infection, virus enter the body via R.tract or conjunctiva.

Incubation period is 7-23 days,

Symptoms includes mild fever, rash starts from scalp to trunk, arms and legs, the rash progress into macule, papule, vesicle, pustule and scab.

In adult chickenpox occur in a severe form.

In pregnant women , the virus crosses the placenta and infects the foetus resulting congenital malformation

A single attack of chickenpox confers lifelong immunity

Shingles (Herpes zoster)

This can be seen above 50 aged people, it occurs after exposure to chickenpox or reactivation of herpes zoster virus, this virus remain s latent in the nerve roots for many years.

It causes numbness, itching or pain followed by the appearance of clusters of little blisters in one side of the body, zoster lesion is similar to varicella

Laboratory diagnosis

Specimens : skin scrapings , vesicle fluid, corneal scrapings, CSF and saliva

Direct microscopy

Smear is prepared from the base of vesicles and stained with 1% toluidine blue and examined under microscope,

Geimsa stained smears are examined for the presence of multinucleated intranuclear inclusion bodies.

Smears is prepared and examined under electron microscope for the presence of virus particles

Immunofluorescent stained antibodies are used for detection of viral antigen in skin scrapings and tissue preparation

Serology

ELISA, neutralization test complement fixation test used for detection of virus specific antibodies

Molecular level diagnosis

PCR is used for detection of viral genome.

Chemotherapy

Acyclovir and vidarabine

Prophylaxis

VZV immunoglobulin prepared from convalescing patients and give to immunocompromised children and pregnant women (passive immunization).

Cytomegalovirus – is a salivary gland virus, largest virus in herpes family, infect man , monkey and guinea pig, it can grow in human fibroblast cultures,

it causes prolonged latency, it causes infection in salivary gland and kidney, the virus is excreted in saliva and urine, other symptoms includes hepatosplenomegaly, jaundice, thrombocytopaenic purpura, haemolytic anaemia, microencephaly and chorioretinitis

Virus enter the body via sexual intercourse, blood transfusion and organ transplantation,

It causes fatal infection in AIDS and COVID -19 patients

Lab diagnosis

Isolation – specimen saliva or urine

Grow in human fibroblast cultures and growth is identified by immunofuorescence test

Serology

ELISA – detection of Ig M antibody from serum

Treatment

Ganciclovir is the drug of choice

No vaccine against CMV.

Epstein - Barr Virus.

It causes infectious mononucleosis (glandular fever,)

This infection is common in children and young adults characterized by sore throat, fever, lymphadenopathy and presence of abnormal B- lymphocytes in peripheral blood. It is transmitted through respiratory route (R. route),, this virus infect the B lymphocytes and multiply continuously within lymphocytes,

Incubation period

4-8 weeks and disease last for 2-3 weeks

Symptoms

It cause lymphoma in AIDS and immunocompromised patients, this is characterized by abnormal proliferation of B. Lymphocytes (blood cancer),

Burkitt,s lymphoma , is characterized by malignant B.lymphocytes, nasopharyngeal carcinoma seen in AIDS and organ transplant patients

Lab diagnosis

Demonstration of antibody, and virus by E. microscope

Serology

IFT, CFT, and ELISA.

Yellow fever

It is more prevalent in tropical Africa and America

The infection occurs in two forms : Urban cycle and forest or sylvatic cycle, in urban cycle man is the reservoir and ***Aedes aegypti***, mosquito is the vector , in forest cycle wild monkeys act as reservoirs and several species of forest mosquitoes act as vectors.

Incubation period

3-6 days

Clinical features includes:

Fever , chills, headache, nausea, vomiting and slow pulse and jaundice, albuminuria,, death may occur due to hepatic or renal failure

Prevention

Urban yellow fever can be controlled by eradicating *Aedes aegypti* mosquitoes .

Vaccination : a live attenuated 17D vaccine is highly effective and subcutaneous, provides immunity for 10 years.

Yellow fever does not exist in India, this may be due to strict vigilance on vaccination and quarantine for travels from endemic areas.

Dengue

Dengue virus is distributed throughout the world

Four types of dengue viruses are recognized , DEN1, DEN2, DEN3 and DEN4.

Transmission

It is **transmitted** from man to man by the bite of mosquito ***Aedes aegypt.***, It is the vector ,

Incubation period : 8-10 days

Clinical features

The illness is characterized by fever, headache, conjunctival infections, pain in the back, joints, lymphoadenopathy and maculopapular rash, it is also known as **break bone fever** it lasts for 5-7 days

Dengue also occurs in more serious forms with hemorrhagic manifestation or with shock hemorrhagic fever or dengue shock syndrome

The virus is difficult to isolate

Lab diagnosis

Demonstration of Ig M antibodies by ELISA

Prevention

Controlling of mosquitoes

There is no vaccine against dengue virus.

Chikungunya virus

Mosquito-borne viral disease, caused by **Chikungunya virus**, it is an RNA virus belong to the family Togaviridae.

This virus was first identified in Tanzania in 1952.

This Mosquitoes bite man during daylight hours.

Common in Africa, southeast Asia , tropical regions of the America, epidemics in Africa and Asia

Transmission

It is **transmitted** from man to man by the bite of infected mosquito ***Aedes aegypti***, and ***A. albopictus*** .

Incubation period : 3-7 days

It causes febrile illness in human characterized by fever, crippling joint pain, lymphadenopathy, conjunctivitis , muscle pain, headache, fatigue and rash

This disease does not often result in death and joint pain may persist for months

Once a person has been infected, he or she is likely to be protected from future infections

In India the virus caused an extensive epidemic in 1963 in Kolkata, Chennai and other parts of the country, Chikungunya outbreak occurred till 1973 in Maharashtra

This virus replicate both mosquito midgut and man

Lab diagnosis

Explanation of symptoms to health care worker or doctor

Treatment

There is no specific treatment

Get plenty of rest,

Drink fluids to prevent dehydration

Take medicine such as paracetamol to reduce fever and pain

Prevention

Eradicate mosquito population

No vaccine available

Reoviruses

These are spherical, non-enveloped viruses with double layered icosahedral symmetry

Size is 60-80nm in size, contain ds RNA as their genetic materials divided into 10-12 segments

These viruses occur frequently in respiratory and enteric tracts, Rota virus is a important family member,

Reovirus

First isolated in 1950 from the stools of children, it has three serotypes 1,2 and 3. It causes mild upper R.tract infection, diarrhoea and rarely meningitis and encephalitis

Rotavirus

This virus cause infantile diarrhoea, These are spherical, non-enveloped viruses with double layered icosahedral symmetry , it has double stranded segmented RNA,

It exist in two forms, 1, complete virus is a double shelled and about 70 nm in diameter with a smooth surface

2, incomplete virus is a single – shelled and about 60 nm in diameter with rough surface

Virus without the RNA core are called as empty particles

It has A-G serotypes, serotype A is a human pathogen

On the basis of ELISA and CFT , serotype A divided into subgroups I and II

This virus is transmitted by faecal –oral route

It is a nosocomial pathogen (human acquired this virus infection from hospital environment)

Incubation period is 2-3 days

This virus multiplies in the epithelial cells of small intestine and is excreted in the stool,

It has worldwide distribution

Diarrhoea is common in children and in adults also

There is no specific antiviral therapy

Treatment

Oral rehydration, recovery is spontaneous in 24 -48 hours

Laboratory diagnosis

Demonstration of viruses from stool by electron microscopy, ELISA

Detection of antibody by ELISA and CFT.

Slow viruses

These are group of viruses that cause slow, progressive neurodegenerative disease in animals and human beings, symptoms appear in infected host long after the original infection.

The diseases are characterised by :

Long incubation period

Slow course of illness.

Affinity with central nervous system

Absence of immune response

This virus can be divided into three groups

A, B and D.

Group A diseases

It has two diseases 1, visna and 2, maedi

Visna – it is a demyelinating disease of sheep, caused by Visna virus belong to family retroviridae and subfamily lentivirinae

Incubation period is 2 years

Symptoms includes pneumonia, inflammation of central nervous system that result in paralysis

Virus can be isolated from saliva, CSF, blood of infected animals

Maedi – it is caused by maedi virus, cause haemorrhagic pneumonia in sheep, I. Period is

2-3 years

Group B diseases

caused by Prion (prion diseases), it has made up of proteins only and devoid of nucleic acid genome., prions proliferate in CNS and causes diseases

Scrapie - is an endemic disease in sheep and goats characterised by irritation and scraping of the animals against trees and rocks

Incubation period is 2 years

Suspension of infected brain is transmitted into mice, mice develop disease

Bovine spongiform encephalopathy **(mad cow disease**)

It is believed that cow acquired this virus after ingestion of feed containing bone meal from infected sheep carcasses.

Human diseases caused by Prion

Crutzfelt – Jacob Disease(CJ disease)

Degenerative disease of the central nervous system of humans, this may be transmitted from one person to other by corneal transplant.

Kuru

This disease seen in tribals of New Guinea.

Incubation period is 5-10 years, symptoms includes cerebral ataxia and tremors leading to death.

Subacute sclerosing panencephalities(SSPE)

It is characterised by slow deterioration of mental and motor function leading to death in 1-3 years

Progressive multilocal leucoencephalopathy

Causative agent is Papovavirus,

This disease is characterised by gradual deterioration of motor functions, speech and vision leading to death in 3-4 months,

Lab diagnosis

Demonstration of viruses from brain materials.

Papovaviruses

These are human papillomaviruses(HPV), these are about 70 types, cannot be grown in cell culture, highly host specific, infect the skin and mucous membranes,, these viruses are transmitted by direct contact, sexually and perinatally, incubation period is 2-8 months

Size is 40-55nm in diameter, icosahedral, non-enveloped, double stranded DNNA.

They cause cutaneous warts, genital warts, oralpapillomatosis and cancer, cutaneous warts in children are caused by HPV types 1,2, 3 and 4 , they are spontaneously disappear within 2 years of onset

Genital warts are caused by HPV-6 and 11 , are called as condyloma acminata common in sexually active adults

HPV-6 and 11 induce recurrent respiratory papillomatosis. Infants acquire the lesion while passing through the infected birth canal while adult get the infection by the orogenital contact

Oral papillomatosis is caused by HPV type 6,7,11,13 ,16 and 32. This condition sometimes prograss to malignancy

HPV is responsible for cervical cancer, hairy leukoplakia on the tongue of HIV infected patients

Lab diagnosis

Detection of viruses by electron microscopy, detection of viral DNA by PCR.

Polyomavirus

Morphologically similar to HPV.

It has two types

1, JC Virus

It is carcinogenic virus, causes infection in children and is usally asymptomatic, but the virus remain latent, reactivation can be seen AIDS patients

Lab diagnosis

Detection of viruses by electron microscopy, detection of viral DNA by PCR.

BK virus

It was first isolated from urine of a patient with kidney transplant, cause infection children below 3-4 years of age and remain latent in the kidney, reactivation can be seen AIDS patients, this virus grow in variety of primary and continuous cell line

Lab diagnosis

Detection of viruses from urine by electron microscopy,

Detection of viral DNA by PCR.

Parvovirus

These are very small in size 20 nm in diameter, they have single stranded DNA as their genetic material, non - enveloped with icosahedral symmetry, medically important human pathogen is parvovirus B19

Parvovirus B19

It was first isolated in 1983 from the blood of an asymptomatic donor,

It is known as **erythrovirus**

It is highly contagious and is transmitted by respiratory secretion or transplantally ot blood transfusion

It has an affinity for immature red blood cells and causes erythema infectiosum , it is also known as fifth disease, because it is in fifth in the list of childwood erythematous fever. It is characterised by a erythematous rash on the cheek.

In pregnant woman the virus causes erythroblastosis foetalis.

Lab diagnosis

Detection of viruses from urine by electron microscopy,

Detection of Ig M antibody in serum by ELISA, RIA, immunoflourescence test

Detection of viral DNA by PCR.

Coronaviruses

These are medium size virus 100-150 nm in diameter, it contain single stranded positive sense RNA, surrounded by lipoprotein envelope carrying large club shaped or petal shaped projection on the surface,

It has two species

1, human coronavirus 229E and 2, OC 43 they infect R, tract causing common cold

Transmission by inhalation of infected droplets or aerosols . about 30% common cold is caused by Coronavirus, incubation period is 2-5 days, virus can be isolated from nasopharyngeal washing,

Lab diagnosis

Detection of viruses from nasopharyngeal washing by electron microscopy,

Detection of Ig M antibody in serum by ELISA, RIA, immunoflourescence test

Detection of viral RNA by RT-PCR.

Severe acute respiratory syndrome (SARS)

it is caused by Coronavirus type 4., the disease is characterised by fever , cough and pneumonia.

First outbreak occur in South China in Nov 2002, by middle 2003 the disease spread more than 30 countries that result in death of more than 800 deaths,

Transmission by inhalation of infected droplets or aerosols, incubation period is 10 days, no treatment available, death occurs due to R,failure, controlled by strict isolation and quarantine

Lab diagnosis

Detection of viruses from nasopharyngeal washing by electron microscopy,

Detection of Ig M antibody in serum by ELISA, RIA, immunoflourescence test

Detection of viral RNA by RT-PCR

Arenaviruses

These are medium sized 50- 300nm in diameter, it is spherical, pleomorphic enveloped virus, having single stranded negative sense RNA, ,

Rodents are natural host for this virus, man get this infection by contact with excreta of infected rodents,

Medically important viruses are1, lymphocytic choriomeningitis virus(LCM)

2, Lassa fever virus

3, South American haemorrhagic fever virus

lymphocytic choriomeningitis virus(LCM)

In human it causes influenza like illness, rarely it cause severe encephalomyelitis

Lassa fever virus

It was first observed in 1969 in Lassa in Nigeria

In humans the virus causes haemorrhagic fever with high mortality rate, rodent excreta is the source of infection

Man acquired this infection after inhalation if infected droplets, the virus is present in throat, urine and blood of patients

Incubation period is 3-16 days, the disease is characterised by high fever, myalgia and haemorrhagic skin rash

South American haemorrhagic fever virus

Three related viruses found in South America cause haemorrhagic fever

Junin virus. Muchupo virus

Guanarito virus

Incubation period 1-2 weeks

Mortality rate is 5-30%

Lab diagnosis

Detection of viruses from nasopharyngeal washing , blood, CSF, urine by electron microscopy,

Detection of Ig M antibody in serum by ELISA, RIA, immunoflourescence test

Detection of viral RNA by RT-PCR

Filovirus

These are enveloped long , thread like virus, it is 80 nm in diameter and 800 – 1000 nm in long,

It has single stranded negative sense RNA,

It has two important viruses

1, Marburg virus

2, Ebola virus

Marburg virus infection was first recognized in Marburg among the laboratory workers exposed to African green monkeys. In 1976 , the virus Ebola virus was isolated in Sudan and Zaire.

Both viruses causes haemorrhagic fever with high fatality rate

Three strains of Ebola virus have been identified

1, Zaire strain has fatality rate about 90%

2, Sudan strain has fatality rate 50%

3, Reston strain has fatality rate is low compared to others

The virus is transmitted to man by aerosol route and by direct contact with blood

Man to man secondary transmission occurs by direct contact with body fluids, sexual intercourse and use of contaminated syringes and needles

Lab diagnosis

Detection of viruses from nasopharyngeal washing , blood, and affected tissues by electron microscopy,

Isolation of viruses from blood in Vero cells

Detection of Ig M antibody in serum by ELISA, RIA, immunoflourescence test

Detection of viral RNA by RT-PCR

Nipah virus infection

This virus is bat - borne virus, this virus infection in humans with a high mortality rate, it occurred in South and southeast Asia.

This virus was first discovered in 1999 following in outbreak of diseases in pigs and people in Malaysia and Singapore.

Zoonotic virus, causes zoonotic illness that is transmitted from animals to humans and can also be transnsmitted through contaminated food.

This virus was first isolated in 1999 in Nipah, Malaysia, belongs to family Paramyxoviridae. This ***virus belong to the genus Henipavirus.***

Nipah henipavirus

A 23 – year old student was admitted into hospital with Nipah virus infection at Kochi in Kerala in 2019: June.

Symptoms

Fever, cough, headache and confusion, inflammation of brain, seizures

Incubation period : 5-14 days

Transmitted by direct contact

Outbreaks

Nipah virus have been reported in Malaysia, Singapore, Bangladesh and India, the highest mortality rate was found in Banglaesh

Diagnosis

Detection of antibodies by ELISA.

RT- PCR specimen includes throat swabs, cerebrospinal fluid, urine and blood.

Based on symptoms confirmed by laboratory testing

Treatment

No specific treatment .

Prevention

There is no effective treatment for Nipah disease,

Avoid exposure to sick pigs, bats

Avoiding exposure to bats and sick pigs and people

To avoid consumption of uncooked fruits and fruit products. Such food contaminated with urine or saliva from infected fruit bats is source of outbreak

Natural host is fruit bats

This virus has caused large outbreaks of diarrhoea resulting in 50% case fatality rate,

Swine flu or Zoonotic swine flu

It is an respiratory infection caused by Influenza strain that is transmitted from pigs, first identified in 1919 pandemic and still circulate as a seasonal flu virus swine flu is caused by Influenza virus HINI strain which started in pigs. Swine flu is caused by several types of swine influenza viruses Influenza A known as HINI, HIN2, H2NI, H3NI, H3N2 andH2N3 . People with regular exposure to pigs are at increased risk of swine flu infection

In August 2010, WHO world health organization declared the swine flu pandemic officially over.

First swine flu in India was reported in 2015, positive cases 31156, death 1841 upto March 2015.

Influenza virus (swine flu HINI strain is single stranded negative sense RNA virus, RNA occur in eight segmented form, spherical, enveloped, envelope is lipoprotein contains two kinds of spikes 1, hemagglutinin(HA) 2, neurominidase(NA) , both are major antigens and glycoprotein

Antigenic variation

Two kind of antigenic variation can be seen in swine flu virus, so difficult to prepare vaccine against this virus.

1, antigenic shift – it means major changes in the gene code for hemagglutinin

2, antigenic drift - it means minor changes in the gene code for haemagglutinin

Hemagglutinin responsible for host cell attachment and agglutinate host RBC cell

Neuraminidase responsible for lysis of host cell during infection

Replication

Pig respiratory tract cell is infected by influenza virus from men, pig and birds. During replication the segmented form RNA exchanged or genetic recombination may occur(**resassortment**) that result in formation of **new strain** that are antigenically differ from parent virus particles.

Transmission

Human getting this infection by inhalation of infected respiratory droplet (coughs and sneezes)

By touching contaminated surface (blanket or doorknob)

By saliva(kissing, shared drinking)

By skin to skin contact (handshake or hugs)

Incubation period – 1-4 days , 7 days for adult and child

Symptoms

Fever, nausea, cough, weakness, sore throat, chills, fatigue, vomiting, lethargy,diarrhoea

In severe cases , it cause shortness of breath and decreased appetite so need respiratory supporters ventilator to breath,

Death may occur due to pneumonia or secondary bacterial infection.

Elder, pregnant women and child are more susceptible to swine flu

Illness persist for three to seven days, but malaise and cough can persist for two weeks

Treatment

Good Rest , taking pain relievers,

Oseltamivir (tamiflu)

Zanamivir (relenza) are effective against swine flu

Amantadine and rimantadine are not effective against swine flu

Epidemiology

Swine flu is transmitted from one person to other by inhalation of or ingestion of droplets contain virus from people while sneezing, coughing, it is not transmitted by eating cooked pork, strain H3N2 v causes outbreak in 2011, the v indicate variant virus, this strain infect both pigs and humans

Death rate of Influenza strain HINI is 0.17%

Spanish flu is 2% -20%

Swine flu strain HINI is 160 death.

Zika virus

A disease caused by Zika virus in humans that is spread through the mosquito bites

This virus belong to family **Flaviviridae**, human getting this virus after biting day time active female mosquito ***Aedes aegypti*** and ***A. Albopictus***,

Morphology

These are enveloped , spherical and small in size 50 nm in diameter

Genome is single stranded RNA as their genetic materials

The name Zika derives from the Ziika forest of Uganda where the virus was first isolated in 1947.

This virus is similar to dengue virus, yellow fever virus, japanese encephalitis and West Nile Fever virus. Since 1950 this virus found only in Africa and Asia.

From 2007 – 2016 the virus spread from pacific region to America,. An epidemic was caused by this virus during 2015-2016 in America.

Zika virus

A disease caused by Zika virus in humans that is spread through the mosquito bites

This virus belong to family **Flaviviridae**, human getting this virus after biting day time active female mosquito ***Aedes aegypti*** and ***A. Albopictus***,

Morphology

These are enveloped , spherical and small in size 50 nm in diameter

Genome is single stranded RNA as their genetic materials

The name Zika derives from the Ziika forest of Uganda where the virus was first isolated in 1947.

This virus is similar to dengue virus, yellow fever virus, japanese encephalitis and West Nile Fever virus. Since 1950 this virus found only in Africa and Asia.

From 2007 – 2016 the virus spread from pacific region to America,. An epidemic was caused by this virus during 2015-2016 in America.

Blood transfusion – this virus transmitted to other by blood transfusion also, so screening is necessary before blood transfusion of blood donors.

Lab diagnosis

Detection of Ig M antibodies by ELISA

Treatment

There is no specific medicine and vaccine for this virus.

Hepatitis viruses

Viral hepatitis is an infection of the liver, caused by hepatitis viruses, they include a range of heterogenous group of small viruses, it consists of hepatitis viruses **A,B,C,D,E,**and **G.** Although unrelated , all viruses causes similar clinical symptoms range from asymptomatic to fatal form, hepatitis infection leads to inflammation and necrosis of liver

Hepatitis A Viruses

This virus belong to family **picornaviridae** and genus **Hepatovirus** causes infective or **infectious hepatitits.** This virus is originally **enterovirus 72**.

Morphology

Size: 27nm in diameter

Shape: spherical, icosahedral symmetry

Nucleic acid : single stranded positive sense RNA

Envelope : absent

Cultivation , physical- chemical properties

This virus can grow in human and monkey cell cultures

Resistant to heat at 60 ^{0}C for one hour

Not affected by ether and acid survive at low temperature for long periods

Pathogenesis

Infectious hepatitis is mostly seen in children and young adults

The virus enter the body via oral route

It multiplies in the intestinal epithelium and reaches liver by blood

Incubation period is 2-6 weeks

The symptoms includes fever, malaise, anorexia, nausea, vomiting and liver tenderness

The virus shed in the faeces during the late incubation period

Laboratory diagnosis

Demonstration of the virus in the faeces by immunoelectron microscope or by ELISA.

Isolation of the virus by growing on continuous cell lines of monkey kidney or human fibroblast cells.

Detection of specific IgM and IgG HAV antibodies in the serum of patients by ELISA

Epidemiology

HAV is the most common cause of hepatitis in children.

It spreads from person to person and by ingestion of contaminated food, water or milk.

Epidemics of this disease are found in overcrowding or where sanitation is poor.

Treatment and Prophylaxis

There is no specific antiviral therapy

Prophylactic measures include:

a) Improved sanitation and prevention of faecal contamination of food and water.

b) Passive immunization with normal human immunoglobulin.

c) A safe and effective formalin inactivated alum conjugated HAV vaccine is available for active immunization.

A single attack of HAV gives life-long immunity.

Hepatitis B Virus (HBV)

Hepatitis B virus belongs to the family Hepadnaviridae and genus Orthohepadnavirus. It causes serum hepatitis or transfusion hepatitis.

Morphology

*Size: Small ,42 nm in diameter.

*Symmetry: Icosahedral

*Nucleic acid: Double stranded DNA.

*Envelope: Present.

Electron microscope observation of sera of hepatitis B patients reveals presence of three types of particles.

a) A spherical particle, 22nm in diameter. This is the most abundant form.

b) A tubular particle of varying length.

c) A double shelled spherical particle ,42nm in diameter. This is the complete hepatitis B virus. It was first described by Dane and co-workers in 1970 and hence it is known as “Dane particle”.

Hepatitis B virus has not been cultivated in the laboratory.

Antigenic Structure

Hepatitis B surface antigen (HBsAg)

HBsAg is present on the outer surface or envelope of the virus.

In 1965, **Blumberg** and co-workers observed that a protein antigen present in the serum of an Australian aborigine who had received multiple transfusions. The antigen was named “Australia antigen”. Later this antigen was found to be the surface component of hepatitis B virus and was renamed as “hepatitis B surface antigen (HBsAg)”.

The envelope antigen present on the surface of the spherical as well as tubular particles continues HBsAg.

Hepatitis B core antigen (HbcAg)

The antigen present on the core of nucleocapsid of the virus is known as “hepatitis B core antigen-HbcAg”.

It is usually not found in human serum.

Hepatitis B e antigen (HBeAg)

It is a hidden nucleocapsid protein and is known as “hepatitis B e antigen” (HBeAg).

Though HBcAg and HBeAg are immunologically distinct, they are coded by same gene.

Viral Genes and Antigens

The genome of the hepatitis B virus is made up of two linear strands of DNA – the plus strand and the minus strand – held in a circular configuration. The plus strand is incomplete whereas the minus strand is complete. The plus strand carries the DNA polymerases which can fill the gap in the incomplete strand and make it fully double stranded.

The genome has four genes coding for different antigens:

*P gene- Polymerase

*S gene- Envelope antigen

*C gene-HbcAg and HBeAg

*X gene-HbxAg(A non-particulated antigen)

Pathogenesis

Hepatitis type B is a blood-borne infection and is transmitted by parenteral, perinatal and sexual modes.

Parenteral transmission: HBV is present in blood, body fluids and secretions such as saliva, breast milk, semen, vaginal secretions, urine and bile. Transmission occurs through accidental inoculation of very minute quantities of these specimens during medical, surgical\and dental procedures, use of contaminated needles syringes, ear and nose piercing, tattooing, acupuncture, sharing of shaving razors, kissing, etc.

Perinatal transmissions: Here transmission occurs when carrier mother's blood contaminates the mucous membrane of the newborn during birth. Infection may also result from breast feeding and close contact between the baby and the infected parents.

Sexual transmission: HBV present in vaginal secretions , semen, etc.., is transmitted by sexual contact. Male homosexuals are at higher risk of getting the injection. The incubation period is 6 weeks to 6 months. The course of HBV infection is divided into three phases.

Pre-icteric phase: During this period the patient develops malaise, anorexia, weakness, myalgia, nausea and vomiting. A few patients develop arthralgia, serum sickness, polyarteritis nodosa and glomerulonephritis.

Icteric phase: This phase is characterized by jaundice, pale stools and dark urine.

Convalescent phase: This phase is long and associated with malaise and fatigue.

Hepatitis B carriers

Super carriers: These are highly infectious virus in the blood.

Simple carriers: These are more common.

Laboratory Diagnosis of HBV infection is carried out by detecting hepatitis B antigens and antibodies by ELISA

HBc antibody presence of IgM anti-HBc indicates recent infection and IgG anti-HBc indicates remote infection.

Detection of viral DNA

Presence of HBV DNA by **PCR.**

Prophylaxis

General preventive measures

Avoiding contact with blood and other body fluids of patients or carriers.

Use of disposable syringes, needles, etc.

Screening of HBsAg and HBeAg in blood.

Hepatitis B immunoglobulin (HB1G) this may not prevent infection but gives protection against illness.

Plasma derived vaccine is a purified 22nm particle of HBsAg derived from the plasma of healthy carriers and inactivated with formaldehyde. It is immunogenic and safe.

Recombinant yeast hepatitis B vaccine is produced by cloning the gene HBV in baker’s yeast. The vaccine is immunogenic and safe without any side effects. It

is absorbed on aluminium hydroxide and stored in cold. It is administered intramuscularly in three doses at 0,1and 6 months.

Treatment

There is no specific treatment. Interferon alpha either alone or in combination with lamivudine and famcyclovir has been used in some chronic cases.

Hepatitis C Virus (HCV)

It belongs to the family Flaviviridae and genus Hepacivirus.

Morphology

* Size: 50-60nm in diameter.

* Symmetry: spherical or cubic

* Nucleic acid: single stranded RNA

* Envelope: present

Cultivation

It is difficult to grow HCV in tissue culture inactivated by exposure to ether, chloroform, organic solvents and detergents.

Antigenic structure

HCV antigenic diversity. Based on these differences ,it has been classified into 11 genotypes.

Pathogenesis

HCV infection occurs by blood transfusion, needle stick injuries, use of contaminated needles and syringes and by sexual intercourse. Direct transmission from mother to baby also takes place.

Incubation period is 15-160 days.

In acute cases, Hepatitis C gives rise to less severe infection with very little or no jaundice.

In chronic cases, about 80% of the HCV patients progress to liver cirrhosis and hepatocellular carcinoma.

Laboratory diagnosis

Detection of antibody of HCV by ELISA.

Detection of HCV RNA by PCR and immunofluorescence.

Prophylaxis

Screening of blood and blood products.

Treatment

Interferon alpha either alone or in combination with antiviral agents like ribavirin is effective in some cases.

Hepatitis D Virus (HDV)

Hepatitis D virus belongs to the genus Deltavirus, It is dependent on HBV for its replication and can survive as long as HBV persists in the body.

Morphology

- Size: 36-38nm in diameter.
- Symmetry: spherical or cubic
- Nucleic acid: single stranded RNA
- Envelope: present.

HDV has not been cultivated.

Antigenic structure

HDV has a special antigenic feature –hepatitis B antigen. The RNA genome encodes "Delta antigen" or HDAg.

Pathogenesis

HDV is transmitted by blood and blood products and also by sexual contact. It can cause infection only in the presence of HBV.

Coinfection: It is the simultaneous infection with both HDV and HBV. This type of an infection is more severe than that produced by HBV alone.

Super infection: It is infection of the HBsAg carrier by HDV. HDV is not associated with hepatocellular carcinoma.

Laboratory diagonosis:

- Detection of IgM anti-delta antibody in the serum by ELISA and RIA.
- Detection of HDV RNA by hybridization using radiolabelled probes.

Prophylaxis

There is no specific prophylaxis.

Hepatitis E Virus

Morphology

- Size: 30-34nm in diameter.
- Shape: spherical icosahedral symmetry
- Nucleic acid: single stranded positive sense RNA.
- Envelope : not present.

Cultivation

It has not been cultivated so far.

Pathogenesis

Hepatitis E is encountered exclusively in developing countries in epidemic, endemic and sporadic forms.

Pigs are reported to be the reservoirs of HEV.

Infection is through ingestion of faecally contaminated water.

Incubation period is 2-8 weeks.

The infection occurs mostly in the age group of 15-40 years.

Laboratory diagnosis.

- Detection of IgM and IgG antibodies by ELISA and Western blot assay.
- Detection of HEV RNA in patient's faeces or acute phase sera by PCR.

Treatment

There is no effective antiviral drug.

Prophylaxis

- Use of chlorinated water.
- Improved standards of sanitation.
- No vaccine is available against HEV.

Hepatitis G Virus (HGV)

Hepatitis G virus belongs to the family Flaviviridae and genus Hepacivirus.

It was first isolated in 1996 from a patient with chronic hepatitis.

It is a blood-borne virus resembling hepatitis C virus.

It has not been grown in cell culture.

Oncogenic viruses

Viruses that produces tumours in their natural hosts known as “Oncogenic viruses” or “Tumor producing viruses”. These viruses contain oncogenes, the genes which can transform normal host cells into cancerous cells. Several DNA viruses and retroviruses are known to be oncogenic.

Oncogenic viruses cause changes in the host cell by “Transformation”.

Oncogenic transformation

- Alternation in cell morphology: Alternation in shape and orientation.
- Alternation in cell metabolism: Growth rate increases with increased production of acids and acid mucopolysaccharides.
- Alternation in growth characteristics: This involves loss of “contact inhibition”, formation of headed-up growth(microtumours) and capacity to grow in liquid or semisolid medium.
- Antigenic alternations: There is a loss of surface antigen, increases in agglutinability by lectins and appearance of new virus specified T antigens (tumour antigen)
- Capacity to produce tumours in susceptible animals.

Oncogenes

The first oncogene was discovered in 1970 in a chicken retrovirus. **Dr Steve Martin** of the **University of California.**

Genes that are responsible for the induction of tumours are known as "**oncogenes".** They are also known as "cancer genes".

They encode proteins that trigger transformation of normal cells into malignant cells. Oncogenes found in viruses are called "viral oncogenes". Genes resembling oncogenes are also present in normal as well as cancer cells. Such genes found in normal cells are known as "**proto-oncogenes**" and those isolated from cancer cells are known as "**cellular oncogenes**".

Cellular oncogenes possess introns characteristic of eukaryotic cells and have important controlling functions in cell growth and regulation.

Proto-oncogenes are found to code for proteins involved in regulating cell growth and differentiation.

A new class of genes, known as tumour suppressor genes, growth suppressor genes or **anti-oncogenes** has been identified.

Retinoblastoma gene is a prototype of suppressor gene. Loss of this gene results in the development of retinoblastoma.

Mechanism of viral oncogenesis

The exact mechanism of viral oncogenesis is not clearly known.

It is believed that in the case of oncogenic DNA viruses, the DNA is integrated with the host cell genome. The integrated viral DNA being incomplete or "defective", no infectious virus is produced and the host cell undergoes malignant transformation.

1). **Papovavirus**: Important member of this group is papillomavirus.

* **Human papillomavirus**(HPV) types 6 and 11 cause benign warts, premalignant lesions and carcinoma of the male and female genital tract.

* HPV types 16,18 and 31 are responsible for 60-100% cervical cancers.

2). Herpesvirus:

* **Herpes simplex types 1 and 2** are associated with cervical carcinoma. Type 1 is also associated with cancer of the lip.

* **Human herpes virus type 8** is linked with kaposi's sarcoma.

* **Cytomegalovirus** causes carcinoma of the prostate and **kaposi's sarcoma.**

* **Epstein-Barr virus** is associated with Burkitt's lymphoma and nasopharyngeal carcinoma. EB virus is also believed to transform normal lymphocytes into lymphoblasts in immune-compromised children.

3). Hepatitis B and C: Cause hepatocellular carcinoma.

4). Poxvirus: **Mollusipox** virus induces molluscum contagiosum.

5). Retrovirus: **Human T cells leukemia virus-1** causes adult T cell leukemia.

* In retroviruses, the RNA is converted into double stranded DNA form known as "provirus".

* The provirus becomes integrated with the cell genome and remains latent for variable periods.

* When activated, the integrated provirus acts as a template for RNA synthesis and induces cell transformation.

Human immunodeficiency virus (HIV)

This virus belong to the family Retroviridae (viruses having reverse transcriptase enzymes – it make RNA copy onto ss DNA,) , sub-family Lentivirinae. It primarily infects vital components of the human immune system and destroys them leading to acquired immunodeficiency syndrome (AIDS), The weakening of the immune system makes the infected person vulnerable to a variety of opportunistic infections and cancers. HIV directly attacks kidneys, heart and brain.

Morphology of HIV virus

Size: medium size : 90-120nm

Shape : spherical

Nucleic acid : ss positive sense RNA

Envelope : present

Cultivation

This virus can be cultivated in human lymphocytes in the presence of interleukin-2

Physical-chemical properties

It can be inactivated at 60^0 C in 10 minutes

This virus can be viable at room temperature up to 8 days

This virus can be inactivated by 1% Lysol,70% ethanol, 35% isopropyl alcohol, 2% glutaraldehyde, 3% hydrogen peroxoide,5% formaldehyde, 2% bleaching powder, 1% sodium hypochlorite , 2.5% Tween -20 and detergents, lyophilized virus remain viable for years.

Viral genes and antigens

Two types of genes 1, structural genes 2, non-structural genes

1, **structural genes** - gag gene code for P15, P18, and P24.(p- refer protein)

These all are core and shell(capsid) of the virus, p24 act as antigen present in serum of HIV infected patients

env gene – code for envelope glycoprotein (gp 160) it can be cleaved into gp120 (forms surface spike- major antigen)and gp 41(transmembrane protein)

2, **non-structural genes**

These are regulatory genes regulate gene expression example nef gene, vpr gene, rev gene, tat gene, LTR gene vpu, vpx , and vif gene.

Pol gene code for reverse transcriptase enzyme.

Antigenic variation

Based on antigenic variation , HIV can be classified into two types

HIV-I, and HIV-II.

HIV-I

Based on geographical distribution , it can be classified into various groups

1, Sub type A – Worldwide

2, Sub type-B – America and Europe

3, Sub-type- A, C and D – Africa

4, Sub-type –E, C and B –Asia

5, Subtype –E – Thailand

6, Subtype –C – India and China

HIV-II.

It is less virulent than HIVI, it more in Africa, first isolated from West Africa in 1986.

Mode of transmission

HIV can not be transmitted by touching, kissing, coughing, sneezing, use common toilets and utensils

Three modes :sexual, parenteral(subcutaneous and intramuscular) and perinatal

Sexual transmission – 80% of infection transmitted by sexually, common among homosexual, heterosexual individuals

Parenteral -(subcutaneous and intramuscular)

Using contaminated needle and syringes, transfusion of blood or blood products and also tissue or organ transplantation.

Perinatal

Transmission of virus from mother to baby

Through the placenta before birth. genital secretion, through mothers milk after birth

Pathogenesis

HIV virus infect the CD4+Lymphocytes, the infected T-cells are loss of both humoral and cell mediated immunity

The virus specifically bind the CD4-T-Lymphocytes with the help of glycoprotein gp 120, T-cell receptor is CXCR4,

This virus also infect macrophages and macrophages of brain , lungs, dendritic cells of skin., monocytes , they have receptor for HIV is CCR5.

In the cells , virus transcribe RNA into single stranded DNA by reverse transcriptase and single stranded DNA again polymerised into double DNA by DNA polymerase of host cell,

This double stranded DNA is now integrated into host cell chromosomal DNA with the help of enzyme integrase,

The period between thev HIV infection and appearance of antibodies in the blood known as Window period , it will last for 2-3 weeks only

HIV infected person remain asymptomatic for a long period

The lytic (lysis of CD T4 cell) infection leads to release of new virus particles that infect other T- cells

As the number of CD4 T cells decreases, there is increased release of virus into the blood. This indicates the development of symptoms of AIDS.

Stages of HIV infection

The Centre for Disease Control (CDC) in Atlanta, USA has classified the clinical course of HIV infection into the following five stages.

1). Acute HIV infection

* Incubation period is 2-6 weeks.

* The clinical symptoms include fever, malaise, sore throat, myalgia, arthralgia, skin rash, lymphadenopathy and sometimes encephalopathy.

* HIV antibody tests are usually negative at the onset of the illness ‘SEROCONVERSION ILLNESS’

* The virus, viral nucleic acid or P24 antigen can be detected during this stage by DNA hybridization, PCR or EIA tests.

2). Asymptomatic or latent infection

* It is also known as period of clinical latency and lasts up to several years.

* All HIV infected persons pass through this phase. They do not exhibit any symptoms and are healthy

* HIV antibody tests are positive

3). Persistent generalized lymphadenopathy(PGL)

* This stage is characterized by the presence of enlarged lymph nodes.

4). AIDS related complex(ARC)

* In this stage, the individual is considerably immunodeficient and the CD4++ T lymphocyte count falls below 400 per microliter

* The patient develops constitutional symptoms like fatigue, unexplained fever, weight loss, night sweats and persistent diarrhoea.

* The patients also acquires opportunistic infections such as oral candidiasis, hairy cell leucoplakia, herpes zoster, salmonellosis, tuberculosis, etc.,

5). AIDS

* It is the terminal stage of HIV infection.

* Incubation period varies for 1-14 years with an average of 6 years.

* CD4+ T cell falls below 200 per microlitre and titre of virus increases significantly.

* There is severe breakdown of immune defence mechanism and the patient suffers from opportunistic infections and malignancies.

* The patient also develops primary CNS lymphoma, dementia, severe encephalopathy, myelopathy, diminished concentration, motor disturbances, etc,

* In children, the risk factors for AIDS are infected mothers and transferred blood or blood products.

Opportunistic infections and malignancies associated with HIV infection

Bacterial

- Mycobacterial infection- Tuberculosis and M.avium complex.
- Salmonellosis.

Viral

- Cytomegalovirus
- Herpes simplex virus
- Varicella-zoster virus
- Epstein-Barr virus
- Human herpes viruses 6 and 8

Fungal

- Pneumocystis carinii pneumonia.
- Candidiasis
- Cryptococcosis
- Aspergillosis
- Histoplasmosis
- Coccidioidomycosis

Parasitic

- Toxoplasmosis
- Cryptosporidiosis
- Isosporiasis
- Strongyloidiasis

Malignancies

- Kaposi's sarcoma
- B cell lymphoma or Non-Hodgkin's lymphoma.

Specific tests

These tests involve detection of antigen, detection of antibody, detection of viral nucleic acid or isolation of virus.

- **Detection of antigen**

The virus antigen P24 and reverse transcriptase RT) by ELISA.

- **Detection of antibody**

Detection of antibody is the most commonly used procedure for the diagnosis of HIV infection.

Antibodies usually appear 2-8 weeks after infection. IgM antibodies appear in about 3-4 weeks after infection.

Screening tests:

ELISA (Enzyme Linked Immuno-Sorbent Assay)-It is the most commonly used method for the diagnosis of HIV infection.

Confirmatory tests:

- **Western blot test:**

In this test, the HIV proteins are broken into fragments by polyacrylamide gel electrophoresis and blotted on to nitrocellulose paper strips. The test serum is allowed to react with these strips. Antibodies to HIV proteins, if present in the serum, combine with the HIV fragments. Strips are washed and treated with enzyme –conjugated anti human gamma globulin on adding a suitable substrate, colour bands are formed.

- **Indirect immunofluorescence:**

HIV infected cells are fixed onto glass slides and treated with serum followed by flurorescein conjugated antihuman gamma globulin. An apple green fluorescence appears in a positive test when examined under a **fluorescent microscope.**

- **Detection of viral nucleic acid**

Viral nucleic acid is detected by **polymerase chain reaction**. It is considered the 'gold standard' for diagnosis of all stages of HIV infection.

- **Isolation of virus**

HIV can be isolated from CD4 lymphocytes of peripheral blood, bone marrow, serum and body fluids.

Epidemiology

- HIV infection is worldwide in distribution.
- HIV is believed to have originated from simian immunodeficiency virus.
- HIV is transmitted through blood, semen, vaginal fluid and infected mother.
- The mode of transmission is sexual, parenteral or perinatal routes.

- About 60% adults with HIV infection develop AIDS within 5-10 years.
- In Africa, AIDS is known as ‘slim disease’.
- The first human infection occurred in Africa in 1930.
- United states in 1981
- In India, the first HIV infection was reported from Chennai in 1986 and also in Mumbai.

Prevention

- Use of condoms
- Use of sterile syringes or needles
- Screening of all blood and blood products, organs and tissue for transplantation or transfusion
- Identification and isolation of AIDS patients and initiation of treatment
- Identification of HIV infected persons by screening.(high risk groups).

Treatment

The antiretroviral drugs in use include:

- Reverse transcriptase inhibitors like zidovudine, zalicitabine, stavudine, lamivudine and nevirapine.
- Protease inhibitors like saquinavir and ritonavir.
- These drugs are used either singly or in combination.
- Because of the side effects and high cost, their use is restricted.