Chapter

1

Introduction to Medical Virology

Even before the realization of existence of virus, diseases caused by viruses have changed the destiny of the mankind; and after the discovery of virus, the field of virology has given a new shape to science.

The diseases caused by the viruses have been recorded in the history which dates back to more than 3000 BC. But the existence of virus was realized only in late nineteenth century when a series of experiments were carried out in several countries to study the disease affecting the tobacco plants. This happened during the era, when Koch's postulate to associate the microbe as the cause of disease was almost accepted.

DISCOVERY OF VIRUS

Adolf Mayer, an agricultural scientist, was instructed by the Netherlands to investigate the tobacco disease. Mayer named the disease as tobacco mosaic based on the mosaic pattern of appearance on the affected tobacco leaves. He took the sap of the diseased leaves and inoculated onto the healthy tobacco plants and demonstrated the development of disease in the healthy plants. His experiment reveals the infectious nature of the disease agent. But as he could not cultivate or detect the agent under microscope, the causative agent remained unidentified. This also indicated, probably the infectious agent is not cultivable in nature and also small enough to be seen under microscope (submicroscopic particle).

The experiment was continued by a Russian scientist Dmitri Ivanovsky when he was ordered by the Russian Department of Agriculture to find out the cause of the tobacco disease. Ivanovsky reconfirmed Mayer's finding of transmissibility of the causative agent and also showed that the agent is filterable by successfully transmitting the infection from infected sap after passing through Chamberland filter to healthy plant. Ivanovsky's experiment added to the information that the causative agent is small enough and not only submicroscopic but also filterable. As he could not culture this filterable agent, he suggested it as a possible toxin and not a living particle.

However, both the scientists failed to satisfy Koch's postulate as they could not cultivate the infective agent.

The experiment was continued by a Dutch scientist Martinus Beijerinck who collaborated with Adolf Mayer and independently showed that the agent is infectious and filterable. He also showed that the infective fluid is not a toxin but a reproducible particle by showing the diluted sap is regaining the strength in the inoculated healthy plant. This indicated the reproducible capacity of the agent which proved that the agent is a living particle and not a toxin. It was also noticed that the agent was able to reproduce in the living tissue and not in the cell free sap. The later revelation also changed the concept of the then Koch's postulate that, there are some infective agents

exists which do not grow outside their host. He named this agent as **contagium vivum fluidum** or contagious living fluid.

The experiments by these three scientists led to the discovery of a novel organism which was smaller than bacteria as it could not be filtered by Chamberland candle filter, could not be visualized by the light microscope and could not be multiplied in artificial media as it could grow only in the living tissue. The agent was then named as **tobacco mosaic virus** as the term **virus (Latin meaning for slimy fluid or poison)** was used for any infective agent.

By end of the experiments that led the discovery of tobacco mosaic virus, the physical characteristics that were discovered for it became part of the definition for "virus"; an infectious filterable agent which is sub-microscopic and grow only within the living tissue. With the further development of technologies, size of the virus was determined to be between 30 and 200 nm and structure, morphology and genomic details were gradually revealed.

MORPHOLOGY OF VIRUSES

The structure of virus is unique and different from other groups of microorganisms like protozoa, bacteria and fungi (Fig. 1.1).

The mature virus particle called "the virion" is comprised of nucleic acid and a protein coat called "capsid" which surrounds the nucleic acid. Capsid is composed of capsomers which are the morphological unit of the capsid. Together both nucleic acid and capsid constitute the nucleocapsid. In addition to this, some of the viruses possess an envelop that surrounds the capsid. Envelop of some viruses possesses surface projections (also called peplomers) which are of glycoprotein in nature. So, from inside outwards, the structure of virus consists of nucleic acid, capsid, and envelop.

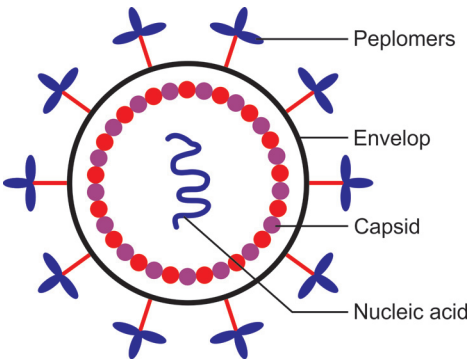

Fig. 1.1: Structure of virus (schematic)

SYMMETRY OF VIRUS

The arrangements of the capsomers in human viruses are of two types; isometric with icosahedral symmetry or helical symmetry.

Icosahedral Symmetry

Icosahedral symmetry is formed when the capsomers are arranged in an icosahedrons shape. Icosahedron is a geometrical figure consists of 20 equilateral triangles. So it has **20 faces** (surface of equilateral triangle), **30 edges** (margins of 20 triangles) and **12 vertices** (corners of triangles). It has 5, 3, and 2 rotational symmetry which are formed when the imaginary axis passes through the vertices, faces and edges, respectively (Fig. 1.2a and b).

The icosahedrons shape permits a particular pattern of arrangement of capsomers so that a proper fitting can occur. Along the edges and faces, the capsomers are surrounded by six capsomers and are called "hexamers", whereas the capsomer that is present at the vertices (corner of the triangle) are

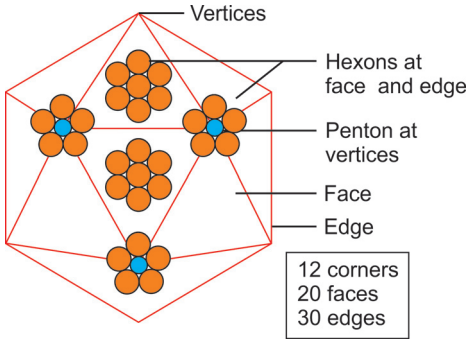

Fig. 1.2a: Icosahedral symmetry showing the location of pentons and hexons

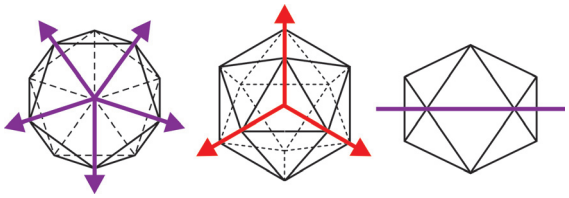

Fig. 1.2b: Purple arrows showing 5-fold symmetry through vertices **red lines** showing 3-fold symmetry through faces

surrounded by five capsomers and are called "pentamers".

The icosahedrons basically a compact shape and thus provides a robust protection to the nucleic acid. Therefore, the presence of envelop is not essential for those viruses. So, viruses with icosahedral symmetry may or may not possess envelops.

Helical Symmetry

Helical symmetry is seen in many of the RNA viruses, but not found in any of the DNA viruses.

The name "helical" is self-explanatory, where the nucleic acid is present in helical manner and surrounded by the protein coat capsid. We can imagine a metal spring or coil with a plastic covering, where the nucleic acid can be compared with the metal spring (present in a coiled fashion) and like the plastic coat, it is covered by the capsid all along it (Fig. 1.3). The entire arrangement, however, appears quite open, so possibly requiring another layer of protection. Therefore, all human viruses with helical symmetry always possess envelop.

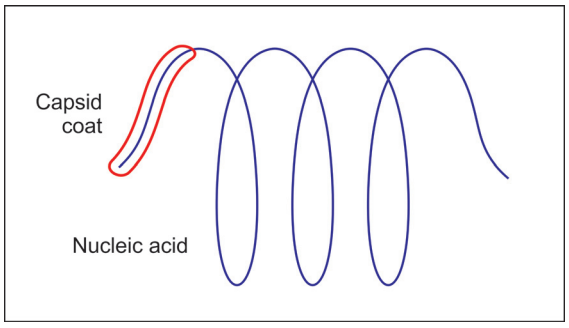

Fig. 1.3: Helical symmetry

Envelop

Viral envelop is the outermost covering of the virus and is of lipoprotein in nature. The lipid component is derived from the host cell membrane, either from the plasma membrane or from the membrane of the cytoplasmic organelle during the process of release of nucleocapsid by budding. The glycoprotein surface peplomers when present on the envelop get incorporated to it by replacing the host cell protein.

As discussed under "helical symmetry", all human viruses with helical symmetry are enveloped and only certain viruses with icosahedral symmetry possess envelops (herpesviruses, togaviruses, retroviruses and flaviviruses).

CHEMICAL COMPOSITION OF VIRUSES

Nucleic Acid

Viruses possess only one type of nucleic acid, i.e. either deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and never both. This also highlights another unique property of the viruses where genetic information is possessed by RNA (RNA viruses) instead of DNA. Based on the type of nucleic acid the virus contains, they are divided broadly into two types—DNA and RNA viruses. Though DNA is double stranded and RNA is single stranded, DNA and RNA viruses are either double stranded or single stranded. The nucleic acid is present as a single molecule in all the viruses except retroviruses which contain two copies of the nucleic acid.

DNA Viruses

All the DNA viruses are double stranded except parvoviruses which are single stranded DNA viruses and hepadnavirus which has a partially double-stranded genome.

DNA molecule is present either in linear form or in circularized form. The latter form helps the virus in integration of the viral genome with the cellular DNA, also protects

the genome against exonucleases. Papillomaviruses, polyomaviruses and hepadnaviruses possess circular DNA.

The genome of DNA viruses contains around 400 to 4000 nucleotides in the smallest (parvoviruses) and largest group of viruses (poxviruses) (Table 1.1).

RNA Viruses

Based on the different properties, RNA viruses can be divided according to the number of strand they possess, number of molecule (single or multiple as segmented), arrangement of nucleic acid (liner or circular) and polarity (positive, negative or ambisense). Table 1.2 gives the list according to each type.

Proteins

The major bulk of the virus is made up of protein. The capsid which is present as a protective coat surrounding the nucleic acid is composed of protein. Thus the major function of protein is to provide the protection. Each capsomer, which is the building blocks of capsid, consists of one to six polypeptide molecules.

The other component of virus that contains protein is the surface projections present on envelop of some of the viruses. Besides the structural component, protein is also present in the form of enzymes which are important for viral replication.

Lipids

The envelop of the virus is of lipoprotein in nature, where the lipid component is derived from the host cell and the protein is of viral origin. The lipid part constitutes of around 30% of the total dry weight of the virus. The major part of the envelop lipid (50–60%) is of phospholipid in nature present in the form of bilayer.

Glycoproteins

The surface projections or peplomers present on some of the enveloped viruses (influenza virus, human immunodeficiency virus) are of glycoprotein in nature which are synthesized by the cellular glycosyltransferase.

CLASSIFICATION OF VIRUSES

Viruses have been classified by several means—based on their epidemiological criteria, organ—system affected, or based on their physicochemical properties. Classification system based on the former criteria though not used as a formal classification system, but useful for clinical practice.

Another type of classification is based on the replication strategy of the viruses called **"Baltimore classification"**. This is based on the observation of Sir David Baltimore that all the viruses must have to generate a positive strand mRNA in order to start their replication. However, this classification is not used for general purpose of virus classification and is discussed under viral replication.

Table 1.1: List of DNA viruses according to different morphological properties

Property	*Viruses*
Number of NA strand	All DNA viruses are double stranded *except* Parvoviridae (single-stranded DNA) and Hepadnaviridae (partially double-stranded DNA)
Arrangement of NA	
• Linear	Adenoviridae, Herpesviridae, Poxviridae, Parvoviridae
• Circular	Papillomaviridae, Polyomaviridae and Hepadnaviridae
Nucleocapsid symmetry	All DNA viruses have icosahedral symmetry *except* Poxviridae which has a complex/ovoid symmetry

NA: Nucleic acid

Table 1.2: List of RNA viruses according to different morphological properties	
Property	*Viruses*
Number of NA strand	All RNA viruses are single stranded except Reoviridae
Number of NA molecule	All RNA viruses contain single molecule of genome *except* retroviruses which contain two molecules of single strand RNA and RNA viruses with segmented genome: Arenaviridae, Reoviridae, Bunyaviridae and Orthomyxoviridae
Arrangement of NA	All RNA viruses are linear *except* arenaviruses and bunyaviruses
Polarity	
• Positive	The RNA genome that acts as mRNA: Picornaviridae, Caliciviridae, Togaviridae, Coronaviridae, Flaviviridae and Retroviridae
• Negative	When the nucleotide sequence of the genome is complementary to that of mRNA: Orthomyxoviruses, paramyxoviruses, rhabdoviruses, arenaviruses and bunyaviruses
• Ambisense	Arenaviruses and bunyaviruses
Nucleocapsid symmetry	
• Helical	Orthomyxoviruses, paramyxoviruses, Coronaviridae, Filoviridae, Rhabdoviridae
• Icosahedral	Astroviridae, Caliciviridae, Reoviridae, Togaviridae
Enveloped	All RNA viruses with helical symmetry are enveloped and Retroviridae, Togaviridae and Flaviviridae are enveloped with icosahedral symmetry

NA: Nucleic acid

The International Committee on Taxonomy of Viruses (ICTV) is the virology division of International Union of Microbiological Societies. ICTV has made the formal classification of viruses based on the group of properties of the viruses which constitutes mainly of the type of nucleic acid, capsid symmetry, and possession of envelop, genomic architecture and nucleotide sequence similarities. However, there is no fixed list of properties to describe all virus groups.

The characters that are considered to distinguish between different families and genera of viruses are; morphology of virion, genome organization, size of structural and non-structural viral proteins and the method of viral replication.

The characters that are considered to distinguish between different species within the single genus are—physicochemical properties, antigenic properties of the viral proteins, natural host range, cell and tissue tropism, mode of transmission, pathogenicity, cytopathology and nucleotide sequence relatedness.

Table 1.3: Nomenclature of viruses according to hierarchy		
Taxon	*Suffix*	*Example*
Order	virales	Herpesvirales
Family	viridae	Herpesviridae
Subfamily	virinae	Alphaherpesvirinae
Genus	virus	Simplexvirus

Table 1.3 gives the nomenclature of virus as per the hierarchy.

SENSITIVITY TO PHYSICAL AND CHEMICAL AGENTS

It is important to know the sensitivity of viruses to various common physical and chemical agents as the knowledge can be

utilized for preservation of viruses and to understand the mode of transmission so that proper preventive measures can be taken.

Temperature Sensitivity

In general, viruses are sensitive to temperature and easily loose the infectivity when exposed to high temperature. Most of the viruses get inactivated or killed when exposed to 60°C. This occurs due to the denaturation of viral surface proteins which is responsible for the attachment to the host cell. Table 1.4 gives the temperature sensitivity of most of the viruses as applicable in various practical situations. (The information is accepted widely in an informal manner.)

As at or above ambient temperature, most of the viruses are destroyed within hours, the storage of viruses is done at lower temperature. In situations where sample for virus diagnosis cannot be sent to the lab immediately or in the lab cannot be processed on the same day, it can be stored in the refrigerator at 4°C for 1–2 days till transportation. This is a common scenario during viral outbreaks when the sample is transported from remote locality to the lab for confirmation.

In the virology testing labs, samples or stock virus when needs to be stored for prolong period, it is kept at or below –70°C or in liquid nitrogen cylinder when possible. It is important to note that virus stock or samples for isolation or molecular diagnosis purpose should never be kept at –20°C/–40°C deep freezer. Storage at this temperature usually leads to the formation of ice vesicles which breaks the virion. This is particularly applicable for respiratory viruses.

In general, the viruses that are transmitted through ingestion of contaminated food and water or infect the gastrointestinal tract such as enteroviruses, hepatitis A virus, rotavirus and other viruses causing diarrhea are relatively resistant to 60°C, usual concentration of chlorination and acidic pH. This indicates how these viruses manage to survive in the environment and are transmitted through food and water.

Sensitivity to Common Chemical Disinfectants

Most of the viruses are susceptible to common chemical agents which act by denaturation of proteins, inactivation of the nucleic acids, amino acids or those which acts as dehydrating agents.

However, enveloped viruses are more susceptible to the lipid solvents like ethyl alcohol, chloroform or chlorine compounds as the lipid component of the envelop gets destroyed by these agents making the virus non-infectious. The use of such agents (hand sanitizers) is important in common practice to prevent infection from respiratory viruses which are transmitted through infected droplets. They also play an important role even for the virus like HIV in case of soiling with blood or body fluids. So, it is important to remember that enveloped viruses can be destroyed more easily by using lipid solvents even if they are highly infectious.

REPLICATION OF VIRUSES

The replication or multiplication of viruses is unique among all the microorganisms as they not only multiply within the host cell but also utilize the host cell machinery for their replication.

Table 1.4: Temperature sensitivity of viruses in various practical situations

Temperature	*Practical scenario*	*Time of half-life*
60°C	Heating temperature of food and liquid	Seconds
37°C	Ambient temperature in tropical countries/summer season	Minutes
18–22°C	Ambient temperature in air-conditioned room/lab	Hours
+4°C (2–8°C)	Refrigerator temperature	Days
≤ –70°C	Deep freezer	Years

Basic steps of viral replication: To begin the process of virus replication, the virus first has to come into the contact of the host cell (attachment), then the virus or its nucleic acid enters inside the cell (entry or uptake) and the nucleic acid of the virus is released by the process of uncoating. At this stage, the early genes are transcribed to mRNA from which regulatory proteins and enzymes required for viral genome replication are formed along with the proteins which shutdown the host cell nucleic acid and protein synthesis. Replication of viral nucleic acid leads to transcription of late mRNA which is then translated to late proteins. These late proteins are structural proteins of the virus. Finally all the synthesized viral components (nucleic acid, structural proteins) get assembled and released from the host cell in large numbers (Fig. 1.4).

Attachment: The attachment between virus and the host cell depends on the cell and tissue tropism of the virus. For example, certain viruses infect only one type of tissue (liver by hepatitis viruses; hepatitis A virus, hepatitis B virus, hepatitis C virus and hepatitis E virus) and certain viruses infect only one particular type of cells (neurotropism by rabies virus and other encephalitis viruses). This is primarily determined by the presence of specific receptor on those cells and tissues.

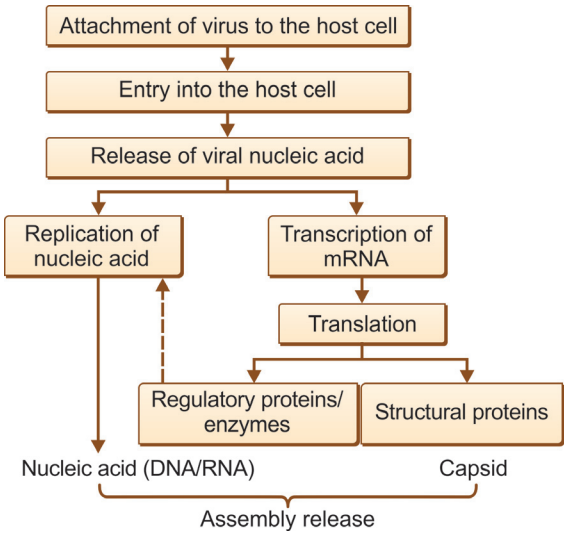

Fig. 1.4: Steps of viral replication

Entry of virus and uncoating: Different types of viruses enter into the host cell by different mechanisms.

Endocytosis: The virus particle after adsorption onto the host cell surface gets invaginated and encircled by endocytic vescicle. The envelop of the virus then fuses with the endocytic vesicle and nucleocapsid is released. It is observed mostly with the enveloped viruses.

Fusion: The viral envelop gets fused with the host cell membrane and nucleic acid is released into the cell. It is observed mostly with the enveloped viruses.

Translocation: The icosahedral capsid of the virus fuses with the host cell membrane. A channel is formed through which the nucleic acid gets translocated to the host cell.

Transcription: After the viral genome is released inside the host cell, the process of transcription is supposed to begin. In molecular biology, the transcription occurs from double-stranded DNA to single-stranded RNA. However, as viruses can be double-stranded DNA, single-stranded DNA, and single-stranded RNA or double-stranded RNA, they have been classified into six types based on their strategy of mRNA transcription. This classification was made by David Baltimore and is known as "Baltimore's classification". According to this principle, mRNA is the beginning point of nucleic acid replication. So, viruses of different types of genome have to first generate a positive mRNA strand. Figure 1.5 shows the transcription strategies of viruses of different types of genome.

Translation: Early proteins are formed from the transcripts of the early genes. Early proteins are viral enzymes, regulatory proteins and the proteins required for nucleic acid replication.

Late proteins are translated from the late mRNA and are structural protein in nature.

Assembly and release: After the replication of nucleic acid and structural proteins, assembly of capsomers occurs to form the

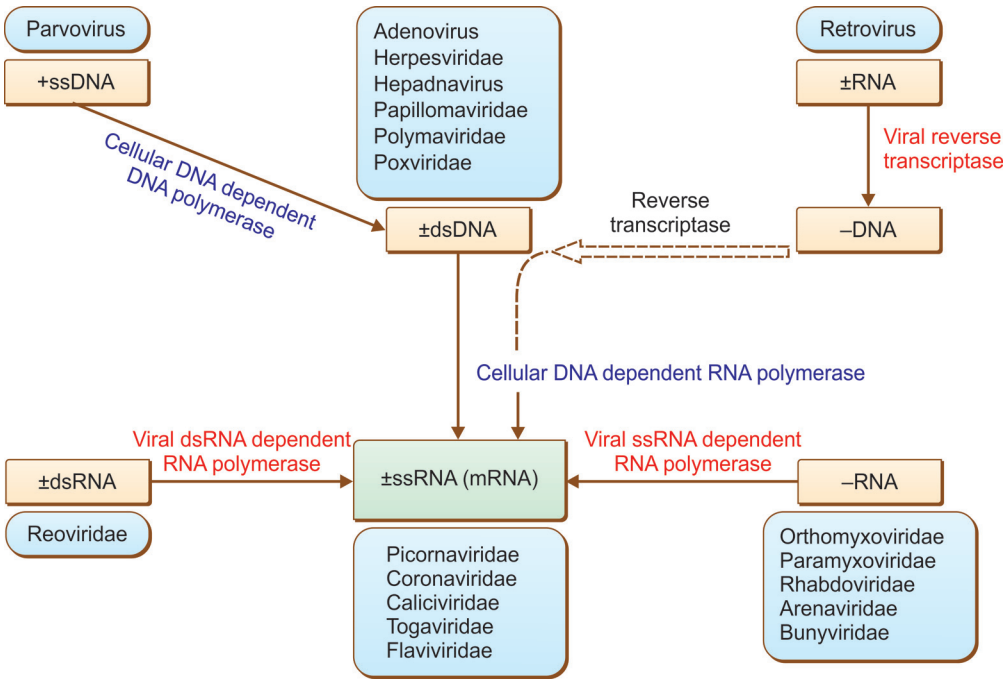

Fig. 1.5: mRNA transcription strategies from different types of viral genomes

procapsid. Packaging of the viral nucleic acid occurs inside the procapsid.

Glycosylation: Glycosylation of the protein occurs that are destined to be incorporated in to the envelop in order to form the peplomers. The process occurs inside the endoplasmic reticulum of the host cell.

Release: After the completion of process of assembly and packaging, the virus particle is released from the host cell either by budding or exocytosis.

VIRAL INFECTIONS

Depending on the cell receptor affinity and tissue tropism, different viruses infect through different routes and involve different organs. According to these properties, viruses can be divided broadly into different groups. This grouping is important from clinical and epidemiological aspects.

Groups of viruses according to their route of infection:

Respiratory viruses: These viruses mainly infect, through infected droplets or aerosol, e.g. influenza virus, parainfluenza viruses, respiratory syncytial virus, human metapneumovirus, rhinoviruses, and coronaviruses. However, some other viruses such as measles virus, mumps virus, parvovirus B19 though infect through respiratory route, manifestations occur through involvement of different organs.

Enteric viruses: These viruses enter the body through ingestion of contaminated food and water, e.g. rotavirus, norovirus, sapoviruses, astrovirus, adenovirus group F (adenovirus 40, 41), enteroviruses and hepatitis A and E viruses. Amongst these, enteroviruses though enter through gastrointestinal tract do not cause gastroenteritis and involve mainly the central nervous system. Similarly HAV and HEV mainly attack the liver tissue and cause hepatitis.

Arboviruses: Viruses transmitted through bite of different arthropods have been clubbed into one group—"arboviruses". This group constitutes of members from different virus families, transmitted by different arthropods. The organ involvement and clinical manifestations are also heterogenous. Table 1.5 gives

Table 1.5: List of important arboviruses

Vector	*Viral agents according to clinical symptoms*
Mosquito-borne	Fever: Dengue virus (1–4), chikungunya virus, zika virus Arthritis: Chikungunya virus Hemorrhagic fever: Dengue virus (1–4), yellow fever virus Encephalitis: JEV, WNV, EEEV, WEEV, VEEV
Tick-borne	Hemorrhagic fever: CCHFV, KFDV, OHFV Encephalitis: TBEV (Western/European subtype, far eastern subtype, Siberian subtype (previous name Russian spring summer EV), louping ill subtype (previous name louping ill virus)
Sandfly	Encephalitis: Chandipura virus

JEV: Japanese encephalitis virus; WNV: West Nile virus; EEEV: Eastern equine encephalitis viruses; WEEV: Western equine encephalitis viruses; VEEV: Venezuelan equine encephalitis viruses; CCHFV: Crimean-Congo hemorrhagic fever virus; KFDV: Kyasanur forest disease virus; OHFV: Omsk hemorrhagic fever; TBEV: Tick-borne encephalitis viruses.

the list of some of the arboviruses with their vectors and major clinical manifestations.

Transfusion transmitted viruses: Viruses that can be transmitted through blood or blood products have been grouped together, so that appropriate preventive measures can be taken to prevent the transmission, e.g. human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), parvovirus B19, cytomegalovirus (CMV). Amongst these, the screening of HIV, HBV and HCV has become mandatory before blood transfusion.

Sexually transmitted viruses: HIV, human papillomavirus (HPV), and herpes simplex virus-2 are the common viruses which are transmitted through sexual route. However, many other viruses which can be transmitted through body fluids can also infect through sexual route.

Congenital viruses: Viruses that can be transmitted either through transplacental route or perinatally through birth canal or breast milk have been largely grouped as congenital virus. It is important from the perspective of prevention when such infections occur during pregnancy. Rubella virus, cytomegalovirus, herpes simplex virus (mostly HSV-2), hepatitis B virus, HIV and parvovirus B19 which causes hydrops fetalis in severe fetal infection are the major causes of congenital viral infections. Recently zika virus which is mainly transmitted through mosquito bite but can be transmitted to fetus through transplacental route and causes fetal microcephaly has also been included in the list.

Viral infections have also been grouped according to the tissue tropism, pathogenesis and clinical manifestations caused by the viruses as described below.

Viral encephalitis: HSV, Japanese encephalitis virus (JEV), West Nile virus (WNV), rabies virus and nipah virus are important viral causes of encephalitis. Several tick-borne viruses are also important cause of encephalitis in certain parts of the globe. Large number of other viruses also can lead to encephalitis in severe form of disease or part of their dissemination which occurs mostly in immunocompromised patients, such as varicella zoster virus, cytomegalovirus, Epstein-Barr virus, influenza virus.

Hepatitis viruses: HAV, HBV, HCV, HEV and hepatitis D virus (HDV) are the major viruses that predominantly target the liver. HAV and HEV are transmitted mainly through enteric route, whereas HBV and HCV enter through parenteral route.

Table 1.6: Human oncogenic viruses

Virus family	*Virus*	*Human cancer*
DNA viruses		
Papillomaviridae	Human papillomavirus	Carcinoma cervix (HPV16,18), genital tumors (HPV6&11), oropharyngeal carcinoma
Hepadnaviridae	Hepatitis B virus	Hepatocellular carcinoma (HCC)
Herpesviridae	Epstein-Barr virus	Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's lymphoma
	HHV-8 (human herpes-virus 8)	Kaposi's sarcoma
Polyomaviridae	Merkel cell polyomavirus	Merkel cell carcinoma
RNA viruses		
Retroviridae	Human T cell lymphotropic virus (HTLV)	Adult T cell leukemia
	HIV	AIDS-related malignancies (KSAV, EBV related tumors, HPV)
Flaviviridae	Hepatitis C virus	Hepatocellular carcinoma (HCC)

Hemorrhagic fever viruses: Some of the arboviruses such as dengue virus, Crimean-Congo hemorrhagic fever (CCHF) virus, Kyasanur forest disease (KFD) virus, members of filovirus like Ebola virus and members of Arenaviridae such as Junin and Machupo virus are the predominant cause of hemorrhagic fever. Most of these viruses are restricted to particular geographical location. However, in 2014, the world has experienced the devastating spread of the fatal Ebola virus infection through man-to-man transmission through contact with contaminated blood and body fluids.

Viral myocarditis: A large number of viruses including enteroviruses, adenovirus, parvovirus, herpesviruses, respiratory viruses and arboviruses like dengue virus and chikungunya virus have been associated with myocarditis. However, coxsackievirus, adenovirus, parvovirus and human herpes virus-6 are the common viral agents that have been associated with myocarditis and cardiomyopathy.

Oncogenic viruses: Some of the viruses have been associated with tumorogenesis and are known as oncogenic viruses. This is important to know, so that virus associated preneoplastic conditions can be screened to prevent the development of neoplasia. Knowledge regarding the association of HBV with hepatocellular carcinoma and HPV with cervical carcinoma has led to the development and implementation of their vaccine. Table 1.6 gives the list of human oncogenic viruses.

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