Research Article

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Monkeypox: A Global Challenge - An Overview

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ABSTRACT

Monkeypox is a zoonosis caused by an orthopoxvirus named monkeypox virus(MPXV), structurally related to smallpox virus, enveloped, brick shaped, replicates in cytoplasm. Currently spreading throughout Africa & different parts of world. Has two distinct clades, West African and Central African (Congo Basin) clades. It manifests as rashes, concentrated on face & extremities like palms & soles. Both humoral and cell mediated immunity are seen in host, diagnosed by polymerase chain reaction (PCR) and various serological tests. Patients receive supportive treatments along with antiviral drugs. Presently FDA approved new Mpox vaccines. Scientific community needs to extend their research work to enhance knowledge in this field. This review briefs about monkeypox in human life in context of ongoing outbreaks around the world.

Keywords : Monkeypox virus (MPXV), Open Reading Frame (ORF), Inverted Terminal Repitition (ITR), single nucleotide polymorphisms (SNPs).

1. INTRODUCTION

A wide spectrum of re-appearance of viral diseases have turn up in twenty-first century viz. Zika virus, Swine flu (H1N1), Ebola, Nipah, Avian influenza (H5N1), Severe acute respiratory syndrome coronavirus 2 (SARS-C0V-2). Monkeypox virus (MPXV) also showed a wave of outbreak which got its own concern on public health point of view in relation to morbidity & mortality. Alarming outbreak was observed in 2022-23. Pandemicity as well as epidemicity involved 110 countries as per WHO (May 2022) list. Morbidity & mortality showed 87,000 & 112 respectively. On 23rd July 2022 MPXV has been taken as public health emergency of international concern (PHEIC) by WHO.[1] First monkeypox reported in India was on July14 in Kerala followed by number of cases in Delhi, Telengana ,Bihar, Uttar Pradesh etc. [2]Govt of India (MoHFW) declared National Institute of virology (NIV) as nodal institute for diagnosis of monkeypox on July 2022. Again on 11th May 2023 WHO declared monkeypox is not a public health emergency(PHE). [3,4]As monkeypox is an emerging zoonotic disease, health care staffs is expected to upgrade their knowledge to combat the zoonotic infectious disease not only on epidemic potential view but also clinical management as well as lab. diagnosis. This review briefs about epidemiology, immunopathogenesis, diagnosis of monkeypox based on various ongoing outbreaks around the world.

2. EPIDEMIOLOGY

2.1. Agent: Monkeypox Virus
2.1.1. Family: Poxviridae.
2.1.2. Subfamily: chordopoxvirinae.
2.1.3. Genus: Orthopoxvirus (OPV).
2.1.4. Species: monkeypox virus. [5]
2.1.5. Genetic clades: Based on epidemiological, molecular and animal evidences, two different clades of monkeypox were suggested in different geographical regions of Africa: [6]

• Clade 1

- Central African or Congo Basin clade.
- Causes more severe disease with more transmissibility.
- Has high morbidity & mortality (10%) .
- Congo Basin strain is ZAI-96.
- Virulence factors are: D14L (complement inhibitor), D10L (host range protein), B14R (IL-1β binding protein), B10R (apoptotic regulator) & B19R (serine protease inhibitor-like protein) genes.

• Clade 2

- West African clade.
- Causes self limited disease, with low case fatality (1%).
- Has minimum virulence.
- This clade is deficient in several genes found in Central African clades and includes: SL-V70, COP-58,

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and WRAIR-6.

- Subdivided into clade IIa: having low mortality (1%) & poor transmissibility & clade IIb : evoluted by human apolipoproteinBm RNA-editing catalytic polypeptide-like3 (APOBEC3) cytosine deaminase enzymes.
- Clade IIb is responsible for ongoing 2022-23 epidemic.
- Based on metagenomic sequencing studies, some have proposed to name this strain as clade 3. [6]

2.1.6. Morphology

It is visualised by both light & electron microscopy (EM).Consists of outer & inner membrane. On electron microscopy (EM), virus is oval or brick-shaped, slightly pleomorphic, 220-250 nm long, 140-260 nm broad, surrounded by a lipoprotein envelope which has a wrinkled or corrugated surface. Outer membrane consist of tubular proteins which are projected outward & interact with host proteins like glycosaminoglycans (GAGs) and many other proteins. Inner membrane is a rigid palisade layer. Inside inner membrane there lies dumb-bell shaped nucleocapsid core. Inside nucleocapsid, there are core fibril, dsDNA, transcription factors. Core contains enzymes. It consist of concavity above & below. There lies lateral bodies. Functions of lateral bodies are unknown, however studies said they suppress immune system of host (Fig:1).[7]

Figure 1

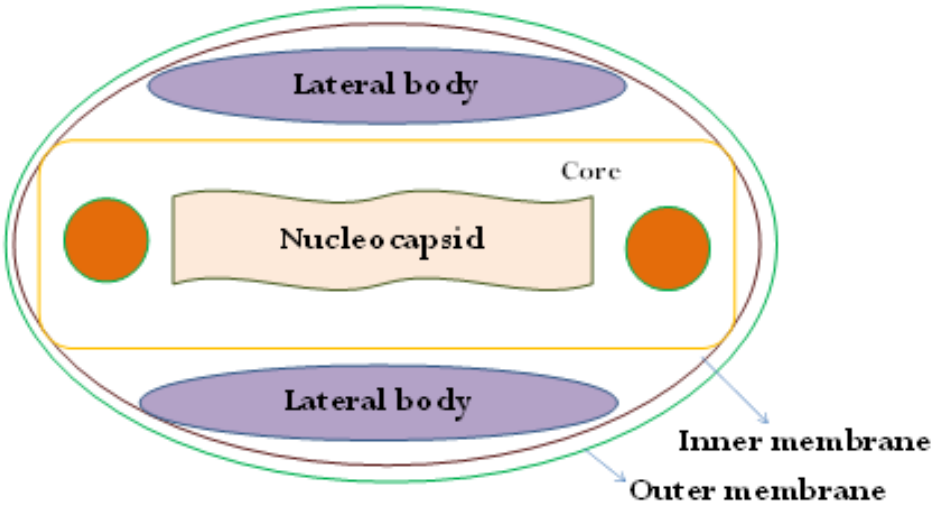

Figure 1. Structure of Monkeypox

2.1.7. Genome

It closely resembles other orthopox viruses. MPXV consists of 197kbp long linear double stranded DNA (dsDNA) genome, encoding about 180 proteins. Has highly conserved central core region (101 kbp) with approximately 190 non-overlapping open reading frames (ORFs),terminal variable regions at either side with 6379 bp inverted terminal repetition(ITR),80 bp long hairpin loop, 54 or 70 short tandem repeats(STR) & unique ITR sequences NR1 and NR2 and coding region[8]. Central core shares more than 90% sequence homology with other orthopoxviruses, particularly within open reading frames(ORF) located between C10L & A25R. Species & strain-specific characteristics of orthopoxviruses are often found in variable regions at ends of the genome.There lies genes responsible for viral replication, transcription, assembly and release expressing virulence and host tropism. Terminal gene is evaded by host immune system by interfering with signalling presentation, antigen recognition & apoptosis [9]. Monkeypox virus has 3'-5' exonuclease activity of its DNA polymerase.[10]

However virus evasion to host immune response & how genetic configuration of strain changes it's transmissibility, is still unclear (Fig.2).

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Figure 2

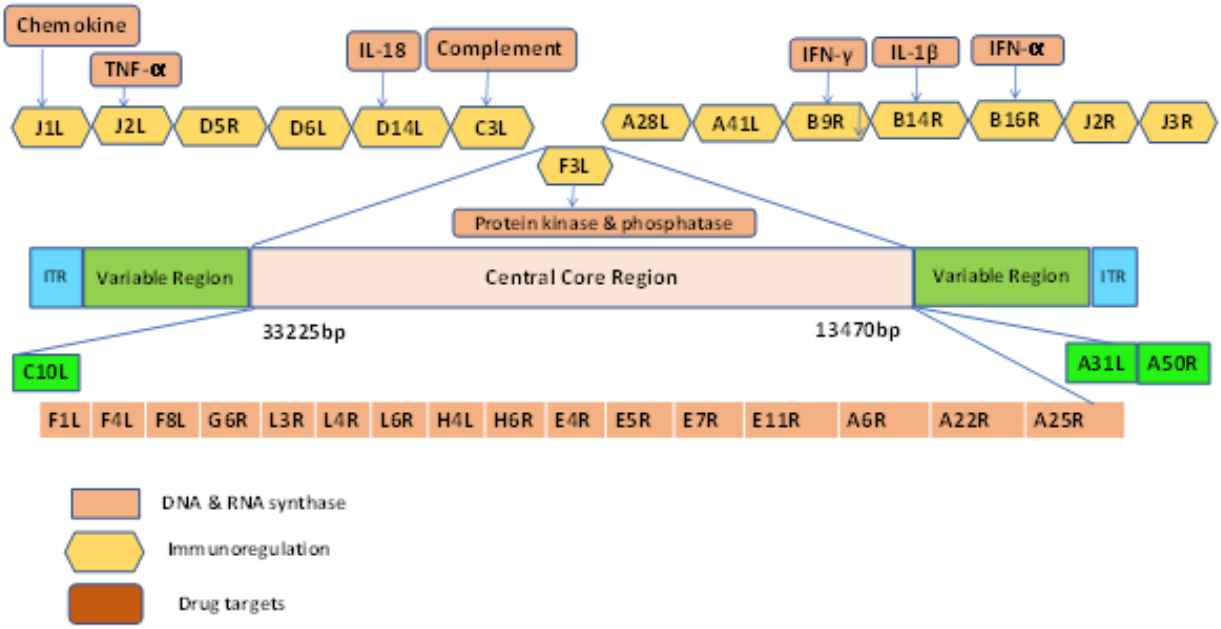

Figure 2. Genome Structure & Potential antiviral targets of MPXV

2.2. Hosts

Viruses can infect a wide range of mammals, commonly rodents, rabbits & other non-human primates like rope squirrels, tree squirrels, Gambian pouched rat (Fig:3). Recently human to human transmission is also reported.[11]

Figure 3

Figure 3. a. Rope squerrel, b. Tree squirrel, c. Gambian pouched rat

2.2.1. Route of transmission

Virus can be transmitted vertically from mother to foetus. The 2022-23 outbreak was mainly reported in gay, bisexual, homosexuals without documented history of travel. Studies showed high affinity for testes.[12] Also can be transmitted by inhalation & inoculation with body fluids, contaminated materials.[13] Transmitted from animal to human in rural areas. Longest documented chain of transmission in a community is from 6 to 9 successive person-to-person infections reflecting declining immunity in communities.

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2.3. Environment

Incidence is high near tropical rainforest and equatorial countries with hot climate among poor people in rural areas.[14] Commonly found in summer & spring in Central and West Africa.[15]

3.EVOLUTION OF MPOX VIRUS

MPXV is one of the four recognised smallpox virus infection after eradication of smallpox. Virus was first discovered in cynomolgus monkeys in 1958 during a smallpox outbreak in Copenhagen kept for research. In 1970, it was first isolated in human in a 9 month old boy in Democratic Republic of Congo (DRC), Africa. Cases rised gradually. In 1970-1979, 54 cases were reported in sub-Saharan Africa mainly in Congo. WHO established an active surveillance program in DRC (detected 338 suspected cases & 33 deaths).Sporadic cases were found in Cameroon, Gaboon & DRC in 1986-92.In 1996-97 there was a large outbreak with 511 suspected cases in Kasai-oriental province of DRC. Another outbreak occur in 2001(Feb-August) in equator province of DRC. First Mpox outbreak outside the endemic regions of Africa with 72 recorded cases seen in USA in 2003.CDC established case definition criteria for human mpox during 2003 outbreak in united states.760 cases were reported from nine health zone of DRC in 2005-7. During 2022-23 outbreak European countries & USA were also infected. Evaluation of mpox in several decades were given in fig: 4.

Figure 4

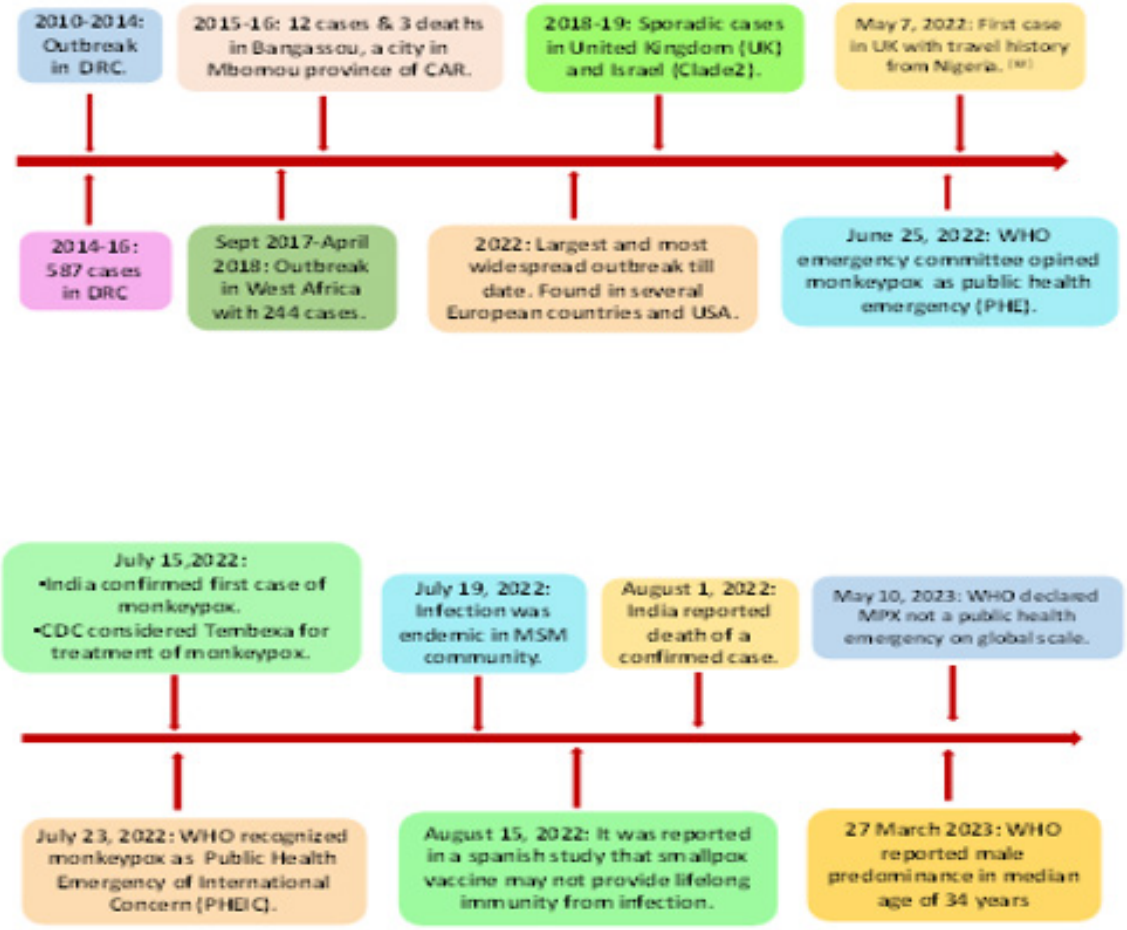

Figure 4. Evolution of monkeypox

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4. VIRAL REPLICATION

4.1.Portal of entry

Clinical menifestations depend on route of entry. Clade 1 causes more severe disease than clade 2. This might be due to site of replication of virus where clade1 was found to replicate more in respiratory, genitourinary, and gastrointestinal tract than skin, lymphoid tissues, and reticuloendothelial system where clade2 replicates.

4.2. Site of primary replication

Initially virus lodges on :

- Malpighian layer of epidermis.
- Fibroblast.
- Histiocytes of dermis.
- Dendritic cells (langerhan type of giant cell)
- Alveolar macrophages.
- Small bronchioles.

4.3. Virus Spread

From site of primary replication, virus enters to lymphatics, then to circulation (lymphohaematogenous route) and causes primary viraemia, then disseminate to distant organs, [16,17] primarily in spleen & liver. From these organs, through infected cells virus re-enters to circulation & causes secondary viraemia, disseminates to lungs, kidneys, intestines, skin & other organs[18]. During viraemic phase of disease virus particles remain cell associated.

4.4.Replication[19]

4.4.1. Site:

Replication occur in cytoplasm.

4.4.2. Receptors:

Specific receptors for monkeypox virus are not identified yet, so GAGs like heparin sulphates, chondroitin, laminin are taken into consideration.

Generally monkeypox exists in two infectious forms: a. Mature virion or intracellular mature virion (IMV) & b. Enveloped virion (EV). EV includes Extracellular Enveloped Virion (EEV) and Cell associated enveloped virion (CEV). Mature virion (MV) is single membraned. When MV is enclosed within endosomal membrane or trans-golgi, it will form triple membrane, called wrapped virion (WV or IEV). MV remain free until cell lyses. EV has additional outer membrane that is cleaved before fusion.

Virions bind to host cell membrane, fuse & enter into cell by endocytosis. In MV, single membrane, in EV extra outer membrane disrupt & fuse to host cell membrane. Virus attaches via 11 to 12 non-glycosylated transmembrane proteins (4 to 43 kDa). After entry DNA synthesis is initiated within 2 hours. MV uncoats & viral materials are released in cytoplasm. Prepackaged viral proteins & enzymatic factors that inactivate cell defence mechanism are generated. Early genes are expressed. Virus encoded multi-subunit DNA dependent RNA polymerase initiate transcription & translation. Early, intermediate & late mRNA are expressed. Early mRNA assists uncoating mechanism, DNA replication & generation of intermediate transcription factors. Late mRNA is translated into structural & non-structural proteins in host ribosome. Translated proteins & DNAs are gathered together & enclosed to form immature virions (IMVs). It transform to MV. These are devoid of external membrane & causes infection when liberated due to disruption of cell. They then travel into inner cell membrane aided by microtubules & eventually fuses to form cell associated virions. Then all these are released to exterior environment by budding (Fig:5).

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Figure 5

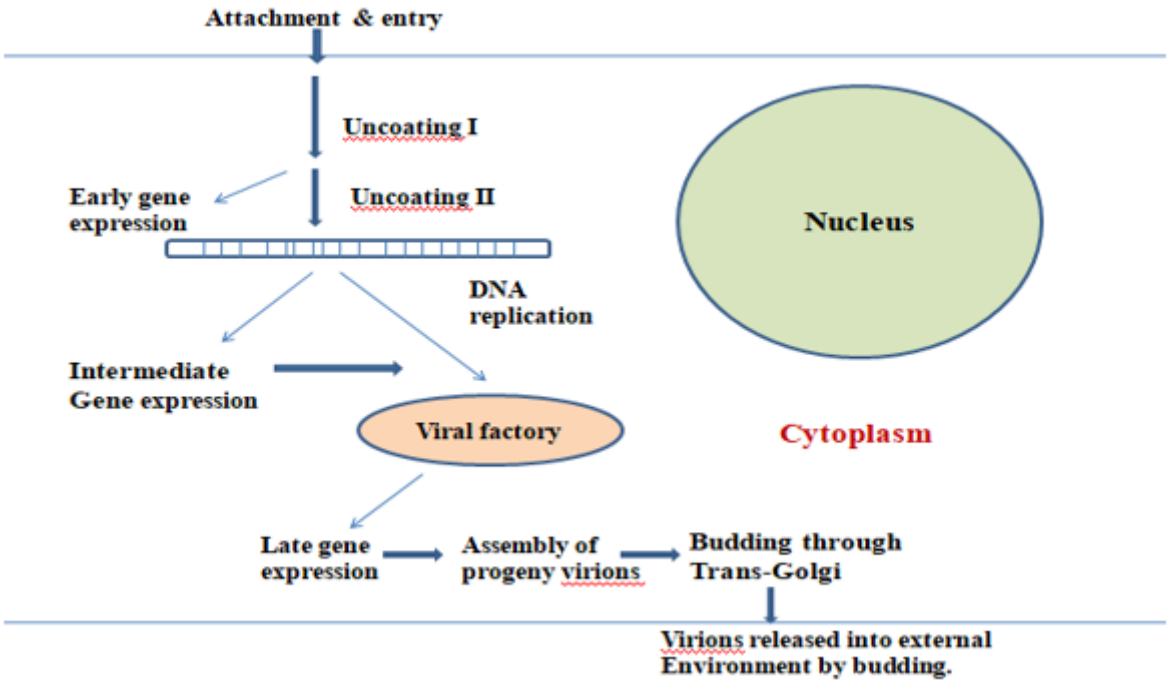

Figure 5. Viral replication

5.PATHOLOGICAL CHANGES

Viruses show inflammatory responses in primary & secondary sites of lodgement.

On Skin: Rashes appear.

In epidermis: Degeneration of reticuloendothelial cells seen in upper & middle layer of stratum spinosum. Nuclear condenses, fragments & vescicles are formed in lower stratum spinosum & basal layer. There is focal hyperplasia, ballooning degeneration, spongiosis, and exocytosis of few neutrophils.

In papillary layer of dermis: Capillaries dilate, endothelial cells swell, blood vessels cuffed with lymphocytes, macrophages, plasma cells, eosinophils leading to edema.

Cellular changes: Cytoplasm enlarges, nuclear materials are lost, vacuoles coalesces to undergo ballooning degeneration, cell ruptures, inclusion bodies are released. Vescicular cavities retain some cellular remnants & polymorphonuclear cells, leading to pustule formation. Lesions contain high virus titer.

In mucosal surface: Epithelial cells proliferate, ballooning degeneration, superficial necrosis and neutrophilic inflammation are seen. Virus proliferates to create necrosis & ulcer.

In ulcer beds: Edema, hemorrhage, vascular necrosis with thrombi & infiltration of neutrophils seen.

In muscles: Necrosis of myofibrils and fibroconnective tissues, with haemorrhage, fibrin, necroinflammatory debris & vascular necrosis are seen.

6.IMMUNE RESPONSE MECHANISM OF HOST CELLS

6.1.Innate immunity

Following active viral infection, innate immune cells come first. MPXV is recognised by PRR(Pattern Recognition Receptor) like TLR (Toll like receptors) & RIG like helicase present in antigen presenting cells(APCs) like monocytes & neutrophils.[20] Monocytes complex with MHC-II and presents to helper-T(TH) cells & Treg cells.TH cells stimulate release of IL-1β,IL-1RA,IL-2R,IL-4,IL-5,IL-6,IL-8,IL-10,IL-13,IL-15,IL-17,CCL2 & CCL5.In severe cases (defined as having >250 lesions) higher concentration of IL-2R,IL-10,GM-CSF,CCL5 and lower concentration of IL-6 are found. Treg cells cause release of IL-2,TNF,IFNα,IFNγ.[21] Migratory capacity of various natural killer cell subsets was significantly impaired. There is down regulation of chemokine receptors like

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CXCR3,CCR5,CCR6,CCR7,CCL25,CCL28,CXCL12β,CXCL13,CXCL14.NK cells loose their ability to degranulate & secrete IFNγ, TNF [22]thus, preventing host conferring an IFN-mediated immune response against viruses.(Fig 6)

Figure 6

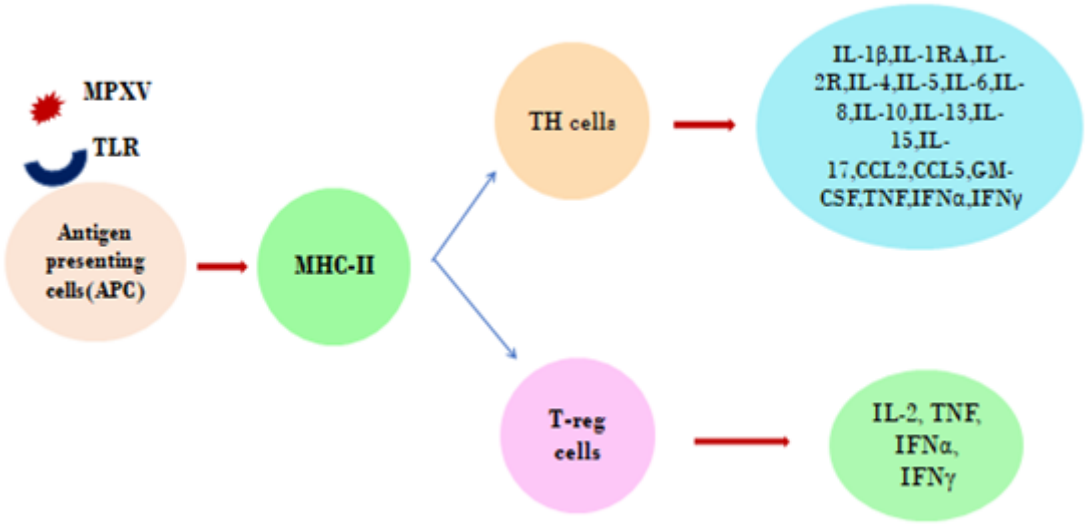

Figure 6. Mechanism of innate immunity

6.2. B cell and antibody production

Activated TH cells secrete cytokines that stimulate B cell growth factor (BCGF), B cell differentiation factor (BCDF) thus activates B cells. B cells differentiate to antibody secreting plasma cells that form IgM which further undergo class switching & produce IgG.[23] IgM has high affinity for proteins C19,A33,A44.[24]IgM typically dominates in primary immune responses, whereas IgG dominates in secondary immune responses.

6.3.T cell immunity

MHC-I complexed with CD8+T cell & MHC-II complexed with CD4+T cell.[25] CD8+T cells depend on γδT cells which upregulate co-stimulatory molecules CD80 and CD86 and secrete IL-1 and IFNα, this further activate CD8+T cells.[26] CD8+T cells causes cytolysis by IFNγ release & perforin secretion.

6.4. Immune Evasion Mechanism of Host Against Mpxv

Here, we discuss some of the evasion mechanisms used by MPXV during active infection.

6.4.1. Preventing cellular signalling

Pattern recognition receptor (PRR) usually toll like receptor (TLR) on host cell surface can trigger intracellular signalling cascades involving numerous host cofactors like MYD88, TRAM, TIRAP & TRIF. This activates important immune transcription factors like NF-ϗβ & interferon regulatory factors (IRFs).IRF3 controls expression of antiviral molecules IFNα & IFNβ [27].The MPXV orthologue B16 can inhibit IFNβ signalling [28,29] In infected children, interferon response is weaker.[30,31].Ankyrin like protein prevent NF-ϗβ activation, this compete with IϗBα for phosphorylation by IKK.MPXV genome encodes eight ankyrin-like genes, J3L, D1L, D7L, D9L, O1L, C1L, B5R, B17R,N4R & J1R, among them J3L,J1R and D1L,N4R are duplicated ORFs in left & right inverted terminal repeats within the viral genome(Fig7) [32]

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Figure 7

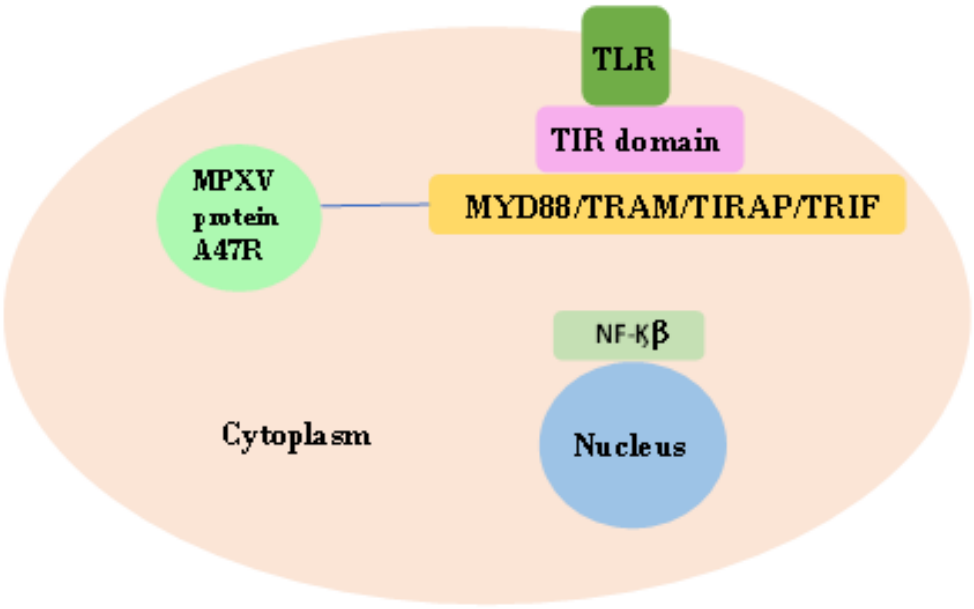

Figure 7. Immune evasion

6.4.2. Regulation of apoptosis

MPXV inhibits caspase1 and 8, thus interfere with pyroptotic or apoptotic pathways in infected cells.[33] MPXV has various anti-apoptotic proteins like A47, B13, P1, C6 and D11.[34]Tumor necrotic factor receptor (TNFR) CrmB in MPXV bind to TNF & TNFβ thus interfere with host inflammation & apoptotic events mainly BCL-2 mediated regulation of the intrinsic apoptotic pathway.[35]

6.4.3.Reduction of cellular activation

MPXVs secrete MHC class-I like protein encoded by N3R gene resembling MHC-I molecule & binds to NKG2D.It overcome T cell-mediated and NK cell-mediated cytotoxicity,[36] trigger a state of T cell unresponsiveness via an MHC-independent mechanism thus suppresses T cell mediated immunity[37].B10R gene encodes an orthologue of CPXVB8 protein, impair peptide loading & MHC-I trafficking within endoplasmic reticulum[38]

Figure 8

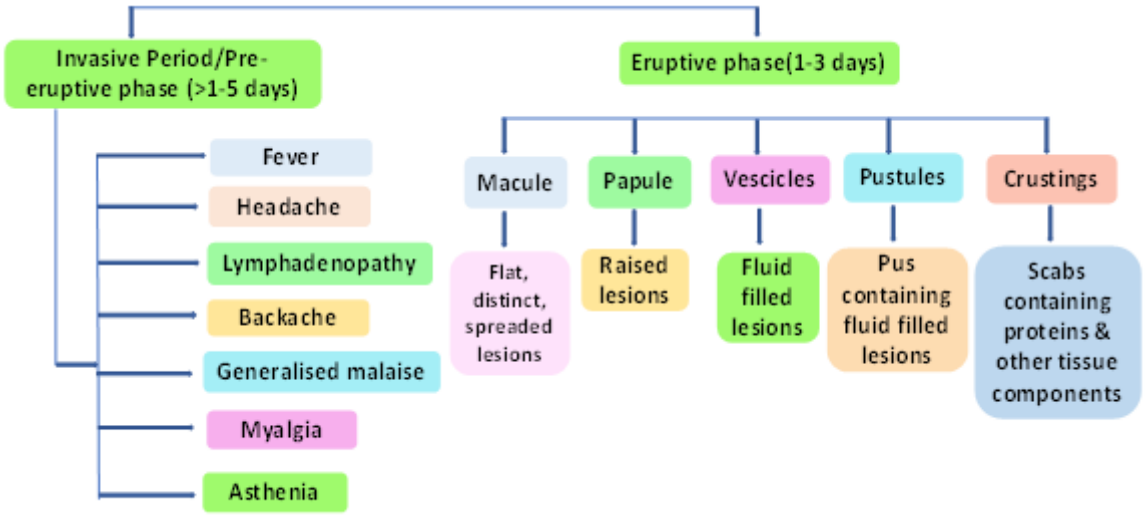

Figure 8. Clinical manife stations of MPXV

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7.CLINICAL PRESENTATION

A mpox patient is contagious till rashes fully healed and a fresh layer of skin has formed. Rash is the commonest symptom (in 80.7% cases), followed by fever (59.1%). Symptomatology of cases are very consistent over time in this outbreak.[39]

Incubation period: Usually 1-2 weeks, non-contagious. Physicians are currently recommended to monitor patients upto 21 days. Self limiting disease, typically lasts for 2-4 weeks. Clinical features are directly proportional to vaccination status irrespective of age & sex. Severity depend upon initial health of individual and route of exposure.

Prodrome Phase: Also called pre-eruptive phase. Patient may develop fever, headache, backache, generalised malaise, sore throat, cough and lymphadenopathy.

Fever: Temperature rises on day2, become 38.5-40.5^{O}C, remain for 1-3 days. Contagious, patients should be isolated if they develop symptoms.

Lymphadenopathy: Inguinal lymph nodes (LN) are commonly involved then maxillary, submandibular, cervical, axillary LNs.

Eruptive phase: Occur 1-3 days after fever. Eruption starts as macule, papule, vescicle, pustules, become dry & fall off as crust. Rashes begin on face, involve conjunctiva, oral cavity, pharynx, larynx & other mucosal epithelium(enanthems),spread to trunk, arms, legs, palms & soles (squamous epithelium-exanthems).Distributed centrifugally. Rashes usually confined to single to few lesions. Significant quantities of MPXV DNA are seen on fallen scabs indicating presence of infectious viral material. [40]In recent 2022-23 outbreak, genital rashes are common. [41] Lesions heal by hyper & hypopigmented atrophic scars, patchy alopecia, hypertrophic skin scarring and contracture/ deformity of facial muscles.[42]

8. COMPLICATION[16]

In severe condition, secondary bacterial skin infections, drug-associated erruption, permanent skin scarring, dehydration, vision loss (permanent corneal scarring), pneumonia, sepsis etc are observed in young patients. Miscarriage in first trimester & stillbirth is one of the feature during pregnancy. Complication are more intense in immunocompromised patient leading to encephalitis & death.

9. LABORATORY DIAGNOSIS

In past two decades, our global society has experienced several public health emergencies caused by viral pathogens like SARS-CoV, Ebola, Zika etc. The spread of these viruses in human population has motivated development of rapid & accurate diagnostic tools. These are conducted in field. This review comprehence the rapidly expanding diagnostic technologies for monkeypoxvirus infection.

9.1. Light microscopy (LM)

Here we see the cytopathic effect(CPE) of monkeypox virus on different cell line cultures.

9.2. Electron microscopy(EM)

Electron microscope(EM) identifies virus particles after isolating in culture-based system.

Evaluate specimens for all progeny virions at various stages of assembly (eg.,immature & mature MPXV particles) in cytoplasm of infected cells. On EM particles exhibit a brick shaped (200-250nm) ovoid structure with double stranded (dsDNA) genome (197 kilo bases) and associated enzymes.

9.3. Viral Culture

Culture based testing is not performed as routine diagnostic procedure. Usually performed in BSL-3. Grown in several cell lines, such as Vero (African green monkey kidney), Vero E6, Vero76, BSC-1, HEP-2(Homo sapiens epithelial carcinoma cells), PEK(pig embryonic kidney), MA-104, HeLa, BSC-40, LLC-MK2 and BaIb/3T3 clone A31.Cytopathic effects are usually visualized 48 hr after inoculation.

9.4. Serological Tests [43]

Enzyme-linked immunosorbent assay(ELISA), western blot(WB), immunohistochemistry (IHC) are commonly used serological tests. They are less specific as various orthopoxvirus strains are serologically cross-reactive. Virus specific IgM and IgG antibodies are detected. IgM remain in circulation for one year, IgG generate after rashes appear, rise gradually in 6 weeks, and lasts for decades.

9.5. Molecular methods

MPXV DNA can be detected by real time PCR in lesions, nasopharyngeal swab, saliva, throat swab, blood, urine, feces, rectal. Targeted genes are G2R, ATI in West African strain, C3L, D14L, F3L, B7R, B6R, hemagglutinin ,acidophilic-type inclusion bodies CrmB gene.DNA polymerase E9L,DNA dependent RNA polymerase subunit 18(RPO18) & N3R[44,45]

Recombinase polymerase amplification (RPA) targeting TNF binding protein gene. whole genome sequencing, loop-mediated isothermal amplification(LAMP), and restriction fragment length polymorphism (RFLP).[46] are various other genotypic methods. Now a days all diagnostic samples from all individuals testing positive for mpox should be subjected to clade confirmation by clade specific testing.

9.6. Genome Sequencing

DNA sequencing has been used for tracking changes in viral

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genome over time & tracing transmission patterns during current epidemiological scenario. Currently, only a small percentage of patient samples are being selected for DNA sequencing. Metagenomic & next generation sequencing (e.g., Illumina & MinION) tools are also used.

10. VACCINES

Smallpox vaccines have cross-protection against monkeypox. Both pre and post-exposure prophylaxis are there. As pre-exposure prophylaxis, two vaccines are approved by US Food and Drug Administration (FDA): a second-generation live VACV vaccine ACAM2000 and attenuated third-generation vaccine based on modified vaccinia Ankara (MVA), JYNNEOS [47].ACAM2000 is associated with Myocarditis & Pericarditis. Risks is high among people with eczema and pregnant women.CDC recommended JYNNEOS for vaccination, two doses of vaccine given 4 weeks apart,[48] has a better safety profile than earlier vaccines. In order to analyse effectiveness, few clinical trials were conducted, most of them are ongoing (https://clinicaltrials.gov/).

11. CONCLUSION

Human monkeypox is a recent re-emerging zoonotic disease. Till date, molecular biologists & research workers are in the quest of getting right clue to enrich knowledge on monkeypox for better diagnostic method with a view of adequate clinical management, guiding health care workers to minimise disease spread in a potential low level and rendering people about importance of monkeypox infection on public health point of view.

12. FUTURE PERSPECTIVES

- Risk factors for severe monkeypox infection need to study.
- Long term disease sequelae in patients & children born to infected mothers need to be followed.
- Immense study on genome structure to provide better information for diagnosis & right therapeutic approach is expected.
- Patient's immunological status following vaccine administration should be analysed.
- Co-infection with MPXV in endemic zone of other viral infection and their sequelae should be ruled out.
- Role of IgA and tissue-resident memory T cells in developing mucosal immunity following MPXV infection need to be studied.

13.Conflicts Of Interest

The author(s) declare that there are no conflicts of interest.

14. Funding

This article did not receive any specific grant from funding agencies in public, commercial, or not-for-profit sectors.

15. Abbreviation

- APC: Antigen Presenting Cells.
- BCGF: B cell growth factor.
- BCDF: B cell differentiation factor.
- CD: Cluster of Differentiation.
- DRC: Democratic Republic of Congo.
- EEV: Extracellular Enveloped Virion.
- ELISA: Enyme Linked Immunosorbent Assay.
- IFN: Interferon.
- IL: Interleukin.
- IMV: Intracellular mature virion.
- IRF: Interferon Regulatory Factor.
- ITR: Inverted Terminal Repeatation.
- LAMP: Loop Mediated Isothermal Amplification.
- MHC: Major Histocompatibility Complex.
- Mpox: Monkeypox.
- NF: Nuclear Factor.
- ORF: Open Reading Frame.
- PRR: Pattern Recognition Receptor.
- PHEIC: Public Health Emergency of International Concern.
- SNP: Single Nucleotide Polymorphism.
- TNF: Tumor Necrotic Factor.
- TRAF: TNF Receptor Associated Factor.
- TRAM: Toll like Receptor Adaptor Molecule.
- RFLP: Restriction Fragment Length Polymorphism.
- VACV: Vaccinia Virus.
- WHO: World Health Organisation.

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