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Review Article

Are We Sure that Drug Can Cure or the Vaccine is Genuine to Restrain Mpox to be Benign: A Brief Review

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Abstract

Earlier monkeypox was considered as a neglected disease of human. Presently it has received media attention due to a growing number of cases being reported in USA and beyond African territory. The first documented case of the illness in monkeys was reported in 1958 in Denmark, accordingly it has been named as monkeypox. To avoid stigmatizing language, WHO has renamed the disease as Mpox in the year 2022. As per scientific report, Democratic Republic of the Congo (DRC) is the country from which first human case of Mpox was detected in the year 1970. In real sense Mpox is a misnomer and in true sense it has very little to do with monkeys. No more monkeys are affected with this virus rather human cases are predominant. Mpox virus originates in rodents, and act as reservoir host for this virus. The experts have opined that cessation of smallpox vaccination may have set the stage for a resurgence of Mpox. Mpox is considered as "little cousin" of smallpox in part, because the illness resembles smallpox and the virus is grouped under the genus Orthopoxvirus; the same genus also includes variola virus (causes smallpox), vaccinia virus (used in the smallpox vaccine), and cowpox virus. Mostly the disease is self-limiting in nature, but the fatality rate may escalate up to 10% under extreme illness with certain clads of virus. Mpox has never been considered as sex transmitted disease, yet in several cases lesions usually appear on genital parts of homosexual individuals. Genital lesions are mostly found among men sex with men that is adding complexity in clinical settings. Invariably smallpox vaccines are recommended to control the Mpox virus infection. Non-replicating modified live attenuated virus are used to prevent Mpox illness in human being. Few antiviral drugs have been approved to treat the cases with moderate efficacy. We have to remember that Mpox does have the potential for a wider spillover. It has been observed that partly due to mutation in viral genome and unusual sexual behaviour of men sex with men is the contributing factor for proliferation of Mpox illness in African population. Contrary to mutational theory researchers have indicated that more than half of the mutations observed in viral genome during 2018 through 2020 are "silent" mutation without any major impact on the severity of the disease. Therefore, mutation in viral genome may not be the sole culprit of viral spread, rather climate change and changes in human behaviour might be playing certain role for the unprecedented spread of this illness. Are there strategies that could eradicate mpox? Answer is not so simple. Once again ring vaccination using smallpox vaccine can be initiated to control the Mpox outbreak is a matter of scientific debate but unform consensus has not been reached among advisory groups of WHO.

Keywords: Antiviral Drug; Dryvax; LC16m8; Monkeypox; MOPICE; PEPV; Tecovirimat

Recent event with past content

It may be nearly two years back, still fresh in our memory an alarming news flashed in electronic media about the first human case of monkeypox in an individual who returned from Nigeria to United Kingdom was declared positive by WHO on 7th May 2022 [1]. Afterwards the family members of the infected man also picked up the infection due to proximity. Since smallpox was deemed to be globally eradicated in 1980, monkeypox has become the most important orthopoxviral disease in terms of public health importance. Presently monkeypox is a re-emerging zoonotic disease caused by a DNA virus. Since 2022 the term monkeypox received criticism as racist and stigmatizing language, therefore, following a series of consultations with global experts, in November 2022, WHO has decided "Mpox" as the preferred term for monkeypox, subsequently the Mpox or mpox is used in research publications as a synonym for monkeypox [2]. According to published report the first Mpox disease in India was recorded on 14th July 2022 from Kerala, the individual returned from the United Arab Emirates [3]. Most of the public across the world never considered Mpox so seriously till the unprecedent global spread of monkeypox (Mpox), reached its peak in summer 2022 [4]. Mpox is considered as self-limiting disease but it causes nasty illness. With clad dependant virus infection, case fatality may vary from 1 to 10% in endemic region. Typically illness begins with fever, followed by the development of multiple papular, vesiculopustular, and ulcerative lesions on the face and body with prominent lymphadenopathy. Outcome of infection may add complication like pneumonitis, encephalitis, keratitis, and secondary bacterial infections [5]. One of the versions of Mpox is quite deadly and mortality may escalate up to 10% among affected people. High transmissibility of this virus is evident but it does not necessarily lethal. As per scientific data Mpox has been around for more than 50 years. Earlier counted as neglected disease, now it has re-emerged in African soil and progressively crossed the African territory. In July, 2022 US President Joe Biden estimated a budget of US$7 billion to control Mpox in US [6]. According to epidemiological data, Mpox endemic countries are: Benin, Cameroon, the Central African Republic, the Democratic Republic of the Congo, Gabon, Ghana (identified in animals only), Ivory Coast, Liberia, Nigeria, the Republic of the Congo, Sierra Leone, and South Sudan. While screening previous disease reporting data, we can find that Nigeria had not reported Mpox for last 39 years whereas cases suddenly reported in 2017 at the Niger Delta University Hospital. Subsequently, the virus continued to remain in circulation and appeared periodically with alarming call. However, suspected cases have declined substantially from 198 cases in 2017 to 35 illness in 2020, out of which only eight cases were confirmed; this may be due to Covid-19 related social distancing restrictions imposed during 2020 [7]. Almost 30 years after the eradication of smallpox the incidence of human monkeypox has increased in the African countries [8]. In first instance, we may suspect monkeys are the prime culprit for Mpox. In Denmark, the disease was first detected in monkeys in the year 1958 [9]. Scientifically monkeypox is a misnomer as monkeys are not major carriers. Mpox virus, although zoonotic, yet exhibit limited human-to-human transmission. But due to adaptation to humans, sustained transmission is now observed. Recent findings suggest that African rodents like rope squirrels, tree squirrels, African giant pouched rats, harbour Mpox virus [10]. Whether these species are true reservoir or amplifying host or not is yet to be authenticated. From rodents the virus can infect people so, it is better to name the virus as rodentpox (Figure 1).

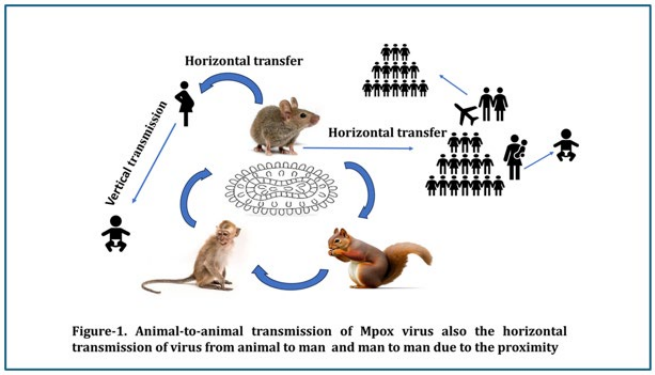

Figure-1. Animal-to-animal transmission of Mpox virus also the horizontal transmission of virus from animal to man and man to man due to the proximity

Figure 1

Considering the enormity of the transboundary transmission of the virus, on 13th August 2024, the Africa Centres for Disease Control and Prevention has declared the outbreak a Public Health Emergency of Continental Security. Simultaneously, Tedros Adhanom Ghebreyesus, the Director General of WHO, on August 14, 2024, decided to declare this outbreak a Public Health Emergency of International Concern [11]. The clinical symptoms of Mpox in human is having similarity with smallpox; but with reduced severity and relatively less contagious than smallpox. More details in this regard can be found in a recent publication [12]. Few antiviral

drugs notably tecovirimat, brincidofovir (brand name Tembexa) and cidofovir are used to treat the severity of Mpox virus. Tecovirimat is approved by FDA as well as received approval from European Medicines Agency for European countries. Synthesis of p37 one of the highly conserved envelop protein of orthopoxviruses, is inhibited by tecovirimat. Subse, afterward the release of virus is abrogated. The safety level of tecovirimat is quite high; even 23 times higher than the recommended dose for human has neither caused any neurotoxic, embryotoxic or teratogenic effects on rabbit. Pharmacokinetics study has shown the presence of tecovirimat in breast milk of animal but no authentic data in this regard from human study is in hand. In US, tecovirimat is recommended for the pregnant women with Mpox infection [13]. Since 1980, as per the data collected from DRC, the efficacy of small pox vaccine (vaccinia virus) has shown significant protective immune response against Mpox. As on date, three vaccines which were initially developed to fight smallpox, are recommended to reduce the Mpox burden. Regardless of endemic appearance of Mpox in African continent, mass immunization against Mpox is not an acceptable norm by WHO [14].

Virus track from Denmark

During summer and fall of 1958, two outbreaks of a non-fatal pox-like disease was detected in the colonies of cynomolgus monkeys (*Macaca cynomolgus*) imported from Singapore for research purpose at the Statens Serum Institute Copenhagen, a governmental public health and research institution under the Danish Ministry of Health, Denmark. Hence the illness was designated as monkeypox [9]. Afterwards in quick succession, animal quarters of Merck, Sharp, and Dohme in Philadelphia suffered an outbreak of Mpox. Next in 1962, the primate colony of the Walter Reed Army Institute of Research, Washington, DC was affected with Mpox. Next, within 1968, eight more outbreaks of Mpox in groups of captive monkeys in the USA and in Netherlands were detected those monkeys were imported from India, Malaysia, and the Philippines. Possibly the virus has moved to US through monkeys procured from these countries for research purpose but lacking scientific evidence. An outbreak of Mpox in the Zoological Garden, Rotterdam, Netherlands has shown that the Mpox virus can affect several animal species like giant anteaters (*Myrmecophaga tridactyla*), orangutans (*Pongo pygmaeus*), chimpanzees (*Pan troglodytes*), gorillas (*Gorilla gorilla*), guenons (*Cercopithecus* sp.), squirrel monkeys (*Siamiri sciurea*), macaques (*Macaca* sp.), gibbons (*Hylobates lar*), and marmosets (*Hapale jacchus*). More details about the history of Mpox outbreaks in animal colonies can be obtained from an excellent review published in 1973 [15]. In 2003, the virus crossed the boundaries of African country and was first detected in the United States. The source of human infection in Wisconsin state was confirmed to be a prairie dog. Prairie dog belonging to *Scuiridae* (squirrel) family offers hope to use this species as laboratory model for tackling Mpox virus [16].

Earliest detection of human infection

It was 1st September 1970; a nine-month-old boy from a small village Bokenda of the Democratic Republic of Congo (DRC) who became ill and later developed rash suspected of having smallpox was admitted to the Basankusu Hospital, Equatorial Province, DRC. Following health guidelines biological materials was collected and referred to the WHO Smallpox Reference Centre, Moscow for confirmation. In medical history it can be considered as first recognized human Mpox case [17]. Next four victims were children of 4-9 years old and sixth case was a 24 years old human being, cited by Cho and Wenner, 1973 [15]. Thereafter in 1970-1971, similar cases were also detected from the Ivory Coast, Liberia, Nigeria, and Sierra Leone and were accredited to be monkeypox infection. Similarly, between 1970 to 1986, as good as 10 cases of monkeypox infection in human being, was reported from Western African countries and 394 cases from Congo Basin countries of Cameroon, and DRC [18].

Virus description and mode of transmission

Mpox virus is a double-stranded DNA virus of the family *Poxviridae*, sub family *Chorodpoxvirinae* and genus *Orthopoxvirus*. This genus also includes variola virus (causes smallpox), vaccinia virus (used in the smallpox vaccine), and cowpox virus. This virus has double-stranded DNA genome comprising of 197,000 base pairs. Both the ends of genome have tight hairpin and capable to produce necessary proteins for transcription and viral replication [17]. Under transmission electron microscope, Mpox is typically brick shape having 280 nm × 220 nm size. The virus can infect Vero cells with cytopathic effect of rounding and cell detachment. As characteristic of pox viruses, the Mpox virus carry DNA dependant RNA polymerase with associated transcriptional enzymes and more than 30 structural and non-structural proteins [19]. Differing in

their surface glycoprotein structure and cell infecting mechanism, there are two distinct forms of virus exist, one is intracellular mature virus and the other one is extracellular enveloped virus but both are equally infectious. The extracellular enveloped virus released early and spread quite fast while the intracellular mature virus (IMV) released late during cell lysis. Intracellular virus lacks the additional outermost membrane layer [19]. Both the forms have different amounts of integrated viral proteins. Entry of virion is dependent on cell types and viral clades. Chemical moieties such as chondroitin sulphate or the heparan sulphate present on host cell membrane act as cell receptor for virus attachment. On post attachment event, either fusion of virus or through endosomal uptake by micropinocytosis virus get an entry inside cell cytoplasm [20]. During in-vitro propagation, several cell lines such as primary chick embryo fibroblast, primary monkey kidney cells and continuous cell lines like VERO, and laryngeal origin Hep-2 cell line are found to be susceptible to Mpox virus. Once we deep dive to analyse the molecular epidemiology data collected from multi centre outbreak of Mpox it gives a hint of dual origin of virus dissemination comprising of two clades. Out of the two clades one is Congo Basin clades also known as Central and East Africa Clad (Clad-I) and West African clad (Clad-II), encompassing two phylogenetically distinct sub-clades, IIa and IIb. West African clad virus (Clad-II) is relatively milder with 3.6 % mortality whereas Clad-I (Congo basin clad/ Central African clad) is more fatal with mortality rate 10.6% [10]. The virus isolated from Democratic Republic of the Congo (DRC) and Sudan is of central African clad whereas, the virus isolated from Nigeria between 2010 and 2019 is of West African clad [5]. Analysis of genome sequence data has revealed 95 to 99% similarity between these two clades. Presently circulating virus affecting non-African countries are caused by the West African clade the less virulent form of pox virus [21]. Homologous to smallpox virus one of the potential gene existing in Mpox virus act as inhibitor of complement enzymes of mammalian host. The complement inhibitors enzyme prevents the complement mediated lysis of virus thus virus is rescued without being lysed and virus load persists in circulation that escalate pathogenicity of pox viruses. Due to presence of gene sequence responsible for Mpox inhibitor of complement enzymes (MOPICE) detected in Congo Basin isolates makes these viruses more pathogenic than West African virus lacking that strip of genes. In an opposite way, the absence of MOPICE gene in the West-African Mpox virus isolates makes them more prone to complement-mediated lysis, so exhibit low virulence [22]. Mpox virus can spread to anyone through close, interpersonal contact including direct touching or sharing items like towels, beds, intimate contact, including: oral, anal, or vaginal sex, or touching the genitals (penis, testicles, labia, and vagina) or anus. A person can spread Mpox virus from the time symptoms start until the rash has fully healed. Due to bites or scratches from infected animals' virus can also be transmitted to human being [23]. Vertical transmission from mother to foetus during pregnancy or to the newborn during and after birth is possible. Interestingly transmission of Mpox from man to animal has not yet been confirmed. Since 2022, the unusual and complicating situation in Mpox outbreaks have been noticed where most of the infected cases were male persons within 20-50 years of age and majority of them are gay or bisexual or have sex with men and don't have any history of visit to Mpox endemic countries. Although Mpox has never been considered as sexually transmitted disease yet due to unnatural sexual activity of human being, the virus was coincidentally introduced into an MSM (men sex with men) community. Surprisingly several infected individuals were not having symptoms like fever, malaise, and headache, but prominent skin lesions at the point of sexual contact in several patients has given definite clue for sexual transmission of Mpox virus. Sexual contact had a role in the transmission of Mpox in Nigeria has already been predicted with scientific evidence. A unique report conducted in USA has shown that cis-gender women Black or African American and Hispanic or Latino women were disproportionately affected with Mpox virus, where most cis-gender women reported recent sexual activity with men confirming sexual transmission of disease [24]. From the insufficient data collected, it has been observed that vertical transmission of Mpox from mother to offspring results in higher risk of miscarriage and stillbirth. A systemic review conducted on paediatric, maternal, and congenital Mpox cases has indicated number of foetal deaths where mothers were infected during pregnancy, along with 11% case fatality rate of Mpox in children [25]. During pregnancy, there is a high risk of adverse pregnancy outcome including spontaneous abortion and stillbirth as perinatal loss due to vertical transmission of Mpox infection have been reported earlier. In macaque monkeys within 6 to 14 days of Mpox infection vertical transmission of virus with foetal death has also been observed. In one of the clinical cases in which post-mortem and pathology findings of one stillborn foetus

delivered at 18 weeks' gestation by a patient with Mpox virus infection showed a level of 10^6 copies of virus per milli litre. This much quantity of virus was equivalent to cycle threshold of 22 and was found within foetal and maternal–foetal interface tissues confirming vertical transmission of virus from mother to offspring [25]. In one of the case, breast milk of lactating infected women was negative for Mpox viral DNA in PCR, that does not provide substantial evidence to conform the release of Mpox virus in breast milk [24].

Mutation and base substitution

Mutation in DNA viruses is not so frequent. Therefore, it is unlikely that the Mpox virus has abruptly mutated to become adept at human transmission. Similarly, it has also not yet ascertained whether Mpox has suddenly jumped from monkeys nor are monkeys major carriers of the virus. Since 1970s, Mpox virus was predominantly affecting infants and children and less frequently adults are affected. Contrary to it, since 2017 the case records from Nigeria had shown the infected individuals were adults aged 20 to 50 (79%). Among the adults, 27% were women. Comparative genome analysis data has given an indication that currently circulating orthopoxvirus are from single ancestral origin; gradually with deletion of a cluster of accessory genes, it has diverged to segregate lineage specific groups. Possibility directs towards resemblance of original virus with cowpox virus [26].

The first draft genome sequence of the Mpox (Monkeypox_PT0001_2022.zip; 52.1 KB)) was from skin lesions of a Portuguese male patient obtained on May 4, 2022 [27]. While comparing the genome sequence of present isolates with parallel sequence of global Mpox virus, it has been confirmed that the virus involves in 2022 outbreaks belongs to Clad -III (earlier designated as West African clad- II type). Mpox virus of clad-II and clad III exhibits <1% case–fatality rate. Scientific literature has shown that the rate of mutation in Mpox virus is much higher than expected. Possibility of error in replication mechanism of virus may not justify such high rate of mutation in DNA genome of virus. Most observed nucleotide changes are centered on a dinucleotide change from TC→TT or its reverse complement, GA→AA nucleotide base. The dinucleotide change from G to A or C to T such as TC to TT; GA to AA is consistent with the editing activity of APOBEC3 (apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3) proteins. The APOBEC3 is an evolutionarily conserved protein grouped under APOBEC/AID (Activation induced cytidine deaminase) family that catalyse the deamination of cytosine to uracil in single-stranded DNA and, to a limited extent, in RNA substrates. The Mpox virus genome is quickly recognised by human APOBEC3 enzyme, once the viral genome is confronted with this enzyme it makes the virus enfeeble or devitalize. While comparing the genome sequences of viruses from Nigeria that spread to European countries during 2018, with the present one just surfaced in Europe and the U. S, as good as 42 nucleotide substitutions had been detected [28]. Therefore, it is hypothesized that APOBEC3 family proteins act as inducer of virus evolution.

Pathogenesis and disease diagnosis

Incubation period of Mpox usually range from 4 days to 3 weeks. The clinical manifestation depends upon the route of viral entry. Skin surface acts as large bed for viral transmission. Vesiculopustular rashes like that of smallpox appear as prominent skin lesions within 1–10 days of infection. For Mpox infection the skin lesions are so characteristics that it rarely goes unnoticed. The lesions in Mpox typically exhibit macular, papular, vesicular and pustular form that ends with crust formation known as scab. Within scab infectious viable virus is detected that act as source of aerosol infection. Initiation of skin lesion takes place once the virus reaches small dermal blood vessels. How the virus is transported to upper skin which is devoid of blood and lymphatic supply is remained hazy. Residential skin dendritic cell/Langerhans cells might be carrying the virus to epidermal layer of skin such possibility can't be ruled out. Monocyte and Langerhans cell in circulation act as vehicle to transport the virus into stratified squamous epithelial cells that is lacking lymphoid tissue to its vicinity has been hypothesized [29]. Aerosol transmission through respiratory tract or ingestion is adequate for viral entry. At the point of viral entry, tissue resident keratinocytes in skin and nearby monocytes, macrophages, Langerhans cells and dendritic cells and natural killer cells uptake virus particle and carry them to draining lymphatics. Finally, virus particles are retained in regional lymph node with enlargement of lymph node [29]. From clinical studies it has been shown that lymphoid tissues nearby to neck and throat region are the site of predilection for Mpox virus multiplication [29]. Myeloid cells such as of monocyte/macrophages, dendritic cells, B cells and activated T-cells express unique receptor to be the target for virus amplification [30]. Post viral entry is followed by low grade viremia and subsequently the virus spreads to the lungs, kidneys, intestines, skin,

and other vital organs. Presumptive identification of Mpox based on clinical symptoms is possible. However, the lesions may be confused with chicken pox. Conventional tests such as isolation of virus from scab material or fluids from pustules grown in chicken embryo or cell culture, immunohistochemistry of biological tissue sample and electron microscopy are sufficiently reliable for diagnosis of Pox disease. Lesions on epithelial surface of oropharyngeal mucosa, tongue, pharynx, larynx, trachea, and oesophagus, can release infectious virus particle in saliva. Isolation of virus particle in semen samples indicates virus tropism for testicular cells. Due to tropism of virus in sperm, all of whom participate in activities with MSM, confirms sexual transmission of Mpox. Further, exclusive genital lesions in infected individuals support virus tropism for testicular cells of males. Although not fully confirmed testicular cells that remain sequestered from the immune system and enjoy privileged immunity may provide a protective cite to support latent infection for Mpox virus. Information gathered from animal experiment with Mpox related vaccinia virus, ovarian and testicular cells have shown as homing site for Mpox virus [31].

Mpox viral DNA is quite stable at ambient temperature and under cold temperature stability of DNA remains for an extended period. Accuracy in diagnosis of Mpox relies upon detection of unique sequences of viral DNA either by real-time polymerase chain reaction (PCR) and/or sequencing [32]. Antibody-based diagnosis preferably identify previous occurrence of diseases in an individual so that identification of retrospective cases can be traced. Members of orthopoxvirus are cross reactive and share common antigenic epitopes. Therefore, presence of anti-orthopoxvirus IgG molecule in blood might be due to previous vaccine related antibody or due to past infection from related members of Orthopoxvirus. Without any animal reservoir for variola, on May 8, 1980, the World Health Assembly announced worldwide cessation of small pox vaccination program [33]. As on date, it is nearly 24 years gap from the cessation of smallpox vaccination, thus there is very feeble chance for detection of circulating IgG against Poxvirus in population. Contrary to it, IgM isotypes represent recent infection or recent vaccination so detection of orthopoxvirus specific IgM is more reliable for detection of Mpox virus infection [34]. The commercially available Orthopox BioThreat® Alert is an antibody-based lateral-flow assay that captures orthopox viral agents in the serum sample is a new arrival in diagnostic platform. With high reproducibility, this test can detect up to 10^7 pfu/ml of Mpox virus. The test sensitivity is quite acceptable as it can detect 9 out of 11 positive samples [35].

Protective immunity and infected community

Immune cells like monocyte and neutrophil can arrest the virus during early stage of infection so detection of poxvirus antigen in both these cells by cytometric staining confirm the role of these innate cells in viral protection [36]. Innate immune cells act as double edges weapon for Mpox virus as these cells are the target for viral replication more specifically monocytes and neutrophil are preferred target of Mpox virus and an early detection of viral antigen in these cells is strong predictor of Mpox infection [36]. Protective role of neutrophil against pox virus infection has already been documented in animal experiment studies [37]. Cells bearing Ly6G+ marker representing monocyte and neutrophil population infiltrate virus infected site thus minimise virus induced tissue damage. Decrease in blood neutrophils count with positive correlation with Mpox related morbidity, supports the contributory role of neutrophil against Mpox virus infection. Similarly, the high levels of viremia and neutropenia along with excessive inflammatory cytokine responses indicate noteworthy role of neutrophils after experimental infection with West African monkeypox virus in a cynomolgus monkey model [38]. Activated macrophages with two distinct phenotypes viz. M1 and M2 exhibiting functional dichotomy is an established finding. The M1 macrophages secrete pro-inflammatory cytokine and involved in killing while M2 macrophages secreting anti-inflammatory cytokines participate in wound healing and tissue repair activities. Earlier report has shown that human monocyte act as permissive cells for vaccinia virus but it ends with an abortive infection whereas M2 macrophage can yield extracellular enveloped virus in culture supernatant; those virions are source of virus dissemination to different location inside body [39]. During initial stage of infection, the innate immune effector cells such as $CD11b^+Ly6C^+Ly6G^-$monocytes accumulate at the site of peripheral pox virus infection, eventually replaced by $CD11b^+Ly6C^+Ly6G^+$ cells resemble neutrophils that has prime role to prevent systemic spread of the virus. To explore the virulence of Mpox virus, CAST and CASA mice has been used. These mice (derived from Southeastern Asian house mice trapped in Thailand) have been used due to the extensive genetic difference exhibited by these mice from other inbreed mice strains. CAST mice are more susceptible to orthopoxvirus infection due to low number of NK cell or a defect in their function. Previous findings have given a clue that depleting NK cells of C57BL/6 mice enhances orthopoxvirus virulence. In-vitro and in-vivo treatment with IL-15,

the CAST mice those are inherently susceptible to Mpox virus can withstand the viral infection and remain protective [40]. An earliest enhanced transcriptional response was observed in cynomolgus macaques (*Macaca fascicularis*) for a large cluster of IFN-associated genes, within 24 hours of infection with Harper or India 7124 variola strains. It has indicated the role of interferon in viral protection [41]. Worldwide success for eradication of small pox from earth through vaccination emphasise the role of B cell mediated protective antibody response against variola [33]. People with smallpox vaccination in the United Kingdom have adequate levels of antibodies that cross-neutralize Mpox virus [42]. Systemic studies conducted on individuals to detect the residual antibody against vaccinia virus have shown persisting antibody in a detectable manner after a gap of nearly 45 years from cessation of immunization programm. Virus specific high titters neutralization IgG antibodies have been detected in post recovery sera from Mpox infected individuals [43]. Contrary to it, Mpox can inhibit activation of CD4 + and CD8 + T cells, therefore, cell mediated immunity against virus is retarded [44]. In Spain one of the sero-surveillance study conducted among adults above 50 years of age who had probably received smallpox vaccination has shown vaccinia virus specific antibodies. According to this study smallpox vaccine induced antibodies among older adults possibly protect them against Mpox [45]. Since long cross neutralization ability between vaccinia and Mpox virus antisera has been recorded [46].

Drug for the bug

Antiviral research against Mpox is still under progress. As of now few FDA approved drugs have been used for treatment of Mpox virus. In clinical application tecovirimat, cidofovir (CDV) and brincidofovir (BCV) trifluridine, and immunoglobulin against vaccinia virus has been tested and recommended for Mpox virus cases [47]. A systemic review on clinical applications of antivirals on Mpox has advocated in favour Tecovirimat for the clinical application as an antiviral with proven beneficial effect in several aggravating cases. Simultaneously topical application of trifluridine as an adjuvant treatment is better option along with tecovirimat mentioned in that literature [48]. Functionally tecovirimat inhibits p37 viral proteins essential for cellular localization and formation of the viral envelopes resulting reduction in production of extracellular forms of Mpox viruses [49]. Pharmacologically tecovirimat decreases the production of extracellular forms of Mpox virus by inhibiting p37 viral proteins, essential for cellular localization and formation of the viral envelope. By inhibiting p37envelope protein on the virus, tecovirimat prevents the systemic spread of the virus from the infected cell, thereby avert further damage to the host cell. Nucleoside analogues that competitively bind to the viral DNA or RNA polymerase, disrupting the viral replication process often exhibit broad-spectrum antiviral activity. Based on this principle in 2022 Cidofovir (CDV), a non-cyclic monophosphate nucleoside analogue was employed in clinical trials for the treatment of Mpox outbreak [48]. CDV has broad-spectrum activity against DNA viruses. The active form of CDV is CDV diphosphoryl (CDVpp). Conversion of CDV to CDVpp is cellular enzyme dependant. CDVpp has an affinity to bind with viral DNA polymerase that leads to termination of the DNA chain synthesis thereby interrupting the virus replication process. In summary, CDVpp behave as a competitive inhibitor. Much details of Cidofovir activity against poxvirus infections have been described by research groups working from Rega Institute for Medical Research, from Belgium [50]. Cidofovir being a divalent anion is less effective with low bioavailability and its metabolites tends to accumulate in kidney resulting renal dysfunction. To overcome the adverse effect of Cidofovir, lipid conjugation technique has been used to design a derivative with trade name Brincidofovir as antiviral drug against small pox with FDA approval since 2021 [51]. Higher bioavailability and reduced nephrotoxicity of Brincidofovir has proved it as a better drug of choice yet hepatic damage and adverse effect on gastrointestinal tract has not been completely abolished. Much more ramification in treatment protocols has been adapted to treat pox diseases to incorporate various risk populations, like children, pregnant women, or other immunocompromised individuals. While screening 35 different strains of variola as well as other orthopoxviruses, preliminary data indicated similar drug sensitivities for cidofovir, cHPMPC, and ribavirin [52].

Vaccine to be genuine

From past evidence it is already confirmed that smallpox vaccine recipients are sufficiently immune against Mpox virus. Accumulated data collected during surveillance studies from central Africa in 1980s and later has shown that small pox vaccine is 85% efficacious against Mpox virus infection [53]. Since 1982, mass immunization against smallpox has been discontinued in DRC. DRC population born after 1982 has never been received small-

pox vaccine; these populations are naïve and surviving without vaccinia specific antibody. Similarly, smallpox vaccine recipients vaccinated before 1983 are surviving with diminishing antibody against smallpox vaccine thus are prone to susceptible towards an Orthopoxvirus infection. Now it is a subject of interest whether discontinuation of small pox vaccination might be the prime cause of Mpox outbreak in endemic countries is a bold question without gold answer [54]. Scientific community with undivided opinion has suspected that the cessation of smallpox vaccination has made favourable condition for Mpox virus to propagate in immunologically naive population resulting surge in monkeypox incidence [8]. Both smallpox and Mpox vaccine are equally capable to prevent Mpox illness. First-generation smallpox vaccines with trade name Dryvax is a lyophilized, live-virus preparation holding infectious vaccinia virus of Wyeth Laboratories, Pennsylvania (Wyeth is now merged with Pfizer) prepared from vaccinia virus infected calf lymph. When Dryvax was infused to different animal species, like chimpanzees, rhesus macaques, and cynomolgus macaques, complete protection against Mpox was observed. Adequate cross protection between vaccinia and Mpox virus has been well documented since long. The Dryvax is now reformulated and available as cell culture-based vaccine containing vaccinia virus. Further detail has been described below. For Mpox two different type of vaccines are available, viz. one is pre-exposure vaccines intended for high-risk groups like medical professionals and hospital workers those are highly prone to Mpox virus infection and another one is post-exposure vaccines to limit fulminating illness [53]. Strategic advisory group of experts on immunization (SAGE) is the principal advisory group to WHO for vaccines and immunization, recommends pre-exposure and post-exposure preventive vaccination (PEPV) for contacts related cases. The PEPV should be ideally given within 4 to14 days of contact (not showing clinical symptoms) [55]. Initially as per decision of SAGE three vaccines were recommended for Mpox, subsequently in March 2024 due to revised decision MVA-BN or LC16m8 vaccines has been recommended in outbreaks and for high-risk groups in non-outbreak countries [56]. Across the world MVA-BN (Bavarian Nordic) and LC16m8 (KM Biologics) are the two vaccines considered as 'third-generation' vaccines for Mpox. In USA, two smallpox vaccines such as (i) JYNNEOS (BavarianNordic, Hellerup, Denmark) and (ii) ACAM2000 (Emergent Product Development Gaithersburg, MD, USA), are received license from FDA in 2019 for human use. ACAM2000 is a vaccine derived from single clonal viral isolate from Dryvax grown in cell culture having reduced neurovirulence in animal models and considered as second-generation vaccine. MVA-BN® is derived from vaccinia Ankara strain at the Turkish Vaccine Institute in Ankara. Originally the ancestor vaccinia virus was propagated on the skin of calves at the Turkish vaccine institute in Ankara for the large-scale production of smallpox vaccine. In 1953, this Ankara strain was brought to the Institute for Infectious Diseases and Tropical Medicine at the University of Munich and it was cultivated on the chorioallantoic membranes (CAM) of embryonated chicken eggs so designated as chorioallantois vaccinia virus Ankara (CVA) [57]. Anton Mayr and his team at the Bavarian State Vaccine Institute in Munich passaged CVA more than 500 times in chick embryo fibroblasts (CEF). After 516th passage in CEF the virus was renamed as MVA (modified vaccinia Ankara) and provided to the Bavarian State Institute for Vaccines for quality control testing before being used as seed lot for smallpox vaccine production [58]. While comparing genomic sequence of MVA with CVA, it has been observed that long deletions of non-essential genes in MVA has made it to be non-replicative in most mammalian cells, including human cells. The resultant MVA is a mutant already lost nearly 15% of its original genome [59]. The replication cycle of virus is blocked at a very late stage; therefore, MVA makes early, intermediate, and late proteins, but synthesize only immature form of virions, therefore progeny virus particles are not released in the vaccinee. If progeny virus is not released, how protective immune response is induced is a subject of interest. Non-replicating virus produces immunogenic effect by completing an already initiated replication cycle, but no further replication steps take place in human cells [60]. MVA is considered as "gold standard" for vaccinia-based vaccine against smallpox. Although MVA is non-replicating, so does not move through replicating cycles in primary human cells but it can synthesize all the essential viral proteins, thereby the MVA can deliver complete antigenic dose required to induce neutralizing antibody against vaccinia virus [61]. In 1998, the Institute of Molecular Virology, a section of the Research Centre for Environment and Health (GSF, Munich), transferred one vial of MVA (at passage level 582) to Bavarian Nordic GmbH, Martinsried, Germany. It was further attenuated to develop a non-replicating live virus, grown in CEF, and marketed as MVA-BN; a proprietary product of Bavarian Nordic. Even intra-cerebral inoculation of MVA in mice model has been found to be safe [62]. Due to non-replicating character of the virus, MVA-BN cannot induce a cutaneous reaction known as

"take". Usually in smallpox vaccination "take" may indicate a certain level of localized or systemic viral replication and "take" is a surrogate of protective immunity whereas non-take is considered as vaccination failure. The MVA-BN consistently induces neutralizing antibodies and cell-mediated immune responses against multiple orthopoxviruses: variola, vaccinia, and Mpox. Since 2022, nearly 2 million people including thousands of children across the globe have received this vaccine. Eighteen years and above are included in immunization program; however, in risk prone area the children are also incorporated. Primary immunization is followed by a booster dose at 28 days is the normal routine practice adapted in vaccination program. This vaccine is considered as safe during pregnancy; therefore, no specific care or precautionary measure has been advised for pregnant mother [56]. In US any person at risk for mpox infection is advised to be included in routine adult immunization schedule with MVA-BN vaccine.

LC16m8 or LC16 'KMB'

In 1960 due to intensified smallpox eradication program, vaccinia-related adverse events (AEs) were not uncommon in vaccine recipients. In 1966 Japanese Ministry of Health formed the Smallpox Vaccine Research Group (SVRG) and was more concerned to reduce AEs. Later in1970, the SVRG recommended for Lister strain (Lister Clone 16) as vaccine candidate in smallpox vaccination program in Japan. Prof. Hashizume of the Chiba Serum Institute in Japan selected a temperature sensitive replication competent clone of Lister strain virus; after serial passages in primary rabbit kidney (PRK) cells that grew well at 30°C but poor growth at 40°C was selected [63]. For attenuation of the virus, initially Lister stain was passaged in PRK cells at low temperature (30°C). At 36th passage, 25 plaque purified temperature-sensitive clones were segregated and were further tested for their proliferating ability in Vero cells. The clone exhibiting lowest titre in Vero cells was taken and named as LC16 (lister 16). Subsequently, LC16 clone was passaged six times more in PRK at low temperature followed by growth on chorioallantoic membrane (CAM) of chicken embryo. On CAM, the virus clones producing pock lesion of medium size (2–3 mm) were isolated and designated as LC16m0 (Lister Clone 16 medium pock size on CAM original clone). The LC16m0 clones were further passaged three times more in PRK, next grown on CAM where small (0.5–1 mm) pocks were picked up, that clone of virus was named as LC16m8 (clone 8) [63]. In summary, Hashizume successfully attempted to tame down original vaccinia strain Lister (Elstree, Lister original; LO) to develop attenuated version of vaccinia virus those are temperature-sensitive and low neurovirulent vaccinia virus variants, named as LC16m0 (Lister clone 16–medium pock size-Original clone) and LC16m8. Immunogenicity and challenge studies in monkeys has shown that LC16m0 can induce protective immunity against Mpox. Limited neurovirulence was quality attributes of LC16m0. Since 1975 in Japan this vaccine has been provisionally licensed as vaccine candidate for mass immunization against smallpox. In 2022, Pharmaceuticals and Medical Devices Agency of Japan has considered LC16 as better choice for Mpox illness as this vaccine is capable to induce cross protection with humoral and cell-mediated immune responses [64]. Recently infusion of approximately 4 µL (1x 0 ^{8} pfu/mL) of LC16m8 vaccine percutaneously in naïve individual was associated with high levels of vaccine "take" and 92 % seroconversion. In Japan, universal smallpox vaccination programs include 3 doses of LC16m8 vaccine first dose at birth, next at age 6 years, and final dose once the child attain 12 years age [65].

Vaccine ACAM2000

ACAM2000 is another kind of Mpox vaccine derived from vaccinia virus (New York City Board of Health strain) that was used to manufacture Dryvax vaccine. This is a "second generation" smallpox vaccine used in United States since August 2007. Vaccinia virus ACAM1000 is the seed strain for ACAM2000. In brief, vaccinia virus Dryvax grown in MRC-5 cells (diploid human embryonic lung fibroblasts), afterward plaque purified clones free from any neurovirulence in suckling mice was selected. Out of six clones, clone 2 was selected as ACAM1000 due to its attenuated character. ACAM1000 is the master seed virus for ACAM2000 vaccine. ACAM1000 master seed virus (passage 7 in MRC-5 cells) grown in Vero cells under serum-free medium, thus the eighth passage of ACAM1000 was designated as the first passage and origin of ACAM2000 in Vero cells. Eighth passage of ACAM1000 was the first passage ACAM2000 in Vero cells [66]. Both ACAM1000 and ACAM2000 vaccines evoke neutralizing antibody and cell-mediated immune-response with visible reactogenicity in the form of cutaneous "take" in vast majority [67]. ACAM2000 was approved by the FDA on August 31, 2007 for people at high risk of exposure to smallpox. ACAM2000 vaccine is a lyophilized preparation of purified live virus suspended

in HEPES buffer (6 mM–8 mM, pH 6.5–7.5, 0.5-07% NaCl) supplemented with 2% human serum albumin, and 5% mannitol along with traces amount of neomycin and polymyxin B [67]. Both MVA-BN and ACAM2000 are FDA approved vaccines for the prevention of both smallpox and Mpox. The major drawback of ACAM2000, is that it is not safe for people who are immunocompromised or who have HIV.

Conclusion

The first human case of Mpox appeared almost 50 years back in DRC and once again that same virus has re-emerged with an alarming call, yet we are not too sure how to control the spread of this virus beyond African continent. In 2024 more than 30000 suspected cases of Mpox in 15 African countries have been detected, that has surpassed previous records. Can we suspect something terribly wrong has happened in causative virus resulting sudden bursting of this disease? Possibility of abrupt mutation in the DNA genome of Mpox has not been detected so it is unlikely to predict antigenic shift in this virus. In 2010 a study published in PNAS by Dr. Anne Rimoin a professor of epidemiology at the UCLA Fielding School of Public Health and a leading Mpox researcher has precisely documented how mpox cases have "dramatically increased." According to Professor Rimoin, smallpox and Mpox are closely related so, immunity to one may help to fight against the other. Thirty years after smallpox vaccination campaigns ended, the world's immunity to Mpox is no more detectable; it has waned, therefore monkeypox incidence has dramatically increased in rural DRC [8]. Scientific literature has suggested that the burden of Mpox in endemic area is influenced by (a) small pox vaccinated population (b) contact or exposure to animal reservoir (c) human-to-human transmission. It was expected that due to urbanization the chance of exposure to wild animals' reservoir of Mpox virus would be lower, but that has not happened. Invariably one gets a chance to be infected with Mpox after handling even little rodents. At present time, virus spreads swiftly due to close contact as well flight mode transmission through infected travellers. Human to human transmission is inevitable. Possibility of transmission of virus from child to mother and vice versa is unavoidable. How can we tell a mother not to hold her sick child? Mothers would like to be with their infected children (Figure 1). As per recommendation of government of India, even detection of single case of mpox will be considered as an outbreak [68]. So, vaccination is must in endemic countries. Interestingly, due to change in sexual behaviour of human being, nearly 90% of cases occurring in men who have sex with men (MSM), not usually observed in heterosexual relationship as reported during 2022-2023 outbreaks. However, the spatiotemporal distribution of Mpox is not well established [68]. Besides global loss of smallpox immunity, the landscape of Mpox has shifted since earlier time. One of the strains is relatively fatal and the other one is casual that has complicated the outcome of present outbreak. Next in 1978 as per WHO's assessment "Mpox is a rare, sporadic and is not highly transmissible" is no longer holds true. We have now observed Mpox is no more endemic in Africa rather crossed the African territory and affecting US and European countries. Vaccines exclusively for Mpox has not been developed but 3rd generation smallpox vaccine prepared from replication deficient live virus is quite safe for mass immunization in endemic countries to control Mpox illness. The pre-exposure and post exposure vaccines are also in hand to tackle the high-risk groups. Presently available antiviral drugs are quite effective to prevent fatality in clinical cases, but treatment is not the solution for infectious disease circulation. As of now Mpox has been considered as neglected disease and, presently we have realised that "the end of smallpox was the beginning for Mpox". It is the appropriate time for public health authority to decide whether once again vaccination using third generation smallpox vaccine in endemic areas is demanding or not.

Conflict of Interest

The authors declare no conflict of interest.

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