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MONKEYPOX VIRUS RECENT BREAKTHROUGH: REVIEW ON EFFICACY OF DIAGNOSIS AND TREATMENT METHODS

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Abstract
Following the devastating pandemic of COVID a new and concerning global threat "re-emergence of the Monkeypox virus" has occurred during May 2022. Viruses-based diseases cannot be ignored so preventive measures have been taken by the scientific community across the globe. Significant breakthroughs have been seen regarding the diagnosis and treatment of monkey virus infections leading to improved efficacy. Monkey virus, also considered as simian virus can affect primates including monkeys and humans. The accuracy and speed of diagnosis methods have been ameliorated by several paramount approaches, serological tests, viral culture, CRISPR-based identification, and PCR. Additionally, antiviral therapy advancements and supportive care strategies have shown significant results in the treatment of monkeypox infection. The efficacy of these diagnosis and treatment methods was discussed and challenges related to these techniques were highlighted. This review aims to provide a thorough insight into current trends in the diagnosis and treatment of the monkeypox virus that can serve as paramount information for public health authorities, researchers, and clinicians pertinent to effective management and control of these emerging viral diseases.
Keywords: *Diagnosis, Emerging viral diseases, Monkeypox virus, Simian virus, Treatment*

INTRODUCTION

The severity of dealing with viruses can be imagined through the previous pandemic experience of COVID-19 which has had a long-lasting effect on Humans across the globe. Soon after this deadly pandemic the re-emergence of the Monkeypox virus occurred as a breakthrough in May 2022 to onwards (1) This specific virus is classified under the family (Poxviridae). Monkeypox virus (MPXV) is considered as oval or brick-shaped, enveloped double-stranded DNA that belongs to the genus Orthopox virus having almost 197 Kb of length (2-5) Due to the phenomena of cross-protection and cross-reactivity, hosts previously infected from any orthodox viruses can be immunized against other members. This factor can serve as a key point to illustrate insights to cope with future epidemiological alertness. The poxviridae is composed of multiple viruses including smallpox, vaccinia, camelpox, etc., as they possess a common antigenic nature, the same preventive measures can be taken for the monkeypox virus to deal with its severity (6) As they are enveloped by DNA virus so most probably the target for virologists will be viral proteins present on the envelope and some enzymes like DNA polymerase. In comparison to RNA, DNA viruses exhibit greater stability and undergo fewer mutations due to their double-stranded DNA structure and the presence of 3′–5′

exonuclease proofreading activity in the poxvirus DNA polymerase (7). Nowadays it is the trend of genomics and proteomics so the 200,000 base pairs genome of a virus can be sequenced to have a deeper insight into virological and pathogenic details.

Monkeypox virus, characterized by its oval-shaped virion measuring approximately 200-250 nm, is regarded as one of the most intricate and largest viruses. The genome of the virus contains almost 190 nonoverlapping ORFs (Open reading frames), moreover, it possesses inverted sequence repeats at the end of terminals on both sides (8-9). These regions may vary in length due to the process of mutation, translocation, and repetition, however, the middle of the genome contains housekeeping genes that remain conserved and are responsible for the different cellular activities including morphogenesis, transcription, and translation. Most commonly the conserved genes that are used for the identification purpose are; E9L (DNA polymerase), HA (haemagglutinin), and B6R (extracellular enveloped protein) (10). HA serves as a functional gene and plays a significant role in the formation of polykaryocytes (syncytia) (11). The B6R gene is present in the C-terminal of the transmembrane domain, moreover, it is also considered as a viral envelope protein. The DNA polymerase gene E9L of the virus is involved in virus replication (12).

The term "monkeypox" may be misleading since the virus is not exclusively transmitted by monkeys. Instead, it can be transmitted through various other sources, including squirrels, rats, mice, and rodents. MPXV was first isolated from an afflicted monkey species known as *Macaca cynomolgus* this species was traveled from Africa to Denmark. Later on, the first case of MPXV in humans was reported in 1970 from Zaire in central Africa. During that period, the majority of cases were concentrated in the two prominent regions of the Congo basin in West Africa and Africa. Additionally, a few sporadic cases were identified in the United States, Nigeria, and the United Kingdom, which were likely the result of individuals contracting the infection while traveling in the aforementioned prevalent regions (13). The viruses' clade representing the Congo basin region elicited more severity (manifested a 10% mortality rate and was considered more pandemic) as compared to the West African clade. This virus does not possess the ability to undergo rapid mutations as it is a DNA virus, but still can be very dangerous and need to be eradicated (14-18
).

EPIDEMIOLOGY

Within Global Health Organizations, the recent outbreak of the MPXV even in regions other than Africa has grabbed the attention of everyone and has raised multiple concerns. According to an up-to-date survey of the current outbreak, the CDC has announced 73,288 confirmed cases of Monkeypox (out of these 72,428 cases were from countries that do not possess a prior history of Monkeypox virus, and the remaining cases were from countries that have a prior history of this virus) virus infection dated on 14 Oct 2022.

TRANSMISSION ROUTES HOSTS AND SYMPTOMS

Various modes of transmission are possible including direct interaction with the skin, body fluids, mucous linings, droplets from the respiratory tract, or contaminated objects. Moreover, consumption of partially cooked meat of infected animals, or some animal-based products being obtained from the affected animals can have a seriously increased rate of infection (Fig. 1. illustrates symptoms and common hosts of Monkey virus) (19). In addition to this Monkeypox virus can also be transmitted from mother to infant or even in the fetus (through the placenta) several reports have documented such cases. Besides this, the prevalence even in the asymptomatic conditions is still unknown. Based on studies, the most common route for transmission and infection is men having sexual contact with other men (20-21). The exact source of infection remains unidentified, except for individuals who have a travel history to endemic areas. Furthermore, there have been indications of the presence of the virus in small rodent populations across various regions of Africa (22).

Once the virus gets entry to the host via any transmission route, its replication begins within two hours of infection. There are specific inoculation sites inside the cytoplasm known as viral factories. Moreover, viral factories also serve as the places for virion assembly transcription and translation. Several paramount virus-encoded proteins are synthesized including protein kinase, single-stranded DNA binding

proteins, DNA ligase, uracil DNA glycosylase, polymerase, processivity factor, and helicase-primase. These essential proteins perform a pivotal function in facilitating the replication and synthesis of the double-stranded genome, without leaving any detectable traces, these are produced normally in a hairpin loop and link the two ends of the linear DNA (23). The first 7-21 days serve as the incubation stage, then the virus begins to replicate in entry areas like respiratory mucosa or the oropharyngeal depending upon the initial contact of the virus with the circulation of viral loads to the lymph nodes and drains the mucosa to be reached towards the spleen with primary viraemia (24-25). The secondary viraemia begins as the virus replicates again in these areas, it is the prodromal stage and during this stage, the clinical illustration of Monkey virus in the tertiary organs and skin is manifested (24).

Monkeypox virus shows similar clinical manifestations to those of chickenpox and smallpox (26). So, any patient who has a similar presentation to smallpox may be affected by the monkeypox virus (27-28). After infection, there is an average of 7-14 days incubation period. Initially, the symptoms include headache, fever, and weakness. On a clinical basis, there is one difference between smallpox and Monkeypox which is the formation of lymphadenopathy, either localized or generalized in the axilla or neck (27-28). Soon after the prodromal phase a rash appears shortly and distributes among the whole body. Before scaling and healing the lesions are developed into four different stages macular, popular, vesicular, muscular, and pustular (13). The whole process is done in 2-3 weeks. The lesions like varicella-zoster lesions that have papules, macules, all kinds of skin rash, and vesicles that can be visualized but such symptoms are not visible in viral infection of monkeypox, Later on, the vesicles are converted into pustules, dry, and eventually fall off (26, 29). These lesions are primarily present at the head, and limbs moreover these can also be present in the genital area, eyes, and mouth. Furthermore, these lesions can finally be turned into bleed or large bullae (26).

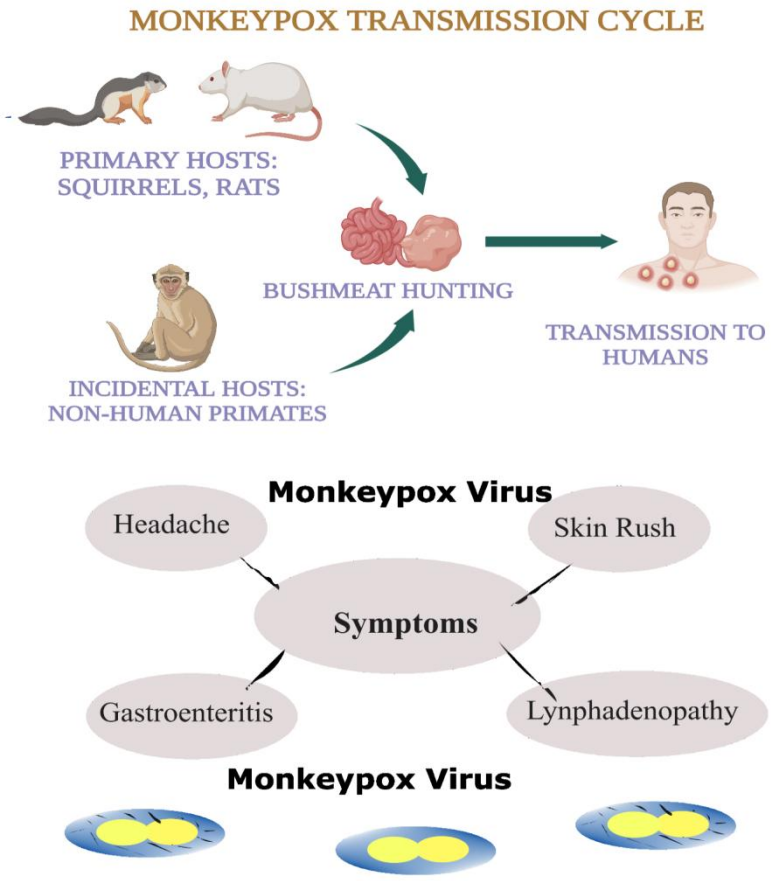

Fig. 1. Monkeypox virus: Hosts, Transmission routes and symptoms

The severity of infection depends upon different factors including virus strain, the root of exposure, and the basic status of the health situation of the patient (30, 19). As mentioned earlier the strain of West African monkeypox is linked to milder disease, limited only to human transmission; meanwhile, the strain of central African monkeypox virus is more disastrous and shows severe effects. They have a higher rate of mortality

in comparison to West Africa. It can be measured at 10.6% in the Central African strain as compared to the West African strain which shows 3.6% severity (31). Even several cases have been reported that confirm the person-to-person transmission of the Central African strain of Monkeypox virus (30, 19).

Previous studies on the reviews of the Monkeypox virus do not give a detailed insight into the methods for treatment and detection of this viral Pathogen. Keeping in mind this study was conducted to gather the maximum available information on the diagnosis and treatment of MPXV. Moreover, their efficacy was compared based on available data. The data could be utilized for the development of better management strategies to cope with the deadly Monkeypox virus.

DETECTION METHODS FOR MONKEYPOX VIRUS

Nowadays, multiple approved tests are considered for the diagnosis of monkeypox. Several PCR-based molecular tests are being used to detect and delineate the wide range of poxviruses, especially orthopoxviruses (32). Poxviruses are larger in size due to this feature they are observable easily under any "light microscope" (33). The isolation methods for the virus comprise inoculation of the vesicular fluid into the chorioallantoic membrane of the chicken embryo that has the MP virus causes hemorrhagic lesions on the specific membrane (34). The primate cells and human cells are the most sensitive cells to be cultured (35). The MP viral agents can be detected by using "immunohistochemical tests" specifically on the tissues and materials collected from the lesions of the skin (36). The MP virus can be detected by checking the presence of antibodies that appear after 7 days by lateral flow immune chromatographic tests or ELISA but there is a limitation that orthopoxviruses cannot be distinguished via any of the serological tests due to the phenomena of cross-reactivity (13). The neutralizing antibodies that are secreted against any specific species of orthopoxviruses can produce cross-protection against the other members within the same genus.

MOLECULAR METHODS FOR MPXV DETECTION

In comparison to other methods for diagnosis, NAATs (Nucleic acid amplification tests) are considered as main methods for delimitation and characterization of MP virus as they are rapid, have specificity, and possess high sensitivity (12). In the last two decades, NAATs have been extensively grown, to attain highly sensitive, efficient, and feasible ways by optimizing many NAATs methods like conventional PCR, real-time PCR, and nested PCR in many detection methods but all methods have advantages and disadvantages as well.

THE RT-PCR METHOD

One of the most effective and reliable approaches for the diagnosis of MPXV is the real-time polymerase chain reaction (RT-PCR) (37, 12). It is more reliable as it possesses the following attributes, rapid, highly sensitive, specific, and yields a high quantity of throughput (12). Moreover, real-time PCR can be actively run to identify the virus load (copy numbers), to check the course of infection, active infection, and virus shedding in animal models and even in humans (19). The use of QPCR or the RT-PCR was limited before the outbreak of COVID-19, moreover, there was no specialized equipment and experts to perform this technique. While upon disastrous dissemination of COVID-19 this technique was used on a wide scale for detection purposes resulting in the generation of experts and specialized equipment across the globe. The melt curve analysis gives us the correct delineation of different strains of viruses, however when melt curve analysis is coupled with the sequencing of PCR product it gives far more accurate results (19). The sequence results from the samples are compared with already available sequences of MP viruses and other viruses that show 99% matching results with two strains of MP virus and more than 90% with other strains of orthopoxviruses (19). The viral terminal regions are being used extensively as ideal regions for sequencing and delimitation of viral strains, these regions have the most reported variations. The reporter gene, Tumor Necrosis Factor (TNF) present at the Inverted Terminal Region (ITR) that has many Single Nucleotide Polymorphism (SNPs), mutations, and deletion/insertions within the course of two strains of MPXV and other viruses of orthopoxviruses (38). Keeping in mind the above characteristics this sequence was utilized to construct probes and primer for detection purposes. This specific sequence was utilized to

construct probes and primers (19). Firstly, in 2006 Li et al., utilized "real-time PCR" to detect the DNA of MPXV in the sample obtained from body rash. They used the primers of two different genes E9L (envelope protein) and B6R (DNA polymerase) for amplification by real-time PCR method (12). In addition to these genes, many other potential genes/sequences are being used to distinguish between West African and Congo Basin strains of MPXV with the help of real-time PCR techniques. The study conducted by Li et al. reported the interpretation of a specific PCR method to isolate two strains of MPXV and the generic reaction (19). The generic MPXV and West African MPXV were detected by using the TNF receptor gene region. This region is not present in the MPXV strain of Congo Basin Viruses, moreover, they do not possess sequence length that can reach the ideal annealing temperatures of the probe, so another gene (C3L) of complement binding protein was targeted, and this specific sequence was deleted in West African MPXV genome (12). Commercially available probes that are mentioned to be used in the above three assays include BHQ1 (30-quencher molecule) (Molecular probes, OR, Eugene) and a fifty reporter molecule (FAM) (Glen Research, Sterling, VA) (19). HA, E9L, and B6R are a few other types of viruses that target those genes that are being utilized for the designing of primers for the delimitation of orthopoxviruses (8,10). According to previous studies HA gene was the only gene that has the power to identify all kinds of pan-orthopoxvirus species in a specific single real-time reaction, but for unidentifiable species, sequencing is required for the exact delineation of species.

REAL-TIME MULTIPLEX PCR

As it is the outburst of the monkeypox virus it is a need of time to correctly identify and differentiate this pathogen from others like variola and varicella-zoster that are clinically not dissimilar. According to a study in 2006, the use of PCR methods like "multiplex real-time PCR" is an efficient candidate for the exact delimitation of these three types of cryptic viruses. The open reading frame gene regions including B12R, ORF38, and F3L regions were first selected and then aligned to design different primers, particularly for variola, monkey-pox viruses, and varicella-zoster accordingly. For varicella, varicella, MPXV, and internal control probes TAMRA/BHQ2, FAM/BHQ1, Cy5/BHQ3, and JOE/BHQ1 were selected as quencher/dye accordingly. The products obtained from these multiplex PCR products reveal the DNA of orthopoxviruses and the DNA of other viruses and even showed 100% accuracy in identifying the DNA of these three viruses (10). Moreover, the samples from healthy persons do not show positive results. Hence the multiplex real-time PCR can be used effectively for exact identification and delineation of the viral pathogens including Monkeypox viruses. An overview of PCR-based detection of monkeypox virus is given in Fig. 2.

PCR/ESI-MS PAN-ORTHOPOXVIRUS

This type of PCR is fascinating as it has the power to distinguish all the viruses that belong to orthopoxviruses even in a single reaction that comes from clinical specimens that do not require sequencing of PCR product. It uses a specific platform of T5000 (10). It does not require previous knowledge of genome sequence and identifies the pathogen more accurately and rapidly. A study conducted by Grant et al., 2010 reported first-time use of this method to evaluate the viral load of MPXV and vaccinia from the clinically available samples and the samples from in vivo models. Therefore, the outcomes of this case study highlight the victory of this specific method to identify and distinguish orthopoxviruses, particularly vaccine-derived "vaccinia viruses", from similar challenging viral pathogens like MPXV. Moreover, this technique has been widely utilized to detect various infectious agents like alphaviruses, coronaviruses, influenza viruses, and other potential species of bacteria like *Streptococcus pyogenes*, *Neisseria meningitides* and *Haemophilus influenza* (12)).

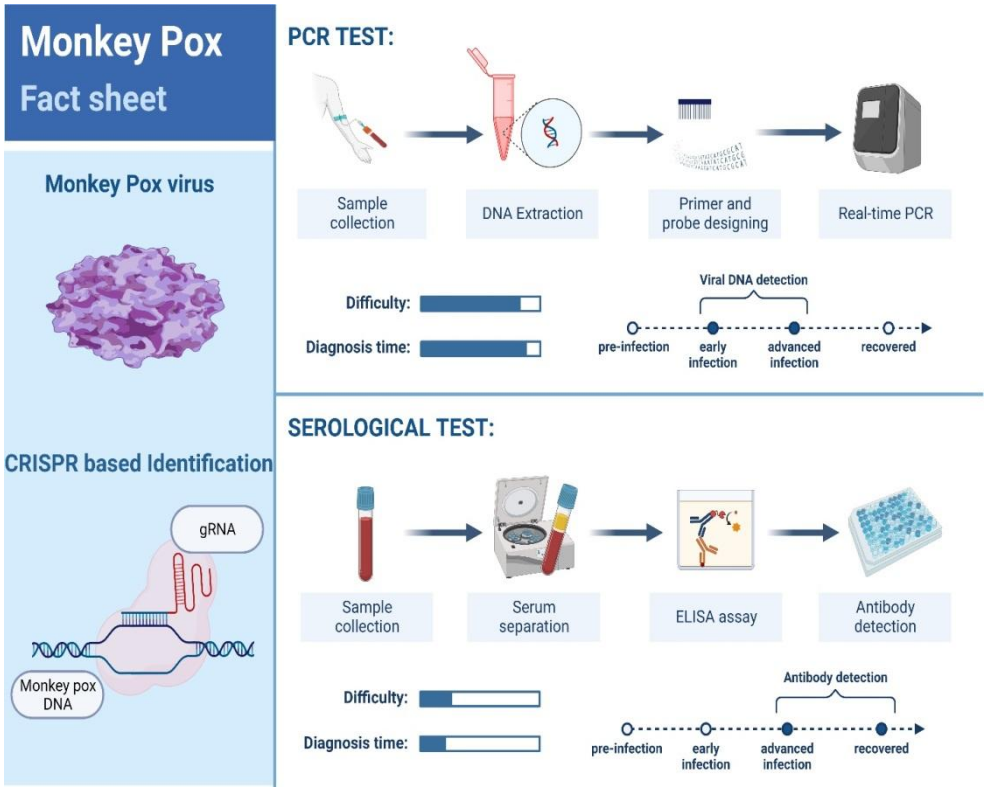

Fig. 2. PCR, CRISPR, and serological-based detection of monkeypox virus

LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP)

This amplification through the isothermal technique is a substitute for nucleic acid amplification which can be used in place of real-time PCR. It is an automated strand displacement DNA synthesis process that involves synthesis by the Bst version of DNA polymerase, which replicates the gene without the need for Light cycler devices (39). This method involves three specific genes that include a specific partial ATI gene for the West African strain, a D14L gene for the Congo Basin and another one is like partial "ATI gene" that is jointly illustrated by groups (both) and thus amplified by using primers (4-6), kept at isothermal (60-65^{o}C) conditions. By comparing the results of LAMP and nested PCR it shows high sensitivity and specificity that is about 70-80 percent & 100 percent accordingly, its accuracy in terms of sensitivity is more than the orthodox PCR technique. Exploring its applications additionally, it can distinguish between different strains of the virus, determine the period of infection of MPXV, and able to identify the level of viremia ultimately linked with the sternness of the clinical indications (39). A study was conducted by Lizuka that reports three oligonucleotides containing LAMPS development in West African strain W-LAMP, for the Congo Basin C-LAMP, and a common COM-LAMP for both of the strains Congo Basin and West African. LAMP possesses many pros over conventional and RT-PCR one of them is ease of handling (39). In addition to this LAMP has the benefit of being cost-effective as compared to others so it can be applied in the poor and relevant affected areas of Monkeypox viruses.

RECOMBINASE POLYMERASE AMPLIFICATION ASSAY (RPA)

Recombinase polymerase amplification assay RPA is also an isothermal-based amplification technique that works on the principle of DNA amplification via enzymatic activity. This method targets a gene named G2R and thus complete reaction is done at the temperature of 37-42^{o} C for almost 3-15 minutes. However, most of the patients of the monkeypox virus are from Africa, and within those regions molecular detection methods are not feasible due to lack of resources. So, in those regions, simple techniques such as RPA can utilized as potential tools for diagnosis purposes. In comparison to real-time PCR, this method has the following advantages: convenience related to time, no complicated thermal cyclers are required, and reagents are not too sensitive and are stable in several environments, even in a temperature of 30° C (37). A study by Davi et al., 2019 showed that the sensitivity of this assay was 95% (16/µl) of DNA molecules in comparison to real-time PCR showing 100% sensitivity (two cases that were reported negative by this

method were weakly positive by real-time PCR [CT value: 38.8 and 39.97]). Furthermore, the specificity for both methods was reported 100% (37).

GENEXPERT FOR MPXV

The scrutiny and effective control of infections by monkeypox virus can be facilitated by field conditions and less sophisticated labs using GeneXpert technology (40). This method is relatively composed of a simple system about the size of a backpack and thus all steps are carried out including preparation of the sample, reaction for real-time, and at last detection, in closed chambers of GeneXpert device. As all of the process is automated and takes place in a closed device there is no or less chance of contamination and the whole process is completed in less than 90 minutes. This method does not require too many samples for processing and there is no need for an expert staff for its monitoring as it is an automated process(40).. After the successful study of Li et al., for characterization and delimitation of monkeypoxvirus that shows 98.8%, and 100% sensitivity and specificity respectively, GeneXpert is being extensively used for the detection of several other infectious agents including *Mycobacterium tuberculosis* coupled with rifampicin resistance test. Methicillin resistance coupled with *Staphylococcus aureus*, is also even used in the detection of EVD (Ebola virus disease). With these great advantages, there are some limitations of GeneXpert, it is a little bit more costly and needs resources like power energy, but these faults can be easily neglected by redesigning this technology in a "multiplex forum" for the identification of MPXV and many other similar infectious agents (40.

PROTEOMICS APPROACHES FOR MPXV DIAGNOSIS

Prediction of protein buildings structure along with their interaction with different cellular factors comprise of wide range in terms of functionality towards all biological systems (41-42). In recent decades application of proteomics in virology studies has been utilized on a large scale (43). Protein-protein interactions are the main step in virus-host contact and such interactions can be manifested by proteomics strategies. So proteomics studies can be considered a powerful tool to illustrate the interactions between hosts and viruses (43). These strategies that are based on proteomics are considered paramount approaches for the identification of pathogens, large-scale analysis of protein structure, their role in the overall outline of regulation, cellular development & phenotypes determination of pathogens (41-42). The antiviral drug targets and delineation of pathogenesis can be illustrated by studying the structure and function of viral proteins (44)). Recently advanced proteomics research has been performed on vaccine strains and the vaccinia virus, which is a prototype orthopoxvirus (45). Many vaccinia proteins have been expressed in baculovirus vectors or *Escherichia coli* moreover the in-vivo expression of all proteomes of the poxviral genome has been done amicably (46). The knowledge of such expressed genes can be very significant for various supreme purposes like diagnosis. In the current era, the exact protein constituents and complete proteome study of the mature virion (MV), a member of the genus "orthodox" that has VACV and MPXV need to be explored. Different studies have reported that viral proteins frequency was 63 and 163 in VACV MV and the frequency of viral proteins in MPXV was about 157 (47-48). The identification of viral proteins can be carried out by using recent advanced approaches to proteomics (49). For this purpose number of methods SILAC, DIGE, and ICAT are being used to lift studies related to proteomics of virus-induced modifications in cells. Such advances in the field of proteomics have made possible a more detailed insight into the characterization of virus-virus, virions, and virus-host interactions, and their role in pathogenesis and infection. Until now, this potential approach has been applied to a few viruses and viral proteins. Proteomics in the field of virology is in its infant stage and there are plenty of uncharacterized pathogens and a vast amount of untapped data that need proper characterization for a better understanding of pathogenesis and viral gene identification. The new cellular functions, life cycles, and pathogenesis can be determined by using wide innovative proteomic approaches (50). Cognate of the implication of CRISPR (Regularly Interspaced Short Palindromic Repeats) together with proteins associated with the CRISPR (Cas) mechanism, proteomics approaches were conceivably not mentioned during the whole detection period of MPXV. Even so, these can play a paramount role in the detailed characterization of the viral proteome.

Furthermore, the viral interactions provide a basis for the deep evaluation of the characterization of viral proteome along with their detailed interactions and links with cell-mediated proteins and many more proteins of orthopox proteins. This novel proteomic approach can play a paramount role in overall relegation and diagnosis methods by using multiple techniques. Keasey et al., 2010, used human smallpox vaccination to elicit an antibody response against the monkeypox virus in monkeys using the proteomics approach. In this regard, a protein microarray representing 92-95% of monkeypox clades of West Africa and Central Africa was evaluated against 92% of the vaccinia proteome. The results obtained from this assay illustrated that immunoglobulins produced can neutralize a definite number of strains that can cause viral infections. This study unveiled several potential viral antigens within the course of its proteome, moreover, these can take part in responses elicited by the immune system and might suggest services of such enclosed proteins together with anti-IgG/IgM against MPXV strategies for analysis (51).

DETECTION THROUGH CRISPR TECHNIQUE

The CRISPR-Cas system is a natural immune system against the foreign DNA present in the genome of *E.Coli* and was first discovered in 1987 (52). Jink et al., 2012 identified this mechanism as a potential technique to cleave target DNA in vitro (53). Nowadays this technique is being used worldwide for multiple research and clinical purposes including gene function prediction, gene editing, the development of vaccines, the pathogenesis of viruses, etc (54). Most often, this technique is considered a treatment method but it can be actively used for the detection of non-infectious as well as infectious diseases (55). Initially, CRISPR-Cas possess 2 groups having six types and a further 22 sub-types depending upon distinctive bacteria and viruses each of them has its applications and characteristics (55-56). Out of the different systems II, V, and VI types were considered for accurate and exact identification of disease-causing agents (56). Nucleic acids are considered major biomarkers for the determination of disease status, the amazing CRISPR-Cas mechanism can detect the disease by targeting nucleic acids DNA/RNA, even after finding the target DNA it can break down the desired sequence and can effectively repair infectious diseases caused by bacteria, viruses and non-infectious diseases (57). No doubt, the first method used for detection and repairing purposes were CRISPR-Cas9, however other CRISPR-Cas systems, including CRISPR-Cas 13a and CRISPR-12a belonging to CRISPR class 2 systems have the potential to diagnose Coronavirus 2 immediately, possessing high sensitivity, and appeared as a high potential technique for detection of several infectious agents (56). Due to recent breakthroughs in virus-based diseases several experiments and research are being carried out on this technique to enhance its specificity, develop simple reagents lateral flow measurements on paper, and develop standard methods for the development of nucleic acid-based POC (point of care) (55). There are several features including high speed, probe sensitivity, DNA-RNA binding or RNA-RNA binding, cost-effectiveness, detection for cancer, SNPs (Single nucleotide polymorphisms), pathogens detection during the pandemic situations, and detection of genetic diseases making it more fascinating to the scientific community (55). The scientific world of diagnosis was revolutionized by the introduction of CRISPR-Cas 12a and CRISPR-Cas 13a as it has the potential to identify several viruses, including dengue virus, Zika virus, type 2 canine parvovirus, influenza virus H7N9, a strain of influenza virus, Ebola virus, mutations (HIV drug resistance), and Lassa virus, etc (56). Identification and characterization of these types of viruses can be widely used via multiple methods like lateral flow assay, and electrochemical techniques for the diagnosis of different viruses (56). The two sensor systems “SHERLOCK” and “DETECTOR” are used for detection purposes in this technique. The SHERLOCK strands for “Specific High- Sensitivity Enzymatic Reporter Unlocking” made in 2017, it is based on the Cas 13a system that possesses a high throughput to detect DNA or RNA from experimental specimens with a high level of sensitivity and specificity (56,55). In 2018 one more potential tool DETECTR (DNA Endonuclease Targeted CRISPR Trans Reporter) was based on the CRISPR-Cas12a system (Chen et al., 2018). These two systems have reporters, present in the form of ssDNA or ssRNA that are labeled with a fluorophore at 5□ end & a quencher dye at 3□ end. Initially, Cas12a or Cas13a recognize the target sequence and specifically bind to them, upon binding they are activated and cause cleavage of the fluorophore, thus resulting in the specific fluorescent signal which renders ease and S/N by a higher signal to noise is analyzed and documented (56). These two

systems have been actively used in the detection of target sequences moreover, they have the capability for direct virus detection collected from different samples like food, urine, mouth swabs, plasma samples, and environmental samples(56).

CRISPR-Cas 13a was used for the diagnosis of "SARS-COV-2" collected from nasal swab samples (59). In another study conducted by Broughton, the CRISPR- Cas12a coupled with an assay like "lateral flow assay", detects SARS-Cov2 with high sensitivity in not more than 5 minutes (60). As there is no effective vaccine available a highly valuable diagnostic mechanism such as CRISPR is required for the exact identification of poxviruses to cope with them. On the other hand, CRISPR is not being extensively used worldwide for diagnosis purposes concerning many reasons, and more research is required.

VIRAL CULTURE/ISOLATION

Viral culture is one more conceivable means of monkeypox infection diagnosis. In this method, the Patient's body fluid is taken as a sample, if the patient is infected the virus is grown and isolated. This strategy is fairly less delicate however it can help with affirmation sometimes (61).

ANTI-ORTHOPOXVIRUS ANTIBODIES-BASED DETECTION

Another possible method for diagnosis includes anti-Orthopoxvirus antibodies (IgG or IgM) based tests. This technique analyzes the patient's blood and anticipates if there was any previous or distant confrontation with Orthopoxvirus (62). If an IgM-based test gives a positive result in CSF or serum, this may illustrate a recent infectious event. Those who are still infected with MPV but are unable to generate an antibody response may result in a negative anti-orthodox IgM test. Other infections, such as syphilis, varicella zoster, herpes simplex, molluscum contagiosum, and acute HIV, should be considered as they may have resemblance or even can be involved with MPV during the course of infection (63).

OTHER SEROLOGICAL TESTS

Lab techniques for recognizing monkeypox likewise include 298, 299 western blotting (WB), ELISA (Enzyme-linked immunosorbent assay), and immunohistochemistry (IHC) tests (64). Upon the unavailability of a virological sample, various serological diagnostic strategies can be used to detect ELISA. In the majority of cases, ELISA is used as a potential serological test (65).

Inconclusive nucleic acid tests can lead to serological testing for the exact detection of MPX (66). Serological testing, though provides evidence for virus infection, yet this specific testing may have some impediments in conclusion as it will recognize other similar viruses as well (67-68). To cope with the pandemic situation, modern highly sensitive immunological techniques can be a paramount tool for MPXV detection, by targeting the fact that T-cell responses and antiviral antibodies are increased during the onset of the disease (28). Meanwhile, the presence of anti-poxvirus antibodies in a person with no previous history of vaccination and having a background of extreme sickness and rash can be suspected as a finding of MPX (69).

DIRECT DETECTION

Direct observation via electron microscope can also be utilized as a helper strategy to recognize the monkeypox virus. However, because of the being labor intensive and expensive sample preparations, it is challenging to utilize on a routine basis (70).

METHODS FOR TREATMENT

ORALLY ADMINISTERED OR TOPICALLY APPLICABLE DRUGS

Supportive care together with an accentuate on nutrition, pain relief, and prevention from superinfection of bacteria on skin lesions is the mainstay of treatment for mild cases of MPV infection, as in most cases MPXV shows self-limiting and mild effects so can be managed without targeted antiviral therapy (71). Skin lesions should be monitored to be kept dry and clean. Oral allergy medicines and topical application of petroleum jelly can be used for the treatment of Pruritis. Moreover, the oral lesions can be

treated by the implementation of magic mouthwash or Anesthetic gels, having antihistamines, lidocaine, steroids, and antimicrobials. NSAIDs (non-steroidal anti-inflammatory drugs), acetaminophen, topical anesthetics neuropathic pain medications, and even opiates can be used as a subsequent treatment for severe cases of painful genital lesions. Proctitis (inflammation of the rectum or anus) likewise can be treated by the addition of agents like sitz bath and stool softeners. Hydration, acetaminophen, nonsteroidal anti-inflammatory drugs (NSAIDs), or some other common treatments for severe headaches can be used to deal with symptoms of headaches (63).

INJECTABLE DRUGS

Considering injectable drugs for MPX treatment, Monkeypox is effectively prevented by vaccination against smallpox. It has the potential to either lessen the severity of a particular disease or even prevent its onset if administered at early stages of infection like (the incubation period) (72). As for now, smallpox vaccines are not accessible to common people, nor are they being utilized in pandemic regions for MPXV endemic regions due to some issues i.e., safety issues, and cost. Safety issues arise as a person is being immunized with a vaccine having live vaccina virus, moreover the obscure impacts of such vaccines in immune-compromised people i.e., those suffering from AIDS (Acquired Immuno-Deficiency Syndrome) (73,74).

GENERAL TREATMENT METHODS

The general treatment strategies for MPXV cases include getting enough rest, eating enough calories and liquids, keeping a balanced water-electrolyte ratio, and homeostasis within the human body. Moreover keeping a close eye on paramount signs like the saturation of fingertip pulse oxygen and the level of discomfort. Poor mood, lethargy, irritability, and paleness—all of which are indicators of a child's mental state including diet must be monitored closely. The complexities should be noted. Premature infants should also have better feeding, nutrition, and nursing. Moreover, the development and growth of infants should also be monitored closely(75).

ANTIVIRAL DRUGS

As smallpox and MPXV share common genetics the antiviral medication used for the treatment of smallpox (cidofovir, brincidofovir, and tecovirimat) can also be effective against MPXV, even though recently there has been no targeted treatment for MPXV (76)(. The viral DNA polymerase is targeted by Cidofovir and its prodrug brincidofovir and hinders it during the process of DNA replication. The VP37 protein is targeted by tecovirimat, this protein has a paramount role in the envelopment of mature infection of intracellular places with Golgi-inferred layers to frame the EV (encompassed infection), inhibit the virus from leaving the infected cell and thus, prevents the dissemination of viral infection to the whole body (77). The administration of Cidofovir is done intravenously at a dosage rate of 5 mg/kg once a week for two weeks (78). According to the Centers for Disease Control and Prevention (CDC) in the United States, tecovirimat is considered the first-line treatment for MPXV infection (79) (CDC, 2022). People having a severe disease or that are at risk of getting the severe disease, and having abnormal functions involving the mouth, eyes, or other anatomical areas (such as genitals or anus); can be considered for tecovirimat treatment. In rare cases, extremely severe infections, progression of disease despite tecovirimat treatment, or due to contradiction or unavailability of tecovirimat cidofovir and its prodrug brincidofovir can be considered (79, 80).

MONKEYPOX PREVENTION

VACCINATION

There is no antibody explicitly intended to forestall monkeypox infection disease. Keeping in mind the phenomena of immunological cross-protection among members of orthopoxviruses (81) smallpox vaccine virus based (vaccinia) was suggested as a potential vaccine to be used in the current endemic of MPXV (82) Moreover the CDC has suggested immunizing the individuals who have been recently exposed

to MPXV or those who have the higher risk to be exposed to MPXV (63). Under the Extended Admittance to Investigational Medications approval, ACAM2000 and JYNNEOS are recognized in the United States for their potential use in treating pediatric cases of MPX (82). The safety and efficacy of the above-mentioned particularly for MPXV in humans, children, or adolescents, is under consideration.

GENERAL PROTECTIVE MEASUREMENTS

To cope with MPXV infection and dissemination of the disease, people are advised to keep a distance from wild animals without protection, especially for ill or dead animals, even from their flesh. Animal flesh being used as food should be properly cooked. People residing in pandemic areas should avoid close contact with animals and sexual contact with the person who is suspected to be infected with MPXV. Hospitalized patients should be kept in strict airtight isolation. While monitoring patients or visiting them should take good PPE (personal protective equipment), like usage of recommended medical masks, disposable clothing for isolation, usage of disposable latex gloves, and avoid close physical contact. If it is mandatory to have contact with MPXV patients, there should be no sharing of household items like, (beds and clothes). Focus on close-by cleanliness and wash hands properly under running tap water with recommended hand sanitizer and cleanser in time(83). Two subcutaneous injections should be given every four weeks to people who have never had the smallpox vaccine, while one subcutaneous injection may be given to people who have had past exposure to the smallpox vaccine (84).

CONCLUSION

Monkeypox has been marked by the WHO (World Health Organization) as one of the top emergency diseases that has now become a worldwide or international pandemic as it previously used to be endemic to Africa. During outbreak of monkeypox in 2022, more than 56000 people were infected. The virus spreads even more speedily due to animal-to-human or human-to-human contact and it is more likely to be caused by a combination of natural or human factors. Nevertheless, efforts are being made to better understand of factors that result in spreading the virus from one person to another. Moreover, strategies are being developed for public awareness to help prevent disease spread thereby preventing future threats of monkeypox. Local, state-level, or international-level health departments i.e., WHO, should be developing and testing new antiviral drugs or vaccines and providing safety guidelines for both patients and healthcare workers to minimize disease spread and control the pandemic situation.

Conflict of Interest:

Authors have no conflict of interest.

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