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Variations in genomic epidemiology and *in-silico* screening of potential phytochemicals to cure Monkeypox

Muhammad Adil, Muhammad Waseem, Abdul Qadeer Haider, Nageen Hussain*

Abstract

Monkeypox virus (MPXV) is passed on when people encounter infectious animals. Before April 2022, the Monkeypox virus was reported only in South Africa and its surrounding region but now it has been spread all over the world. This Monkeypox virus has an incubation period of five to twenty-one days and can be communicated through direct contact, breathing, contaminated towels, bedding, and so on. The *Orthopoxvirus* variety is a subfamily of the *Poxviridae* family that incorporates the Monkeypox infection. Their unique property is to suppress the host defense system and to exploit host immunity. Treatment of Monkeypox involves two vaccines named JYNNEOSTM and ACAM2000. Antiviral medications can be considered for serious diseases, immunocompromised patients, pediatrics, pregnant and lactating ladies, complex sores, and when injuries happen close to the mouth, eyes, and privates. This review article gives a basic information ofA48R, a thymidine kinase, which is involved in DNA replication pathways in the Monkeypox virus. The potential drugs for A48R inhibition like NMCT and rutaecarpine are considered good synthetic drugs. The maximum affinity -18 was shown by phytochemical dictamnine, amentoflavone -7.5, citral -7.8, and naringin -6.6 which can be isolated from different plants. The purpose of this review article is to describe variations in genomic epidemiology and *in-silico* screening of potential phytochemicals to cure Monkeypox.

Introduction

General health specialists are concerned that another scourge caused by the Monkeypox infection could represent another danger while the world keeps on being tested by the COVID-19 (Coronavirus) illness 2019 pandemic [1]. A DNA virus having a place with the family *Orthopoxvirus* is the Monkeypox infection. The Monkeypox infection was at first found in primates, however, it likewise normally contaminates rope squirrels, tree squirrels, Gambian pouched rodents, and dormice. One of every ten cases of Monkeypox brings about death. The Monkeypox infection can cause more extreme cases in youngsters and pregnant ladies. As per reports, 3-6% of instances of Monkeypox bring about death [2]. It is to a great extent accepted to be a self-restricted sickness that, in most cases, may sort itself out without clinical mediation [3]. In 1958, during two flare-ups of a non-lethal rash disease among hostage cynomolgus macaques brought from Singapore, the Monkeypox infection was first found in Copenhagen, Denmark. Comparative episodes in primate provinces in Europe and the US were recorded on various occasions during the accompanying decade. The first human case was not found and Monkeypox was not recognized as a human disease until 1970 [4]. Thousands of laboratories affirmed examples of human Monkeypox which were recorded all around the world. The greater part (96%) of these cases were in the DRC (Democratic Republic of Congo), with the excess cases scattered among seven other Focal or West African countries [5]. The number of cases was expanded, and their geographic spread has developed, in the twenty-first 100 years. The frequency pace of DRC grew multiple times between the 1980s and the mid-2000s [6]. Thought cases kept in the DRC for the many years 2000-2009 and 2010-2019 were more than 10,000 and North of 8,000, separately. 10,545 thought cases and 362 related fatalities have been kept in the DRC between 2020 and May 2022. In 2003, the Republic of Congo encountered its most memorable instance of human Monkeypox, though South Sudan did not face Monkeypox attack cases (East Africa) [7].

Methods

Literature Search and Selection Criteria

In this article we collected past information of Monkeypox virus by reviewing more than 100 research articles. Its genetics, mode of transmission, antiviral chemotherapy, and vaccination has been discussed. Its phylogenetic relation has been discussed by CLAD. A protein A48R in Monkeypox has been targeted *in-silico* by phytochemical inhibitors. Computational tools for docking included PYMOL and Auto Dock Vina were used. The 3D structure of protein was retrieved from PDB (NCBI) while 3D structures of ligands were downloaded from freely available online chemical databases like PubChem and Zinc15. The binding affinities of the compounds were observed in PYMOL. The higher the binding affinity, higher will be chances of that compound to block the target protein.

Discussion

Monkeypox virus classification

The *Orthopoxvirus* variety is a subfamily of the *Poxviridae* family that incorporates the Monkeypox infection. Huge, wrapping infections are *poxviruses*. Their genome has 200kb sets of straight, twofold abandoned DNA (dsDNA), firmly loaded with 200 qualities [8]. A 0.5% genomic grouping contrast isolates the two clades of Monkeypox strains, which are tracked down in different pieces of Africa. In people and cynomolgus monkeys, the Congo Bowl (focal African) clade is more destructive than the West African clade; the assessed case casualty rates for people are 10.6% and 3.6%, separately. So a member of *Orthpoxivirus* belongs to the geographical area of Africa. This virus hasa genome of 196858-genome size. Both sides of genomic stricture cantoning 6379-bp IR(Inverted Repeats). Computational analysis shows that among the whole open reading frame it is found that more than 60 elements are of amino acid out of 190 elements [9].

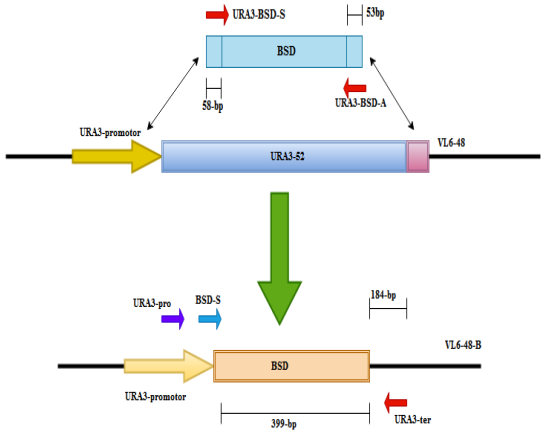


Figure 1: A larger piece of *Monkeypox* virus genome fragment, developed phase of URA-3-52, strains VL6-48-B.

Monkeypox virus (MPXV) consumes incubation period is about five to twenty-one days. The natural reservoirs of Monkeypoxvirus are still obscure but non-human primates and rodents are considered to be habitat of it. Due to this viral attack in humans almost 3,000 cases have been reported over the globe. The first sequencing was exposed publicly on May 2022 by Portugal, but further sequencing material was added in previous sequencing by National Center Biotechnology Information (NCBI) at the end of month May 2022 have shown in table 1 and table 2. A travelling history

hasshown that a patient with MPXV moved from Nigeria to the USA with accession no. ON676708 [10]. Three amino acid changes (D209N, P722S, and M1741I) occurred in the immunogenic surface glycoprotein B21 (MPXV-UK_P2-182) [3]. During the clinical analysis, it is observed that RT-PCR is being used for gene identification rpo18. The first draft of sequencing was 92% the same as to reference sequence [11]. The viral genome consisting of 200 proteins and 200kb size is occupied with linear double standard DNA structure with no 3` and 5` end free but a hairpin structure is attached.The gene in the viral genome is strongly, covalently packed inside. A specified role of naming a gene was introduced in 2021 under "unified nomenclature for *Orthopoxvirus* genes". The mutation rate is 10^{-5} and 10^{-6} / replication site. It is a zoonotic *Orthopoxivirus* having the property of a biothreat agent with varying morbidity and mortality rate as well. Their unique property is to suppress the host defense system and to exploit host immunity [12]. After fully sequencing it was observed that not only single nucleotide polymorphism was detected but frameshift and stop codon was also detected which might be the reason forthe loss of gene initially [13]. A new Monkeypox viral CLAD has been also identified with the name of clad-3 which develop human outbreaks from 2017 to 2022 across the globe. This form is subdivided into lineages A, A.1, A.1.1, and B.1.Currently most cases of the Monkeypox virus has are detected in Portugal and UK. The viral also has changed symptoms new symptoms are fever, rash, and swollen lymph nodes are included. It was an alarming situation when the two first cases were detected in USA and Argentina in May 2022 patient from Argentina shows MPXV B.1 genomic structure after a viral attack having 942bp genome length. The complete MPXV genome sequence BR0001 is also available for public use under (NCBI GenBank) Accession Number "ON751962.1". The consensus sequence and raw data can also be found in the dedicated GitHub repository of our project (GitHub- CADDE-CENTRE /Monkeypox: Monkeypox data) [14]. Before April 2022, the Monkeypox virus was reported only in South Africa and its surrounding region but now it has been spread all over the world including USA, and Portugal and its spreading rate is getting high day by day. It is considered the largest non-endemic outbreak that ever happened due to Monkeypoxvirus. It is suggested that the 2022 outbreak of MPXV is linked with the CLAD-3 type of it.MPXV is more virulent than *Congovirus*. The current ongoing outbreak has a divergent branch than B.1., which is A.1 [15]. The pedigree of MPXV discovered in 2022 shows that it has the same phylogenic as the strain identified in 2018. This shows that it has the same family history. Till 2022 new more than 45 strains have been identified in case of mutation (Figure 2). The phylogenetic tree was generated according to Duque *et al.,* [16]. at different periods, Monkeypox strains are shown in figure 2. Due todiversenature, a largenumber of strains were found across the globe, in which some strains are being shown. The B.1-CLAD shows the current outbreak that occurred in 2022 over the world which has caused a huge death rate and infection rate as well [17].

Monkeypox treatment

ACAM2000, the principal cutting-edge smallpox immunization, is like the now-ceased Dryvax antibody since it is made in cell culture utilizing a Dryvax clone [18]. ACAM2000 is convey2d through cutaneous scarification and incorporates replication-skilled VACV. At the point when immunization is effectively managed, a take at the immunization site is made that contains an infection that can spread via autoinoculation and unexpected vaccination of close contacts [19]. The inoculation isn't suggested for people who are pregnant, have atopic dermatitis, or have safe framework deficiencies, among different circumstances. It is guessed that its security profile will be like that of the Dryvax immunization, which is known to be associated with specific significant incidental effects. A few inoculations have been connected to myopericarditis [20].

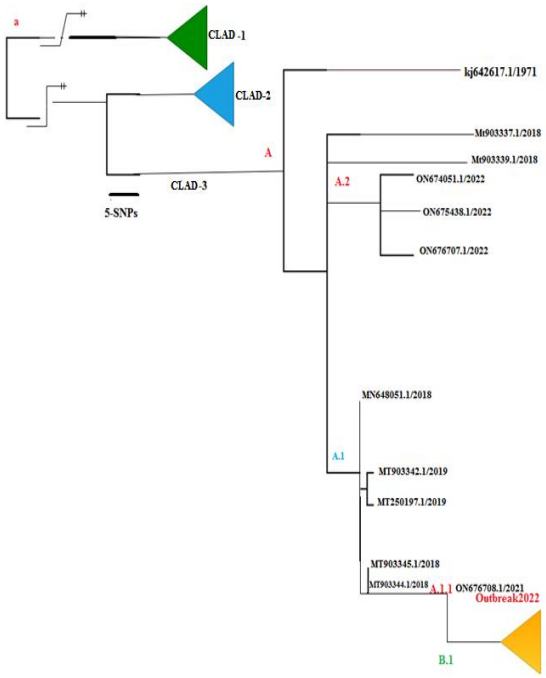


Figure 2: Showing phylogeny outbreaks in 2018 to 2022 of *Monkeypox* over the globe lineage B.1 connected with CLAD-3. (NCBI: 15 June 2022).

MVA-BN (JYNNEOS in the US), the second cutting-edge smallpox immunization, is delivered utilizing the

Altered vaccinia Ankara strain (MVA). Due to some degree to the cancellation of two host range qualities referenced in the previous segments, MVA replication is hampered in most mammalian cells. The MVA-BN inoculation is given as two subcutaneous infusions separated a month, and it has not produced results. There is no gamble of autoinoculation or accidental vaccination, and no significant incidental effects are expected. Given immunogenicity information from clinical examinations as well as adequacy data from creature challenge tests, the immunization has been approved for use in the US against both smallpox and Monkeypox. Nonetheless, clinical preliminaries have not been directed to show human adequacy [21].

Antiviral drugs

Brincidofovir and ST-246 (Tecovirimat) are two antivirals that have gotten U.S. endorsements for the treatment of smallpox. An exceptionally rationed OPXV envelope protein (F13L) is the objective of ST-246, which forestalls virion discharge. Brincidofovir is an orally open lipid compound of cidofovir, a supported medication for the treatment of human cytomegalovirus contamination. Cidofovir is a non-cyclic nucleoside simple. The concealment of poxvirus DNA replication is the system of activity of cidofovir. The efficacy of the drugs in a few instances of human Monkeypox suggests that tecovirimat is strong, while Brincidofovir is not [22]. OPXV can become drug-safe when passaged in cell culture with either ST-246 or cidofovir because of changes in F13L or E9L (DNA polymerase), separately.

Targets of neutralizing antibodies

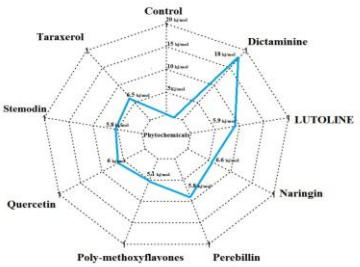
For insurance against smallpox, counteracting agent responses to inoculations are fundamental. Smallpox and issues from the inoculation are effectively treated utilizing immunization-resistant globulin, which is extricated from the plasma of antibody beneficiaries. For the best safeguard, antibodies against orthopoxviral' virions' novel surface antigens are essential. While the epitomized virion is comprised of an MV with a second external layer including eight unmistakable proteins, the experienced virion is comprised of a solitary film inserted with more than 20 proteins. It is known that two EV-explicit proteins (A33 and B5) and seven MV proteins (A13, A17, A27, A28, D8, H3, and L1) might be killed. While the MV killing antibodies are not separately important for MV balance or prevailing in completely vaccinated people, hostile to B5 antibodies is the transcendent EV killing antibodies. All things considered; the smallpox immunization's very repetitive killing neutralizer reactions could be a characteristic that guarantees security in different human populations. A significant number of these antigens are killed by antibodies in a supplement subordinate way [23].

Profilin-like protein A48R thymidine kinase and *In-silico* blocking of A48R

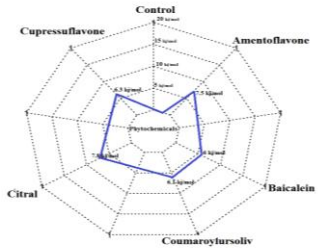
Thymidylate kinase A48R acts as phosphorylate thymidine monophosphate by making diphospahte bond. Its active sites are quite different from humans while making it a potential source of working for thymidine analogous with no involvement of human analogous. Eight drugs are considered under sequencing alignment and A48R is one of them. It was found that A48R with thymine diphosphate hasa 7.4 kJ/mol energy level. A gene A48R working as thymidylate kinase with protein reference number PDB-2V54 with novel drug NMCT and Rutaecarpine with structural nature of single and four benzene rings are attached respectively [20]. The two-component of A48R (Rutaecarpine and NMCT) containsthe largest cluster with middle RMSD/nm which is 0.172 for NMCT and 0.173 for Rutaecarpine with 100% cluster proportion. It is also observed that NMCT hasa 3.7h half-life in vitro cells while the half-life of Rutaecarpine is still unknown. Presently drugs used for NMCT are potent viral inhibitor which is unusable for the human species and Rutaecarpine drugs is COX-2 working as an inhibitor to reduce pain in the upper portion of the human body, especially the head. COX-2 acts as an anti-inflammatory drug. In both of these elements, NMCT shows contact hydrogen strong bonding,but Rutaecarpine shows transient hydrogen bonding. The gene A48R hasan active residue of Ala-107, Asn-65, and Lys-105. A48R was found to be bonded in the amino acid Tyr-144 [9].

A48R is a thymidine kinase that is involved in DNA replication in different pathways in the Monkeypox virus. This protein is a profilin-like protein involved in cytoskeleton regulation in eukaryotic cells. The profilin-like protein binds to actin molecules. The potential drugs for A48R inhibition, NMCT, and rutaecarpine are considered good synthetic drugs. NMCT has potential potent antiviral activity in HSV-2-infected mice, with little apparent cytotoxicity in uninfected Vero cells. A48R has two chains with 133 amino acid sequence lengths. The 3D structure was retrieved in PDB format from NCBI-PDB with ID 2V54. The unique inhibitor ligands were removed in PyMol (version 2.5.2), and the active site was determined in this review article. Additional water molecules were removed from the protein and exported. Then the protein was changed into PDBQT format in auto dock vina (version 4.5.1) and THR 55 and Ser 56 amino residues were targeted by making a grid box. The 3D structures of the ligands were downloaded from PubChem in SDF format and converted into PDB format by using an online tool. Then the ligands were converted into PBQT in auto dock vina.

Then docking was carried out by running commands in the command prompt and the results were saved in the log file. The interactions between ligand and amino residues were observed in PyMol. The maximum binding energies were recorded which have been given in Figure 3 (a, b).



A



B

Figure 3: Binding affinities of phytochemicals to the profilin protein A48R (3a) Dictaminine showed the highest binding affinity -18 KJ/mol to block A48R protein (3b) citral showed -7.8 KJ/mol binding affinity and amentoflavone showed -7.5 KJ/mol binding affinity.

Conclusion

Nowadays, Monkeypox is an emerging zoonotic disease. A48R is involved in the replication process of the Monkeypox virus but its exact mechanism is still unknown. In this study, different phytochemicals have been used to block A48R. The active side in the B chain was targeted by the ligands. Dictaminine binding energy at THR55 was the maximum18KJ/mol. This ligand has also shown a great affinityof about20KJ/mol to block the spike protein of COVID-19. The other ligands including citral with -7.8, taraxerol with-6.6, luteolin with -6.5, and stemodin with binding energy -5.5 showed statistically significant affinities. These compounds interfere with the replication process and stop further virion division.

Competing Interest

The authors declare that there is no conflict of interest.

Author Contributions

Muhammad Adil:
Contributed data and analysis tools
Performed the *Insilico* analysis on bioactive compounds
Wrote the paper

Muhammad Waseem:
Preparation of genomic CLAD
Wrote the paper

Abdul Qadeer Haider:
Epidemiological study of Monkeypox
Wrote the paper

Dr. Nageen Hussain:
Design the review article
Contributed to article writing

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