Advanced Studies in Biology, Vol. 16, 2024, no. 1, 125 - 138
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2024.91909

Relative Cellular Organelle Damage Caused by Wuhan and Delta Strains of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)

Poorna Khaneja [1], Annette Angel [1,*], Vinod Joshi [1], Bennet Angel [1], Shareef Mohammed Buvvaji [1], Shilpa Barthwal [1], Bhawna Sharma [1], Neha Singh [1], Monika Dheer [1], Khushbu Kumari [1], Aarya Chitransh [1], Reshu Chauhan [1], Lalmalsawmi Sailo [1], Nuzhat Maqbool Peer [1] and Ambreen Shafaat Khan [1]

[1] Centre of Excellence in Virology & Immunology
Sharda University, Greater Noida, UP, India-201310
[*] *Corresponding author*

Abstract

The third deadliest respiratory viral infection after Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS) appeared as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) causing severe morbidity and mortality worldwide. During the course of its pandemic travel, SARS-Co-V-2 has also modified itself from one to other genotype with specific clinical severities associated with its infection. While the original strain, Wuhan was considered to cause severe pneumonia, and Respiratory distress like conditions, the Delta strain was attributed to increased transmission rate and spread. This highlight the necessity to study the cellular level happenings in order to understand the cellular causality caused by these different strains such that treatment and prevention strategies can be modified accordingly. Present study highlights the same during Electron microscopy imaging done. The Vero cell lines were inoculated with different strains of SARS-CoV-2 virus such as Wuhan and Delta and further submitted to Electron microscopic analysis. The study highlight gross modifications and changes that appeared in Delta infected strain in-vitro within Vero cell lines

compared to the Wuhan infected cells. Also numerically more virions were transported out of the cell in Delta infection which clearly explain the reason of high transmission capacity despite cellular exacerbations. We infer enhanced intracellular transport of Delta strain comparatively as the cause of high casualty irrespective of mass vaccinations going on during the time of its existence.

Keywords: Cellular exacerbations, Double Membrane Vesicle, mitochondria damage, Nuclear membrane, Golgi bodies

1 Introduction

The end of year 2019 marked the beginning of deadliest form of Coronavirus i.e. the SARS-CoV-2 which affected almost the entire world. SARS-CoV-2 has appeared as one of the worst pandemics causing severe morbidities and enormous mortality. The mortality rate due to SARS-CoV-1 was only (10%), however high mortality rate was reported due to SARS-CoV2 (Pormohammad et al., 2020). Moreover, during the course of few months of transmission, new variants like Wuhan, Beta, Delta, Gamma, Omicron, emerged (Andre et al., 2023). SARS-CoV-2 contains a single stranded positive sense RNA virus genome which produce polyproteins to code sixteen nonstructural and four structural proteins. The four structural protein includes; Spike glycoprotein (S protein), Membrane glycoprotein (M protein), Envelope protein (E protein) and Nucleocapsid protein (N protein) encoded by 3' end (Naqvi et al., 2020).

Due to mutations in its one structural spike protein, the virus has been reported to mutate into number of pathologically significant strains viz; Wuhan, Delta, Omicron and JM.1 strains. The epidemiological data suggest that morbidity and mortality caused by Wuhan and Delta stains during first and second wave of COVID-19 respectively manifested different levels of clinical severities with later being more fatal as compared to former strain. Clinical severities/fatalities caused by any viral pathogen is determined by the extent to which the virus damages crucial cell organelles of infected organ/organelles. Electron microscopic studies of cells infected by Wuhan and Delta Strains of SARS-CoV-2 and relative cell organelles damage is reported as basis of strain specific clinical severities caused by two strains.

If the fate of SARS-CoV-2 is seen, then in merely a period of one year, approximately 20,000 mutations have been verified (Wu et al., 2021). These

mutations have established variants of concerns in the form of Alpha B.1.1.7, Beta B.1.351, Delta B.1.617.2, Gamma P.1 and Omicron (BA.1) (Davies et al., 2021; ECDC, 2021; Madhi et al., 2021; Shinde et al., 2021; Hoffmann et al., 2021). The major mutations reported is of the spike glycoprotein (Alkhatib et al., 2021) in all these variants. The replication and multiplication of the virus particles can be summed up in two stages; in the first stage, the ACE-2 receptor of the host binds with the S protein of virus, the S protein gets cleaved into S1 and S2 subunit making a fusion pore in the host cell to release the viral genome inside along with structural proteins (Fehr and Perlman, 2015). The genome after entering the cell binds with the ribosomes and derives NSPs where NSP3 and NSP4 efficiently helps in replication. In the later stage of viral cycle, the organelles like Endoplasmic Reticulum (ER), Mitochondria, Golgi Bodies, Nucleus, ER-Golgi Intermediate Compartment (ERGIC), Lysosomes etc. are involved (Novoa et al., 2005). Thus, it can be summed up that once the viral progenies are out from the cell, its modification and newness in the form of variants is determined. Hence it is very important to understand what are the underlying mechanism within the host cell that leads to development of new variant and further determine its pathogenicity and transmissibility. It has been studied that the virus induces membrane remodeling, once it is within the cell cytoplasm (Caldas et al., 2021). Present study is an attempt to understand the changes caused due to presence of variants of concerns of SARS-CoV-2.

2 Materials and Methods

Vero E6 cells were procured from National Centre for Cell Sciences (NCCS), Pune. In three T25 flasks, Vero E6 cells were transferred and cultured in DMEM media supplemented with 10% FBS. These were then incubated at 37°C and 5% CO2 for 24 hours in a CO2 incubator. The cells were then infected with 100µl of samples of Wuhan, Delta and Omicron strains and incubated for one hour in CO2 incubator. The infected cultures were then taken out for and subjected to sample preparation for Electron microscopy studies.

The infected cells within each flask were then tapped to loosen the suspended cells. The culture media (with infected cells suspended) were then centrifuged to allow the cells concentrate in the bottom of the Eppendorf tubes. The media was discarded and cells (pellet) was then fixed using Karnovsky fixative containing 2.5% Paraformaldehyde, 2% Glutaraldehyde (pH 7.2) for 24 hours at 4°C. Post fixation, the cells were washed with 0.1M PBS (Phosphate

Buffer Saline) for 45 minutes. The three Eppendorf containing the infected cells were then taken to AIIMS, Delhi (All Indian Institute of Medical Sciences, New Delhi) for Electron microscopy specimen preparation and visualization (outsourced) (Sophisticated Analytical Instrumentation Facility, SAIF Scheme, AIIMS, Delhi). Ultra-thin sections were obtained using ultra microtome and then after double staining were observed under Transmission Electron Microscope (TECNAI G20 HR-TEM 200kV, m/s Thermo Scientific, USA present in the SAIF, AIIMS, Delhi).

3 Results

The Electron Microscopy images were obtained successfully for the Wuhan and Delta infected cells. The images are displayed in Figures 1 to 8. The virus after internalization assembles the viral proteins from Endoplasmic Reticulum for formation of complete daughter virions. Post 1-hour infection in Vero E6 cells by Wuhan strain, the ER got detached from the nucleus and was damaged which appeared as a Double Membrane Vesicle (DMV) having virus particles inside it (Figure 1, 2). However, in case of the Delta strain a totally ruptured ER (Figure 3, 4) was observed.

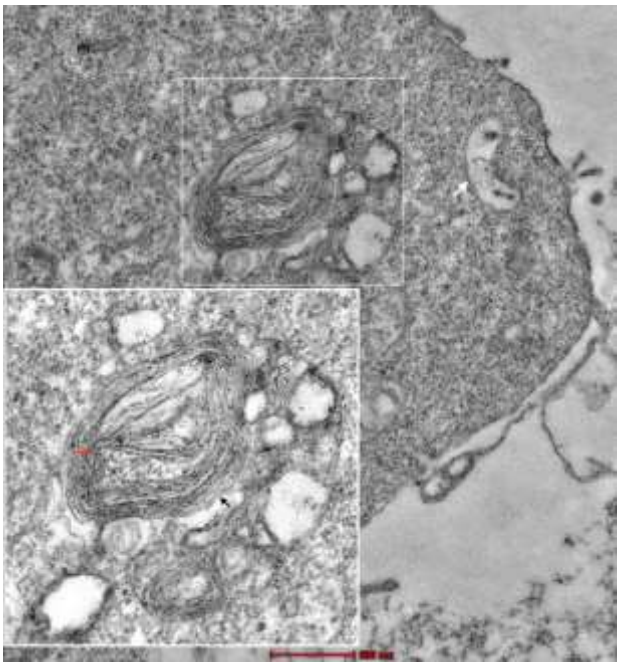

Figure 1. Formation of Double membrane vesicle (black and red arrow) followed by damaged endoplasmic reticulum along with exocytosis in Wuhan variant.

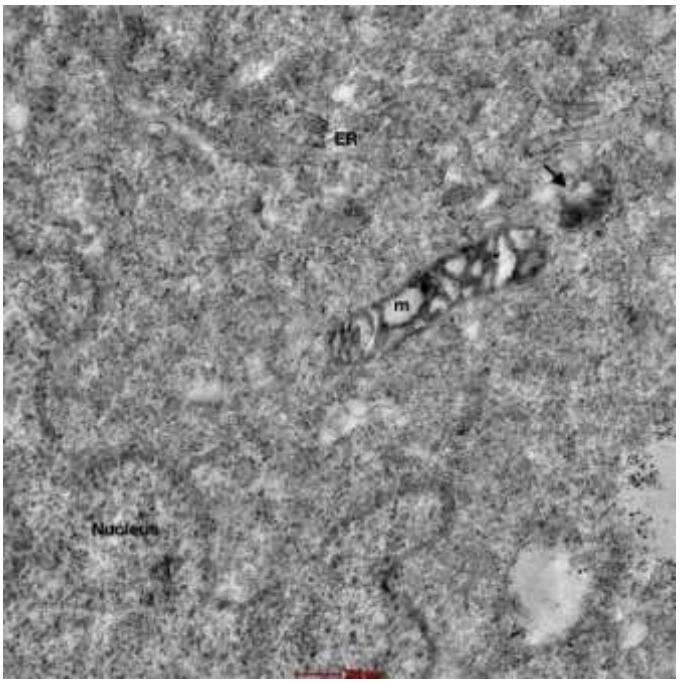

Figure 2. Electro microscopic visuals of Wuhan strain: completely disrupted endoplasmic reticulum (ER), damaged mitochondria (m), and disrupted nucleus with visible nuclear membrane.

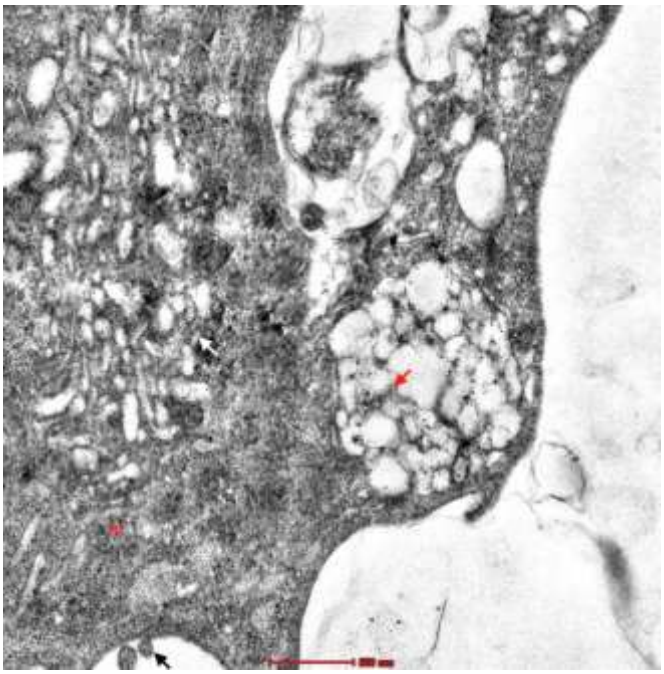

Figure 3. Ruptured mitochondria (m), virions (white arrow) and transmembrane proteins (red arrow) in Delta strain

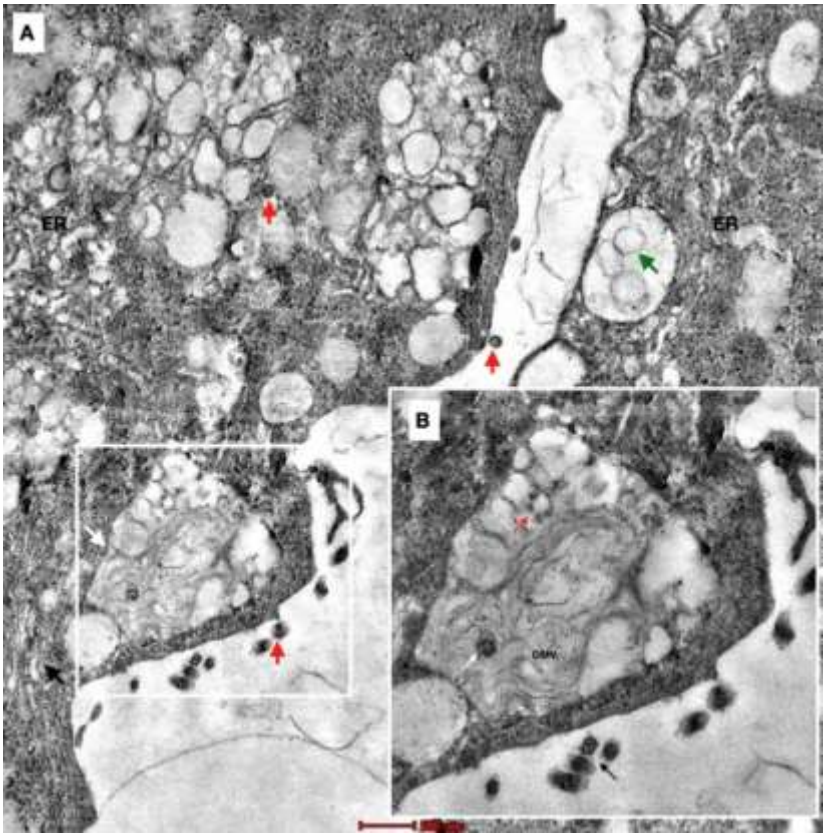

Figure 4. Delta strain (A) ruptured ER, virions in extracellular matrix (red arrow), double membrane vesicle (white arrow) (B) exocytosis of virion from double membrane vesicle

The Nucleus and the nuclear membrane were found to be intact in case of Wuhan strain, except some shape irregularities the membrane appeared irregular in shape (Figure 2). In case of Delta strain, there was disruption of nuclear membrane resulting into dispersal of all nuclear contents (Figure 3, 4). There was complete dissociation of Golgi Bodies when infected with Wuhan strain but a healthy Golgi complex was observed in Delta strain post 1-hour infection (Figure 1 to 4). As far as mitochondria was studied in both the strains, slight disruption was seen in cells infected by Wuhan Strain while massive disruption was seen in case of Delta strain (Figure 2, 4). The pattern of exocytosis was also studied. Some of the newly formed virion followed exocytosis while some remained inside in case of Wuhan strain while in case of Delta strain, exocytosis of virion was visible at many places via double membrane vesicle (Figure 1 to 4). When the post infection number of virions exiting out of cell was studied, it was observed that more number of virions appeared in Delta stain compared to that of Wuhan strain (Figure 1 to 4 and 6).

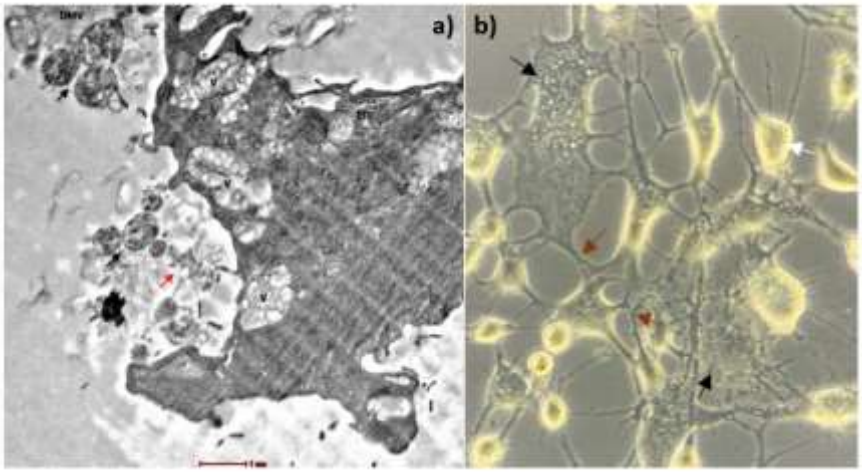

Figure 5. a) Electron microscopic image of Delta strain representing necrosis (red arrow) with virion filled lysosomes (black arrow) along with ruptured endoplasmic reticulum (ER), vesicles/vacuoles (v) and double membrane vesicle (DMV) b) Vero E6 cells infected with Delta strain post 1 hour under fluorescent microscopic representing necrosis (black arrow), syncytium formation (brown arrow) and ruptured cells (white arrow).

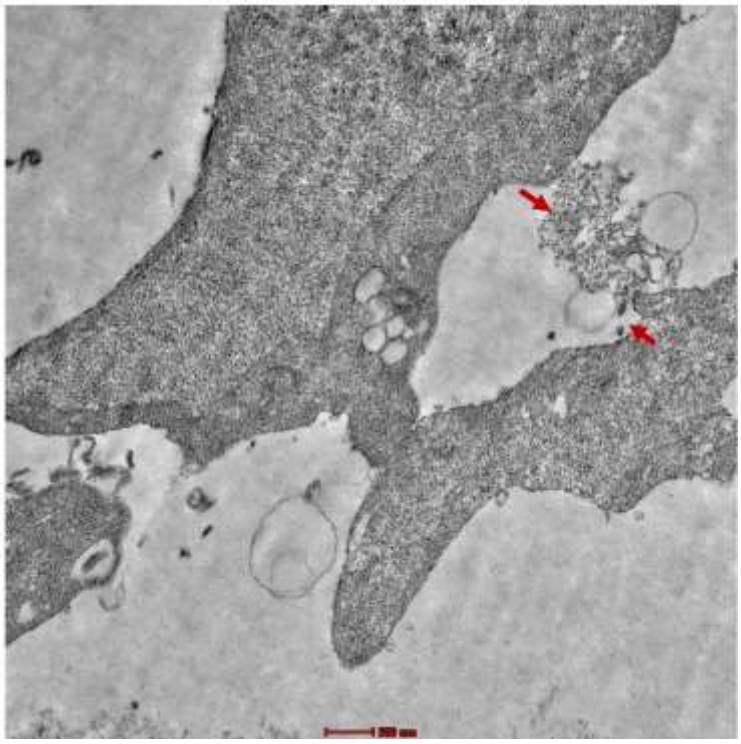

Figure 6. Exocytosis in Wuhan strain infected cells. Red arrow showing the virions initiating exocytosis.

Besides viewing individual cell organelles, lysosomes were also observed in Delta strain infected cells followed by syncytium formation and necrosis (Figure 5a & b). The patterns of exocytosis were also interesting. In case of Wuhan strain, there was rupturing of the cell membrane and no other specific way of exocytosis (Figure 6), while in case of Delta strain infection, there were vacuole like bodies within which the daughter virions were observed (Figure 7, 8).

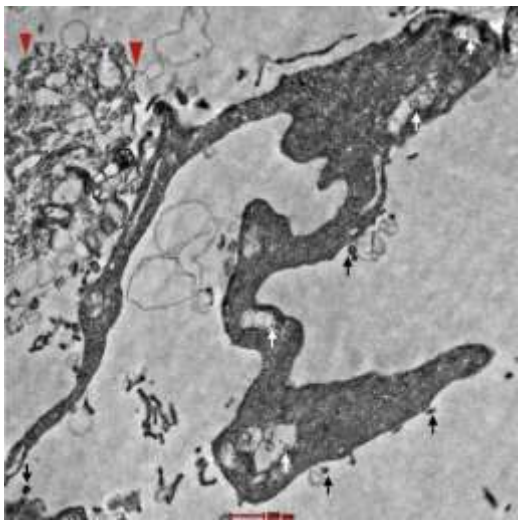

Figure 7. Exocytosis in Delta strain. White arrow showing the virions initiating exocytosis, black arrow showing assembly of viral proteins to form virions extracellularly, red head showing necrosis with virions present inside the dead cell

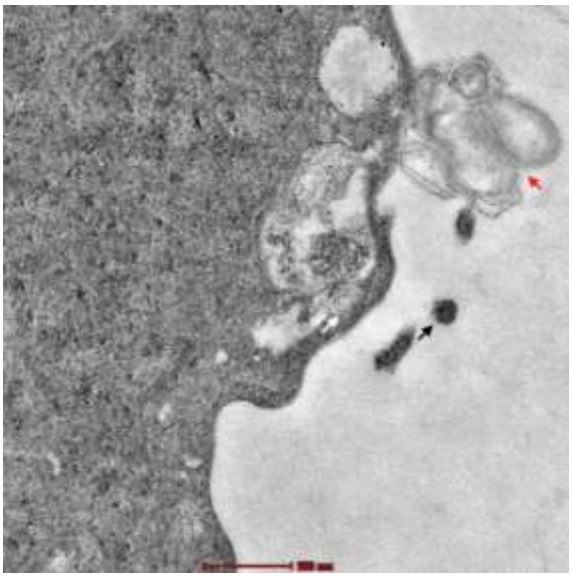

Figure 8. Exocytosis in Delta strain. White arrow showing double membrane vesicle in the extreme end of the cell, red arrow showing double membrane vesicle coming out of the cell with viral proteins present in it, black arrow showing formation of viral particle in the extracellular membrane

4 Discussion

Of the reported Respiratory viral infections till date, SARS-CoV-2 infection appeared to be the most vicious one leading to various casualties and complicated mortalities. Huge number of studies have been reported from all part of the world representing epidemiological, virological, immunological and pathological aspects of the disease (Petersen et al., 2021; Sharma et al., 2020). The cellular damage caused by SARS-CoV-2 have also been reported in few studies, however, the crucial issue of comparative account of infection caused by the different variants of SARS-CoV-2 has not been made. Present study was an attempt to understand the cellular basis of different clinical severities caused by Wuhan and Delta strains.

This relative study of Electron microscopy images of the cells infected with Wuhan strain and the Delta strains showed more damage in the Endoplasmic Reticulum (ER) in Delta strain infected cells compared to that of Wuhan wherein Double membrane vesicle (DMV) were observed. Double membrane vesicle formation is considered to be the end stage of intracellular pathogenicity hindering the viral replication activity. The Delta infection resulted in totally ruptured ER which means a huge number of viral particles in ER lumen led to protein mis-folding increasing stress in ER and resulting in compromised ER functioning. Prolonged ER stress triggers the release of Ca+ ions in the cytoplasm where excess Ca+ ions enter the mitochondria and induce the activation of cytochrome C. Programmed cell death and apoptosis are induced by the Cytochrome C activation of Caspases 3 and 9 (Hughes and Mallucci, 2019).

The Golgi apparatus in general, contributes to viral protein assembly and maturation of virion particles. This is then followed by exocytosis releasing the mature virus particles from the cell by transporting them to lysosomes. A study by Hackstadt and co-workers in the year 2021 has revealed that Vero E6 cells post 6 hours' infection partially dissociate the Golgi apparatus and fully dissociate post 12 hours' infection transiting immature glycoproteins (Hackstadt et al., 2021). The proteins involved in the dispersal of Golgi apparatus include Spike Glycoprotein, Membrane Glycoprotein, ORF6, and NSP3. In the present study, complete dissociation of Golgi complex was seen in case of Wuhan infection within one hour but it was not observed in Delta infection. Same observation was made in nuclear region too.

The Vero E6 cells showed minor disruption in mitochondria by Wuhan infection compared to Delta infection where mitochondria damage was more

pronounced. The mitochondria following the oxidative phosphorylation synthesize the ATP and due to infection an imbalance in ATP production may take place. This observation highlights that among patients infected by Delta strain, more mitochondrial damage and apoptosis may happen leading to severity of the disease. Our observation of more mitochondrial damage appear to be the possible reason of more mortality reported among patients infected by Delta strain.

Lysosomes are essential for the cell as they maintain cell motility, cellular metabolism, initiate autophagy and release cellular proteolytic cells for Tc cells to kill infected cells also are the hub for viral particles in infected cells (Castro et al., 2020). Some studies reported that SARS-CoV-2 infected cells were de-acidified and malfunctioned (Ghosh et al., 2020).

Comparing the number of virion particles released by the infected cells, it was seen that there was less number of progeny released in Wuhan infection than Delta within one hour of infection which supports that fact that faster disease transmission, reinfection and more mortality was observed in Delta strain (ECDC, 2021). The weight of one single mammalian cell has been calculated to be 3-4ng (Sims and Allbritton, 2007). And the overall mass of the SARS-CoV-2 virion has been calculated to be 1-100ug per infected person (Sender et al., 2021).

5 Conclusion

The Electron microscopy images showed more number of particles in case of Delta infection comparatively which further highlights overall increase in the mass of infected cell owing to inability of the cell to maintain itself. We report first comparative study of cellular organelle damage caused by Wuhan and Delta strains which may be useful in addressing the clinical severities. The study could be important from the point of view of patient care and also will help in understanding the course of specific treatment to be applied.

Acknowledgements. The authors would like to thank Sophisticated Analytical Instrumentation Facility at the All India Institute of Medical Sciences, New Delhi, India for helping us with the Electron Microscopy images and to National Centre for Cell Sciences, Pune, India for providing us with the Vero Cell lines.

References

[1] A. Pormohammad, S. Ghorbani, A. Khatami, R. Farzi, B. Baradaran, D.L. Turner, R.J. Turner, N.C. Bahr and J.P. Idrovo, Comparison of confirmed COVID-19 with SARS and MERS cases-Clinical characteristics, laboratory findings, radiographic signs and outcomes: a systematic review and meta-analysis, *Reviews in Medical Virology*, **30** (2020), e2112. https://doi.org/10.1002/rmv.2112

[2] A. Sharma, S. Tiwari, M.K. Deb and J.L. Marty, Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2): a global pandemic and treatment strategies, *International Journal of Antimicrobial Agents*, **56** (2020), 106054. https://doi.org/10.1016/j.ijantimicag.2020.106054

[3] A. Wu, L. Wang, H.Y. Zhou, C.Y. Ji, S.Z. Xia, Y. Cao, J. Meng, X. Ding, S. Gold, T. Jiang and G. Cheng, One year of SARS-CoV-2 evolution, *Cell Host Microbe*, **29** (2021), 503–505. https://doi.org/10.1016/j.chom.2021.02.017

[4] A.A.T. Naqvi, K. Fatima, T. Mohammad, U. Fatima, I.K. Singh, A. Singh, S.M. Atif, G. Hariprasad, G.M. Hasan and M.I. Hassan, Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach, *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, **1866** (2020), 165878. https://doi.org/10.1016/j.bbadis.2020.165878

[5] A.R. Fehr and S. Perlman, Coronaviruses: an overview of their replication and pathogenesis, *Coronaviruses: Methods and Protocols*, 2015, 1-23. https://doi.org/10.1007/978-1-4939-2438-7_1

[6] C.E. Sims and N.L. Allbritton, Analysis of single mammalian cells on-chip, *Lab Chip*, **7** (2007), 423 – 440. https://doi.org/10.1039/b615235j

[7] D. Hughes and G.R. Mallucci, The unfolded protein response in neurodegenerative disorders–therapeutic modulation of the PERK pathway, *The FEBS Journal*, **286** (2019), 342-355. https://doi.org/10.1111/febs.14422

[8] E. Petersen, M. Koopmans, U. Go, D.H. Hamer, N. Petrosillo, F. Castelli, F., Storgaard, M., Al Khalili, S. and Simonsen, L. Comparing SARS-CoV-2 with SARS-CoV and influenza pandemics, *The Lancet Infectious Diseases*, **20** (2020), e238-e244. https://doi.org/10.1016/s1473-3099(20)30484-9

[9] Euopean Centre for Disease Prevention and Control. Implications for the EU/EEA on the spread of the SARS-CoV-2 Delta (B.1.617.2) variant of concern. ECDC, Stockholm, Sweden. 2021.
https://www.ecdc.europa.eu/sites/default/files/documents/Implications-for-the-EU-EEA-on-the-spread-of-SARS-CoV-2-Delta-VOC-23-June-2021_2.pdf.

[10] F.I. de Castro, R. Tenorio, P. Ortega-González, J.J. Knowlton, P.F. Zamora, C.H. Lee, J.J. Fernández, T.S. Dermody, and C. Risco, A modified lysosomal organelle mediates nonlytic egress of reovirus, *Journal of Cell Biology*, **219** (2020), no. 7, 201910131. https://doi.org/10.1083/jcb.201910131

[11] L.A. Caldas, F.A. Carneiro, F.L. Monteiro, I. Augusto, L.M. Higa, K. Miranda, A. Tanuri and W. de Souza, Intracellular host cell membrane remodelling induced by SARS-CoV-2 infection in vitro, *Biology of Cell*, **113** (2021), 281-293. https://doi.org/10.1111/boc.202000146

[12] M. Alkhatib, V. Svicher, R. Salpini, F.A. Ambrosio, M.C. Bellocchi, L. Carioti, L. Piermatteo, R. Scutari, G. Costa, A. Artese, S. Alcaro, R. Shafer and F. Ceccherini-Silberstein, SARS-CoV-2 variants and their relevant mutational profiles: update Summer 2021, *Microbiology Spectrum*, **9** (2021), e01096-21. https://doi.org/10.1128/spectrum.01096-21

[13] M. Andre, L.S. Lau, M.D. Pokharel, J. Ramelow, F. Owens, J. Souchak, J. Akkaoui, E. Ales, H. Brown, R. Shil, and V. Nazaire, From alpha to omicron: how different variants of concern of the SARS-Coronavirus-2 impacted the world, *Biology*, **12** (2023), 1267. https://doi.org/10.3390/biology12091267

[14] M. Hoffmann, P. Arora, R. Groß, A. Seidel, B.F. Hörnich, A.S. Hahn, N. Krüger, L. Graichen, H. Hofmann-Winkler, A. Kempf, M.S. Winkler, S. Schulz, H.M. Jäck, B. Jahrsdörfer, H. Schrezenmeier, M. Müller, A. Kleger, J. Münch and S. Pöhlmann, SARS-CoV-2 variants B.1.351 and P.1 escape from neutralizing antibodies, *Cell*, **184** (2021), 2384–2393.e12.
https://doi.org/10.1016/j.cell.2021.03.036

[15] N.G. Davies, CMMID COVID-19 Working Group, S. Abbott, R.C. Barnard, C.I. Jarvis, A.J. Kucharski, J.D. Munday, C.A.B. Pearson, T.W. Russell, D.C. Tully, A.D. Washburne, T. Wenseleers, A. Gimm, W. Waites, K.L.M. Wong, K. vanZandvoort, J.D. Silverman, K. Diaz-Ordaz, R. Keogh, R.M. Eggo, S. Funk, M. Jit, K.E. Atkins and W.J. Edmunds, Estimated transmissibility and impact of SARS-CoV-2 lineage B.1.1.7 in England, *Science*, **372** (2021), eabg3055.

[16] R. Sender, Y.M. Bar-On, S. Gleizer, B. Bernsthein, A. Flamholz, R. Phillips and R. Milo, The total number and mass of SARS-CoV-2 virions. *Proceedings of the National Academy of Sciences*, (2021), 2020.11.16.20232009. https://doi.org/10.1073/pnas.2024815118

[17] R.R. Novoa, R. Calderita, R. Arranz, J. Fontana, H. Granzow and C. Risco, Virus factories: associations of cell organelles for viral replication and morphogenesis, *Biology of the Cell*, **97** (2005), 147-172. https://doi.org/10.1042/bc20040058

[18] S. Ghosh, T.A. Dellibovi-Ragheb, E. Pak, Q. Qiu, M. Fisher, P.M. Takvorian, C. Bleck, V. Hsu, A.R. Fehr, S. Perlman and S.R. Achar, β-Coronaviruses use lysosomal organelles for cellular egress. bioRxiv, 2020, 2020-07. https://doi.org/10.1101/2020.07.25.192310

[19] S.A. Madhi, Wits-VIDA COVID Group, Baillie, C.L. Cutland, M. Voysey, A.L. Koen, S.D. Fairlie, D. Padayachee, K. Kheda, S.L. Barnabas, Q.E. Bhorat, C. Briner, G. Kwatra, K.P Ahmed, P. Aley, S. Bhikha, J.N. Bhiman, A.E. Bhorat, J. Du Plessis, A. Esmail, M. Groenewald, E. Horne, S.H. Hwa, A. Jose, T. Lambe, M. Laubscher, Malahleha, M. Masenya, M. Masilela, S. McKenzie, K. Molapo, A. Moultrie, S. Oelofse, F. Patel, S. Pillay, S. Rhead, H. Rodel, L. Rossouw, C. Taoushanis, H. Tegally, A. Thombrayil, S. van Eck, C.K. Wibmer, N.M. Durham, E.J. Kelly, T.L. Villafana, S. Gilbert, A.J. Pollard, T. de Oliveira, P.L. Moore, A. Sigal and A. Izu, Efficacy of the ChAdOx1 nCoV-19 Covid-19 vaccine against theB.1.351 variant, *New England Journal of Medicine*, **384** (2021), 1885–1898. https://doi.org/10.32388/n3mvo3

[20] T. Hackstadt, A.I. Chiramel, F.H. Hoyt, B.N. Williamson, C.A. Dooley, P.A. Beare, E. de Wit, S.M. Best and E.R. Fischer, Disruption of the Golgi apparatus and contribution of the endoplasmic reticulum to the SARS-CoV-2 replication complex, *Viruses*, **13** (2020), no. 9, 1798. https://doi.org/10.3390/v13091798

[21] V. Shinde, 2019nCoV-501 Study Group, S. Bhikha, Z. Hoosain, M. Archary, Q. Bhorat, L. Fairlie, U. Lalloo, M.S.L. Masilela, D. Moodley, S. Hanley, L. Fouche, C. Louw, M. Tameris, N. Singh, A. Goga, K. Dheda, C. Grobbelaar, G. Kruger, N. Carrim-Ganey, V. Baillie, T. de Oliveira, K.A. Lombard, J.J. Lombaard, R. Mngqibisa, A.E. Bhorat, G. Benadé, N. Lalloo, A. Pitsi, P.L. Vollgraaff, A. Luabeya, A. Esmail, F.G. Petrick, A. Oommen-Jose, S. Foulkes, K. Ahmed, A. Thombrayil, L. Fries, S. Cloney-Clark, M. Zhu, C. Bennett, G. Albert,

E. Faust, J.S. Plested, A. Robertson, S. Neal, I. Cho, G.M. Glenn, F. Dubovsky and S.A. Madhi, Efficacy of NVX-CoV2373 Covid-19 vaccine against the B.1.351 variant, *New England Journal of Medicine*, **384** (2021), 1899–1909.

Received: October 1, 2024; Published: October 15, 2024