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Original Article

Enhanced Endoplasmic Reticulum Stress due to Severe Acute Respiratory Syndrome Coronavirus -2 Delta Strain could Induce Cytokine Storm Causing More Clinical Severities/Mortalities

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Abstract

Severe Acute Respiratory Distress Syndrome Coronavirus 2 (SARS-CoV-2) is one of the fastest-replicating viruses known to date, with numerous variants reported worldwide. During its replication and multiplication within the host cell, it induces Endoplasmic Reticulum (ER) stress and activates the host's intracellular defence mechanism, the Unfolded Protein Response (UPR), in an attempt to limit replication and protein synthesis processes. However, the intricate pathophysiology of IRE1alpha during viral infection and its role in the intracellular defence mechanism is not yet fully understood. Given the different variants of SARS-CoV-2 and the diversity of hosts, it was hypothesised that these events may vary. To investigate the variant-specific cellular pathogenicity of SARS-CoV-2, the Vero E6 cells were inoculated with swab samples positive for the Wuhan, Delta, and Omicron variants. One hour post-infection, the cells were extracted and quantitative ELISA for IRE-1 antigen was conducted. The results revealed that the highest ER stress (IRE-1 value) was observed in cells infected with the Delta strain, followed by the Wuhan strain, and the lowest in cells infected with the Omicron strain. This was further confirmed using bioinformatic tools. The study conclude that SARS-CoV-2 variants not only infect host cells from a symptomatic perspective but also disrupt ongoing cellular pathways, one of which is the protein synthesis pathway.

Keywords: *Delta Strain; Endoplasmic Reticulum; IRE-1; SARS-CoV-2*

Introduction

Epidemiological studies reporting morbidity and mortality due to strain-specific waves of COVID-19 have shown the highest mortality associated with the Delta strain of the virus, compared to the Wuhan and Omicron strains (Zhan, Yin & Yin, 2022; Hedberg *et al*., 2024). The Delta variant B.1.617.2 demonstrated faster disease transmission, with approximately 60% of the deaths being linked to infection with this strain, in contrast to other variants (Lovelace, 2021; Shiehzadegan *et al*., 2021). Our previous studies on the relative intracellular damage caused by three strains of SARS-CoV-2 have shown that the Delta variant induced higher cell-to-cell connectivity and caused the most severe damage to the Endoplasmic Reticulum (ER) in experimentally infected Vero E6 cells, as visualised under electron microscopy (Khaneja *et al*., 2024).

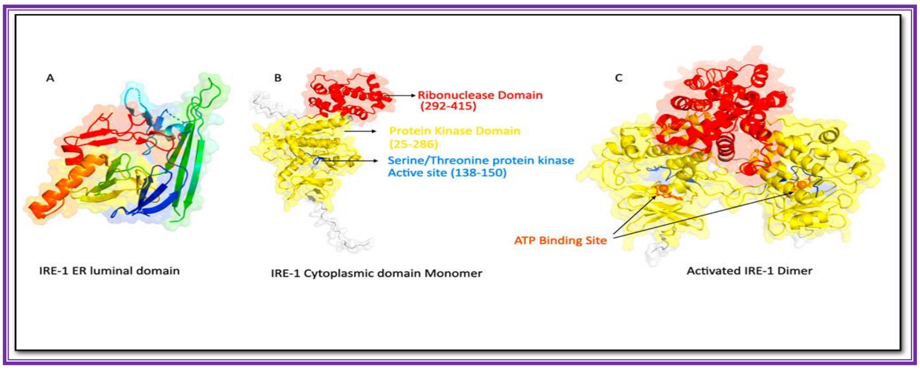

Figure 1: 3D Structure of IRE-1 Alpha Domains, Subdomains and ATP Binding Sites

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As the previous study reported the severe damage of ER in Delta strain infected cells (Khaneja *et al.,* 2024), the possible loss of corresponding cellular function of ER was studied as one of the strong causes of more mortalities of patients infected by Delta strain. The present study assessed the relative quantity of the important transmembrane IRE-1 protein (Inositol-requiring enzyme 1) of the ER (Figure 1), as the cellular and biochemical basis for the relatively higher mortality in patients infected with the Delta strain compared to those infected with the Wuhan and Omicron strains. The in vitro studies were complemented by in silico analyses. This paper reports the detailed findings of our study.

Material & Methods

Cell culture

The Vero-E6 cell line (African green monkey kidney cells) was obtained from the National Centre for Cell Science (NCCS), Pune, India. The cells were detached from the flask using the trypsinisation method and subsequently transferred to 24-well culture plates (200 µl per well) with fresh media (DMEM). The plates were then incubated in a CO2 incubator at 37°C, with 5% CO2 and 95% humidity, for 24 hours.

Viral Inoculation

Nasopharyngeal samples tested positive for SARS-CoV-2 were selected. Six samples were chosen, each representing one of the three variants of SARS-CoV-2: Wuhan, Delta, and Omicron strains. These samples were centrifuged individually, and their supernatants were passed through a 0.2µm syringe filter to remove any contamination. A volume of 100µl from each filtrate was then inoculated into the respective wells containing Vero cells (with 90% confluence of mature and well-differentiated cell lines), as described in the previous section. The plates were then returned to the CO2 incubator at 37°C, with 5% CO2 and 95% humidity, for 1 hour. The experiment was conducted in six sets (Set I to Set VI), each representing one variant.

Quantitative analysis of IRE1-alpha by ELISA

The infected cells (as described in the previous step) were then used for the quantitative estimation of Human ERN1 (Serine/threonine-protein kinase/endoribonuclease, IRE1) using the ELISA assay (m/s FineTest, EH2360). The test range is 62.5-4000 pg/ml. The assay consisted of wells pre-coated with Anti-ERN1 antibody and utilised biotin-conjugated anti-ERN1 antibody to detect the presence of IRE1 in the samples. The test was performed according to the manufacturer's protocol.

In-Silico Analysis of IRE1-Alpha

To understand the interaction of IRE1-alpha (indicator of ER stress) with SARS-CoV-2, In-silico study was done. The secondary structure of the SARS-CoV-2 genome was predicted using the CLC-Workbench. The UNAFold Web Server was used for the prediction of the specific nucleotide site of the IRE1-alpha endoribonuclease recognition.

Ethical Approval

The study received ethical approval by the Institutional Ethical Committee Sharda University, India with reference number SU/SMS&R/ 76-A/2021/08 on 2nd February, 2021.

Results

The values obtained from the ELISA assay indicated an increased concentration of IRE-1 alpha in the cells infected with the Delta strain, followed by those infected with the Wuhan and Omicron variants of SARS-CoV-2. In Set I of the experimentally infected cells, the cells infected with the Delta strain showed the highest concentration (428.1 pg/ml), followed by the Wuhan strain (316.8 pg/ml), and the lowest (86.8 pg/ml) in the cells infected with the Omicron strain. Similarly, in Set II, the highest IRE-1 concentration (841.8 pg/ml) was observed in the Delta strain, with the lowest concentration (145.6 pg/ml) in the Omicron strain. In all experimental settings, the maximum ER stress, in terms of IRE-1 concentration, was observed in cells infected with the Delta strain (SD 156.55), followed by the Wuhan strain (SD 102.461), and the least in the cells infected with the Omicron strain (SD 46.41) (Table 1).

Table 1: Quantitative estimation of IRE-1 in Cells Infected by three Variants of SARS-CoV-2

	Quantity of IRE-1 alpha (in pg/ml) in Vero cells infected by Wuhan strain	**Quantity of IRE-1 alpha (in pg/ml) in Vero cells infected by Delta strain**	**Quantity of IRE-1 alpha (in pg/ml) in Vero cells infected by Omicron strain**
Set I	316.875	428.125	86.875
Set II	303.125	841.875	145.625
Set III	99.375	795.625	140.625
Set IV	280.625	666.875	86.875
Set V	320.625	534.375	28.125
Set VI	409.375	608.125	54.375
Mean	288.333333	645.833333	90.4166667
SD	102.461629	156.559527	46.4180505
cv	35.54%	24.24%	51.34%

The In-silico study suggests that the endoribonuclease activity of the cytoplasmic domain of IRE-1 recognises the nucleotide site (5 'CUGCAG 3'). There were 7 places which indicated presence of IRE-1 alpha endo ribonuclease recognition sites in the genome of SARS-CoV-2. These are positions 183-188, 8109-8114, 8829-8834, 14195-14200, 22347-22352, 24606-24611 and 29463-29468 (Figure 2, 3).

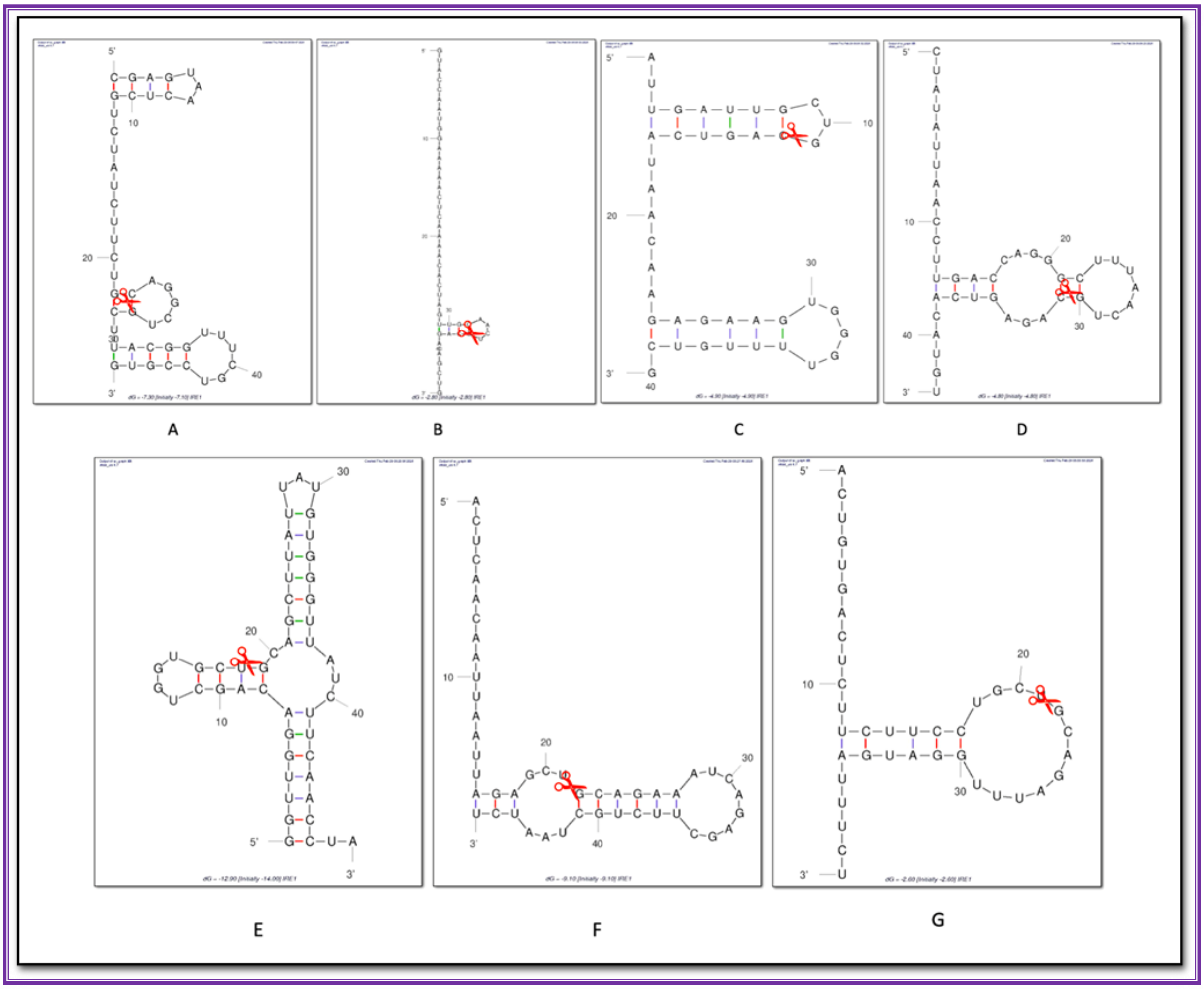

Figure 2: The activated IRE1 endoribonuclease domain cleaves the mRNA recognition site (5 ' CUGCAG 3'). SARS-CoV-2 viral mRNA contains the seven IRE1 endoribonuclease recognition sites; from 183 to 188 (A), from 8109 to 8114 (B), from 8829 to 8834 (C), from 14195 to 14200 (D), from 22347 to 22352 (E), from 24606 to 24611 (F) and from 29463 to 29468 (G).

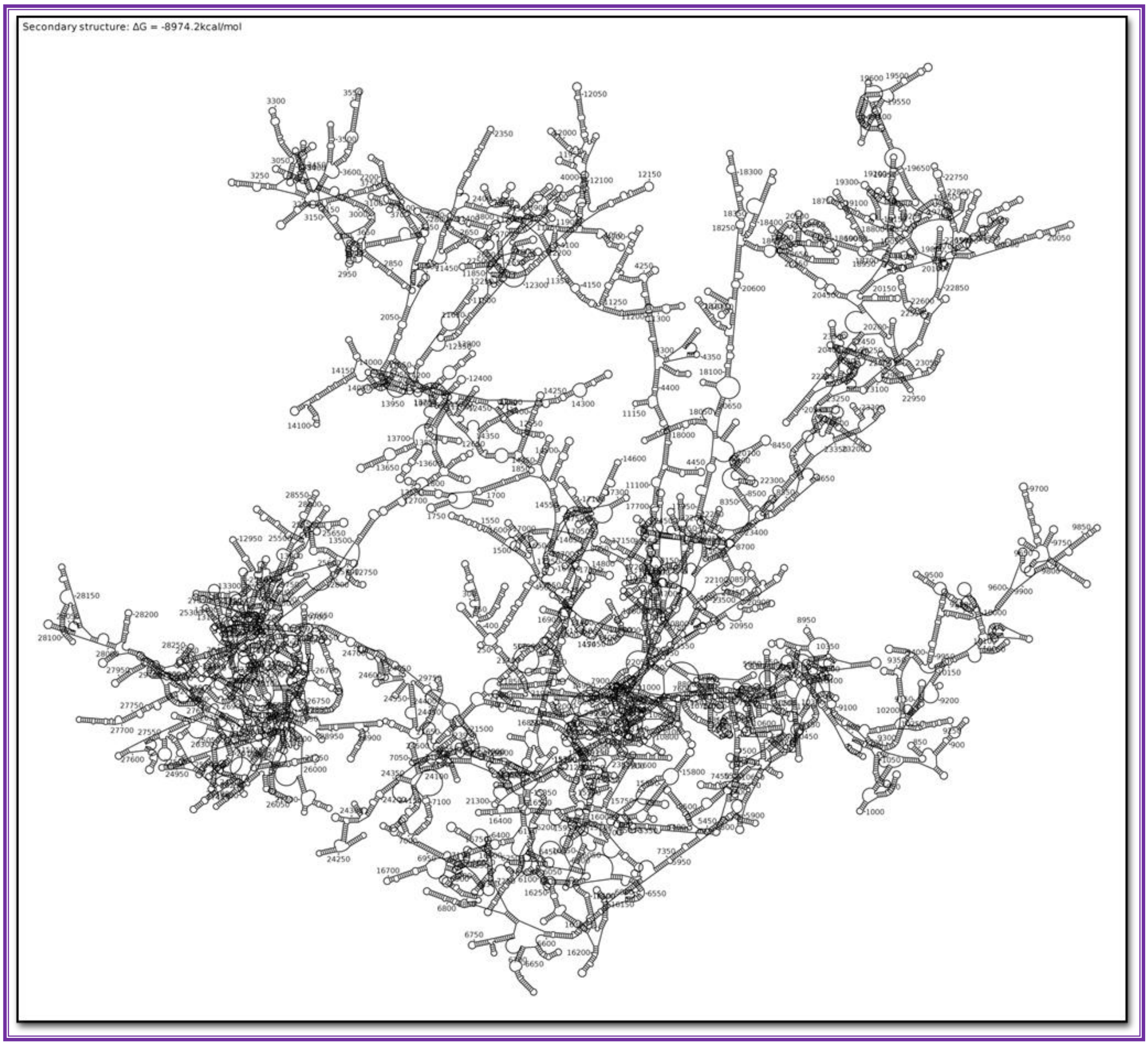

Figure 3: Secondary Structure of SARS-CoV-2 mRNA Predicted Using CLC-Workbench

Discussion

Our experience with the COVID-19 pandemic has demonstrated that SARS-CoV-2, across its different waves, caused a high transmission rate and a significant number of clinical severities and mortalities among patients (Dao *et al*., 2021; Liu Wei & He, 2023). While the speed of disease transmission in the case of respiratory viruses depends on the salivary transfer of infected individuals to healthy ones—which, in turn, is influenced by a range of spatial, behavioural, and social factors hence the severity and mortality due to the infection must be studied at the cellular level. At this level, the specific effects of the virus on cell organelles need to be examined, and corresponding functional abnormalities must be identified to guide therapeutic interventions. Our earlier studies on experimentally infected cells with all three variants have clearly shown a strong association between the mortality caused by the Delta strain and the corresponding damage to the Endoplasmic Reticulum (ER) caused by this strain (Khaneja *et al*., 2024). Further studies on this aspect revealed that in cells infected by the Delta strain, the quantity of IRE-1 was significantly enhanced.

The Endoplasmic Reticulum (ER) is a specialised organelle in mammalian cells where secretory and membrane proteins are synthesised and folded (Alberts *et al*., 2002; Schwarz & Blower, 2016). The IRE1 alpha monomers are transmembrane proteins located on the ER membrane (Li *et al*., 2010; Cho *et al*., 2019). In the luminal domain of IRE1 alpha, there is a BiP (Binding Immunoglobulin Protein) binding site that binds to BiP, while in the cytosolic domain, there is an inactive form of a specific RNA endonuclease (Siwecka *et al*., 2021). Unfolded proteins accumulate in the lumen of the ER and bind to the BiP molecule. This binding causes the release of the BiP molecule from the IRE1 domain into the ER lumen. The two IRE1 monomers then undergo dimerisation to form an IRE1 dimer. This dimerisation activates the RNA endonuclease. The activated RNA endonuclease cuts the unspliced HAC-1/XBP1 mRNA, and the sliced exons are joined to form functional HAC-1 mRNA. The functional HAC-1 mRNA is translated to produce proteins known as HAC-1 transcription factors. These transcription factors move towards the nucleus, bind to specific regions of DNA, activate transcription, and produce mRNA (Figure 4). The mRNAs then translate and produce protein-translating catalysts. Activated IRE-1 and XBP1 promote the production of type I interferon (Hollien *et al*., 2009).

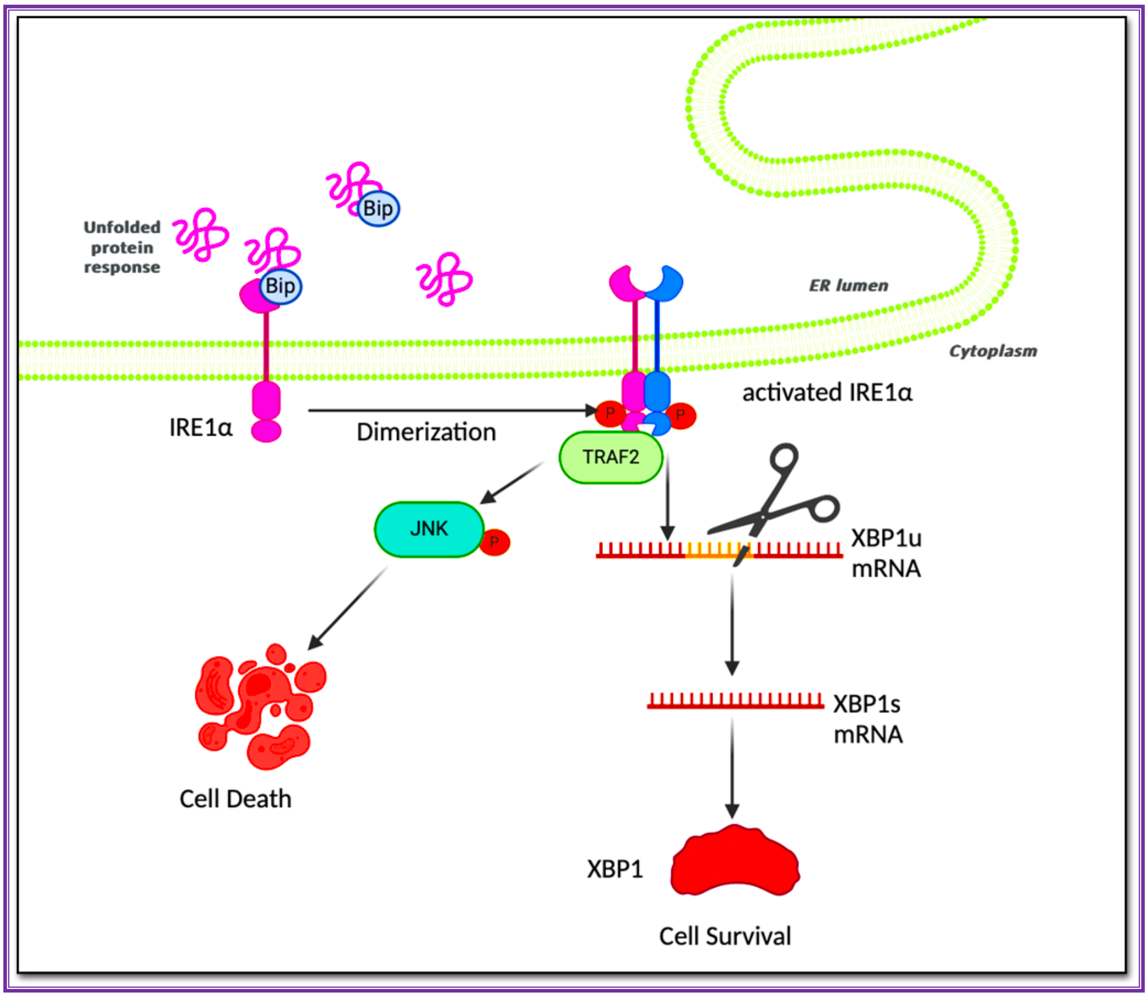

Figure 4: Signaling Pathway of IRE-1 Dependent Cell Survival and Death

The routine protein synthesis occurring in our cells and the protein synthesis performed by the virus, if occurring simultaneously in a single cell, can lead to the massive accumulation of both cellular and viral proteins in the ER. This accumulation of excess proteins in the ER lumen activates the unfolded protein response (Walsh, Mathews & Mohr, 2013). IRE-1 is a highly conserved UPR sensor that activates the ribonuclease in its cytoplasmic domain. RNase processes XBP1 through unconventional mRNA splicing. The spliced XBP1 mRNA encodes transcription factors that regulate protein folding, processing, and maintain protein homeostasis in the host (Le Goupil *et al*., 2024). The ER stress then upregulates the innate immune response, including toll-like receptors (TLRs), and activates cytokine production. Activated XBP1 enhances the secretion of pro-inflammatory cytokines.

Upon activation of IRE-1 Alpha, there is also activation of another viral infection sensor, Retinoic acid-inducible gene I (RIG)-like receptors (RLRs) which mediates the transcription action of type I interferons and collectively causes the innate immune response in viral infected host cells (Vedagiri *et al*., 2021).

Thus, in mammalian cells, the signaling of IRE1 alpha depends on RNase activity and its specificity towards the nucleotide sequence cleavage site.

IRE-1 is also upregulated during Metabolic disorders, Inflammatory responses, Cancer, Fatty liver disease, Alzheimer disease, Parkinson disease, Amyotrophic Lateral Sclerosis, Huntington disease, Apinocerebellar Ataxia and Artherosclerosis (Wang & Kaufman, 2012). Viruses like the Hepatitis C virus and Cytomegalovirus-encoded proteins have been reported to alter the IRE-1-XBP pathway, enhance the translation of viral proteins and inhibit the synthesis of type 1 Interferons (Tardif *et al.,* 2004). So, in case of a person having such prior health coupled with infection by SARS-CoV-2, the role of IRE-1 may become more conspicuous and aggressive in terms of enhancing innate immune response through secretion of Interferons and possibly leading to cytokine storm. The correction and modulation of this IRE-1 as a new drug target may prove a promising intervention for recovery of cytokine storm like severities (Raymundo *et al.,* 2020). Our findings showed that the higher level of IRE-1 alpha in cells infected with the Delta variant of SARS-CoV-2 than the Wuhan and Omicron variants, is mainly involved in the ribonuclease activity in the cytoplasmic domain. It recognises the sequence to cleave the XBP1 protein by unconventional RNA splicing.

Conclusion

SARS-CoV-2 infections reported globally have witnessed numerous mutations, along with the emergence of Variants of Concern and Variants of Interest. These variants have significantly infected host cells, not only from a symptomatic perspective but also by disrupting ongoing cellular pathways, one of which is the protein synthesis pathway. Since viruses lack ribosomes, they hijack the host's protein synthesis machinery to produce proteins for their daughter virions. However, this can interfere with or impair the ongoing protein synthesis necessary for the individual. The protein requirements of our body vary from person to person and depend on factors such as health status and age. Despite these differences, the synthesis of viral proteins by commandeering the protein synthesis machinery may impose a burden on the Endoplasmic Reticulum, potentially leading to the activation of IRE1.

The study concludes that enhanced IRE-1 leads to hyper immune response by non-specific immune system of antibody through excessive secretion of Interferons and such a hyper immune inflammatory consequence when occur in infected lung cells may lead to clinical severities.

The only limitation to this study is that the observations made need to be expanded horizontally to achieve reproducible results, although In-Silico and In-Vitro studies have proved the concept. Further, similar approach needs to be done for other respiratory viruses as well, so that during cases of co-occurrence of more than one respiratory virus, the severity may be studied and addressed clinically.

Conflict of Interest

The authors declare that they have no competing interest.

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References

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Molecular Biology of the Cell. 4th edition. New York: Garland Science; The Endoplasmic Reticulum. Available at: https://www.ncbi.nlm.nih.gov/books/NBK26841

Cho, H., Stanzione, F., Oak, A., Kim, G. H., Yerneni, S., Qi, L., ... & Chan, C. (2019). Intrinsic structural features of the human IRE1α transmembrane domain sense membrane lipid saturation. *Cell Reports*, *27*(1), 307-320. https://doi.org/10.1016/j.celrep.2019.03.017

Dao, T. L., Hoang, V. T., Colson, P., Lagier, J. C., Million, M., Raoult, D., ... & Gautret, P. (2021). SARS-CoV-2 infectivity and severity of COVID-19 according to SARS-CoV-2 variants: current evidence. *Journal of Clinical Medicine*, *10*(12), 2635. https://doi.org/10.3390/jcm10122635

Hedberg, P., Parczewski, M., Serwin, K., Marchetti, G., Bai, F., Jensen, B. E. O., ... & Nauclér, P. (2024). In-hospital mortality during the wild-type, alpha, delta, and omicron SARS-CoV-2 waves: a multinational cohort study in the EuCARE project. *The Lancet Regional Health–Europe*, *38,* 100855. https://doi.org/10.1016/j.lanepe.2024.100855

Hollien, J., Lin, J. H., Li, H., Stevens, N., Walter, P., & Weissman, J. S. (2009). Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *Journal of Cell Biology, 186*(3), 323-331. https://doi.org/10.1083/jcb.200903014

Khaneja, P., Angel, A., Joshi, V., Angel, B., Shareef, B.M., Barthwal, S., Sharma, B., Singh, N., Dheer, M., Kumari, K., Chitransh, A., Chauhan, A., Sailo, C., Peer, N.M., & Khan, A.S. (2024). Relative cellular organelle damage caused by Wuhan and Delta strains of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). *Advanced Studies in Biology, 16* (1), 125-138. https://doi.org/10.12988/asb.2024.91909

Le Goupil, S., Laprade, H., Aubry, M., & Chevet, E. (2024). Exploring the IRE1 interactome: from canonical signaling functions to unexpected roles. *Journal of Biological Chemistry*, *300*(4), 107169. https://doi.org/10.1016/j.jbc.2024.107169

Li, H., Korennykh, A. V., Behrman, S. L., & Walter, P. (2010). Mammalian endoplasmic reticulum stress sensor IRE1 signals by dynamic clustering. *Proceedings of the National Academy of Sciences*, *107*(37), 16113-16118. https://doi.org/10.1073/pnas.101058010

Liu, J., Wei, H., & He, D. (2023). Differences in case-fatality-rate of emerging SARS-CoV-2 variants. *Public Health in Practice*, *5,* 100350. https://doi.org/10.1016/j.puhip.2022.100350

Lovelace Jr., B. (2021, June 18). WHO says delta is becoming the dominant Covid variant globally. *CNBC*. Available at: https://www.cnbc.com/2021/06/18/who-says-delta-is-becoming-the-dominant-covid-variant-globally.html

Raymundo, D. P., Doultsinos, D., Guillory, X., Carlesso, A., Eriksson, L. A., & Chevet, E. (2020). Pharmacological targeting of IRE1 in cancer. *Trends in Cancer*, *6*(12), 1018-1030. https://doi.org/10.1016/j.trecan.2020.07.006

Schwarz, D. S., & Blower, M. D. (2016). The endoplasmic reticulum: structure, function and response to cellular signaling. *Cellular and Molecular Life Sciences*, *73*, 79-94. https://doi.org/10.1007/s00018-015-2052-6

Shiehzadegan, S., Alaghemand, N., Fox, M. and Venketaraman, V., 2021. Analysis of the delta variant B. 1.617. *Clinics and Practice*, *11*(4), 778–784. https://doi.org/10.3390/clinpract11040093

Siwecka, N., Rozpędek-Kamińska, W., Wawrzynkiewicz, A., Pytel, D., Diehl, J. A., & Majsterek, I. (2021). The structure, activation and signaling of IRE1 and its role in determining cell fate. *Biomedicines*, *9*(2), 156. https://doi.org/10.3390/biomedicines9020156

Tardif, K. D., Mori, K., Kaufman, R. J., & Siddiqui, A. (2004). Hepatitis C virus suppresses the IRE1-XBP1 pathway of the unfolded protein response. *Journal of Biological Chemistry*, *279*(17), 17158-17164. Available at: https://www.jbc.org/article/S0021-9258(19)75537-9/fulltext

Vedagiri, D., Gupta, D., Mishra, A., Krishna, G., Bhaskar, M., Sah, V., Basu, A., Nayak, D., Kalia, M., Valiya Veettil, M., & Harshan, K.H. (2021). Retinoic acid-inducible gene I-like receptors activate snail to limit RNA viral infections. *Journal of Virology*, *95*(21), 10-1128. https://doi.org/10.1128/JVI.01216-21

Walsh, D., Mathews, M.B., & Mohr, I. (2013). Tinkering with translation: protein synthesis in virus-infected cells. *Cold Spring Harbor Perspectives in Biology*, *5*(1), a012351. https://doi.org/10.1101/cshperspect.a012351

Wang, S., & Kaufman, R. J. (2012). The impact of the unfolded protein response on human disease. *Journal of Cell Biology*, *197*(7), 857-867. https://doi.org/10.1083/jcb.201110131

Zhan, Y., Yin, H., & Yin, J. Y. (2022). B. 1.617. 2 (Delta) Variant of SARS-CoV-2: features, transmission and potential strategies. *International Journal of Biological Sciences*, *18*(5), 1844. https://doi.org/10.7150/ijbs.66881