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Review article

Cryo-Electron

Cryo-Electron Microscopy for Cancer

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Abstract: A review paper on cryo-electron microscopy (cryo-EM) is essential to assess the recent advancements in this revolutionary imaging technique. As cryo-EM continues to revolutionize structural biology, a comprehensive review can consolidate the knowledge, highlight technical challenges, and offer insights into future developments, promoting better understanding and wider adoption of this cutting-edge technology. The absence of a review paper on the recent innovative approach of applying cryo-electron microscopy for cancer research hinders knowledge dissemination and impedes potential breakthroughs. A comprehensive review would bridge the gap, elucidating the successes and challenges of cryo-EM in cancer studies, fostering collaboration, and inspiring further investigations to combat cancer effectively." Cryo-electron microscopy enables examining biomolecular structures at almost atomic precision while capturing multiple dynamic states more than 30 years after developing the industry's preferred method for cryo-embedding biological macromolecules under their native conditions. Techniques and equipment have significantly improved. Advanced image-processing methods are employed in research to facilitate the study of biological macromolecular structures and analyze their dynamics; for this, cryo-EM is a potent tool. Cryo-EM must effectively investigate the cellular macromolecular structure, including dynamic analysis using cutting-edge image-processing methods. Modern Analysis of individual particles using electron tomography is even more easily applicable to the procedure. With the development of single particle analysis and electron tomography, it is now more broadly applicable. These techniques have increased the method's applicability even further. Due to its ease of use and capacity to produce intricate and sophisticated data that can be used to comprehend biological structures better, cryo-EM is currently a more well-liked and available research tool. As a result, it serves as a useful tool for both academic and industrial research. Protein complexes, molecular pathways, and viral structures have also been studied using cryo-EM. Due to its adaptability, it has become a useful tool for illness and drug development. Its low cost and simplicity of usage have also made it a crucial research tool.

Keywords: Cryo-Electron Microscopy, Cancer, Electron Microscopy, Cryo-EM for Cancer

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1. INTRODUCTION

The study of biological macromolecules in their natural environments has undergone a radical change due to the extraordinary advancements made in cryo-EM over the past three decades. These developments have enabled capturing the dynamic states of biomolecules and revealing their structures with near-atomic detail. Researchers can now probe the complex structures of biological macromolecules using cryo-EM while using cutting-edge image-processing methods to examine their dynamics. Cryo- EM has become a significant and potent tool. Scientists have acknowledged Cryo-EM as a highly beneficial method for examining the structures of biological macromolecules from various angles, applying complex algorithmic methods to understand their dynamics based on the images collected. Cryo-EM's ground-breaking capabilities have transformed structural biology by enabling the imaging of macromolecular structures at previously unheard-of degrees of detail. This discovery has allowed researchers better to understand the intricate relationships between proteins and DNA, opening up new channels for cellular activity. As a result, this newly discovered information has paved the path for cutting-edge medical treatments and a deeper comprehension of the underlying biological mechanisms that sustain life. Cryo-EM has also given us a view into the molecular workings of nature, opening up a wide range of opportunities for medical research developments, from gene therapy to drug discovery and beyond. It can transform medical procedures and eventually save lives. Cryo-electron microscopy has emerged as an indispensable method for investigating the dynamics of macromolecules and cellular structures. It is especially true in light of the enormous expansion of cryo-EM's research applications and accessibility by single particle analysis and electron tomography developments. Its success in academic and industrial research environments can be attributed to its simplicity of use and the wealth of intricate and detailed data it offers. Cryo-EM has transformed structural biology and provided new ways for scientists to understand better the structure and function of proteins and other complex molecules. This finding has advanced the search for new drugs, understanding the disease, and numerous other fields. Cryo-EM advances our understanding of cellular mechanisms and the onset of disease by exploring how proteins are affected by mutations and the environment. Unquestionably, the science of structural biology has undergone a paradigm shift due to cryo-EM microscopy, which has presented deep new insights into the complex molecular mechanisms underlying biological activities. This newly discovered information has opened up many opportunities for medical therapies and disease diagnosis, catapulting science to previously unimaginable heights. Cryo-EM has set the door for ground-breaking discoveries by digging into the molecular substrate of life, deepening our grasp of the intricacies that underlie biological processes. Cryo-EM has had a significant impact, uncovering the molecular basis of life itself and inspiring a deep respect for its complexity. This information has completely transformed the discipline of structural biology, and its long-lasting effects will influence future studies. Understanding the molecular basis of life has allowed us to create new illness therapies and given us insights into how biological systems have evolved. Cryo-EM has also given scientists the ability to precisely construct medications and proteins, opening up opportunities for medical breakthroughs and scientific discoveries. Cryo-EM has proven invaluable for scientists, offering previously unattainable insights into the molecular mechanisms driving various biological processes. It has opened up fresh avenues for investigation and learning that look to change biology research in the future. It could lead to the creation of novel illness treatments and more effective and economical medical technology. Cryo- EM also impacts environmental sustainability because it helps researchers find creative ways to cut pollutants and protect the environment.It has revolutionized structural biology and made it possible to investigate biological processes at the molecular level in great detail. It significantly affects disease diagnosis, medical treatments, and scientific developments. Cryo-EM has laid a strong platform for future study and can potentially change several fields by revealing the molecular details of life. In addition to deepening our understanding of biology, it paved the path for a better future characterized by advances in science, environmental sustainability, and healthcare. In numerous fields of scientific inquiry, cryo-EM has played a critical and pivotal role. It has been crucial in deciphering complex biochemical pathways, examining the complexity of protein complexes, and researching viral shapes. Cryo-EM has become an essential tool for drug discovery and illness research due to its versatility, which has led to substantial advancements in these domains. Cryo-EM has several benefits, including being affordable and user-friendly, which raises its value in scientific research. By offering priceless insights into the three-dimensional structures of proteins and other molecules, this technique has completely changed the area of structural biology. By viewing these structures, scientists can better understand the mechanisms of action of medicines and other biomolecules. This understanding has significantly influenced the creation of novel, better treatments for various illnesses and medical disorders. Cryo-EM technology, researchers may now obtain precise three-dimensional models of the molecular structures of proteins, enzymes, and other biomolecules. This knowledge is essential to create novel medications with improved specificity and efficacy. Cryo-EM has also proved crucial in revealing the structures of viruses, which is necessary to create vaccinations and antiviral medications [1]. Cryo-EM has also made it easier to analyze cell membrane architecture, showing how medications and other substances affect cells. This information is crucial for comprehending cellular functions and creating focused therapies [2].Cryo-EM has made a major contribution to the development of medical research and improving public health. It has proven revolutionary in its capacity to produce intricate 3D models of molecular structures, identify virus structures, and investigate cell membrane dynamics [3]. Cryo-EM has revolutionized structural biology and improved our understanding of complex biological systems by supplying vital insights that have aided in the creation of new medications, vaccines, and therapies. Since its invention, cryo-electron microscopy (cryo-EM) has advanced significantly. Permitting near-atomic resolution imaging and capturing dynamic behavior has revolutionized the study of biological macromolecules. Its ease of use, diverse uses, and provision of complex data have made it an essential tool in academic and industrial research contexts, fostering progress in various scientific fields. Software for cryo-EM has many benefits. First, because of its simplicity, it can be used and adopted widely by researchers with different degrees of competence. The software can also be used in various study fields, allowing for investigating complicated natural occurrences and clarifying underlying dynamics [4]. Cryo-EM software assists in improving our understanding of a variety of phenomena, accelerating the advancement of research. Cryo-EM software has the benefit of enabling virtual experimentation in addition to its simulation capabilities. This function lowers the expense of performing physical experiments and considerably shortens the experimental period, allowing researchers to advance their work quickly. Researchers can also foresee experiment results thanks to the software's capacity to imitate real-world situations. It not only saves time and money but also secures the security of the researchers and maintains the reliability of the data that has been acquired. With significant advancements in cryo-EM software, biological macromolecules may now be studied with extraordinary resolution

and dynamic visualization. It has become indispensable in academic and commercial research and user-friendly interface, a wide range of applications, and provision of complex data. The software's virtual experimentation, simulation, and prediction features provide several benefits, from increasing research productivity to guaranteeing experiment integrity and safety [5].

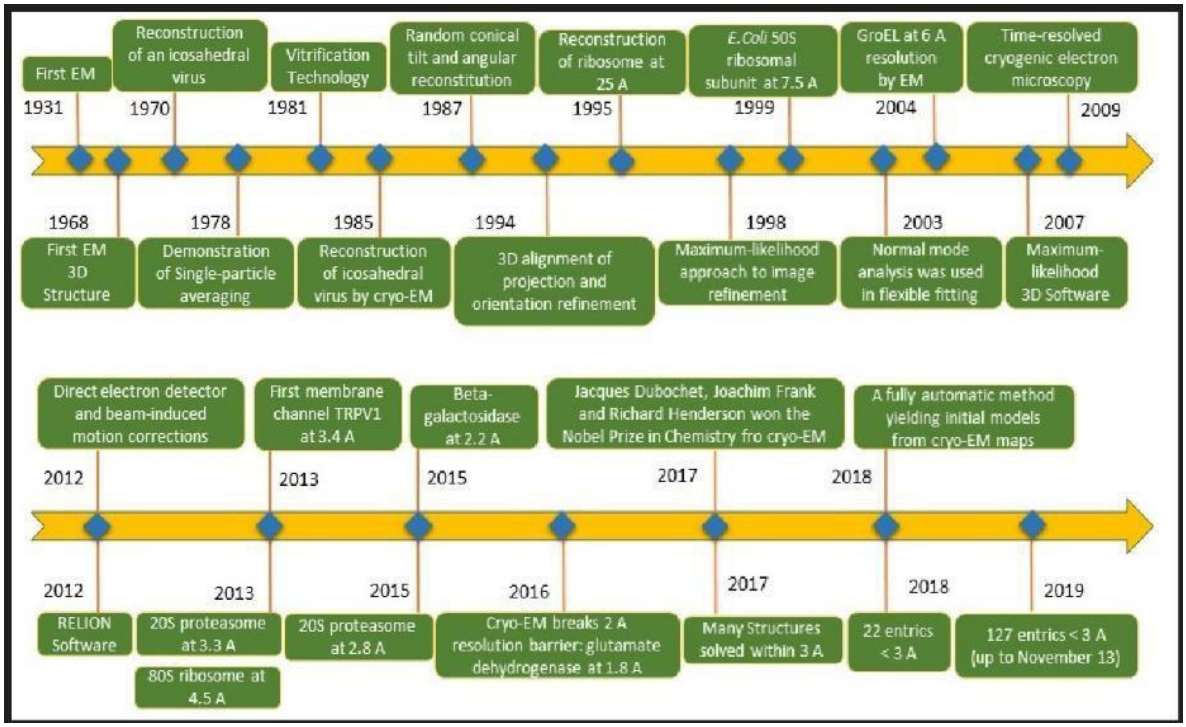

Fig 1: Timeline of key events in the development of cryo-EM [6].

2. BREAKTHROUGHS IN CRYO-EM

Through the potent use of Cryo-electron microscopy, commonly known as cryo-EM, alongside three-dimensional (3D) reconstruction techniques, structural biology has recently experienced several notable discoveries. These innovations are the result of outstanding developments in both hardware and software, each of which has significantly improved the capabilities of cryo-EM [7]. The development of cryo-EM apparatus has significantly pushed the limits of structural biology. One example is creating extremely complex electron microscopes with enhanced stability, sensitivity, and resolution. Modern electron microscopes come with cutting-edge imaging detectors that allow taking of effective, high-quality pictures of biological samples while frozen [8]. These advances in hardware technology have successfully made it possible for cryo-EM to visualize the intricate structures of macromolecules and cellular complexes. The software innovations that have transformed cryo-EM are equally significant. A game-changer has been the creation of complex 3D reconstruction algorithms. These algorithms enable the creation of precise 3D models of the examined biological specimens from the 2D pictures recorded by cryo-EM in combination with cutting-edge image processing techniques [9]. This reconstruction procedure entails the alignment and fusion of many 2D pictures to produce a thorough 3D representation, offering vital insights into macromolecule molecular organization and architecture. Additionally, the software utilized for image data processing has been essential in helping researchers extract useful data from cryo-EM datasets. Using these software tools, the collected images can be effectively aligned, denoised, and particle- picked. They allow the massive amount of raw data from cryo-EM investigations to be preprocessed and ready for subsequent 3D reconstruction and analysis [10]. The science of structural biology has advanced to new heights due to these cryo-EM hardware and software developments. Higher resolution photographs have been able to be captured by those improved hardware, and more accurate and detailed 3D models have been created to enhance software techniques. Understanding the structures and functions of different biological macromolecules, such as proteins, nucleic acids, and their complexes, has advanced. Through the fusion of cryo-EM and 3D reconstruction techniques, great strides in structural biology have been made in recent years. Significant developments in both hardware and software components have made these discoveries possible. The capabilities of cryo-EM have been expanded, giving researchers a better understanding of the complex molecular world. It has been made possible by the ongoing development of electron microscopes, imaging detectors, 3D reconstruction algorithms, and image processing software. These developments have paved the way for fascinating discoveries and advances across various scientific disciplines, from aiding medication discovery and development to comprehending basic biological processes.

3. ANALYSIS OF MICROSCALE PROTEINS' STRUCTURES

Cryo-EM found it challenging to determine the structure of very small molecule complexes for a very long period due to the weak contrast transfer function (CTF). As a result, it has been assumed that cryo-EM is only helpful for determining cryo-EM and has been extensively used to investigate the structures of macromolecular complexes with molecular weights exceeding 500 kDa. However, numerous pharmacological targets have relatively modest molecular weights. Nevertheless, cryo-EM has shown remarkable advancements in recent times, allowing for the resolution of an increasing number of high-resolution structures, even those with molecular weights below 150 kDa

4. FROM 'BIOLOGY' TO HIGH-RESOLUTION STRUCTURES

Cryo-EM has transformed over the last decade from a low-resolution biology tool to a high-throughput, high-resolution

structural biology approach. The widespread adoption of direct electron detectors drove this progress, improving signal-to- noise ratio and imaging speed. Concurrent advancements in image processing techniques were equally crucial. This integration of cutting-edge detectors and sophisticated algorithms expanded structural biology's scope, allowing visualization of complex molecular structures with unprecedented clarity. Cryo-EM's higher throughput facilitated dynamic biological process observation and disease research, contributing to drug development. With ongoing advancements, Cryo-EM's potential for unraveling biological mysteries and driving scientific progress remains unparalleled.

5. DECIPHERING THE STRUCTURES OF PROTEINS

Scientists at Japan's University of Tsukuba use Cryo-EM to analyze previously unobservable biomolecular structures, expediting cancer research and new drug creation. Kenji Iwasaki, [11], a passionate structural biochemist, is deeply invested due to personal reasons. Understanding synovial sarcoma requires further investigation [12], compound screening, and medication development. Through Cryo-EM's insights, the team aims to revolutionize cancer knowledge and drug development, with Iwasaki's commitment reflecting the potentially life-changing impact. Hopeful for effective treatments and a cure, their dedication brings us closer to defeating synovial sarcoma.

6. CRYO-EM FOR SPOP (SPECKLE-TYPE POZ PROTEIN)

The researchers employed cryo-electron microscopy to investigate the three-dimensional architecture of SPOP, which allowed them to acquire a more profound understanding of the protein's functionality in its regular form and how it changes in the presence of cancer mutations. Their investigation of the SPOP oligomer and its assembly made significant findings about vital interactions occurring in previously uncharted protein regions. Intriguingly, these particular regions were observed to be influenced by mutations associated with endometrial carcinoma. Cryo-electron microscopy facilitated the visualization of SPOP's structural intricacies and shed light on the molecular mechanisms underlying its role in cancer development. This groundbreaking research could drive advancements in treating endometrial carcinoma and other related diseases[13]

7. A RADICALLY DIFFERENT WAY TO STUDY THE STRUCTURE OF LARGE BIOMOLECULES

Synovial sarcoma, a rare cancer affecting the soft tissue near joints, is associated with an abnormal protein, SS18-SSX. The complex structure of SS18-SSX makes its analysis difficult using conventional methods and cryo-EM. Cryo-EM, a cutting-edge imaging technique, allows scientists to visualize biomolecules at near-atomic resolution. Despite its challenges, cryo-EM holds promise in understanding SS18-SSX's structure and behavior [14]. By gaining insights into this protein, researchers can advance treatment development for synovial sarcoma and design targeted therapies to combat the disease effectively.

8. CRYO-EM SPA (SINGLE PARTICLE ANALYSIS)

Using SPA, isolated macromolecules, and complexes are studied in vitro for their structural composition. It is based on 3D reconstruction after aligning and averaging thousands of photos of distinct complexity. Cryo-EM SPA is a powerful and advanced technique used in structural biology to visualize and study the three-dimensional structures of biological macromolecules. This method involves freezing the sample in a thin layer of vitreous ice, preserving its native state, and avoiding crystallization. Through Cryo-EM SPA, scientists can obtain high-resolution images of individual particles, such as proteins or complexes, in various conformations and environments. These images are then computationally processed to align and combine, generating a three-dimensional reconstruction of the molecule's structure. Cryo-EM SPA has significantly revolutionized the field of structural biology by providing insights into intricate molecular details and revealing essential mechanisms underlying various biological processes. Its ability to analyze flexible and heterogeneous samples makes it particularly valuable in studying dynamic biomolecular systems that were previously challenging to investigate using other methods. As a result, Cryo-EM SPA has become an indispensable tool for researchers seeking to unravel the mysteries of the molecular world and contribute to advances in biomedical research and drug development.

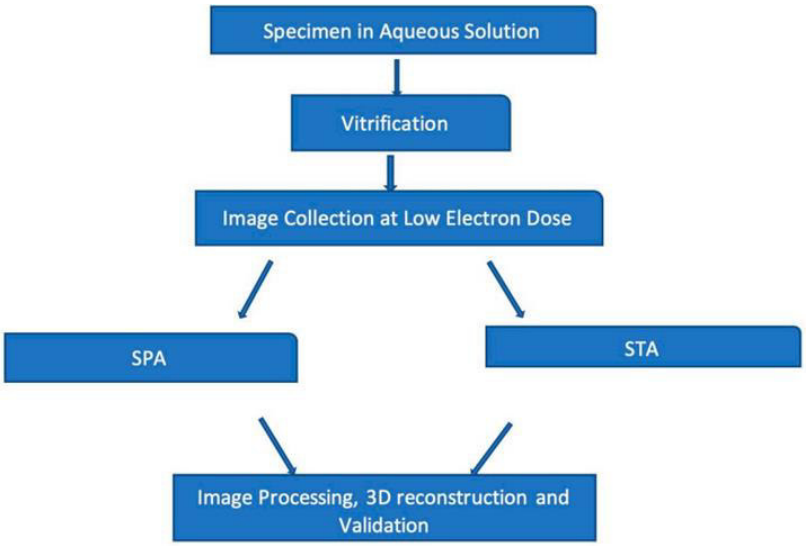

Fig 2: Image collected at low dose electron can be analyzed by Cryo-EM single-particle Analysis [14]

9. CRYO-ELECTRON TOMOGRAPHY

Australian researchers have studied the structure of the protein anchor chains that hold cells in place in the human body using cryo-electron microscopy. These minuscule objects, 1/10,000th the breadth of a human hair, aid cells and exert pressure on their surroundings [15]. They are also crucial for cell migration. Because they have fewer anchor chains, some cancer cells have the ability to metastasize, which is the spread of cancerous cells throughout the body. Thus, it is possible to determine if cancer cells are likely to spread by looking at the quantity and distribution of these anchors. Most cells in the human body are joined together by tiny connections known as "focal adhesions" to the extracellular matrix. This protein framework provides the body with its shape. Cells can detect the stiffness of the surrounding matrix to microscopic motors that move along the fibers connected to these adhesions. Normal cells either multiply given the right stiffness circumstances or are destined to die. However, it is known that cancer cells can avoid scheduled death and increase and spread throughout the body. Lastra Cagigas and co-authors evaluated the presence and distribution of several variations of the protein tropomyosin a crucial component of these anchor chains in healthy mouse cells and human cells using specialized cryo-electron microscopy [16].

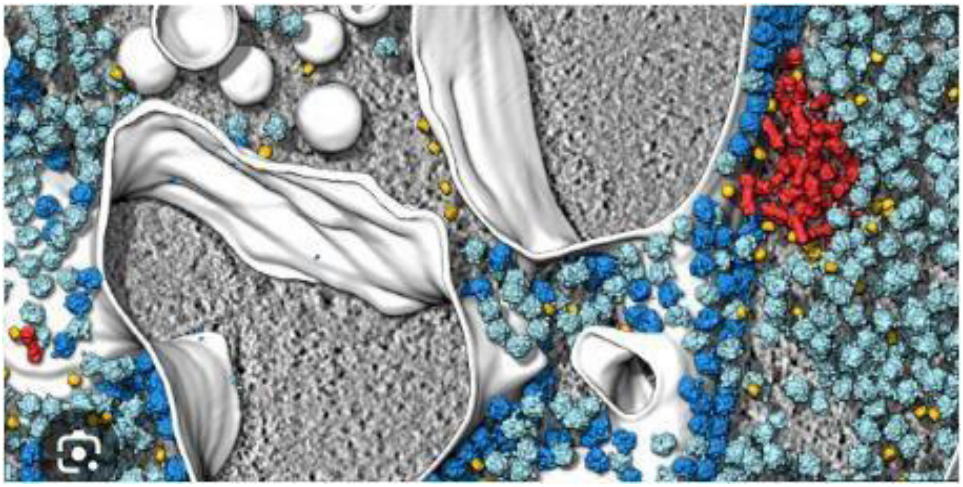

Fig 3: Cryo-electron tomography [17]

10. ANTI-CANCER THERAPY TARGET

Researchers from Weill Cornell Medicine and Memorial Sloan Kettering in the US have used cryo-electron microscopy to observe the complete structure of a glucocorticoid-induced tumor necrosis factor receptor (GITR), a target for cancer immunotherapy. This breakthrough sheds light on how GITR interacts with the therapeutic antibody and receptor ligand[18], providing valuable insights for the development of cancer treatments. The findings also open doors for similar research on other tumor necrosis factor (TNF) receptors, crucial immune system components. Previous studies on TNF receptors primarily relied on X-ray crystallography [19], but cryo-EM allowed the first examination of a full-length TNF receptor, as stated by Professor Loel Meyerson of Cornell Medicine. This advancement could impact future therapies and disease understanding.

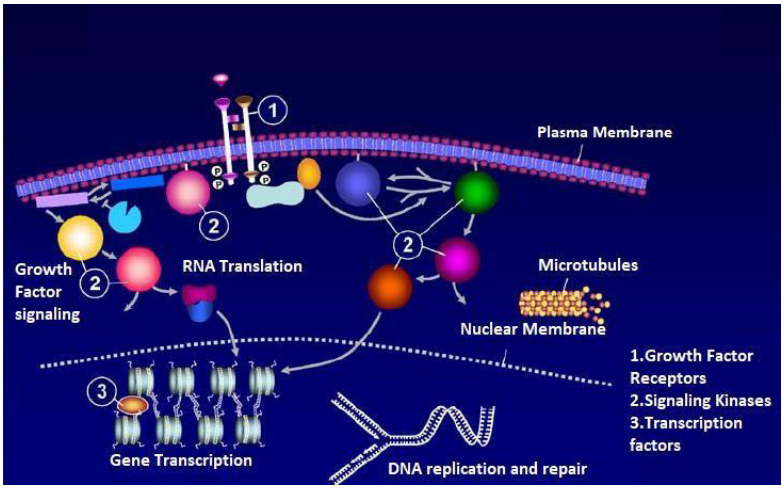

Fig 4: Potential targets of the targeted anti-cancer therapies [20]

11. CONCLUSION

Cryo-electron microscopy (cryo-EM) has emerged as a groundbreaking and transformative technique within cancer research. This technology has provided unparalleled insights into the mechanisms driving cancer immunotherapy by enabling the visualization of complete receptor structures, including the vital glucocorticoid-induced tumor necrosis factor receptor (GITR). This newfound understanding of GITR's interactions with therapeutic antibodies and receptor ligands equips researchers with enhanced tools for developing and optimizing targeted cancer treatments. Furthermore, the potential of cryo-EM to extend its application to the study of other tumor necrosis factor (TNF) receptors holds promise for deepening our

comprehension of the immune system's role in cancer and human health. As this cutting-edge technology continues to evolve, its impact on cancer research and treatment is poised to be profound, ushering in an era of more effective and individualized therapeutic approaches in the ongoing battle against cance.

12. AUTHORS CONTRIBUTION STATEMENT

Dr. Bablee Jyoti, Marai Dr. Anand Mohan Jha, and Dr. Jagadeesh Dhamodharan conceptualized the manuscript and gathered the data. Dr. Aswinprakash Subramanian, analyzed the data and provided the necessary information regarding the research design. All the authors discussed the methodology and results and contributed to the final manuscript. All the authors revised the manuscript critically and approved it before submission.

13. CONFLICT OF INTEREST

Conflict of interest declared none.

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