

HUMAN METAPNEUMOVIRUS: AN OVERVIEW OF A SIGNIFICANT RESPIRATORY PATHOGEN

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Abstract

Discovered in 2001, the human metapneumovirus (hMPV) primarily causes upper and lower respiratory tract infections in young children but also affects older individuals and those with weakened immune systems. The virus accounts for 5% to 10% of pediatric hospitalizations for acute respiratory infections. Clinical presentations range from mild respiratory symptoms to severe conditions such as bronchiolitis and pneumonia. Advances in genomics have shed light on the molecular biology of hMPV, including its classification under the Paramyxoviridae family. hMPV's genomic features and its impact on the immune system are critical to understanding its pathogenesis.

Despite promising vaccine candidates, none are currently approved, underscoring the urgent need for effective treatment and prevention strategies. This aligns with the United Nations Sustainable Development Goal SDG 3: Good Health and Well-being, which emphasizes reducing mortality from communicable diseases and ensuring access to essential healthcare services. Furthermore, ongoing research and innovation in vaccine design and diagnostic technologies support SDG 9: Industry, Innovation, and Infrastructure, highlighting the importance of scientific advancement and global collaboration in combating infectious diseases.

This review explores the genomics, molecular virology, impact on diseased individuals, control strategies, statistical approaches, and recent advancements in hMPV treatment and diagnosis within the context of these global health objectives.

Key words: hMPV, Reverse transcriptase PCR, Bronchiolitis, Pneumonia, hRSV infection.

Introduction:

Globally, acute respiratory tract infections (ARI) are a major source of morbidity and mortality. In 2000 alone, ARIs were responsible for 20% of all deaths in children under the age of five worldwide; additionally, roughly 70% of these deaths took place in southern Asia and Sub-Saharan Africa.[1] ARIs have comparable incidence rates in affluent and developing nations, but a greater fatality rate in developing nations. They impact children irrespective of their financial situation.[2] Children in developing nations are more likely to get pneumonia (10–20%), whereas in industrialised nations, the risk is 3–4%.[3]

Children's respiratory issues can be caused by a variety of etiological causes.[4] Even while upper respiratory tract infections are typically less severe, they nevertheless have a substantial negative impact on society due to missed work, missed school, and higher medical expenses. Determining the etiological agents of these infections is crucial because of this. We have established the significance of recognised viral infections such as the coronavirus, rhinovirus, influenza virus, parainfluenza virus, and human respiratory syncytial virus (hRSV) via decades of study and epidemiological investigations. Nevertheless, a significant percentage of respiratory tract infections remain unattributable to any recognised pathogen despite these investigations.

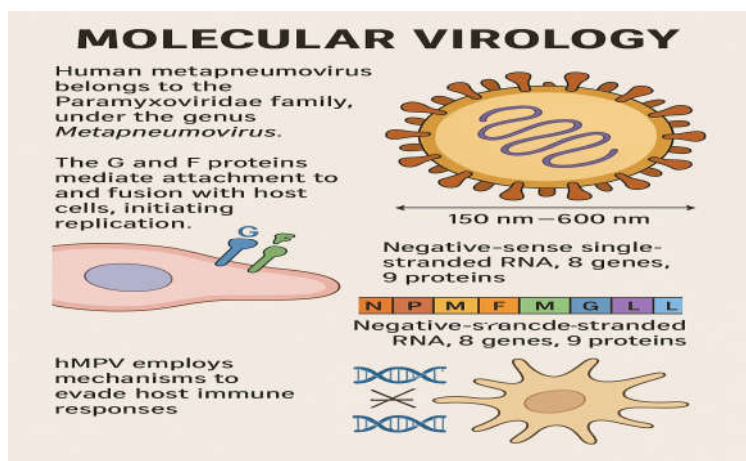
The human metapneumovirus, or hMPV, was initially identified in the Netherlands in 2001 after being recovered from a paediatric child with symptoms resembling those of an infection with hRSV.[5] Since then, 4–16% of ARI patients have been found to have hMPV.[6,7,8] In the same region, the incidence of hMPV may change from year to year.[9] hMPV mainly affects youngsters, although it can also infect adults and those with weakened immune systems. The sickness brought on by a hMPV infection might manifest clinically as anything from a minor upper respiratory tract infection to potentially fatal acute pneumonia and bronchiolitis.

The Paramyxoviridae family, which is a member of the order Mononegavirales, is further subdivided into the Paramyxovirinae and Pneumovirinae subfamilies. Pneumovirus and Metapneumovirus are the two genera that make up the Pneumovirinae subfamily. Whereas hMPV is classified under the genus Metapneumovirus, hRSV is classified under the genus Pneumovirus. According to a whole genome study, there are two genotypes of hMPV: A and B. Both of these genotypes are further separated into subgroups A1, A2, B1, and B2 according to the sequence variability of the attachment (G) and fusion (F) surface glycoproteins. Once more, subgroup A2 is separated into A2a and A2b.[10,11] There may be a new subgroup developing inside the A major subgroup since one study described a strain under main subgroup A but does not belong to subgroups A1 or A2.[12] With the development of reverse genetics platforms, research into the molecular biology of hMPV made considerable strides, however a

trustworthy vaccine to prevent hMPV infection is still lacking. This review covers recent discoveries in hMPV molecular virology, diagnosis, and control methods.

Molecular Virology:

Human metapneumovirus belongs to the Paramyxoviridae family, under the genus Metapneumovirus. The pleomorphic virion, ranging from 150 nm to 600 nm, contains a negative-sense single-stranded RNA genome comprising eight genes encoding nine proteins: N, P, M, F, M2, SH, G, and L. The G and F proteins mediate attachment to and fusion with host cells, initiating replication. Viral proteins such as M2-2 inhibit innate immunity, while the P protein stabilizes the viral polymerase for efficient replication.



Figure_1: Molecular Structure and Replication Cycle of Human Metapneumovirus (hMPV)

hMPV employs mechanisms to evade host immune responses, including interfering with toll-like receptor signalling and reducing antigen presentation by dendritic cells. The genetic variability within its genotypes and subgroups influences its pathogenicity and antigenicity, complicating vaccine development.[13]

Viral structure and genomic arrangements:

Eight genes that code for nine proteins make up the negative-sense single-stranded RNA that makes up the hMPV genome. The genes in the genome are arranged as follows (from 3' to 5' end): N–P–M–F–M2–SH–G–L. The proteins are the following: the matrix protein (M protein), the fusion glycoprotein (F protein), the phosphoprotein (P protein), the nucleoprotein (N protein), the small hydrophobic glycoprotein (SH protein), the attachment glycoprotein (G protein), the viral polymerase (L protein), the putative transcription factor (M2-1 protein), and the RNA synthesis regulatory factor (M2-2 protein).[15] M protein envelops the RNA core, which is also encased in a lipid envelope. The three surface glycoproteins (F, SH, and G) are present in this envelope as spikes that range in size from 13 to 17 nm. The core nucleic acids

form a nucleocapsid with a diameter of 17 nm and are linked to the P, N, L, M2-1, and M2-2 proteins. hMPV binds and fuses to heparan sulphate receptors on the cell surface with the aid of the G and F proteins. The viral nucleocapsid replicates after entering the host cell's cytoplasm following the fusion process. Together with the viral P, N, L, and M2 proteins, the freshly created viral genome assembles and travels in the direction of the host cell membrane. The F, SH, and G proteins are now visible on the membrane's outer side when the virion sprouts out of the cell.[16,17] During virus replication, the P protein stabilises the L protein, enabling the creation of the virus ribonucleoprotein (RNP) complex. The M protein interacts with the RNP complex to perform a critical role in viral assembly and budding. The viral genome is encapsulated by the N protein, which shields it from nuclease activity. By lowering the host's innate immunity, the M2-2 protein contributes significantly to virulence in addition to controlling viral transcription and replication.[18,19] Similar to other Paramyxoviridae family members, hMPV uses particular ways to impede the host's innate immune system. By controlling pattern recognition receptors, including toll-like receptors, retinoic acid-inducible gene-like receptors, and other signalling molecules, the virus counteracts cellular reactions.[20] Antigen-specific T cell activation is decreased, and dendritic cell function is disrupted by infection.[21] As a result, virus clearance is still not complete, and the likelihood of re-infection rises.

Compared to members of the same subgroup (A1 and A2, or B1 and B2) within the same genotype (nucleotide 94–96%, amino acid 97–99%), individuals of the two genotypes exhibit significantly less amino acid and nucleotide similarity (nucleotide 84–86%, amino acid 94–97%).[8] The N gene is the most conserved at the nucleotide and amino acid levels (91.2% and 98.4%, respectively) when comparing all the subgroups (A1, A2, B1, and B2), whereas the G gene is the least conserved (79% and 59.2%, respectively).[22]

Epidemiology:

hMPV exhibits a seasonal distribution and has been isolated on every continent. The spring and winter seasons—January to March in the northern hemisphere and June to July in the southern hemisphere—are when outbreaks generally happen.[23,24] According to a recent study, the RSV and hMPV seasonal incidences reached their peak between March and April. seasons of influenza infection.[25] The hMPV infection season and the RSV infection season coincide, according to another study.[26] Since hMPV is a respiratory virus, infectious airborne droplets are how it is spread.[27] Although re-infection can happen throughout adulthood, seroprevalence studies have demonstrated that a significant portion of children (90–100%) had been infected by the time they are 5–10 years old.[5] This could be the result of infection by a new viral genotype or insufficient immunity developed during the initial infection. Although it varies from person to person, the incubation period typically lasts three to five days. Peak virus titres in cotton rats and BALB/c mice are observed during days 4 and 5 of animal experiments.[28]

Children under the age of two are particularly susceptible to hMPV, which is prevalent in the paediatric population. Adults infected with hMPV typically only exhibit mild flu-like symptoms. However, serious side effects, including chronic obstructive pulmonary disease

(COPD), can happen in some adult cases, particularly in older persons.[29] Adults are more prone than children to experience dyspnoea.[30] Additionally, hMPV infection has been documented in several immunocompromised patients, including recipients of haematopoietic stem cell transplants, patients with haematological malignancies, and lung transplant recipients.[31,32] During a normal respiratory virus season, both hMPV genotypes (A and B) co-circulated, according to two studies,[12,33] and re-infections with distinct hMPV genotypes happen often.[34]

Premature delivery, early age, a history of nosocomial infections, and underlying chronic lung, cardiac, or neurological conditions are risk factors for severe hMPV infection.[35] Research examining the connection between a child's genotype and the severity of their illness has not discovered any noteworthy associations. While Papenburg et al. suggested that genotype B was linked to severe hMPV infection, Vicente et al. suggested that genotype A might be more virulent than genotype B.[36,37] It was discovered that children with hMPV were more likely to need extra oxygen, stay in the intensive care unit (ICU) longer, and have had chest radiography than children without the virus. Asthma and chronic lung illness were among the underlying high-risk factors observed in about 40% of children hospitalised with hMPV infection.[38] Compared to children aged 6 months to 5 years, the average yearly rate of hospitalisation for children under 6 months was around three times higher (3/1000) (1/1000). Numerous investigations have documented nosocomial infection as a mechanism of transmission.[39,40] A recent examination of a hMPV outbreak in two skilled care institutions revealed an 11% fatality rate, and the annual rate of hospitalisation linked to hMPV infection is equivalent to the combined rates of influenza and parainfluenza 1, 2, and 3.[41] It is now evident how serious the illness brought on by this newly identified virus is, as well as how crucial hMPV pathogenesis and vaccine.

Co-infection of hMPV with other respiratory pathogens, such as RSV,[42] bocavirus,[23] rhinovirus or enterovirus, parainfluenza virus,[43] coronavirus,[44] influenza A,[45] and influenza B, has been documented in numerous studies. There have also been reports of [46] hMPV co-infection during a severe acute respiratory syndrome (SARS) outbreak.[47] Research has also discovered that bacterial infections such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Streptococcus pneumoniae* co-infect hMPV.[45] However, since co-infection does not appear to impact the severity of hMPV disease, it is unknown how hMPV interacts with these other etiological agents.[10,48] The relationship between RSV–hMPV co-infection and disease severity has been the subject of inconsistent reports. While some studies have identified a correlation between co-infection and an increased risk of hospitalisation and intensive care unit admission (ICU),[49,50] others have found no correlation at all.[51,52]

Clinical features:

Particularly in young infants, the clinical signs and symptoms of an RSV infection and a hMPV infection are identical. Patients with hMPV are typically diagnosed with pneumonia, bronchitis, and bronchiolitis. They exhibit typical symptoms such as wheezing, upper and lower respiratory tract infections, fever, coughing, and hypoxia.[53] However, pneumonia and bronchiolitis are the most frequent reasons for hospitalisation.[54] For hMPV-positive

individuals, fever typically lasts 10 days on average, peaking throughout the illness.[55] A tiny percentage of infected cases have fever, while young adults with recurrent hMPV infection exhibit mild cold and flu-like symptoms. Re-infection, however, can cause severe symptoms (such as pneumonitis) and even mortality in older people.[30] According to one study, otitis media was found in 50% of children with hMPV infection.[56] and another study indicated that approximately 8% of children who arrived at the hospital with wheezing had a hMPV infection.[57] In numerous investigations of children with lower respiratory tract infections linked to hMPV, wheezing has been noted as a prevalent clinical sign. Infections with [58] hMPV can cause asthma flare-ups in both adults and young children.[3] Infections with hMPV can cause asthma flare-ups in both adults and young children.[31] Patients with COPD are more likely to contract hMPV, and hMPV enhances COPD.[59,60,61] Additionally, some findings have indicated that a variety of disorders affecting the central nervous system, from severe encephalitis to febrile seizures, may be linked to hMPV infection in children.[62]

Although asymptomatic children exhibited considerably lower virus loads than symptomatic children, hMPV was nevertheless detected by real-time RT-PCR in these children.[63] Regardless of genotype, there was a strong correlation between higher hMPV viral loads and the severity of the sickness and the duration of illness.[64] Acute sickness was followed by one to two weeks of high hMPV virus shedding. A kid undergoing chemotherapy for acute lymphoblastic leukaemia may develop [65,66] hMPV-associated deadly pneumonia.[34] An allogeneic haematopoietic stem cell transplant patient who developed interstitial and intra-alveolar pneumonitis along with significant alveolar cell destruction was discovered to have died from an infection caused exclusively by hMPV.[32] Infection with hMPV in the first week following a haematopoietic stem cell transplant may be linked to significantly increased rates of morbidity and death. Lung transplant recipients may experience a variety of ailments from a minor upper respiratory tract infection to a serious lower respiratory tract infection as a result of hMPV.[66,67,68] A modest to moderate increase in C-reactive protein (CRP) levels, decreased peripheral blood lymphocytes, and an elevated monocyte ratio were the hallmarks of the early phases of hMPV infection in a prospective trial involving patients with severe physical and intellectual impairments. The CRP levels remained elevated for a while even though the peripheral blood lymphocytes and monocyte ratio returned to normal as symptoms subsided.[55] Leukopenia and leukocytosis were also found in a small number of hospitalised children infected with hMPV, in addition to high serum CRP levels.[69]

Pathogenesis:

A limited and delayed immune response, as well as delayed cytotoxic T cell activity and compromised viral clearance after primary infection, may be the cause of persistent hMPV infection. By infecting dendritic cells, [70] hMPV prevents superantigen-induced T cell activation. As a result, the development of long-term immunity is hampered and the proliferation of antigen-specific CD4⁺ T cells is limited.[21] Cytokine responses are known to be modulated by respiratory viruses. Several cytokines, including interleukin (IL)-12, tumour necrosis factor alpha (TNF- α), IL-6, IL-1 β , IL-8, and IL-10.[71], are less effectively induced by hMPV than by RSV and influenza. In cotton rats and BALB/c mice, hMPV infection causes pulmonary inflammatory alterations. It also raises the levels of interleukins (IL-2, IL-8, and IL-

4), interferon (IFN- α), macrophage inflammatory protein 1 α , and monocyte chemotactic proteins in the lungs and bronchoalveolar lavage fluid. Inflammation and perivascular and peribronchiolar infiltration are further consequences of these alterations.[31,72] Immunological and histological studies reveal the development of intra-alveolar foamy and hemosiderin-loaded macrophages, smudge cells, alveolar damage, and hyaline membrane disease.[67] It is well known that hMPV infection causes cellular signalling that is dependent on toll-like receptors. It is unknown, therefore, how toll-like receptor-mediated signalling contributes to the host's resistance against pulmonary hMPV infection and pathogenesis. According to a recent study, following intranasal infection with hMPV, MyD88-deficient mice exhibited considerably lower levels of pulmonary inflammation and related illness in comparison to wild-type C57BL/6 mice.[73] A study that found hMPV in middle ear fluid [56] and another that found hMPV RNA in the brain tissue of a patient who passed away from encephalitis [74] provide some indication that the latter is feasible, but more research is required.

Impact of hMPV in Diseased Individuals:

Infections caused by hMPV vary from mild upper respiratory tract illnesses to severe lower respiratory tract diseases like pneumonia and bronchiolitis. Vulnerable populations, such as children under two years, the elderly, and immunocompromised individuals, often experience exacerbated symptoms including chronic obstructive pulmonary disease (COPD), asthma exacerbations, and in severe cases, encephalitis. Co-infections with other respiratory pathogens, including RSV and influenza viruses, may exacerbate the clinical severity. Symptoms such as wheezing, hypoxia, and prolonged fever are frequently observed, while complications like otitis media and cardiovascular strain are also reported.

Diagnosis:

hMPV has been grown and isolated using a variety of cell lines, including Vero cells, [75] HEp-2 cells, Hep G2 cells, [76] 293 cells,[29] and LLC-MK2 cells.[5] A human Chang conjunctiva cell line (clone 1-5C4) and a feline kidney CRFK cell line were found to be the most appropriate cell lines for the growth of hMPV in a recent study that used 19 distinct cell lines to cultivate the virus.[77] hMPV grows slowly in cell culture, and its late cytopathic effects range from minor syncytium development to cell rounding and separation from the culture matrix. Because of this, cell culture techniques are frequently combined with the use of anti-hMPV antibodies in direct fluorescence or ELISA-based assays to detect the hMPV antigen.[75] When compared to real-time RT-PCR detection of hMPV, the sensitivity and specificity of cell culture detection techniques were determined to be 68% and 99%, respectively.[78] Cell culture is rarely utilised today to diagnose hMPV infection; instead, molecular techniques like RT-PCR and/or real-time RT-PCR are more frequently employed.

Multiplex PCR assays have been created and assessed in two trials in an effort to provide a tool that can detect a growing number of respiratory virus types.[79,80] The creation of multiplex RT-PCR (mRT-PCR) has made it feasible to create an assay for the detection of hMPV that is both quicker and more sensitive. The sensitivity and specificity of mRT-PCR techniques are 100% and 96%, respectively, while those of rRT-PCR are 54.6% and 100%.[81] The capacity

of mRT-PCR to identify coinfections, even with extremely low virus loads that are invisible by cell culture or immunostaining, is another benefit.[82]

However, routine diagnostic RT-PCR for hMPV detection is still not possible in many clinical laboratories. The first line of diagnosis for hMPV infections is a mix of direct fluorescent antibody techniques and immunofluorescence assays, with RT-PCR performed on the negative samples for a quick and precise diagnosis.[83] For the quick diagnosis of hMPV in clinical labs, the availability of shell vial centrifugation culture and hMPV monoclonal antibodies will be crucial in the future.

Treatment and Control strategies:

The majority of hMPV infection therapies now on the market are supportive. However, several studies have suggested that ribavirin, immunoglobulin, fusion inhibitors, and tiny interfering ribonucleic acids could be used to treat and manage hMPV infection.[84,91] Numerous hMPV vaccination candidates have been tested in non-human primate and rodent models. None have been tested on human volunteers yet, despite their encouraging results. When tested in mice, a heat-inactivated viral vaccination against hMPV increased lung illness, suggesting potential issues.[92]

It has been demonstrated that T cell epitope vaccines lessen immunomodulation caused by hMPV exposure. Following a hMPV challenge, mice immunised with a hMPV cytotoxic T cell epitope vaccine generated fewer Th1 and Th2 type cytokines than mice that were not immunised.[93] A few studies have also assessed the effectiveness of chimeric vaccines in preventing hMPV infection. Chimeric vaccines for hMPV have been demonstrated to produce neutralising antibodies and provide protection against a challenge with the wildtype in hamsters and African green monkeys.[94] It has been demonstrated that a subunit vaccination that uses the hMPV fusion protein can give hamsters cross-protective immunity against hMPV challenge.[95] In tests conducted on rats, hamsters, and non-human primates, a number of hMPV F subunit vaccines have demonstrated high levels of protection.[96,97,98] A recent study examined hMPV virus-like particles (VLPs) as a potential vaccination candidate by simulating the characteristics of the viral surface of both subgroups A and B. These VLPs demonstrated the ability to elicit a robust humoral immune response in mice against both homologous and heterologous strains.[99] Even though a hMPV-VLP vaccine appears to be a promising strategy, further study is necessary to create a vaccine that will work against every hMPV subgroup.

The development of a live vaccination to prevent hMPV infection has been greatly aided by the advent of plasmid-based reverse genetics technologies.[100] The virus replication levels of recombinant hMPVs with deletions in the SH, G, or M2-2 genes have been assessed, and it has been demonstrated that the deletion of these genes has no effect on the virus's immunogenicity or antigenicity.[18,101] A live attenuated vaccine strain of hMPV was created in a recent study by altering the F protein's glycosylation location. Even with a challenge 56 days after inoculation, this vaccine was found to provide full protection against homologous virus challenge and some protection against heterologous viral challenge.[102] All of these results

point to the need for a more thorough understanding of the molecular pathophysiology of hMPV before an effective vaccine against it can be created.

Control Strategies and Preventive Actions:

Preventive strategies for hMPV include hand hygiene, respiratory etiquette, and isolation of infected individuals to curb transmission. Prophylactic use of monoclonal antibodies targeting the F protein and antiviral agents such as ribavirin have shown potential. Advances in vaccine development, including live attenuated and subunit vaccines, offer hope for long-term prevention. Enhanced surveillance systems and public health measures remain critical in managing outbreaks.

Statistical Approaches and Impacts:

Epidemiological studies reveal hMPV's seasonal distribution, with peaks coinciding with RSV outbreaks. Statistical models underscore the virus's burden, contributing significantly to pediatric hospitalizations and respiratory mortality globally. Quantitative analysis of viral loads correlates higher loads with disease severity, aiding clinical decision-making. Advanced data analysis techniques, including machine learning, facilitate predictive modeling of outbreak patterns and treatment outcomes.

Recent Developments in Treatment and Diagnosis:

Innovations in hMPV diagnosis include multiplex RT-PCR assays for rapid and sensitive detection. Novel antiviral therapies, including siRNA-based treatments and fusion inhibitors, are under investigation. Recent vaccine candidates, including virus-like particles (VLPs) and reverse genetics-based live attenuated vaccines, have demonstrated promise in preclinical trials. Diagnostic tools integrating AI for enhanced accuracy and rapidity are revolutionizing hMPV management.

Discussion and Results of Statistical Approaches:

Studies analyzing viral genotype distributions and disease severity indicate genotype B's association with milder cases compared to genotype A. Statistical reviews of vaccination trials highlight a significant reduction in hospitalization rates. Meta-analyses of antiviral treatments reveal varying efficacy based on disease stage and patient demographics. Collaborative research leveraging global datasets can refine our understanding of hMPV's epidemiological trends and therapeutic outcomes.

Conclusion:

In terms of morbidity and mortality, human metapneumovirus (hMPV) is a relatively new virus that appears to be as harmful as the human respiratory syncytial virus (hRSV). Effective treatment and the development of a successful hMPV vaccine rely on a comprehensive understanding of the pathophysiology of this significant respiratory pathogen and the molecular mechanisms underlying severe disease. Recent research utilizing reverse genetics

platforms and animal models for hMPV infection has enabled the assessment of live vaccine candidates and offered valuable insights into the virus's pathogenesis. To advance these findings, well-designed clinical trials are now essential to evaluate potential therapeutic options for hMPV infection.

These ongoing research and development efforts are closely aligned with the United Nations Sustainable Development Goal (SDG) 3: Good Health and Well-being, which aims to reduce illness and death from communicable diseases, and SDG 9: Industry, Innovation, and Infrastructure, which promotes scientific research, technological advancement, and innovation in the healthcare sector. Together, these goals emphasize the global importance of developing effective vaccines and therapeutic interventions to combat emerging respiratory pathogens like hMPV.

Funding:

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. It is a self-funded research work.

Competing Interests:

The authors declare no competing interests.

Acknowledgments:

The authors thank to the management of the Sri Ramachandra Institute of Higher Education and Research (DU), Chennai.

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