ABSTRACT

Rotaviruses (RVs) are one of the most common causes of acute gastroenteritis in children under five-year-old worldwide. RVs are non-enveloped RNA viruses and belong to the family Reoviridae. The 11 dsRNA genome segments of rotavirus encode six structural and six non-structural proteins. Group A rotaviruses is one of the primary cause of severe childhood diarrhoea. Currently, two indigenous, oral and live-attenuated rotavirus vaccines; Rotavac (Bharat Biotech) and RotaSIIL (Serum Institute of India) have been introduced in the national immunization schedule since 2016. Vaccines prevent the most severe form of the disease by mediating a combination of serotype-specific and heterotypic immunity to the prevalent rotavirus serotypes or genotypes.

Thesis is divided into seven chapters which are discussed briefly below.

Chapter 1 presents an introduction on gastroenteritis and rotavirus. The chapter includes the history of rotavirus, burden of rotavirus diarrhoea: hospitalization, mortality and economic burden. The circulating rotavirus strains globally and in India are also discussed along with research status in north-eastern states (NER) of India and the objectives of the doctoral research work.

Chapter 2 provides literature review on rotavirus and previous studies on rotavirus genome organization and structure of rotavirus. The chapter discusses the classification of group A rotaviruses (RVAs) into group, subgroup, serotype, genotype, and electropherotype. Regarding its life cycle, rotaviruses enter the body through mouth, travel through the alimentary canal and infect the lining of the small intestines and it is followed by reproducing new progeny viruses. Further, rotavirus pathogenesis and pathology, epidemiology, symptoms, diagnosis, treatment, control and prevention are reviewed elaborately in this chapter.

Chapter 3 reports a hospital-based surveillance of rotavirus diarrhoea conducted in children under five-year-old from Imphal, Manipur, India prior to the introduction of rotavirus vaccine into the national immunization program. Children less than 5 years of age admitted at RIMS, Manipur from December 2015 to March 2019 were enrolled for the study from. Of the total 517 patients enrolled during the 3 years surveillance,

~68.86% (356/517) was positive for rotavirus antigen. Viral RNA was extracted from EIA positive samples and genotyped using RT-PCR to identify the genotypes. Further, co-infection of rotavirus with other enteric viruses Adenovirus, Astrovirus and Norovirus were also detected. Rotavirus cases were highest in children aged 12-23 months (40.78%), and 35.2% in 6-12 months. A peak increase in rotavirus diarrhoea was seen during the cooler months, with the highest positivity between December and February. The most prevalent rotavirus G-genotype was G3 (40%) followed by G1 (15%), G2 (8%), G9 (5%) and G8 (3%) which is different from another previous study in the region. Among P-types, P[6] (20%) accounted for the highest prevalence followed by P[8] (10%) and P[4] (4%), P[11] (4%), and P[10] (3%). Further, co-infection of rotavirus with other enteric viruses (Adenovirus, Astrovirus and Norovirus) was observed in 24% (124/517) of the children. The findings revealed the high burden of rotavirus gastroenteritis and shifting in the prevalent genotype strains in the region. The findings highlight the need for continuous surveillance and to assess the impact of the vaccine among children of Manipur, India and also the effect of enteric viruses on rotavirus infection.

Chapter 4 is study on genetic analysis of capsid protein VP7 and VP4 of the vaccine strains available in India and the circulating rotavirus strains for assessment of rotavirus vaccine efficacy and rotavirus diversity in the study region. Antigenic epitopes of VP7 and VP4 were compared between the strains from Imphal and the vaccine strains. G9 lineage showed upto three amino acid changes with respect to the G9 lineage 2 of Rotavac strain. Whereas, in VP4 (VP8*) epitope, P[8] lineage showed upto six amino acid changes with respect to the P[8] lineage 1 and 2 of Rotarix and RotaTeq. Furthermore, structural analysis showed changes in three residues in the epitope region of G9 led to substantial change in interaction between the VP7 and the Fab region of IgG. Continuous monitoring of the antigenic epitopes of prevalent strains in Imphal and the vaccine strains is necessary to assess vaccine(s) efficacy.

Chapter 5 presents rotavirus whole genome sequencing information. Few rotavirus isolates showing unusual migration pattern upon RNA PAGE analysis were subjected for whole genome sequencing. We report the near-complete draft genome characterization of one human G3P[8] RV strain RM251122016 isolated from a 12-month child infected with rotavirus in 2016. The strain showed a Wa-like genotype constellation I1-R1-C1-M1-A1-N1-T1-E1-H1. Phylogenetic analysis revealed a configuration of genes of human

origin, with two genes having close similarity with animal RV strains; VP1 showed a close similarity with porcine and equine RVs and VP7 found having similarity with a bovine RV strain. The gene segments have common related strains; Kenya G3P[8] strain KCH1187, Tokyo17-21 (G3P[8]) from Japan, Italian strain ME659 (G12P[8]) and Nigerian strain NGR_Ref (G1P[8]). The antigenic regions of outer capsid proteins VP7, VP4, and VP6 of circulating strains were compared with vaccine. The results showed high conservation between the G3P[8] strain and strains from their respective phylogenetic tree and revealed many substitutions when compared with the RotaTeq™, Rotarix™, and Rotavac™ vaccine strains. The genome sequence will serve as a part of baseline data of the region and the country to assess vaccine efficacy and also to monitor strain diversity.

Chapter 6 is a study on the functional correlation of B- and T-cells to long-lasting immunity against rotavirus infection in the literature is limited. Next, a series of computational immuno-informatics approaches were applied and identified 28 linear B-cells, 26 conformational B-cell, 44 Tc cell and 40 Th cell binding epitopes for structural and non-structural proteins of rotavirus. Further selection of putative B and T cell epitopes in the multi-epitope vaccine construct was carried out based on immunogenicity, conservancy, allergenicity and the helical content of predicted epitopes. An in-silico vaccine constructs was developed using an N-terminal adjuvant (RGD motif) followed by Tc and Th cell epitopes and B-cell epitope with an appropriate linker. Multi-threading models of multi-epitope vaccine construct with B- and T-cell epitopes were generated and molecular dynamics simulation was performed to determine the stability of designed vaccine. Codon optimized multi-epitope vaccine antigens was expressed and affinity purified using the E. coli expression system. Further the T cell epitope presentation assay using the recombinant multi-epitope constructs and the T cell epitope predicted and identified in this study have not been investigated. Multiepitope vaccine construct encompassing predicted B- and T-cell epitopes may help to generate long-term immune responses against rotavirus. The computational findings reported in this study may provide information in developing epitope-based vaccine and diagnostic assay for rotavirus-led diarrhoea in children.

Chapter 7 is the summary and the future prospects of the doctoral research work.