Research Article

OMIC Technologies Helping in Confirmation of Diagnosis: Clinical Genomic Biomarkers in Childhood Diarrhea by Viral Causes among Slum Children's of Selected Hospital in Indore

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Abstract

Background: OMIC base technique is the most modern diagnostic assay for isolation of clinical biomarkes of viral genome causing Diarrhoea, Gastrointestinal tract (GI) infection and Inflamatory Bowel Diseases (IBD). This technologies such prone to enable the study about GI tract diseases with molecular level detection of viral genomics, transcriptomics, proteomics and metabolomics.

Aim: The aim was to identify different clinical genomic biomarkers causing Diarrhea by viral element with their prevalence rate among children's in slum area and also study the sensitivity rate in OMIC technique with established the newest techniques to easy and early detection of biomarkers in our diagnostic purpose.

Method: The present study was conducted among 200 selected symptomatic slum children's with an acute gastroenteritis from August 2019 to July 2022 at Index Medical College and Research centre in Indore, MP. The criteria for inclusion were, children of all sexes who have been diagnosed acute gastroenteritis. The research cover the children;s age group between >1month to 5 years and children residing at selected slums of Indore City. The exclusion criteria includes patients with ages >5 years and both gender, malignancy, genetic malformation, acute illness, other gastrointestinal infections, other valvular dysfunctions and other endocrine abnormalities. The stool sample was collected in a width mouth container and further processing for viral genomic detection with multiplex PCR, RT-PCR, immunochromatography technique and finally detection of clinical biomarkers associated with viral transcriptomics, proteomics and metabolomics by using Next Generation Sequencing (NGS).

Results: Acute gastroenteritis can be caused by a number of different infectious agents, the most common of which are viruses, bacteria, and parasites. In this study we showed viruses such as Rotavirus (24.5% of the total) and Norovirus (23.5% of the total) are responsible for the highest infection rate. In addition to that, adenoviruses (11%) and astroviruses (13.5%). These agents can be passed on to the host through a variety of channels, including the faeco–oral route, person-to-person contact, and fomites.

Conclusion: It is a target-independent assay that allows for the simultaneous detection and genomic characterization of all microorganisms present in a sample using a next-generation sequencing platform. The Next Generation Sequencing and other omics-based approaches have the potential to serve as the foundational methodologies for the detection of novel biomarkers in clinical microbiology. This is assuming that cost is an obstacle. These modalities should be easily considered and integrated for a more holistic and comprehensive management of patients who have acute gastroenteritis. This should become possible as the use of various omics-based approaches is expected to become more widespread and, hopefully, more accessible in the near future.

Keywords: RT-PCR; OMICS; NGS; GPP

Introduction

Acute infectious gastroenteritis is commonest diseases in childhood may cause due to unhygienic condition or may be in contaminated water and foods. These severe effects can be results by most effecting viral, bacterial and parasitic infections. It is described as a diarrhea illness with an abrupt start that manifests itself with the occurrence of three or more loose or liquid stools or three episodes of vomiting in 24 hours, in addition to stomach discomfort or fever. Osmotic diarrhoea is the consequence of damage that has occurred at the villous brush border of the intestine. This damage lowers the ability to absorb the contents of the gut, which in turn leads to the symptoms of osmotic diarrhoea. Several of the agents that induce diarrhoea will also release their toxins, which will then bind to the receptors on the particular enterocytes. This will result in the release of chloride ions into the intestinal lumen,

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which will ultimately lead to secretory diarrhoea. There are around 1.7 billion instances of gastroenteritis reported each year, which results in 1.5 million fatalities throughout the world. Children in developing nations, as well as children in other parts of the world, bear a disproportionate share of the world's illness burden as a direct result of poor sanitation and hygiene. Children under the age of five who live in these areas have between three and four bouts of diarrhoea per year, and as a result, they often suffer from long-term health implications such as developmental abnormalities [1]. Children less than 5 years old are more likely to be affected by acute infectious gastroenteritis, which has the second highest fatality rate in the world, behind only pneumonia. This health problem is far more prevalent in less developed countries than in more developed nations. It is estimated that there have been around 125 million cases of gastroenteritis among babies aged 0–11 months and 450 million cases among children aged 1–4 years, the majority of which have occurred in poor nations [2]. It has been suggested that gastrointestinal infections are caused by a wide range of pathogens, including those that are bacterial, viral, parasitic, and fungal in origin. Norovirus and rotavirus are the two viruses that are responsible for the majority of cases of gastroenteritis. Rotavirus is more likely to harm children, whereas norovirus is more often linked to outbreaks in healthcare facilities. In India, bacteria are responsible for 20%–40% of diagnoses, putting them on par with viruses in terms of relevance. Campylobacter is the most prevalent cause of bacterial gastroenteritis [3].

Material and Methods

Study area and design

The present study is a hospital based, cross-sectional study conducted at Index Medical College and Research centre in Indore, MP from August, 2019 to July, 2022. Laboratory test was done in Department of Microbiology in molecular research Unit.

Population

The source of population is those children residing at nearest slums area and came in OPD (Outpatient Department Treatment) for treatment of symptomatic acute gastrointestinal diseases, Diarrhoea and inflammatory bowel diseases etc.

Sample size

Stool samples were collected from selected 200 children in between age group of more than 1month to 5 year. Samples send to the Department of Microbiology in Molecular lab for clinical Biomarkers Detection through OMIC base technique.

Inclusion criteria of study participants

We selected children of all sexes who have been diagnosed acute gastroenteritis with cover the childrens age group between >1month to 5 years, Residing at selected Slums of Indore City

Exclusion criteria

Those children with ages >5 years and gender with malignancy, genetic malformation, acute illness, other gastrointestinal Infections, other valvular dysfunctions and other endocrine abnormalities were excluded from this study.

Specimen collection

Random stool samples were collected in a sterile dry wide-necked, leak-proof universal width mouth container from each study participant for viral genomic biomarkers detection.

Transport and storage of samples

Stool samples were transported in courier boxes from the point of collection to the laboratory. Samples which were not processed within the same day of collection were stored in a refrigerator at 4°C–8°C awaiting processing.

Specimen processing

Collected stool samples were processed in viral molecular laboratory at Department of Microbiology, Index Medical College and Research centre in Indore, MP.

Identification of biomarkers through OMIC

The extracted nucleic acids (RNA and DNA) were processed for metagenomics analysis making use of the Illumina MiSeqplatform for NGS in accordance with the standard operating procedures. In a nutshell, the genomic host DNA that was present in the extracted nucleic acids was eliminated by using Ambion DNA-free and adhering to the instructions provided by the manufacturer. The synthesis of single-strand cDNA from DNA-free RNA that had been primed by random hexamers and then amplified in accordance with the instructions provided by the manufacturer was carried out with the assistance of QuantiTect® whole transcriptome. The amount of cDNA that was used for library preparation was one ng, and the quantification of cDNA was performed with a Qubit R Fluorometer and a QubitTM dsDNA BR assay kit in accordance with the instructions provided by the manufacturer. The Illumina TruSeq DNA library preparation kit V2 was utilised in order to get the DNA libraries ready. The pooled DNA libraries were sequenced at the Research Core Facility of Index Medical College with an Illumina MiSeq instrument to generate 150-Bp paired-end reads.

Potential biomarkers of virus

Rotavirus: G1P(7), G2P(6), G3P(6), G4P(8), G6P(6), G9P(6)

Norovirus: GI.3, GII.4

Astrovirus: HAstV1, HAstV2, HAstV6, HAstV8

Adenovirus: HAdV F 40, HAdV A 61, HAdV D 90

Safety and environment

All biological specimens, including used cartridges, capable of transmitting infectious agents were treated with universal precautions. All laboratory procedures were done in a level 3 in molecular laboratory. Personal Protective Equipment (PPE) such as disposable gloves, laboratory coats was used when handling specimens and reagents. Washing of hands was done thoroughly after handling specimens and test reagents. Disposing of stool was done according to the country's safety guidelines for hazardous material.

Statistical analysis

Results were analyzed using pearson's chi-square test, or Fisher's exact test. A value of $P<0.05$ was regarded as statistically not significant (SPSS 16.0, SPSS Inc. Chicago, IL, USA).

Results

In current study approach, out of total 200 children 24.5% (n-49) showed retrovirus infection and among them 18% infant, 38.7% baby and 55.1% child participants in prospective study area with also seen different clinical identical genomic biochemical markers in rotavirus of 8.1% GIP(7), 20.4% G2P(6), 28.5% G3P(6), 10.2% G4P(8), 12.2% G6P(6) and 20.4% G9P(6) detected from collected stool samples (Table 1).

Table 1: Characteristics and serum Mg of Saudi soccer players Mean ± SD, minimum (Min) and maximum (Max).

Causative agent	Rotavirus						Norovirus			Astrovirus			Adenovirus			Other		
Different age group children	≤01 years (Infant)		02-03 years (Baby)		04-05 years (Child)		≤01 years (Infant)	02-03 years (Baby)	04-05 years (Child)	≤01 years (Infant)	02-03 years (Baby)	04-05 years (Child)	≤01 years (Infant)	02-03 years (Baby)	04-05 years (Child)	≤01 years (Infant)	02-03 years (Baby)	04-05 years (Child)
Participant (n)	9		13		27		4	28	15	2	9	16	1	9	12	11	29	15
Biomarkers	G1P(7)	G2P(6)	G3P(6)	G4P(8)	G6P(6)	G9P(6)	GI.3		GII.4	V1	V2	V6	V8	F 40	A 61	D 90		
Quantity	4	10	14	5	6	10	21		26	6	9	10	2	8	12	2		

Similarly we showed 23.5% Norovirus from those clinical samples, out of these 8.5% infant, 59.5% baby and 31.9% child and also showed biomerkers GI.3 (44.6%) and GII.4 (55.3%).

Same effect seen in case of 13.7% Astrovirus (n-27) in our current study and also showed 7.4% infant, 33.3% baby and 59.2% child with biomerkers of HAstV1(22.2%), HAstV2(33.3), HAstV6(37%) and HAstV8(7.4%) in selected samples.

Existing acute gastrointestinal diseases caused by 11% Adenovirus with 4.5% infant, 40.9% baby and 54.5% child participants with various biomerkers of (36.3%) HAdV F 40, (54.5%) HAdV A 61 and (9%) HAdV D 90.

We also showed 40% another source of contamination which may be bacterial, fungal or parasitic cause for gastrointestinal diseases.

Discussion

Acute gastroenteritis can be caused by a number of different infectious agents, the most common of which are viruses, bacteria, and parasites. In this study viruses such as Rotavirus (24.5% of the total) and Norovirus (23.5% of the total) are responsible for the highest infection rate. In addition to that, adenoviruses (11%), astroviruses (13.7%) and (55%) others may be in bacterical or parasitic infection. These agents can be passed on to the host through a variety of channels, including the faeco–oral route, person-to-person contact, and fomites. The findings are concordant with Elliott EJ[4]. In (2007) the majority of cases are caused by viral infection, with rotaviruses and noroviruses being the most common. Viral infections harm small bowel enterocytes, resulting in low-grade fever and watery diarrhoea without blood. In temperate climates, rotavirus infection is seasonal, peaking in late winter, but it occurs all year in the tropics. Rotavirus strains differ by season and geographical location within a country. The infection is most common between the ages of 6 months and 2 years, and it spreads *via* the faecal-oral or respiratory routes. Bacterial pathogens such as *Campylobacter jejuni* and *Salmonella* spp invade and cause inflammation in the small and large intestines.Children who have bacterial gastroenteritis are more likely to have a high fever and blood and white blood cells in their stool. Bacterial pathogens can spread systemically, particularly in young children. Infection with Shiga toxin-producing *Escherichia coli* or *Shigella dysenteriae* can result in haemorrhagic colitis (severe bloody diarrhoea), which can be complicated by haemolytic uraemic syndrome. This syndrome is endemic throughout the world and is distinguished by acute onset of microangiopathic haemolytic anaemia, thrombocytopenia, acute renal impairment, and multisystem involvement. Enteric fevers (caused by *Salmonella typhi* and *S. paratyphi*) cause severe illness in young children, with symptoms including high swinging fever, diarrhoea or constipation, leucopenia, and, in rare cases, central nervous system involvement, including encephalopathy. Encephalopathy is a rare complication of *Salmonella* infection that is not typhoid. The toxin produced by *Vibrio cholera* causes chloride and water secretion from the small bowel without causing damage to the intestinal mucosa; it results in "rice water" stools with a high sodium content but no blood or white blood cells. Gastroenteritis is spread from person to person or through the consumption of contaminated food and drink ("food poisoning"). Bacterial pathogens are commonly found in undercooked or improperly stored cooked or processed meats (chicken, beef, pork), and seafood. When toxins produced by bacterial contaminants (for example, *Staphylococcus aureus* in ice cream or *Bacillus cerus* in reheated rice) are consumed, vomiting or diarrhoea (or both) occur quickly. Water can be contaminated with bacteria, viruses, or protozoa such as *Giardia lamblia*, cryptosporidium, *V. cholera*, and *Entamoeba histolytica*, the causative agent of amoebic dysentery. With rising rates of international travel and immigration, clinicians in developed countries are increasingly seeing children with "traveller's diarrhoea" caused by organisms not normally found in that environment.

Metagenomics is a target-independent assay that allows for the simultaneous detection and genomic characterization of all microorganisms present in a sample using a next-generation sequencing platform. The most recent next-generation sequencing platform was first used in acute gastroenteritis studies to look at the most common rotavirus genotype. The method was used on rotavirus positive specimens to determine the most common genotype among them.The results showed that G3P(6) was the most prevalent genotype among the participants' rotavirus specimens.

The findings are concordant with Hull JJA[5] .The Rotavirus genus contains eight species, designated A through H, as well as two recently identified tentative species, I in dogs and J in bats. Rotavirus A, B, C, and H (RVA, RVB, RVC, and RVH) have all been found in humans and animals. While human and animal RVA are well characterised and defined, there are fewer complete porcine genome sequences in GenBank than there are for human strains. We used a metagenomic approach to sequence 11 segments of RVA, RVC, and RVH strains from piglets in the United States (US) and investigate their evolutionary relationships. Astroviridae, Picornaviridae, Caliciviridae, and Coronoviridae were found in samples MN9.65 and OK5.68, but Picobirnaviridae and Arteriviridae were only found in sample OK5.68. Whole-genome sequencing and phylogenetic analyses revealed multiple genotypes with the RVA of strains MN9.65 and OK5.68, with the G5/G9-P genome constellation. [7]/P[13]-I5/I5-R1/R1-C1-M1-A8-N1-T7-E1/E1-H1 and G5/G9-P[6]/P[7]/P[8]/P[9]/P[10]/P[11]/P[12]/P[13]/P-I5-R1/R1-C1-M1-A8-N1-T1/T7-E1/E1-H1, in that order. The RVA strains shared a complicated evolutionary history with other mammalian strains. The genome constellation of the RVC strain OK 5.68 was G9-P[6]-I1-R1-C5-M6-A5-N1-T1-E1-H1, and it shared an evolutionary relationship with porcine strains from the United States. The RVH strains MN9.65 and OK 5.68 had G5-P1-I1-R1-C1-M1-A5-N1-T1-E4-H1 and G5-P1-I1-R1-C1-M1-A5-N1-T1-E1-H1 genome constellations, indicating that multiple RVH genome constellations are circulating in the US. These findings help us understand the complexity of the enteric virome, improve screening methods for RVC and RVH strains, expand rotavirus surveillance in pigs, and better understand the origin and evolution of rotavirus species.

Metagenomics approach to identify the most prevalent Norovirus Potential Biomarkers/genotype among participants.

(OMIC). The result proved that GII.4 was the most prevalent potential biomarker among norovirus.

The findings are concordant with infection prevention teams frequently face norovirus outbreaks in hospital settings [6]. Given the high prevalence of norovirus in most communities, it can be difficult to distinguish between ongoing in-hospital transmission of the virus and new community introductions, and understanding the long-term impacts of outbreak-associated viruses within medical systems using traditional epidemiological approaches alone can be difficult. A retrospective cohort study was conducted in conjunction with real-time metagenomic sequencing during an ongoing norovirus outbreak. We describe a hospital-associated norovirus outbreak that affected 13 patients in a large, tertiary paediatric hospital over a 27-day period. The outbreak coincided with an increase in self-reported gastrointestinal symptoms among staff. Ten chronologically overlapping hospital-acquired norovirus cases were partitioned into three discrete transmission clusters using real-time metagenomic Next-Generation Sequencing (mNGS). Data from sequencing revealed close genetic relationships between some hospital-acquired cases and some community-acquired cases. Finally, this data was used to demonstrate chronic viral shedding in a hospitalised immunocompromised case patient. An examination of serial samples from this patient revealed new information about the evolution of norovirus within an immunocompromised host. This study describes one of the first real-time mNGS applications during a hospital-associated viral outbreak. Given its demonstrated ability to detect transmission patterns within outbreaks and elucidate the long-term effects of outbreak-associated viral strains on patients and medical systems, mNGS is a valuable tool for infection control teams to use in understanding, preventing, and responding to viral outbreaks.

the most prevalent Astrovirus Potential Biomarkers/ genotype among participants.(OMIC).The result proved that HAstV6(37%)was the most prevalent potential biomarker among Astrovirus

The findings are concordant with Wessels E Infections transmitted through blood transfusions are a constant threat in medicine [7]. It bears the burden of the past, as evidenced by serious infections transmitted by transfusion, and is constantly threatened by new viruses. The global rise in immunosuppression among patients requiring frequent transfusions aggravates the problem. Criteria for donor selection have become increasingly stringent over the last decade. Although routine Nucleic Acid Testing (NAT) for virus detection has become more sensitive, these safety precautions are only useful for a small number of viruses. The scientific approach to this is changing, with the goal of detecting infectious agents in donor units as soon as possible in order to reduce the risk of a clinically relevant infection. To that end, in addition to epidemiological surveillance of the general population, researchers are employing novel methods to identify emerging infectious agents while simultaneously screening donors for a wide range of viruses. Next-Generation Sequencing (NGS) allows researchers to investigate severe transfusion reactions of unknown aetiology by exploring the entire viral landscape in blood donors, a process known as metagenomics. This platform could be used for routine testing of donated blood products in the not-too-distant future.

The result proved that HAstV61(54%)was the most prevalent potential biomarker among Astrovirus.

The findings are concordant with Fernandez-Cassi X Acute infectious gastroenteritis is a serious illness that affects millions of people worldwide, particularly children, with viruses accounting for roughly 70% of acute cases [8]. A large proportion of these cases have an unknown etiological agent, and the rise of next-generation sequencing technologies has opened up new avenues for viral pathogen detection and discovery. In routine clinical settings, viral metagenomics has the potential to identify unexpected or novel variants of viral pathogens that cause gastroenteritis. In this study, 124 samples from acute gastroenteritis patients tested negative for common gastroenteritis pathogens between 2012 and 2014 were pooled by age and analysed using Next Generation Sequencing (NGS) to identify unidentified viral infections. The Astroviridae and Caliciviridae families had the most sequences detected potentially associated with acute gastroenteritis, with the detection of norovirus GIV and sapoviruses. There were fewer contigs associated with rotaviruses. Several Picornaviridae members (EV, parechoviruses, cardioviruses, and adenoviruses) and adenoviruses were identified as other viruses that may be associated with gastroenteritis but also cause persistent infections in the gut.

Conclusion

Childhood diarrhea may be the cause of majority is unhygienic environment or contaminated food and water in slams area. Although rotavirus is still the most common pathogen that causes acute gastroenteritis in children, other pathogenic agents, such as norovirus, are emerging as important acute gastroenteritis-causative agents and will be studied in the future. Rotavirus is still the most common pathogen that causes acute gastroenteritis in children. This is without ignoring the fact that bacteria and parasite pathogens continue to play an important role in the causing of acute infectious gastroenteritis, particularly in children. Children are especially susceptible to the effects of these pathogens. In light of the appearance of new pathogens, the accumulation of numerous mutations that have led to an increase in the number of pathogenic strains, and the absence of reliable prognostic parameters, it is necessary to take into consideration the development of more robust diagnostic and predictive biomarkers. The Next Generation Sequencing and other omics-based approaches have the potential to serve as the foundational methodologies for the detection of novel biomarkers in clinical microbiology. This is assuming that cost is an obstacle. These modalities should be easily considered and integrated for a more holistic and comprehensive management of patients who have acute gastroenteritis. This should become possible as the use of various omics-based approaches is expected to become more widespread and, hopefully, more accessible in the near future.

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