Detection of Norwalk-Like Virus in Cases of Acute Gastroenteritis

Thesis

Submitted in Partial Fulfillment for Master Degree

in Clinical Pathology

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الكشف عن الفيروس المشابه للنوروك فى حالات النزلات المعوية الحادة

رسالـة
توطئة للحصول على درجة الماجستير
فـــي الباثولوجيا الإكلينيكية والكيميائيـــة

مقدمـة من
الطبيبة / نـدا السيد أحـمـد سيـد
بكالوريوس الطب والجراحة – جامعة عين شمس

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2007

List of Abbreviations

Bp	:	Base pair
CDC	:	Centers for Disease Control and Prevention
CPE	:	Cytopathic effect
Cscl	:	Cesium chloride
cDNA	:	Complementary deoxy ribonucleic acid
dNTP	:	Deoxynucleoside triphosphates
DTT	:	Dithiothreitol
E.coli	:	Escherichia coli
EIA	:	Enzyme immunoassays
ELISA	:	Enzyme linked immunosorbent assay
EM	:	Electron microscopy
EPEC	:	Enteropathogenic E.coli
GI, GII	:	Genogroup I, genogroup II
IEM	:	Immune electron microscopy
Ig	:	Immunoglobulin
Kb	:	Kilo base
KDa	:	Kilo dalton
NLVs	:	Norwalk like viruses
OD	:	Optical density
ORF	:	Open reading frame
P	:	Probe
P/N	:	Positive / negative

PBS	:	Phosphate buffer saline
PPM	:	Part per million
Pt	:	Precipitate
RIA	:	Radioimmunoassays
RNA	:	Ribonucleic acid
rNV	:	Recombinant Norwalk virus
RT-PCR	:	Reverse transcriptase - polymerase chain reaction
SLVs	:	Sapporo-like viruses
SPIEM	:	Solid phase immune electron microscopy
SRSV	:	Small round-structured viruses
S.S	:	Salmonella shigella
TP	:	Taq probe
UK	:	United Kingdom
US	:	United States
VPg	:	Genome linked viral protein
XLD	:	Xylose lysine deoxy cholate

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Introduction

Since the initial description of Norwalk virus in 1972, Norwalk-like viruses (NLVs) have come to be recognized as the most common causes of outbreaks of acute non bacterial gastroenteritis *(Mounts et al., 2000).*

Norwalk-like viruses, are genus of genetically diverse, non enveloped single stranded RNA viruses belonging to the family caliciviridae *(Green et al., 2001)*. They are approximately 27 to 35 nm in diameter and have amorphous structure with ragged edge *(Liue et al., 1999).* They are classified into 2 genogroups I and II. The genogroups are further divided into various genotypes *(Johansson et al., 2002).*

In industried countries, NLVs may be responsible for 68-80% of outbreaks of gastroenteritis. These outbreaks occur in all age groups and in many settings, including schools, nursing homes, hospitals, cruise ships and restaurants *(Fankhauser et al., 2002)*.

Norwalk-like viruses can be transmitted through contaminated food or water, directly from person to person, and occasionally by airborn droplets produced-during vomiting. Food handlers are often suspected as the source

of foodborn outbreaks. Many local and state health departments require that food handler with gastroenteritis do not return to work until 2 or 3 days after they feel better. In addition, because the virus continues to be present in the stool for as long as 2 to 3 weeks after person feels better, strict hand washing after using the bath room and before handling food items is important in preventing the spread of this virus *(Daniels et al., 2000 and CDC, 2006a).*

The disease is self limiting, mild and characterized by nausea, vomiting, diarrhea and abdominal pain. Headache and low grade fever may occur *(Bresee et al., 2002 and Mathner et al., 2005).* The incubation period is generally reported to be 24-48 hours, but longer duration of incubation has been described *(Bull et al., 2006).*

The major obstacle in the laboratory diagnosis of NLV infection is the lack of tissue culture system for propagating the viruses. Therefore, electron microscopy (EM) has been routinely used to detect NLV particles in stool specimens. However, the sensitivity of EM detection is low, requiring at least 10^6 particles per ml stool. Positive identification of the virus relies on immune electron microscopy which is not well suited for large scale screening *(Kageyama et al., 2003).* Other serological methods for detection include enzyme

linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) for demonstration of a rising antibody titre against virus in the patients serum *(Moe et al., 2004).* Reverse transcription-PCR (RT-PCR) has been increasingly used for detection of viruses and would be an attractive alternative for NLV detection. It is highly sensitive to a broad range of NLVs *(Kageyama et al., 2003).*

Aim of the Work

The aim of this work is the detection and identification of Norwalk-like virus in stool specimens of children suffering from acute diarrhea by reverse transcriptase-polymerase chain reaction (RT-PCR).

Norwalk-Like Viruses

Caliciviruses are recognized as one of the most important causes of acute non bacterial gastroenteritis in sporadic community cases as well as in outbreaks in different settings *(Hedlund et al., 2000).*

The confusing early names of these viruses, which were determined on the basis of the location of their discovery (e.g. Norwalk, Hawaii) or their appearance by EM (e.g. small round structured virus (SRSV), classic viruses), have been clarified *(Glass et al., 2000).* The human caliciviruses have been divided into 2 major groups on the basis of genome organization, morphology, genetic and antigenic properties. Norwalk virus is the prototype strain for the genus Norwalk like viruses (NLVs). These are small round-structured viruses which are commonly found in association with all ages gastroenteritis. Sapporo virus is the prototype strain for the genus Sapporo like viruses (SLVs) also known as classic calicivirus which causes human gastroenteritis as well *(Rochx et al., 2002).*

Norovirus is recently approved as the official genus name for group of viruses provisionally described as "Norwalk-like viruses" *(Burton-Macleod et al., 2004).*

Caliciviruses in animals are classified into vesivirus and lagovirus. Vesivirus is represented by swin vesicular exanthema virus and feline calicivirus. However, lagovirus is represented by rabbit hemorrhagic disease virus and European brown hare syndrome virus *(Nakata et al., 2000).*

A. Historical Aspect:

NLVs are group of related viruses named after the prototype strain Norwalk virus, which was first discovered in 1972 by Albert Kapikian at National Institutes of Health. He used immune electron microscopy to examine fecal samples obtained from infected human volunteers. The volunteers had been given filterates of stool samples collected by Centers for Disease Control and Prevention (CDC) epidemiologists during an outbreak of vomiting at an elementary school in Norwalk, Ohio, in 1968. Initially, no agent could be identified in these specimens, but the filterates given to the volunteers induced gastrointestinal disease, indicating the likelihood of infectious causes. Dr. Kapikian's discovery marked the first time that a virus had definitively demonstrated to cause diarrheal disease in humans. Research conducted during the following decade confirmed that Norwalk virus was significant cause of epidemics of gastroenteritis in a wide variety of settings, and the distinct clinical and epidemiologic features of these outbreaks served as a diagnostic guide in absence of

routine laboratory testing *(Bresee et al., 2002)*.

B. Morphology:

The common feature of the family caliciviridae included the presence of a single major structural protein from which the capsid was constructed and the appearance of 32 cup - shaped depression on the surface of the virion arranged in icosahedral symmetry. The name of the new family was derived from the Latin word calix, which mean cup or chalice. Another major feature of this new family was the absence of a methylated cap at the $\overline{5}$ terminus of the virion RNA. Instead, a small protein (VPg) of 10-12 $x10^3$ KDa was shown to be covalently linked to the virion RNA and was described as essential for the infectivity of the RNA *(Green et al., 2000)*.

NLVs are approximately 27 to 35 nm in diameter and have an amorphous structure with a ragged edge (Figure 1) *(Liue et al., 1999)*.