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RESEARCH ARTICLE

Detection Norovirus and Rotavirus using Transmission Electronic Microscope

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Manuscript Info *Abstract*

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Transmission Electronic Microscope (TEM) continues to play a role in the understanding of viral gastroenteritis.
Ten samples proven to be positive *Norovirus* by real time PCR and two ELISA *Rotaviruse* positive sample were selected and examine under Transmission Electrone microscope magnified 80000-100000 time .we found that *Rotaviruse* easy detected because the virusesshedding in large quantities and has a circular shape size of 65-70 nm ranges.We found that *Norovirus* diagnosis still difficult and needs to concentrate the sample to increase the number of virus particles and its size ranged from approximately 38-40 nm.Because there is not a previous studyof Norovirusesin Iraq,wedecidedto takeamicrograph ofthevirus to find outsize and shape ofthevirus we previously isolated.

Introduction

It was only with the development of Transmission ElectronMicroscopy (TEM) that virus structure could be studied in detail(Finnerand White,1976),and TEM has had a significant impact on the development ofvirology ingeneral(Palmer and Martin, 1988;Marshall, 2005).The accumulated knowledge on virus ultrastructure formed the grounds for the first classification of viruses based onvirus intrinsic properties rather than on host characteristics and diseases produced by them(Finner et al., 1974).
The technique of transmission electron microscopy(TEM) was crucial in the discovery of the major viralcauses of gastroenteritis in humans (*Norovirus, Rotavirus,Astrovirus, Sapovirus, Adenovirus*) and subsequentlyplayed a valuable role in the detection of these viruses.In the 21st century, TEM continues to play a role in theunderstanding of viral gastroenteritis but chiefly in aresearch role rather than in a diagnostic context(Marshall, 2012).
TEM continues to play a valuableresearch role in the identification and characterization of viruses as associated with the gastrointestinal tract in both humans andanimals.(Marshall, 2012)

Negative staining TEM has proved to be a valuable approach forthe detection of fecal viruses in a variety of animals13-15 and thisapproach remains relevant today. Negative staining TEM was also used to identify *Rotavirus*in an English red squirrel19 and has also been used to identify*Norovirus*-like particles in monkeys (Wang et al., 2007).
Negative staining TEM is a valuable technique to monitorthe presence of recombinant Norovirus virus-like particles(Wang et al., 2007;Taube et al., 2005).
The method is also useful in evaluating the sensitivity andspecificity of commercial virus detection kits(Curry et al., 2006). The family Reoviridae includes the genus Rotavirus. Human and animal rotaviruses areknownStructure. Rotavirus was first identified by electron microscopy in 1973 from duodenal biopsies of children with diarrhea(Vale et al., 2010). Rotaviruses are non-enveloped viruses with icosahedral symmetry and a double capsid. Their electron microscopic appearance shows a 60-80nm wheel with radiating spokes (Latin, Rota = wheel) .The rotavirus genome contains double stranded (ds) RNA in 11 segments.(Narayan and Albercht, 2013).

Rotaviruses are found worldwide, causing major diarrhea-associated hospitalization and 600,000-850,000 deaths per year. Seroprevalence studies show that antibody is present in most infants by age 3 years.

In the U.S., .about 2.7 to 3.5 million people affected by *Rotavirus* infections each year and 20 - 40 deaths per year with 50-70,000 hospitalizations and 500,000 physician visits per year. In about 1-2.5% of case there is severe dehydration. In all, there are probably about 2.7 to 3.5 million people in the US affected by *Rotavirus* infections each year.*Noroviruse* belong to Human caliciviruses were first described in 1976. Norwalk virus was first detected in stools of patients with gastroenteritis (winter vomiting disease) in Norwalk, Ohio in 1968. They cause 40 per cent of non-bacterial gastroenteritis epidemics. Forty five per cent are food-borne and 52 percent are raw shell-fish associated. They tend to cause rapid (explosive) epidemics in places of close contact such as cruise ships, nursing homes, hospitals and camps(Eckardt& Baumgart, 2011).Noroviruses are found world-wide and cause more than 23 million cases of gastroenteritis very year in the United States and are non-enveloped, single stand, positive sense RNA viruses.. They appear round in shape with icosahedral symmetry and ragged surface contain a single capsid protein.. (Narayan and Albercht, 2013). The virus particles demonstrate an amorphous surface structure when visualized using electron microscopy and are between 27-38 nm in size.(Prasad et al., 2001).*Norovirus* affects around 267 million people and causes over 200,000 deaths each year; these deaths are usually in less developed countries and in the very young, elderly and immuno-suppressed.(Debbink et al., 2012;Marshall, 2011).

Material and Methods

About 20% of stool count for10 Noroviruse positive results by Real Time PCR positive,and only two ELISA Rotavirus were suspended phosphate – buffered saline (PBS).

Fecal suspension was clarified at 2000g for 10 min twice .For negative staining, a drop of about 10 µl of the virus suspension to be studied is applied to the hydrophobic surfaceof a parafilm square in a Petri dish.. A formvar-coated grid is floated onto this drop for one minuteto hold the small particles, with the formvarside of the grid in contact with the liquid. The excess liquid is removed from the grid by touching its border with a cutpiece of filter paper. The grid is immediately floated in a drop of 1.5% phosphotungsic acid, and1% aqueous uranyl acetate, For a better assessment of the samples, three gridsshould be prepared, each stained with one of these stains. After staining for one minute, the excess stain is removedwith filter paper and the grid left to dry for a few minutes, before insertion into the microscope column Transmission Electron Microscopy(Zeiss EM10 OCR from Germany)(Goldsmith and Miller, 2009).

Concentration methods for Norovirus detection usingAmmonium sulphate precipitation.

Fecal suspension 30% PBS were centrifuged at 25oo g for 2o min at 2 °C, then 5 ml of supernatant were added to equal volumeof 5 ml of pre-cooled saturated ammonium sulphate. After incubation on ice for 90 min, the mixtures were centrifuged at 2300 g for 30 min at 2 °C. The pooled precipitates were dissolved in 0.5 ml of 10 mM-tris-HC1 buffer, pH 7,2 o.1 M-NaC1, 1 mMEDTA (TNE buffer)(Tamura,1978).

Result and Discussion

The minimum virus concentration necessary for successful detection by TEM is about 10^6 particles per ml. This is a rather concentrated suspension and viruses are often present in diseased tissues and organic fluids at considerably lower concentrations. Another problem in fecal samples is the presence of other structures like bacteria and cell debris that may make virus identification difficult. In these cases, viruses must be separated from contaminating debris using centrifugation twice and concentrated of Noroviruse samples using ammonium sulphate because of the low shedding number of virus to get more than 10^6 particles which is a workable preparation for electron microscopy.Negative staining is a rapid procedure used for viewing small particles, such as viruses, in fluids.Phosphotungsicacid(PTA) sometimes outlines fringe/spikes on enveloped viruses better and does not cause positive staining. Uranyl acetate acts as a fixative as well as a stain, and viruses can be viewed intact long after the initial diagnosis. PTA actively degrades some viruses, and while immediate visualization is possible, viruses fall apart in just a few hours after stainingas mention in (Hayat and miller ,1990).samples selected from positive ELISA and PCR Rotaviruses and Noroviruses previously diagnosed .Molecular or immunological methods, such as Real time PCR and ELISA, are very effective in detecting known viruses and can be used to scrutinize a large amount of sample ,Diagnostic electron microscopy has two advantages over enzyme-linked immunosorbent assay and nucleic acid amplification tests. After a simple and fast negative stain preparation, the undirected, “open view” of electron microscopy allows rapid

morphologic identification and differential diagnosis of different agents contained in the specimenso Electron Microscopy remains the only method that can provide a quick assessment of all pathogens present in a sample, providing an "open view" Electron microscopy is still a major and indispensable inspection technique for Noroviruses, which cannot still be cultured as mention in (Gurry., et al 2006; Utagaw,2004; Vieler and Herbst,1995).TEM also play a valuable role in the identification and characterization of both Noroviruses and Rotaviruses in stool sample which collected from Iraqi children .as show in figure 1,2,and 3 the Noroviruse particle have icosahedral symmetry and ragged surface contain a single capsid protein 32-37nm. We found in figure 4 and5 the Rotaviruses particles have icosahedral symmetry and a double capsid with spikes 76 nm wheel with radiating spokes comparison with similar result of(Marshall 2005;Narayan and Albercht,2013)

Fig.1:- Noroviruse two particles stained with negative staining under TEM

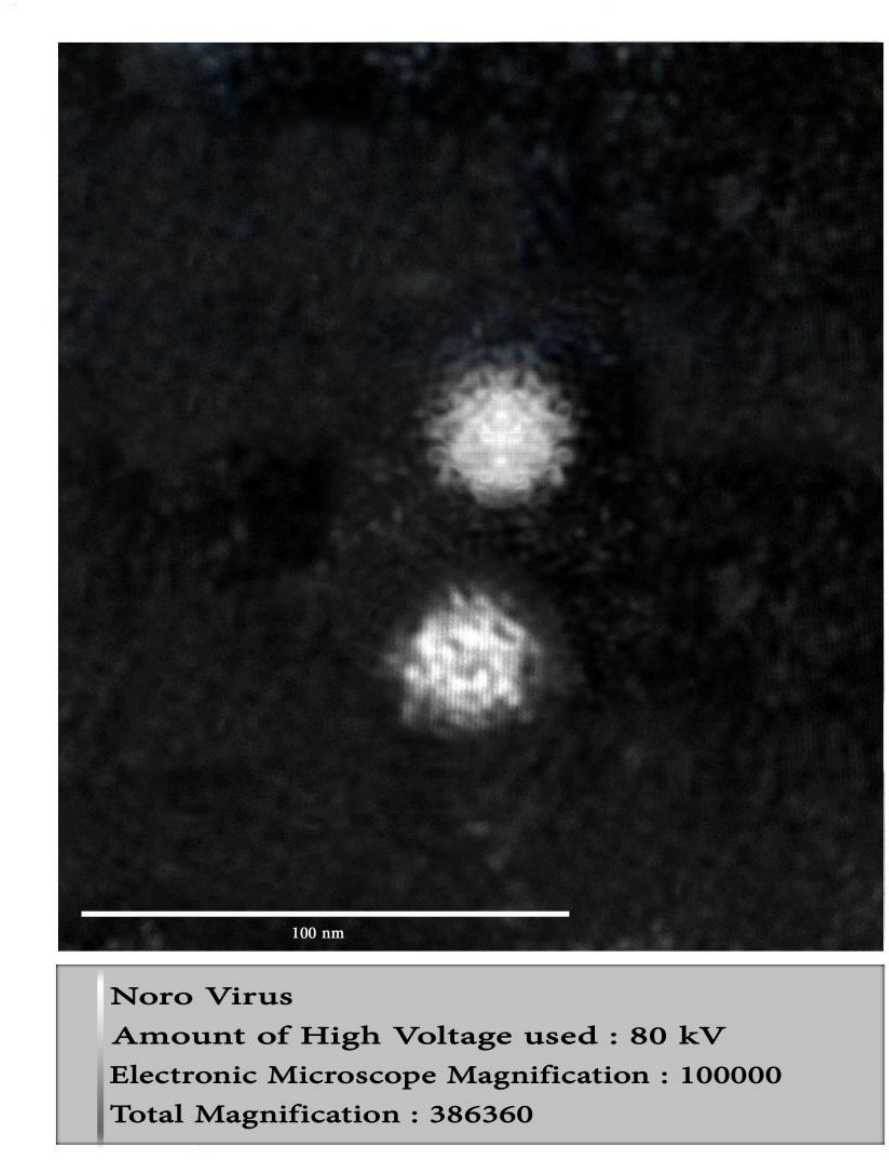


Fig. 2:- Noroviruse particles stained with negative staining under TEM

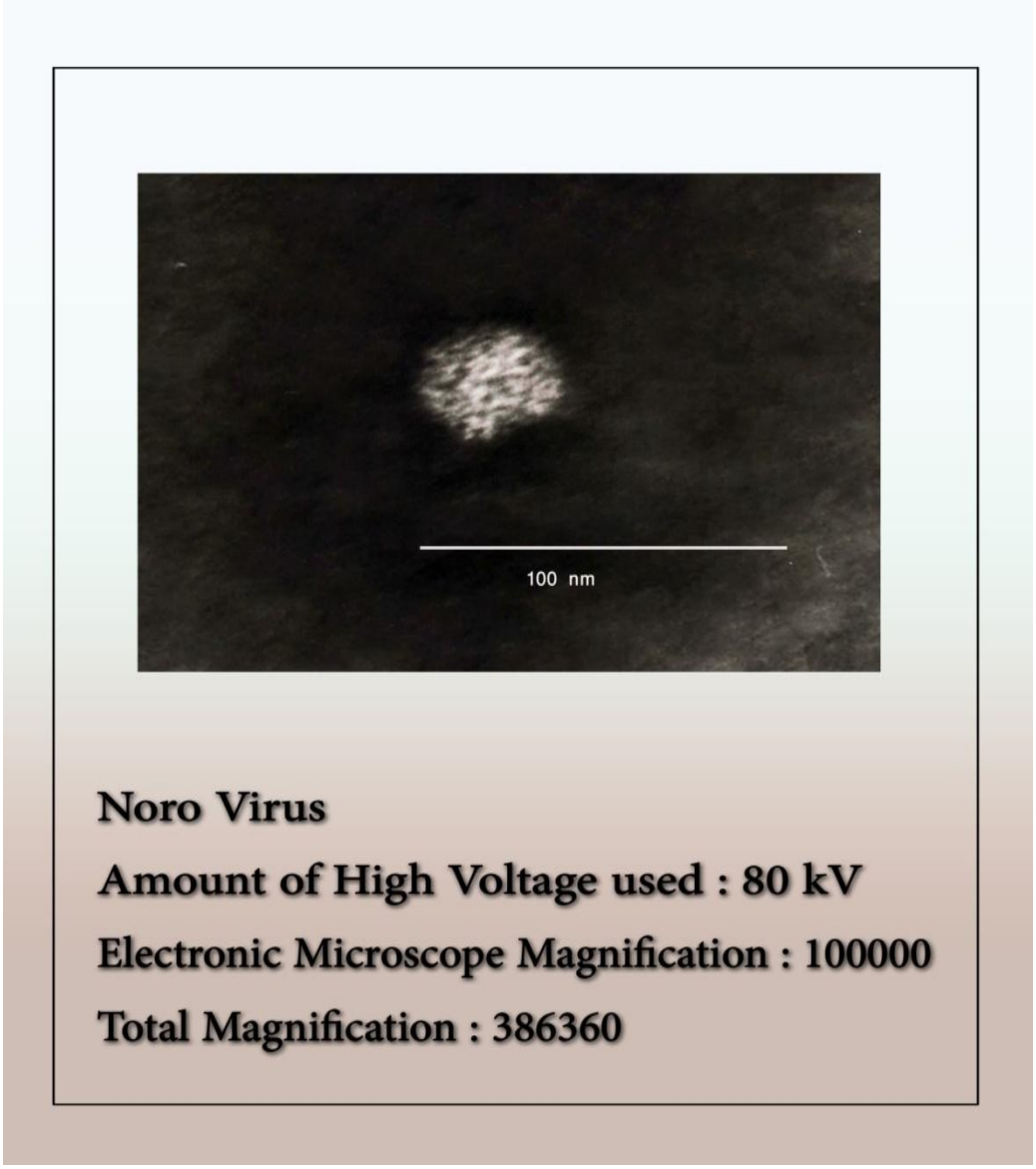
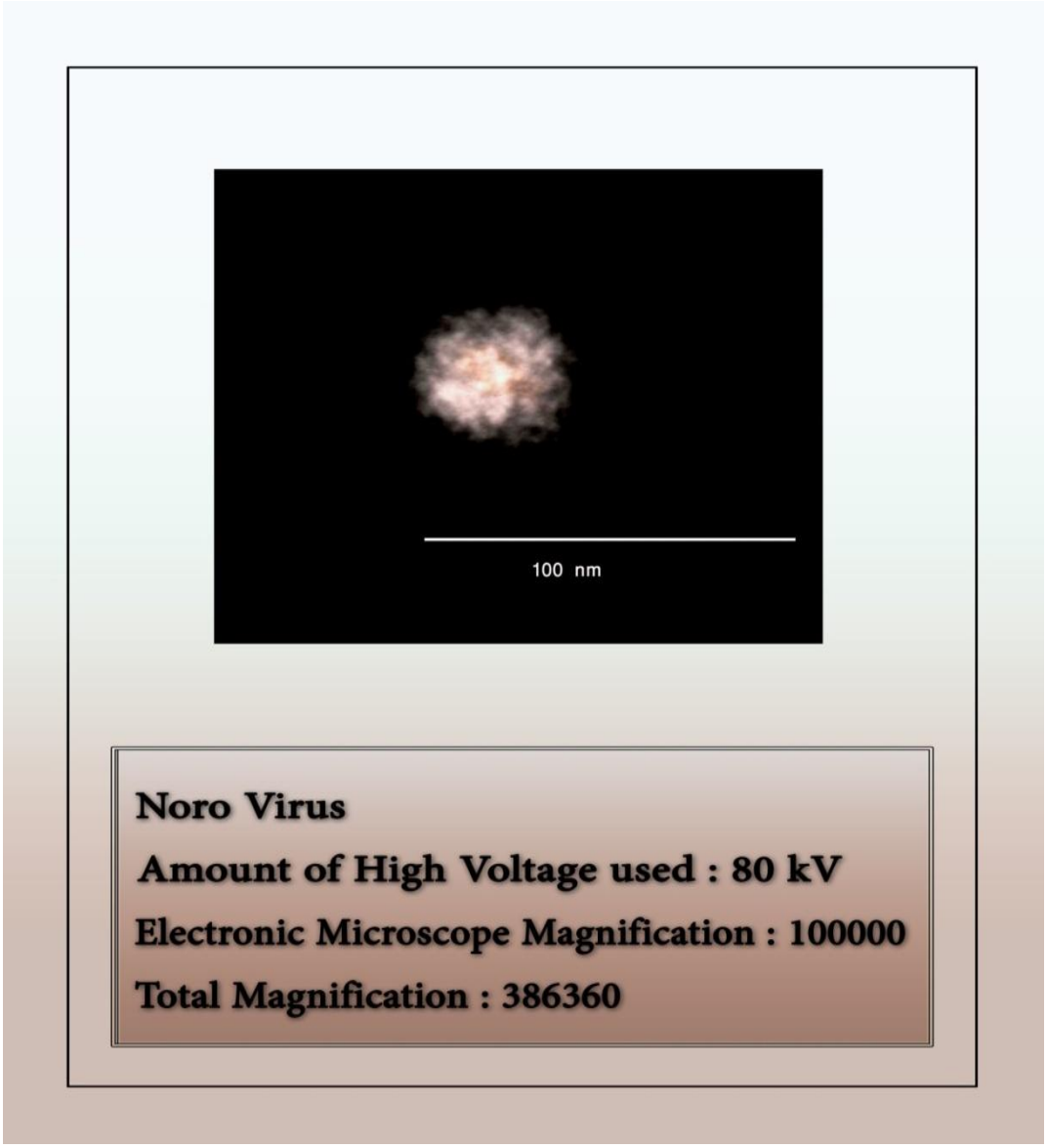


Fig. 3:- Noroviruse particles stained with negative staining under TEM.



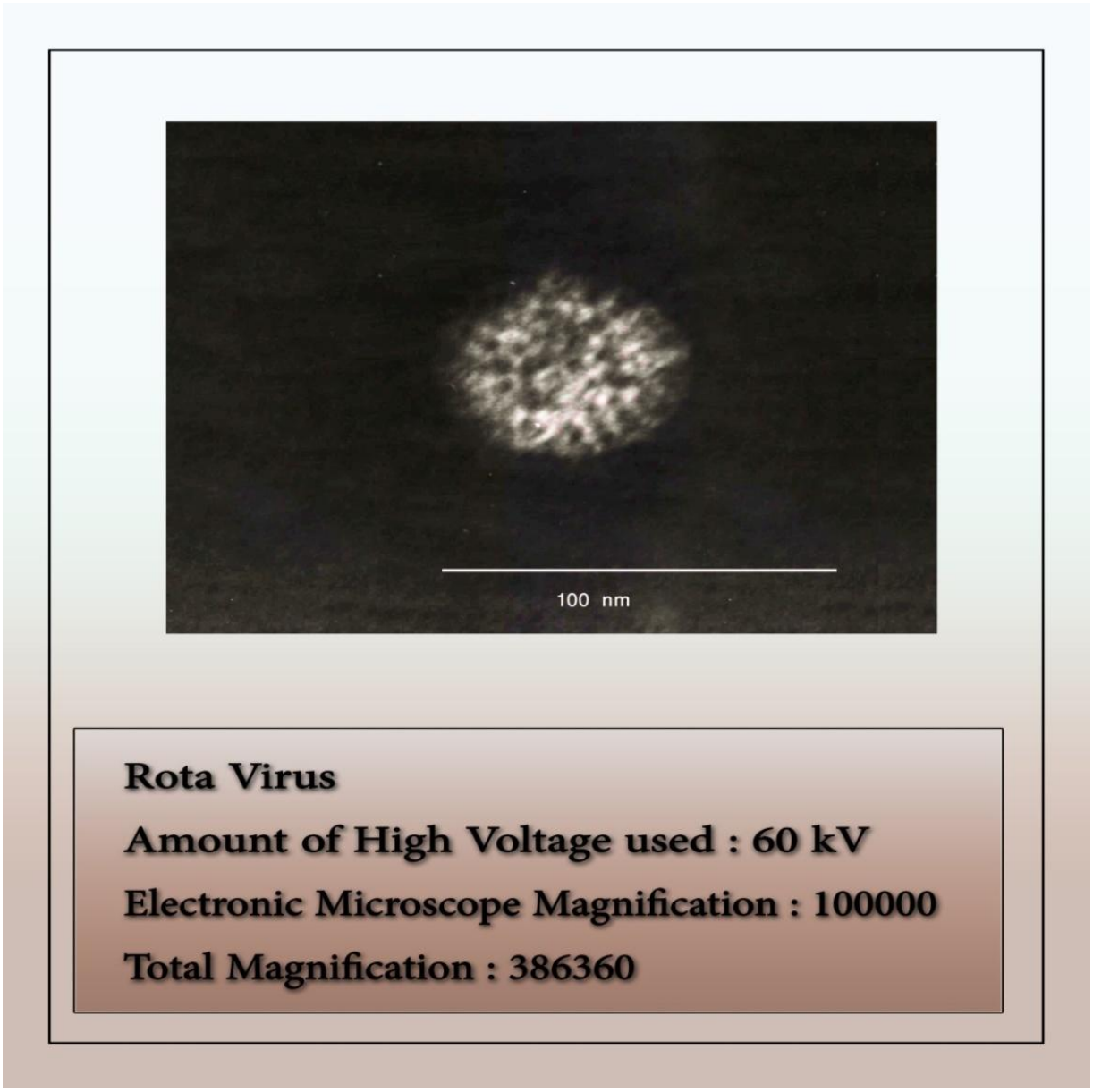


Fig. 4:- Rotaviruse particles stained with negative staining under TEM.

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