

DETECTION OF ROTAVIRUS AND ADENOVIRUS IN ELDERLY PATIENTS WITH AND WITHOUT ACUTE GASTROENTERITIS IN TAIWAN

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Abstract. Up till now, there has been no report on coinfection of adenovirus (ADV) and rotavirus (RV) in elderly patients with and without acute gastroenteritis (AGE) in Taiwan. We investigated the proportion, epidemiological features of concurrent infection by ADV and RV in stools of patients with and without AGE. Among 565 specimens, immunochromatographic assay detected 6% infected by at least one of the two enteric viruses and 26% coinfecting. Rate of coinfection between AGE and non-AGE patients is statistically significant ($p < 0.05$) during a season. To the best of our knowledge, this is the first report of RV and ADV coinfection in elderly patients with and without AGE in Taiwan. Systematic surveillance and evidence-based studies are required to determine the transmission pathway(s) and spread of ADV and RV in Taiwan.

Keywords: adenovirus, coinfection, elderly patient, gastroenteritis, rotavirus, Taiwan

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INTRODUCTION

Viral gastroenteritis is one of the most frequently encountered medical illnesses in children and adults worldwide (Eckardt and Baumgart, 2011). It is estimated that viral gastroenteritis is the cause of 30-40% of infectious cases in developed countries (Hodges and Gill, 2010). An estimated 211-375 million episodes of acute gastroenteritis (AGE) occur annually in the United States, the majority of which are considered to have a viral

etiology (Thielman and Guerrant, 2004; Ismaeel *et al*, 2007). There are more than 20 viruses known to cause AGE, among which adenovirus (ADV) and rotavirus (RV) are frequently associated (Chhabra *et al*, 2013; Li *et al*, 2014).

ADV belongs to the family Adenoviridae and usually affects children <2 years of age and symptomatic adult infections are uncommon (Blacklow and Greenberg, 1991). Although ADVs are primarily recognized as pathogens for respiratory, ocular or genitourinary infections, serotypes 31, 40 and 41 can cause AGE (Matsushima *et al*, 2011) and ADV-related cases occur more commonly in the second half of the year, especially in children (Filho *et al*, 2007). Based on antigenic and genetic properties, RV, a member of the family Reoviridae, is classified into five serological species (A to G) and two unassigned groups (F and G) according to the International Committee on Taxonomy of Viruses (Ramig *et al*, 2005). RVs have 11 segmented double stranded RNA genomes encoding six structural (VP1, VP2, VP3, VP4, VP6, and VP7) and six non-structural proteins (NSP1-NSP6). The inner most layer of the virus is composed of VP1, VP2 and VP3 proteins, the intermediate layer of VP6 protein and the outer-most shell of VP4 and VP7 proteins (Jain *et al*, 2014). RV is involved in epidemics and sporadic AGE cases across all age groups and cause more persistent infection in young children and the elderly than in other age groups (Markkula *et al*, 2017). Both ADV and RV are common in asymptomatic and AGE patients (Zhang *et al*, 2011).

Until now, there has been no report on co-prevalence of ADV and RV in elderly patients with and without AGE, especially in developing countries, Taiwan. Hence, this study sought to identify single and coinfection prevalence of ADV and RV in

elderly patients with and without AGE and to evaluate the effects of gender, age distribution and seasonal trends of gastrointestinal viral infections in Taiwan.

MATERIALS AND METHODS

Case definition and sample collection

AGE patients are defined as patients with clinical diarrhea (≥ 3 loose stools within a 24-hour period), which may be accompanied by abdominal pain, fever, nausea, and vomiting. The study was conducted from January 2016 to December 2016 at Wei-Gong Memorial Hospital, Miaoli County, Taiwan. Stools collected from 565 elderly patients (>60 years of age) with and without AGE were stored at -20°C before transfer on ice to the Department of Public Health, National Defense Medical Center, Miaoli County, where they were examined for the presence of ADV and RV before storage as 10% suspensions in a balanced salt solution at -70°C until used.

The study was approved by the Human Subject Research Ethics Committee, Wei-Gong Memorial Hospital (approval no. 105-I-002). Prior written informed consent was obtained from each participant.

Questionnaire

Participants were sent a follow-up questionnaire the week after enrolment to collect epidemiological features and clinical symptoms.

ADV and RV antigens detection

ADV and RV antigen were investigated in stool samples using an immunochromatographic RIDAQUICK RV/ADV Combi Test Kit (R-Biopharm AG, Darmstadt, Germany). Positive or negative RV and ADV diagnostic results were scored as either positive or negative by at least two independent investigators.

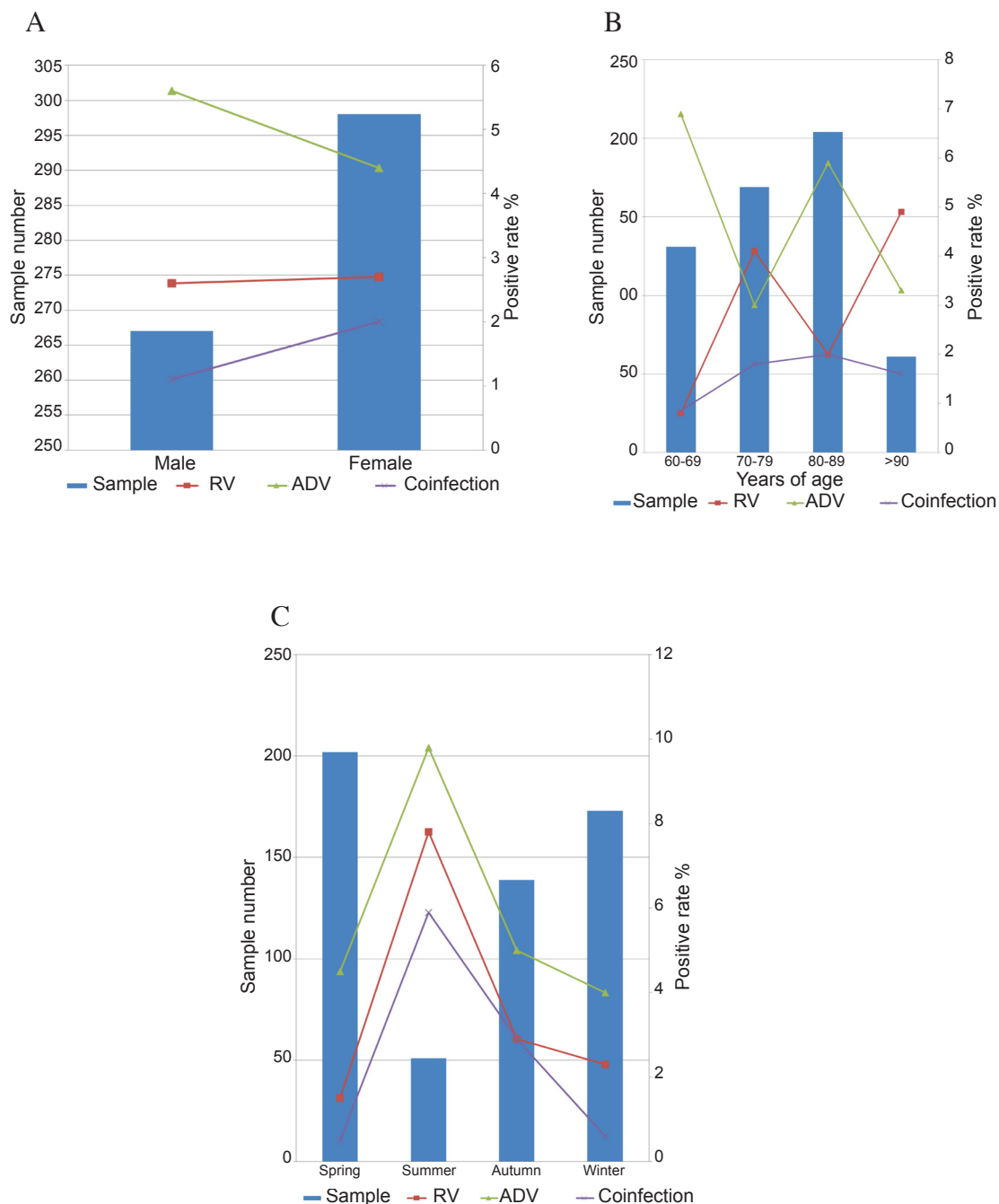


Fig 1–Distribution of adenovirus (ADV) and rotavirus (RV) and ADV infections in elderly patients with and without acute gastroenteritis at Wei-Gong Memorial Hospital, Miaoli county, Taiwan according to (A) gender, (B) age group and (C) season of the year. Virus was detected in stool sample using an immunochromatographic assay. Spring, March to May; summer, June to August; autumn, September to November; winter, December to February.

Table 1
Demographical and clinical data of elderly patients with and without acute gastroenteritis at Wei-Gong Memorial Hospital, Miaoli county, Taiwan.

Demographical data	Coinfection	Monoinfection	<i>p</i> -value ^a	OR	95% CI
Gender					
Male	33% (3/9)	64% (16/25)	0.122	-	-
Female	67% (6/9)	36% (9/25)	0.122	-	-
Age group					
60-69 years	11% (1/9)	32% (8/25)	0.247	-	-
70-79 years	33% (3/9)	24% (6/25)	0.588	-	-
80-90 years	44% (4/9)	32% (8/25)	0.505	-	-
>90 years	11% (1/9)	12% (3/25)	0.943	-	-
Spring	11% (1/9)	40% (10/25)	0.141	-	-
Summer	33% (3/9)	12% (3/25)	0.166	-	-
Autumn	44% (4/9)	12% (3/25)	0.016	8.800	1.502-51.556
Winter	11% (1/9)	36% (9/25)	0.187	-	-

^aSignificance at $p < 0.05$. OR, odds ratio; CI, confidence interval. Spring, March to May; summer, June to August; autumn, September to November; winter, December to February.

Statistical analysis

Samples were classified as coinfection or monoinfection for viral pathogens. For categorical variables, chi-square test was used to examine differences in proportion between groups. Fisher's exact test was used when the expected value for a cell was < 5 . A $p < 0.05$ is considered statistically significant.

RESULTS

The two target enteric viruses were detected in 34/565 (6%) cases, including 25 (4%) single viral infections and 9 (2%) viral coinfections. ADV and RV was identified in 28 (5%) and 15 (3%) samples, respectively.

Although there were more female patients ($n = 298$) ADV infection was less prevalent and coinfection more prevalent in females, and RV infection was of equal prevalence between the two gender groups (Fig 1A). The majority of

the patients were in 80-89 years group, but ADV infection was more prevalent in the 60-69 years group, RV infection in the >90 years group and coinfection equally (low) among all age groups (Fig 1B). ADV and RV infections were more prevalent in summer but that of coinfection sporadic throughout the four seasons without any obvious seasonal trend (Fig 1C). Demographic data analysis revealed a significantly higher prevalence of coinfection compared to monoinfection during the autumn season (Table 1).

DISCUSSION

To the best of our knowledge, this is first survey in Taiwan of ADV, RV and their coinfections from stool samples of elderly patients with and without AGE. Previous studies indicate ADV infection is associated with 1.5-5.4% of patients with AGE worldwide (Huhulescu *et al*, 2009; Kittigul *et al*, 2009), results in line with our

report, as were those of RV single infection in Singapore (1%) (Chau *et al*, 2016) and in UK (2.5%) (Borrows and Turner, 2014). Similar to the previous report of Wolak *et al* (2014) our study highlights the fecal-oral route of ADV and RV infection and transmission. However, no light was shed on the role of gender or age of elderly patients on ADV and RV infection.

Although the prevalence of coinfection was low, it is similar to that (1.9%) reported in Canada (Pang *et al*, 2014). Clinicians and epidemiologists should consider prevention and control of viral coinfections, and the monitoring of a broader range of enteric viruses among patients with or without AGE.

Climate change is likely to affect infectious disease burden from exposure to pathogens in certain environmental niches. ADV and RV monoinfections occurred during summer as were observed in previous studies from other countries (Mladenova *et al*, 2015). However, it is worth noting that coinfection is significantly higher than monoinfection in the autumn season.

A rapid immunochromatographic ADV/RV point-of-care detection assay was used with stool samples as the method is cheap, easy to perform, provides results within 5 minutes, and does not require the use of sophisticated equipment or skilled training. Such point-of-care methods have been deemed a powerful potential solution to fill the current diagnostics gap in decentralized and low-resource settings (Duchesne and Lacombe, 2018). The Rida Quick[®] ADV/RV detection method also is a useful alternative to ELISA and PCR assay (Artiran *et al*, 2017). Compared to PCR assay, specificity and sensitivity of the immunochromatographic method has been evaluated to be 95% and 100%,

respectively (Weitzel *et al*, 2007). This type of test may enhance early detection of ADV and RV and assist to guide clinical management.

There are two strengths in this study. First, all clinical departments of the study hospital adopted a uniform case definition in the enrollment of AGE patients and in the collection of stool samples, and a formatted questionnaire was used to record patients' information. Second, the two enteric viruses of interest were analyzed individually for each sample, allowing identification of mono- and coinfections. However, the study has a number of weaknesses. First, the total proportion of viral coinfection was likely underestimated as we did not analyze other pathogens causing AGE, and this needs to be corrected in future studies. Second, lack of other important virological information, such as nucleic acid sequence, viral concentration and phylogenetic analysis, made it difficult to elucidate the evolution of ADV and RV in greater detail.

In conclusion, this study highlights ADV and RV coinfection in elderly patients with and without AGE in Taiwan. This study also indicates the significance of seasons on the prevalence of mono- and coinfections. A simultaneous investigation of enteric viruses coinfection and viral quantity in patients with gastrointestinal symptoms should prove useful in future studies to better identify viral agents responsible for AGE and to understand the potential route of transmission and interaction of these enteric viruses.

ACKNOWLEDGEMENTS

The study was supported by the Wei-Gong Memorial Hospital (grant no. WG 105-I-002). The authors are grateful to all

colleagues in the Department of Laboratory in Wei-Gong Memorial Hospital for their help in the collection of specimens. We thank Szu-Ying Wang and Su-Chuen Lin, for assistance in data collection and entry, and Wen-Ho Chang and Hung-Yin Chao for technical assistance.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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