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PROJECT TITLE: Viral Co-Morbidity as a Factor Effecting Acute Respiratory Distress Syndrome in H1N1 Influenza Cases Requiring Intensive Care Treatment

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SUMMARY:

The 2009 H1N1 pandemic caused severe disease around the world. H1N1 caused disease in younger, healthier people than typically observed for seasonal influenza. Another difference between H1N1 and the seasonal variant is that H1N1-infected patients presented with high rates of diarrhea. It is currently unclear whether the diarrhea is caused by the H1N1 virus directly, or by some secondary pathogen. Co-infection between H1N1 and a second pathogen could lead to a more severe clinical course due to a synergistic pathologic effect, leading to worse outcomes.

Screening of fecal and respiratory samples of ICU patients with laboratory-confirmed H1N1 showed that 9 patients out of 15 (60%) had a positive result for adenovirus. ICU patients were divided into an H1N1 alone group or an H1N1/adenovirus co-infection group, and after a retrospective chart review, these groups were compared with respect to a number of characteristics. In addition, 2 pairs of matched acute and convalescent sera were examined by immunoelectron microscopy for seroconversion with respect to adenovirus, and both samples showed a significant increase in titre.

The two groups were compared in terms of demographics, complaints of diarrhea, nausea, and vomiting on initial presentation, course in hospital, time course of illness, co-morbidities, complications, and mortality. There were no significant differences between the groups. However, given the limited availability of fecal, respiratory, and blood samples for adenovirus testing, we suspect that a number of adenovirus cases were missed. Identification of these cases could potentially alter the results of our analysis.

In conclusion, patients with H1N1 alone and H1N1/adenovirus co-infection are similar in demographics, clinical course, and outcomes.

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INTRODUCTION

The 2009 H1N1 swine-origin influenza virus that first emerged in North America spread rapidly around the world. This virus differed clinically from its seasonal counterpart in that it caused severe disease in younger, healthier people rather than affecting the older population more commonly considered to be at high risk for infections of this type. In addition, the proportion of cases requiring intensive care support was higher than typically observed for seasonal influenza. One further difference is the unusually high proportion of patients that exhibited gastrointestinal symptoms at the onset of their disease, noted at up to and over 25% (1-6).

It is currently unclear whether the gastrointestinal symptoms are directly caused by the H1N1 virus, or if they may be related to a secondary pathogen. To date, treatment of the disease has generally been based on the former assumption. To determine which of these possibilities is the case, it is necessary to screen fecal material for the presence of a second pathogen associated with the acute intestinal diarrhea (AcID). If present, it would also be necessary to completely elucidate its effects on other organ systems. The presence of a secondary pathogen in the respiratory system could result in a synergistic pathologic effect, resulting in disease of a higher severity with additional clinical features that could impact patient outcomes (7,8). In the case that a secondary pathogen is present, it would also be important to determine the mechanism by which it came to infect the host. It has been shown (9,10) that viral respiratory pathogens can lie dormant in tonsilar lymphatic tissue, with a possibility for reactivation upon the introduction of some unknown trigger. Another possibility would be that the secondary pathogen is simply acquired in the community, especially in the setting of an outbreak. Based on our screening of fecal and respiratory samples for viral AcID-causing pathogens and on the results of this hypothesis generation study, we have developed the hypothesis that upon infection with H1N1 influenza, infection with a second viral respiratory pathogen occurs in some patients, resulting in co-infection, leading to higher morbidity and worse outcomes.

MATERIALS AND METHODS

A retrospective review was performed on charts of adult patients with nucleic acid detection (NAD) confirmed H1N1 infection from the intensive care units (ICUs), wards, and Emergency Rooms (ERs) of Health Sciences Centre (HSC) and St. Boniface General Hospital (SBGH) in Winnipeg, Manitoba, Canada. H1N1 NAD testing was performed on respiratory and stool specimens by Cadham Provincial Laboratory (CPL) and the National Microbiology Laboratories (NML). From the charts, data was collected concerning demographics (age, sex, and postal code), clinical respiratory symptoms, clinical gastrointestinal symptoms (nausea, vomiting, and diarrhea), diagnostic test results, length of ICU/hospital admissions, co-morbidities, complications during the course in hospital, and past medical history. Clinical severity of each case was inferred by length of ICU and hospital stay.

Fecal samples were collected from ICU patients on different days during their period of hospitalization. Patients for whom samples were available had between one and four samples each. Screening of fecal samples from ICU patients was conducted by the Viral Gastroenteritis Study Group (VGSG, University of Manitoba) for conventional viral gastroenteritis pathogens (adenovirus, astrovirus, human calicivirus,

hepatitis A virus, rotavirus, reovirus) by NAD. Briefly, viral Nucleic Acids (vNA) were purified using the QiaAmp viral RNA mini kit (Qiagen, ON) in accordance with the manufacturer's instructions and vNAs were eluted in Elution Buffer (Qiagen) with 0.5U RNasin/µl. Samples were tested in duplicate. vNAs were extracted from serum and respiratory samples by the NML for all ICU cases for which samples had been collected, and provided for testing by the VGSG. These samples were tested for the presence of adenovirus genome sequences by NAD assay. NAD assays were conducted using primers and conditions in Table 1. Reverse transcription was conducted using SuperScript II reverse transcriptase(Invitrogen, ON) and polymerase chain reaction assay conducted using Expand Long Template DNA Polymerase(Roche, QC). Products were separated in 2% Agarose gels in 0.5% Tris Borate EDTA buffer (TBE), amplicons of the appropriate target size were extracted and the sequence determined using an ABI 3100 Genetic Sequencer (Perkin Elmer, ON). Where molecular tests identified a pathogen they also provided genome sequence for that pathogen. Genome sequences were submitted to GenBank (National Center for BioInformatics) to confirm the identity of the pathogens. Sequences for pathogens identified were aligned and phylogenetic trees established using the program Clustal W to determine how related the different isolates are, both to each other and to those prevalent in Manitoba during the time of the pandemic. Blood samples from the ICU patients were examined by immunoelectron microscopy to test for seroconversion with respect to adenovirus serotypes 1 and 41 (HAd1, HAd41). Briefly, stock virus samples of HAd1 and HAd41 were provided by Virus Detection, CPL, and quantified by direct particle count using the Beckman Airfuge with EM-90 rotor (Beckman, CA), and optimal stock virus dilutions were determined. Serial 2-fold dilutions were prepared for acute and convalescent sera from two cases, mixed with stock virus, and allowed to incubate at 37°C for 90 minutes. Reaction products were stabilized by the addition of 5% Glutaraldehyde to a final concentration of 0.2%, incubated for 10 minutes at 20°C, and then the addition of Glycine to limit nonspecific crosslinking and aggregation of viral particles. The reactions were deposited directly to formvar coated copper grids using the Beckman Airfuge as above (11). A significant increase in antibody titre was defined as a change of at least four times.

Descriptive statistics were performed by means of frequency analysis (percentages) for categorical variables, and using means and standard deviations (SD) for continuous variables. Two types of statistical tests were used to compare the group with H1N1 infection alone to the group with H1N1 and adenovirus co-infection. A one-tailed Fisher exact test was performed to detect differences in co-morbidities, rates of complications, and some categorical demographic variables. A one-tailed unpaired two-sample *t* test was used to compare continuous variables including age, length of ICU and hospital stay, and diagnostic lab test results.

RESULTS

There were a total of 331 suspected adult H1N1 cases hospitalized in both HSC and SBGH, of which 106 tested positive for H1N1. Where the required information to locate the chart was provided by NML/CPL, patient charts were reviewed. From HSC, charts of 29 ICU patients, 10 ward patients and 13 ER patients were examined. 29 ICU patient charts from SBGH have been analyzed, as well as 9 ward charts and 28 ER charts. Charts of patients with a positive H1N1 PCR result were selected for analysis.

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Viral Testing

28 fecal samples from 11 ICU patients from HSC and SBGH were screened for viral pathogens that commonly cause AcID. Seven of the 11 patients (63%) had at least one sample that produced a positive stool PCR test result for adenovirus. None of the samples tested showed any evidence of the presence of astrovirus, human calicivirus, hepatitis A virus, reovirus, or rotavirus. Of the 7 patients that had a positive result for adenovirus in their stool, 2 had at least one sample that produced a positive result for H1N1 by PCR. The samples that produced a positive result were collected on ICU day 4 for one patient, with a day 3 sample being negative, and on ICU day 5 for the other patient, with the test results from a day 3 sample being rejected for failure to confirm the quality assurance test for extraction of vNAs. Both of these patients also had a positive H1N1 PCR result from their respiratory secretions.

27 samples of respiratory secretions from 15 ICU patients from HSC and SBGH were subsequently tested for the presence of adenovirus. Six of these patients (40%) had at least one sample that produced a positive PCR test result.

In total, there were 11 patients out of a total of 17 (65%) that had at least one sample with a positive PCR test result for adenovirus in either respiratory or stool samples. Of this group of 17 patients, two had a negative test result for H1N1 and a positive test result for adenovirus. These two cases were excluded from the analysis. Overall, 9 patients (60%) of the 15 included in the analysis had a positive result for H1N1 and adenovirus, and thus comprise the co-infection group. The remaining 6 H1N1 positive cases were placed in the H1N1 infection alone group for subsequent analysis.

Chart Analysis

As all of the charts of patients with a positive test for adenovirus were from the ICU, non-ICU charts were excluded from this analysis. Charts from ICU patients who did not test positive for H1N1 were also excluded from the analysis. Therefore, a total of 33 charts from patients in the ICUs at HSC and SBGH were analyzed. As above, 9 of these charts came from patients that had co-infection of H1N1 and adenovirus (heretofore referred to as the co-infection group), and the remaining 24 charts came from ICU patients who only had a positive test for H1N1 (heretofore referred to as the H1N1 group). Not all of the patients in the H1N1 group had samples available for testing for adenovirus.

Immunoassay for Seroconversion

For only 2 cases, acute and convalescent sera were made available for testing for seroconversion with respect to adenovirus serotypes 1 and 41. Both cases showed a significant increase in antibody titre using cultured adenovirus serotype 1 (Table 2). However, both of these serum samples came from the two patients that were excluded from the analysis above due to a negative H1N1 test result.

Characteristics of Patients

In the H1N1 group, the mean age was 38.9 years (SD ± 13.1); 14 patients were female (58%) and the most common postal code region was R0B (7 cases, 29%). The R0B postal code corresponds to a region in Manitoba, Canada that contains St. Theresa Point, the location of a major H1N1 outbreak in the

summer of 2009. 9 patients (37.5%) reported having contact with someone who had a flu-like illness. 5 patients (21%) complained of diarrhea on presentation to the hospital, and 7 (29%) complained of nausea and vomiting. Two cases had both diarrhea and nausea/vomiting, and are included in both counts above. Diarrhea began an average of 3 days (SD ± 0.8) prior to initial presentation to hospital, and the nausea/vomiting began an average of 3 days (SD ± 2.3) prior to initial presentation. 18 cases (75%) suffered from diarrhea at some point during their hospital admission. On average, when gastrointestinal symptoms (nausea/vomiting or diarrhea) were present on initial presentation, they started 3 days after flu symptoms (SD ± 5.8). The average time between onset of flu symptoms and admission to hospital was 5.4 days (SD ± 8.7). 96% of patients were treated with oseltamivir during their hospital stay. The average length of ICU stay was 21 days (SD ± 15.9) and the average length of stay in hospital (including time in ICU, Ward, and ER where applicable) was 44 days (SD ± 34.8). 2 patients (8%) died during their hospital stay.

The co-infection group had a mean age of 36.8 years (SD 11.8); 7 patients were female (77.8%) and the 2 most common postal codes were R0B (2 cases, 22%) and R0E (2 cases, 22%). The R0E postal code region consists of the area just north of Winnipeg, near the south part of Lake Winnipeg. One patient (11%) reported having a sick contact with flu-like illness. Two patients (22%) complained of diarrhea on initial presentation, and 3 (33%) complained of nausea and vomiting. 1 case had both diarrhea and nausea/vomiting and is included in both counts above. The diarrhea began an average of 5 days (SD 0) prior to initial presentation, while the nausea/vomiting began an average of 2.5 days (SD ± 1.5) before initial presentation. All 9 cases had diarrhea at some point during their admission to hospital. When gastrointestinal symptoms were present at initial presentation, they began an average of 2 days (SD ± 2.8) after flu symptoms. The mean amount of time between onset of flu symptoms and admission to hospital for this group was 5.1 days (SD ± 2.3). 100% of patients in this group were treated with oseltamivir. The average length of ICU stay was 28 days (SD ± 18.4), and the average length of hospital stay was 46 days (SD ± 26.2). 2 patients in this group (22%) died from their illness.

Comparison of co-infection and H1N1 groups

Further to the description of each group individually, it was necessary to compare the groups in a number of aspects. In terms of demographic characteristics (Table 3), there appeared to be no significant differences between the mean age, nor sex distribution of the two groups. Although the R0B postal code was more common in both groups, it was not significantly more likely to encounter a co-infection case from that region. Analysis of clinical information yielded that there were no significant differences between the two groups in rates of diarrhea on admission to hospital, nor in the rates of diarrhea during the hospital stay. As the R0B postal code was more common, the average rate of diarrhea from this postal code was compared to the average rate from all other postal codes, but there was no significant difference. The average length of ICU stay for both groups was compared, however the difference between the means was not statistically significantly different ($P = 0.16$). Overall length of hospital stay was not significantly different between the two groups. In addition, there was no significant difference in mortality between the two groups.

The two groups were also compared in terms of the presence of co-morbidities (Table 4), including pregnancy, diabetes mellitus, cardiovascular disease, obesity, renal disease, pulmonary disease, smoking history, and substance abuse. None of the co-morbidities tested were significantly associated with an increased likelihood in the co-infection group. However, a higher proportion of asthma in the co-infection group neared significance (P = 0.11).

Rates of complications and organ failure (Table 5) among the ICU patients were also recorded and compared between the two cohorts. Complications tested included acute kidney injury, leukopenia, circulatory collapse, cardiac arrest, need for tracheostomy, ventilator-associated pneumonia, and MRSA/VRE nosocomial infection. Rates of these complications were not found to be statistically significantly different between the two groups.

DISCUSSION

The 2009 H1N1 pandemic caused severe disease in a large number of people around the world. According to a previous study (12), the pandemic presented with severe initial disease (mean Acute Physiology and Chronic Health Evaluation II [APACHE II] score on presentation of 19.7 [SD ± 8.7]), a high requirement for mechanical ventilation (81% of patients on the first day in ICU), high rates of complications (24.4% acquired secondary bacterial pneumonia, 32.7% required vasopressors on day 1, 7.1% had acute kidney injury on day 1), organ failure (mean Sequential Organ Failure Assessment [SOFA] score of 6.8 on day 1), and most importantly, mortality (17.3%). Our findings corroborate the findings in this previous study. In addition to the unusual severity presented by H1N1, an unusual preponderance of gastrointestinal symptoms (particularly diarrhea) was observed in a number of studies, with rates such as 25% in hospitalized adult patients in the USA (1), 25% in hospitalized and non-hospitalized patients in the USA (3), 29.4% of hospitalized and non-hospitalized patients in Chile (2), and 29% of a sample of hospitalized patients in New York (5). These findings are in agreement with the rates of diarrhea found in both H1N1 (21%) and co-infection (22%) groups. The mechanism of the diarrhea associated with H1N1 infection is unclear. In testing a number of samples from ICU patients, it was discovered that a high proportion (52%) of the patients for whom we had samples had a positive result for adenovirus in addition to a positive result for H1N1. Two patients from the co-infection group had H1N1 positive PCR results from their stools. These results came from samples that were collected a few days into the hospital stay, with samples that were collected prior being either rejected or negative. Being a respiratory pathogen, it is unlikely that H1N1 was actively causing infection in the gut, and it is possible that the H1N1 was present in the fecal material because of patients swallowing respiratory secretions. Of the patients who had a positive test result for adenovirus, 7 (41%) had adenovirus in their stool and 6 (35%) had adenovirus in their respiratory secretions. This unexpected result suggests that a second pathogen was present in a fraction of the total number of H1N1 ICU cases. Further to this, acute and convalescent sera for 2 cases were available for testing and both showed a significant increase in adenovirus serotype 1-reactive antibody titre. These results indicate that these patients had been infected with adenovirus, and not merely colonized. Although these results came from the 2 patients who had negative H1N1 PCR results, these patients had positive adenovirus stool PCR results. Therefore, it is likely that a positive adenovirus PCR of the stool or respiratory secretion is linked to these immunological findings.

These results lead to new questions: How did the patients acquire a concurrent adenovirus infection? and What is the clinical significance of these findings? It is unclear how patients would have acquired an adenovirus infection. It has been demonstrated that it is possible for dormant adenovirus to reactivate from lymphatic tissue in response to some unknown trigger (9). Another possibility is that patients could have acquired the second virus from the community. However, at that particular time there were no known outbreaks of adenovirus in Manitoba, Canada at the time of the pandemic, so this possibility is unlikely. As to the clinical significance, it is conceivable that co-infection with adenovirus could lead to a worsening of respiratory symptoms, or gastrointestinal symptoms, depending on the serotype of the virus.

However, in comparing the co-infection group to the H1N1 group, the data suggest that we cannot prove that there is a difference between these two cohorts. The demographic data demonstrate that there is not a specific subset of the population that is more likely to be infected with both viruses. The demographics of the co-infection cohort are similar to those previously described for H1N1 patients in other studies. Although a large number of cases in both groups came from a single postal code region (R0B), patients with co-infection were not more likely to come from that postal code region. As well, there appear to be no significant differences between the groups in terms of types of co-morbidities encountered in both groups. However, with the result of asthma being more common in the co-infection group nearing significance, this specific co-morbidity may warrant further investigation, especially in the context of co-infection between H1N1 and a respiratory strain of adenovirus. It is conceivable that asthma could increase a patient's susceptibility to co-infection with the two viruses. Also, rates of in-hospital complications were not significantly different between the two groups. This may suggest that any complications encountered were due solely to H1N1, with adenovirus playing no significant part. As previously noted, mortality rates were not significantly different, suggesting that even if there had been some clinical effect of a secondary pathogen, it had no effect on survival. Overall, the data obtained suggest that positivity for adenovirus in addition to H1N1 infection did not have a significant impact on the clinical course of ICU patients.

This study had some limitations. First, being a retrospective chart review, there were some inherent difficulties including incomplete documentation (and some missing charts), difficulty interpreting some illegible information, inconsistency of information recorded by medical professionals, variable quality of recorded information, and difficulty interpreting cause and effect. In addition, the number of stool, respiratory, and blood samples made available from ICU patients for testing for adenovirus was very limited, and this resulted in a very small co-infection group, limiting the power of the study to find differences between the two groups. As well, only 9 patients had both stool and respiratory samples available for testing, with the remainder having either one or the other sample type. These two limitations could be corrected with a prospective study, but there was no second wave of patients from which to collect information and samples. In addition, because stool, respiratory, and blood samples were not obtained for adenovirus testing from all ICU patients studied, it is not possible to say with certainty whether or not these patients were concurrently infected with adenovirus. Again, this was a limitation of the retrospective format of this hypothesis generation study. However, given the high rate (60%) of adenovirus in patients for whom samples were available for testing, it is reasonable to expect

that there may have been additional cases of adenovirus that were missed due to a lack of direct testing. In future outbreaks such as these, we suggest that multiple stool samples, as well as acute and convalescent sera be collected from all patients so that viral studies may be conducted.

In conclusion, our findings suggest that patients with positive test results for both pandemic H1N1 and adenovirus are similar in demographics, and undergo a similar clinical course to patients infected with H1N1 alone.

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Table 1. Primers and probes for detection of gastroenteric viruses from H1N1 cases

Virus	Primer/ Probe	Sense	Location[a]	Sequence[b]	Amplicon size (bp)	Annealing Temperature (C)
Adenovirus[e]	nehexAA	1893	18937-18960	ΓXX AXX ΓAΓ AXΓ TAX TTX AΓX XTΓ	142	55
	nehexAA	1905	19051-19079	TTΓ TAX ΓAΓ TAX ΓXΓ ΓTA TXX TXΓ XΓΓ TX		
Astrovirus[c]	Mon 348	-	1450-1470	AXA TΓT ΓXT ΓXT ΓTT AXT ATΓ		
	Mon 340	+	1182-1203	XΓT XAT TAT TTΓ TTΓ TXA TAX T	289	50
Norovirus[d]	*Assay 1 – GGI*					
	SR33	-	4856-4876	TΓT XAX ΓAT XTX ATX ATX AXX		
	SR48	+	4754-4773	TΓΓ AAT TXX ATX ΓXX XAX TΓΓ		
	SR50	+	4754-4773	ΓTΓ AAX AΓX ATA AAT XAX TΓΓ		
	SR52	+	4754-4773	ΓTΓ AAX AΓT ATA AAX XAX TΓΓ	123	55
	Assay 2 – GGII					
			4754-4773	ΓTΓ AAX AΓT ATA AAX XAT TΓΓ	123	55
	Assay 3 – GGI & GGII Confirmatory					
	MON431	+	5285-5305	TΓΓ AXI AΓP ΓΓI XXΨ AAΨ XA		
	MON432	+	5285-5305	TΓΓ AXI XΓΨ ΓΓI XXΨ AAΨ XA		
	MON433	-	5093-5112	ΓAA ΨXT XAT XXA ΨXT ΓAA XAT	213	50
	MON434	-	5093-5112	ΓAA ΣXΓ XAT XXA PXΓ ΓAA XAT	213	50
Hepatitis A[f]	HAV 3	-	2204-2229	XXT XXA ΓAA TXA TXT XXA AXT TΓT T		
	HAV 1	+	2022-2048	AXA ΓΓT ATA XAA AΓT XAΓ XAX ATX AΓ	208	54
Reovirus	Reo L1-2	-	2248-2230	AXX ΓXT ΓXX AXT ΓTA AXX XAT XA		
	Reo L1-1	+	1622-1643	ΓΓΓ ΓΓT TXX TΓX ATX XAT TΓT AAA	627	60
Rotavirus	VP6-R	-	1106-1126	ΓTX XAA TTX ATN XXT ΓΓT ΓΓ		
	VP6-F	+	747-766	ΓAX ΓΓς ΓXP AXT AXA TΓΓ T	379	50

a. Location of sequence in viral genome.
b. Abbreviations: D = A,T,G; I = Inosine; N = A,C,G,T; R= A,G; W = A,T; Y = C,T.; V=A+C+G
c. Belliot et al, 1997%%321(Belliot et al., 1997)%%.
d. LeGuyader et al, 1996%%214(Le Guyader et al., 1996)%%.
e. Puig et al, 1994%%142(Puig et al., 1994)%%.
f. Hep A.

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Table 2. Results of immunoelectron microscope study testing for seroconversion with respect to adenovirus serotype 1 in matched acute and convalescent sera

	Case A	**Case B**
Sample 1, titre	<1:25	1:50
Sample 2, titre	1:200	1:800
Change in titre	+8x	+16x

Table 3. Comparison of characteristics of H1N1 and co-infection groups

	H1N1 group (n = 24)	**Co-infection group (n = 9)**	***P* value**
Age, mean (SD), years	38.9 (13.3)	36.8 (11.8)	0.34
Female sex, No. (%)	14 (58)	7 (78)	0.27
Presenting complaints/lab results			
Diarrhea, No. (%)	5 (21)	2 (22)	0.64
Nausea/Vomiting, No. (%)	7 (29)	3 (33)	0.57
Na, mmol/L (SD)	139 (5.3)	138 (3.5)	0.20
K, mmol/L (SD)	3.7 (0.5)	4.4 (0.9)	0.007
Cl, mmol/L (SD)	104 (7.3)	102 (5.9)	0.28
Course in hospital			
Diarrhea, No. (%)	18 (75)	9 (100)	0.64
Treatment with oseltamivir, No. (%)	23 (96)	9 (100)	-
Time course of illness, mean (SD), days			
Flu symptoms until hospital admission	5.4 (8.7)	5.1 (2.3)	-
Flu symptoms until GI symptoms	2.8 (5.8)	2 (2.8)	-
Length of ICU stay	21.3 (15.9)	27.9 (18.4)	0.16
Length of hospital stay	43.6 (34.8)	45.8 (26.2)	0.44
Mortality, No. (%)	2 (8)	2 (22)	0.29

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Table 4. Comparison of pre-existing co-morbidities of H1N1 and co-infection groups

	H1N1 group (n = 24)	**Co-infection group (n = 9)**	***P* value**
Pregnancy, No. (%)	4 (17)	1 (11)	0.58
Type 1 Diabetes Mellitus, No. (%)	0 (0)	1 (11)	0.27
Type 2 Diabetes Mellitus, No. (%)	8 (33)	3 (33)	0.46
Hypertension, No. (%)	6 (25)	3 (33)	0.47
Congestive Heart Failure, No. (%)	2 (8)	0 (0)	0.52
Cerebrovascular Accident, No. (%)	2 (8)	1 (11)	0.63
Obesity, No. (%)	11 (46)	3 (33)	0.40
Chronic Kidney Disease*, No. (%)	2 (8)	1 (11)	0.63
End-Stage Renal Disease, No. (%)	2 (8)	2 (22)	0.29
Psychiatric Disorder**, No. (%)	3 (12.5)	2 (22)	0.42
Substance Abuse, No. (%)	2 (8)	1 (11)	0.63
Ever smoker, No. (%)	5 (21)	2 (22)	0.64
Restrictive Lung Disease, No. (%)	2 (8)	1 (11)	0.63
Asthma, No. (%)	2 (8)	3 (33)	0.11

*Excluding End-Stage Renal Disease

**Includes depression, anxiety disorders, schizoaffective disorder

Table 5. Comparison of rates of complications of H1N1 and co-infection groups

	H1N1 group (n = 24)	**Co-infection group (n = 9)**	***P* value**
Acute Kidney Injury, No. (%)	7 (29)	5 (56)	0.16
Ventilator-Associated Pneumonia, No. (%)	6 (25)	2 (22)	0.63
Leukopenia, No. (%)	2 (8)	2 (22)	0.29
Circulatory Collapse, No. (%)	9 (38)	4 (44)	0.58
MRSA/VRE infection, No. (%)	5 (21)	4 (44)	0.18
Cardiac Arrest, No. (%)	2 (8)	3 (33)	0.11
Requirement of tracheostomy, No. (%)	8 (33)	3 (33)	0.67

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Student's signature **Supervisor's signature**