Screening for influenza viruses in 7804 patients with influenza-like symptoms

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ABSTRACT

الأهداف: عمل مسح شامل لعدد كبير من المرضى الذين يشكون من أعراض مشابهة لأعراض الأنفلونزا وذلك عن طريق اختبار بطاقة الاستشراب المناعي باستخدام الذهب لتعيين مولدات الأجسام المضادة الموجودة في عينات الدم (gold-immunochromatographic assay)

الطريقة: أُجريت هذه الدراسة في قسم العيادات الخارجية بالمستشفى العام التابع لمنطقة بكين العسكرية، بكين، الصين وذلك خلال الفترة من مايو 2009م إلى يناير 2010م، وشملت كافة المرضى الذين بدت عليهم أعراض مشابهة لأعراض الأنفلونزا. لقد قمنا بجمع المسحات البلعومية الأنفية بعد زيارة المرضى مباشرة، ومن ثم تم عمل اختبار بطاقة الاستشراب المناعي باستخدام الذهب من أجل البحث عن فيروسات الأنفلونزا أ و ب وذلك باتباع التعليمات التي ينص عليها الاختبار.

النتائج: أشارت النتائج إلى أن عدد الحالات المُصابة بفيروس الأنفلونزا كان 202 مريضاً من أصل 7804 مريضاً حيث بلغت نسبتهم 2.6% من أصل جميع الحالات التي تم تعيينها، ومن بين 202 مريضاً الذين يعانون من فيروس الأنفلونزا كان عدد المرضى الذين يحملون فيروس الأنفلونزا أ 171 مريضاً، فيما كان عدد المرضى الذين يحملون فيروس الأنفلونزا ب 24 مريضاً، وبلغ عدد المرضى الذين يحملون فيروسي الأنفلونزا أ و ب معاً 7 مرضى. ووصل عدد المرضى الذين يحملون فيروس المرض والذين تقل أعمارهم عن 30 عاماً إلى أكثر من 57%. تمثلت أعراض الأنفلونزا في ظهور الحمى، والتهاب الحلق، واحتقان الأنف، والسعال، وسيلان الأنف، وألم المفاصل وكان ظهورها في المرضى الذين يحملون فيروس الأنفلونزا أ أكثر من المرضى الذين يحملون فيروس الأنفلونزا ب والمرضى الذين لا يحملون فيروس المرض.

خاتمة: أظهرت الدراسة فعالية اختبار بطاقة الاستشراب المناعي باستخدام الذهب لتعيين مولدات الأجسام المضادة الموجودة في عينات الدم وذلك عند المسح عن أعداد كبيرة من المرضى الذين تظهر عليهم أعراض مشابهة لأعراض الأنفلونزا. بالإضافة إلى ذلك فقد كان ظهور أعراض الأنفلونزا المتمثلة في الحمى، والتهاب الحلق، واحتقان الأنف، والسعال، وسيلان الأنف، وألم المفاصل في المرضى الذين يحملون فيروس الأنفلونزا أ أكثر من المرضى الذين يحملون فيروس الأنفلونزا ب والمرضى الذين لا يحملون فيروس المرض.

Objectives: To screen a large number of patients with influenza-like symptoms by using the gold-immunochromatographic assay kit.

Methods: All patients with influenza-like symptoms visiting the outpatient department of the General Hospital of Beijing Military Region, Beijing, China between May 2009 and January 2010 were enrolled in the study. Nasopharyngeal swabs were collected immediately after the patient visited, then a gold-immunochromatographic assay was performed for screening of influenza A and B viruses according to the kit protocol.

Results: Among the 7804 patients enrolled in this study, 202 patients were influenza virus-positive; the positive cases accounted for 2.6% of all cases detected. Among the 202 influenza virus-positive patients, 171 patients were influenza virus A-positive, 24 were influenza virus B-positive, and 7 were co-infected with influenza virus A and B. More than 57% of the virus-positive patients were younger than 30 years old. Symptoms such as fever, sore throat, nasal congestion, sneezing, runny nose, and joint pain were more frequently observed in influenza virus A-positive patients than in influenza virus B-positive and influenza virus-negative patients.

Conclusion: The gold immunochromatographic assay kit is very useful for screening a large number of patients with influenza-like symptoms. A higher number of influenza virus A-positive patients have sore throat, nasal congestion, sneezing, runny nose, and joint pain than influenza virus B-positive and influenza virus-negative patients.

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The outbreak of influenza on June 11, 2009 was the first global influenza pandemic in the 21st century, and the World Health Organization (WHO) raised the swine-originated influenza H1N1 alert level to 6. In China, the number of outpatients with influenza-like symptoms has dramatically increased. The gold immunochromatographic method is a new immunochromatographic technique in which a cellulose membrane is used as the carrier, and a colloidal gold-labeled antigen or antibody is used as the tracer. This method detects the relatively stable core protein of the influenza virus.[1] The methods that are used for the diagnosis of influenza include: virus culture, immunofluorescence, nucleic acid detection, serological testing, antigen detection, and so on. Most of these methods are difficult to carry out extensively as they require special techniques and equipment, complicated operation, are time-consuming, and costly. Virus culture and detection of sero-conversion are the gold standard methods for diagnosis, however, they all need long periods, and there are no guidelines for treatment except for retrospective diagnosis. The reverse transcriptase-polymerase chain reaction (RT-PCR) for the M gene or nucleoprotein gene is the most widely used rapid test,[2,3] but the PCR test requires expensive laboratory instruments and professional operation, and cannot be used in primary care units, especially in developing countries. The rapid influenza virus antigen test based on the gold immunochromatographic assay has a good consistency with viral culture, RT-PCR, and immunofluorescence, with reasonable sensitivity (57-90%) and specificity (78-100%).[4] Meanwhile, the gold immunochromatographic assay operation is simple and can give results in 15 minutes, so it is most useful for testing in the field. Influenza virus A H1N1 treatment program of Ministry of Health of China, developed during the outbreak of influenza, a patient who has influenza-like symptoms and positive for the influenza virus is accepted as a suspicious influenza virus A H1N1 case. The result of gold immunochromatographic assay for the influenza virus provides diagnostic evidence to clinicians and is beneficial for the treatment approach of respiratory infectious diseases.[5,6] In this study, we summarized and analyzed clinical data and results of the gold immunochromatographic assay in the General Hospital of Beijing Military Region.

Methods. All patients who visited the outpatient department with influenza-like symptoms (fever and/or at least one of the following symptoms: sore throat, cough, rhinorrhea, or nasal congestion) and screened by performing gold immunochromatographic assay were enrolled in this study. Between May 2009 and January 2010, more than 8000 adult outpatients visited the outpatient department with influenza-like symptoms, including, but not limited to, fever, chills, headache, sore throat, cough, sneezing, running nose, weakness, malaise, and myalgia. During this time, 7804 patients with influenza-like symptoms were screened for influenza virus A and B by performing the gold immunochromatographic assay. The gold immunochromatographic assay kits for influenza virus are made in Beijing (ASCLE BioEngineering Company, Beijing, China). Influenza virus A test kits (S20063095) and influenza virus B test kits (S20063135), specification one cent/bag×10 bags/box, were used to perform the assay, which was performed according to the manufacturer's instructions using fresh clinical specimens. This test is a double antibody sandwich immunoassay that includes a core protein monoclonal antibody and a colloidal gold-labeled core antigen monoclonal antibody. The method for the detection of the influenza virus is as follows: Samples were collected immediately after the patient visit. A nasopharyngeal swab was collected using sterile swabs, and these swabs were stored in a plastic pipe, including 15 tips diluents, let the nasopharyngeal swab full pressed with diluents to solve the secretions; subsequently, the nasopharyngeal swabs were wrung as much as possible and the diluents, including the nasopharyngeal secretions drip were removed. The solution sample was placed on the test card, and the result was noted after 15 minutes at room temperature. Red color of only the quality control line indicates a negative result; red color of both the quality control line and the test line indicates a positive result (**Figure 1**).

Statistical analysis. A database was established, and statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) for windows 15.0, continuous variables are presented as mean±standard deviation (SD). For categorical variables, the percentages of patients in each group were calculated. We compared the clinical characteristics between subgroups using chi-square test or t-test, as appropriate. A p-value of less than 0.05 was considered significant.

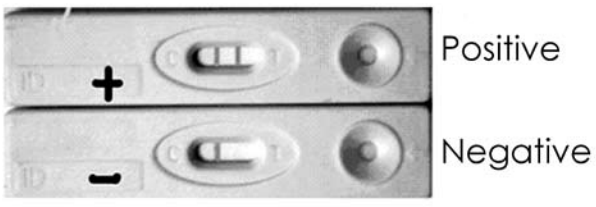

Figure 1 - Visual representation of gold immunochromatographic assay result.

Results. ***Detection rate and time distribution.*** Out of the 7804 enrolled patients, 202 patients were positive for the influenza virus, among whom 171 were influenza virus A-positive, 24 were influenza virus B-positive, and 7 patients were co-infected with influenza A and B. The influenza virus-positive patients accounted for 2.6% of all the detected cases. The number of virus-positive patients is shown in **Table 1**. Most of the virus-positive patients had upper respiratory tract infection, but 9 patients had pneumonia. All the virus-positive patients showed mild symptoms, and they completely recovered after symptomatic treatment, with no serious complications.

Clinical manifestations and laboratory examinations. The most common clinical manifestations of the patients examined included fever and flu-like symptoms; the body temperature of most of the patients was between 37.4-39.4°C, followed by chills, sore throat, headache, cough, sputum, nasal congestion, runny nose, sneezing, and joint pain. Influenza virus A-positive patients showed sore throat , nasal congestion, sneezing, runny nose, and joint pain more often than the influenza virus-negative patients, and the difference among these 2 groups was statistically significant (sore throat p=0.012, rhinoclesis p=0.000, sneezing p=0.00, and joint pain p=0.000). Some patients were co-infected with influenza virus A and B, these patients had a higher incidence of chills, cough, headache, joint pain and malaise/myalgia than influenza virus-negative patients. The clinical symptoms of influenza virus B-positive patients are milder than those of the influenza virus A-positive and influenza virus-negative patients. The prevalence of influenza was no different between men and women. The influenza virus-positive patients were mainly younger people, and we observed no remarkable difference between the ages of patients in both groups (**Table 1**). More than 80% tested patients visited the hospital within 2 days after disease onset; the longest interval is 4 days among the influenza virus-positive patients. Among the influenza virus-negative patients, 28.4% showed increased white blood cell (WBC) count (namely, greater than 10 × 10^9/L); among the influenza virus A-positive group, 21.3% showed increased WBC count increased; among the influenza virus B-positive group, 21.7% showed an increased WBC count, and among the influenza virus A- and B-coinfection group, 14.3% showed increased WBC count. The number of WBC, and the percentage of granulocytes were not significantly different among the groups (p>0.05). The positive influenza patients was identified at any time of the year, but mainly during the summer and autumn of 2009 (**Figure 2**).

Table 1 - Demographic and clinical data of the patients.

Demographic and clinical data	Influenza virus-negative	Influenza virus A-positive	Influenza virus B-positive	Influenza virus A- and B-positive
Male (n)	3656	88	14	4
Female (n)	4148	83	10	3
Ages (mean ±SD)	32.3±15.4	35.1±17.9	30.1±11.8	28.0±14.5
Chills (%)	12.2	15.7	11.1	42.9
Sore throat (%)	48.8	58.8*	38.9	42.9
Headache (%)	45.1	51.0	38.9	71.4
Cough (%)	53.4	56.9	38.9	71.4
Expectoration (%)	25.1	28.1	16.7	28.6
Rhinocleisis (%)	11.5	24.8*	11.1	14.3
Sneezing (%)	14.6	35.9*	16.7	14.3
Runny nose (%)	24.9	53.6*	22.2	28.6
Malaise/myalgia (%)	32.9	37.2*	11.1	42.9
Joint pain (%)	17.5	28.8*	11.1	28.6

*compared with influenza virus-negative group p<0.05.

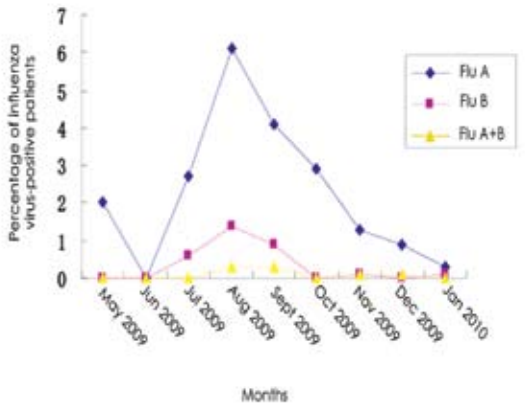

Figure 2 - The positive rate of every month.

Discussion. The operation of the rapid influenza virus test based on the gold immunochromatographic assay is simple and can give results in 15 minutes, so it is most useful for testing in the field. The use of this rapid influenza virus test can reduce numbers of tests ordered, decrease antibiotic use for proven viral illness,[7] and it is helpful in the rapid testing for influenza diagnosis,[8] and it is helpful in the rapid testing for influenza diagnosis. However, currently, this method can only be used to determine the type of the influenza virus (type A, type B, or type C), and it cannot distinguish between the specific influenza A subtypes.[9] Therefore, if the test is positive for influenza A virus, it may indicate seasonal influenza viruses, bird flu, or H1N1. Quick results can be obtained using this test, and it can be performed with simple training; and the result of this test has no statistical significance compared with the results of indirect immunofluorescence assay. With reference to indirect immunofluorescence assay, the sensitivity of the influenza virus A test kit and Influenza virus B test kit are 88.3% and 87.5% separately, and the specificity are 99.4% and 99.6% separately.[10] Therefore, this method is extremely suitable for screening large numbers of

patients with influenza-like symptoms. The influenza virus is divided into A, B, and C types on the basis of the antigenicity of viral nucleoproteins and matrix proteins.[11] Only influenza virus A has a large variation, and it causes severe damage to humans, followed by influenza B.[12] Our study shows that most influenza virus-positive persons are infected with influenza virus A. The clinical symptoms of influenza virus A-positive patients are more serious than those of patients positive for other types of the influenza virus. Our study also showed that the number of influenza virus-positive patients is not as high as the early reports. According to a previous study, respiratory infections in both adults and children, especially in young children, elderly adults, and persons with chronic diseases are caused by the influenza virus;[13] however, our study shows that influenza virus-positive patients are mainly young people around 30 years of age, similar to patients susceptible to the influenza virus A H1N1 variant.[14] The positive rate of influenza virus was high in August, September, October, and November 2009, and the influenza virus A and B-co-infected cases were also found during that period. In addition, our study showed that 19.1% of influenza virus-positive and 28.4% of influenza virus-negative patients had high WBC counts, suggesting that similar to infants, adults who are positive for the influenza virus may have mixed bacterial infection.[15,16] Fever is an important predictor for influenza virus infection. The influenza virus A-positive patients have higher incidence of sore throat, nasal congestion, sneezing, runny nose, joint pain than influenza virus B-positive and influenza virus-negative patients. Therefore, clinicians should carefully examine these patients.

The limitation of this study is the number of influenza virus B-positive and patients co-infected with influenza A and B is small, it needs further study to expand sample size. The gold immunochromatographic assay kit is very useful for screening a large number of patients with influenza-like symptoms. A higher number of influenza virus A-positive patients have sore throat, nasal congestion, sneezing, runny nose, and joint pain than influenza virus B-positive and influenza virus-negative patients.

For better monitor of influenza virus infection, we will expand the application of this rapid influenza test kit.

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