IMMUNOFLUORESCENT DIAGNOSIS OF INFLUENZA INFECTIONS

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Influenza is still one of the most serious problems of the infectious pathology. No other infectious disease can be distributed in such scales for such short period of time. The influenza infections affect all age groups and cause certain health and economic damages. Nearly 15—20% of temporary labour disability of the working population is due to influenza and its complications.

The periodically repeated influenza pandemies and epidemies provided long ago the profound investigation of their etiology. The precise identification of influenza viruses has certain importance for the clinical and epidemiological practice. Therefore, the express methods of diagnosis of any influenza infections are suggested as most suitable. At the present moment one of the most effective method for an express and precise diagnosis of influenza is the immunofluorescent one, which allows a proper and rapid identification of at least 60—80% of all cases in epidemic wave (1, 2, 4, 5, 6). This method is one of the most perspective ways of orientative diagnosis due to the combination of quickness of morphological studies with the specificity of serological reactions.

The method of fluorescent antibodies provides visuality of the reaction antigen-antibody. Due to its specificity, high sensitivity, easiness, quickness and relative simplicity of proceeding it is possible to be used in the morphological investigation of the process of interrelations between virus and cell, dynamics of accumulation of viral antigen in the latter and application for the diagnosis of influenza, even some other, infections.

The object of the present work was to study the effectiveness and quickness of the immunofluorescent method (IM) for the diagnosis of influenza and influenza-like diseases.

Materials and methods

During the last influenza epidemy in Varna and Varna District, Winter-Spring 1979—1980, a total number of 349 patients with a preliminary diagnosis influenza were studied.

The material was taken in the first hours after admiting to the hospital and as for the ambulatory cases — in the time of the examination of the patients. It was a naso-pharyngeal smear taken by a sterile tampon. The working of the materials was after the routine methods (6).

The direct immunofluorescent technique was applied. In order to suppress the nonspecific immunofluorescence dilutions of Ewans'blue 1:60 000—1:80 000 were used.

Conjugated antisera with fluorescin-iso-thyocyanate against influenza virus type A, A+B, B, parainfluenza viruses type 1, 2, 3, Adenoviruses (polyvalent), Respiratory-scincitial virus and Mycoplasma pneumoniae were applied. All conjugated antisera were produced in NIZPB (Sofia).

The investigations were carried out under the microscope "Fluoval" (DDR).

To prove the positive results for influenza 10—12 day old chicken embryos were inoculated with the same materials and by using RHA was established the presence of the influenza virus.

The specific lightening was read according to the intensity with ++++, +++, ++, or +.

Results and discussion

From all 349 investigated cases 112 (32.09%) shew influenza-positive results, as table 1 indicates:

Table 1

Total number of cases	Negative Iniluenza-results	Positive Influenza-results
349	237	112
%	67.91	32.09

The presented data can outline the dynamics of the epidemic wave. Most considerable number of positive results for influenza was established during February (42%) when the peak of the epidemy was. During January, preceded the epidemic peak, the percent of the positive cases was 32.5% and as for the months following the climax, the influenza-positive results gradually decreased in number and percent: March — 20.5%, April — 5.0%.

By virological study on chicken embryos about 80% coincidence was established and by serological methods (RHA, RDHA) — 65—70%. Based on the present data it is obvious that the IM has a middle position between the virological and serological ones, concerning sensitiveness. The preparations (Influenza-positive) were rich (or relatively rich) of cylindrical-epithelial cells from naso-pharyngeal mucosa. Small percent of the preparations contented the minimum of 3—5 cells, necessary to consider the preparation itself a positive one for influenza, according to the accepted criterion. A great variety of morphology was established. Cells from the superficial layer of the cylindrical epithelium, cells of the profound layers with oval or polygoneal forms, were simultaneously detected. From the three types of fluorescence localisation (only nuclear, only cytoplasmatical, combined nuclear-cytoplasmatical) most often was found the third one, then — the second and the first one. The fluorescence intensity was relatively well expressed with marks of +++ or even ++++. The control cells did not show a specific luminescence. Various stages of entering the cell shew different morphology of cells according to the viral antigen and localization of luminescence.

The duration of the IM was about 3—4 hours.

The results of our study prove that IM with all its advantages (cited above) is one valuable method in the virological practice. Certain disadvantage of IM is that it is with a type-specific character, i. g. it can not determine the antigen variant of the studied virus. Therefore, the immunofluorescent diagnosis of influenza is considered as orientative. There exist also certain subjectiveness in analysing the experimental and control preparatins. Deprive to

all aforementioned disadvantages, having in mind the cited excellent sides of it, we suggest that IM can be applied widely in the diagnosis of influenza infections and epidemics.

REFERENCES

1. Дрейзин, Р. С., В. М. Жданов. Аденовирусные инфекции. М., 1962, 137—141. — 2. Капрелян, Г. и др. Етиологична характеристика на респираторните вирусни инфекции в някои райони на Североизточна България и Черноморието. *Проблеми на заразните и паразитните болести.* 1975, 3, 53—67. — 3. Кармышева, В. Я. Применение метода флюоресцирующих антител в вирусологии. М., Медицина, 1979, 115. — 4. Колесникова, И. А. Материалы сессии Грипп и ОРЗ. М., 1967, 26—27. — 5. Левинная, Е. Н. Иммунолюминисценция в медицине. М., Медицина, 1977, 80—145. — 6. Ритова, А. А. Острые респираторные вирусные инфекции у детей раннего возраста. М., 1969, 91—127. — 7. Тунчева, К. Имунофлуоресцентни изследвания в диагностиката на острите респираторни вирусни инфекции при деца от 0—3 години. V Научна сесия. ВМИ, Варна, 1973, 145—147.

ИММУНОФЛЮОРИСЦЕНТНЫЕ ИССЛЕДОВАНИЯ ОРЗ ЗА ПЕРИОД С 1979 ПО 1980 Г. Г.

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РЕЗЮМЕ

Вопрос о быстрой диагностике вирусных инфекций всегда был особенно актуальным. В наше время иммунофлюорисцентный метод, обеспечивающий быструю диагностику вирусных инфекций, занимает первое место, что связано с его доступностью и применимостью. Он обеспечивает визуальность реакции антиген-антитело в пораженной клетке и дает возможность работать над непосредственно взятом от больного материалом.

При исследовании гриппа 1979—1980 г. г. авторами был использован прямой метод иммунофлюорисценции. Было исследовано 355 материалов от стационарно и амбулаторно больных с предварительной диагностикой ОРЗ. Использовались коньюгированные антисыворотки при исследовании гриппозного вируса типа А, парагриппозного вируса типов 1, 2, 3, поливалентных аденовирусов, коронавирусов.

Устанавливается, что первое место при ОРЗ занимают гриппозные вирусы. Существенное значение имеют и аденовирусы, коронавирусы и парагриппозные вирусы.