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Rapid Detection of Pneumocystis Carini in Spiratory Specimens of Rats by Calcofluor White Staining

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Abstract:

The present study was carried out for evaluation of calcoflour white staining (CWS) as a rapid method for detection of *Pneumocystis carinii* in respiratory specimens of rats as an animal model for human infection.

A total of 35 Spraque – Dawley rats were divided into two groups. Group1 (20 rats) received increasing doses of dexamethasone subcutaneously, and Group 2(15 rats)as control group that received no immunosuppressive drugs. After immunosuppressant, all of the rats were killed and necropsy was performed. Broncho-alveolar lavage (BAL) and impression smears from the lungs prepared and stained by CWS .The results were compared with a few standard staining methods which have already been used for *P.carinii.*

The calcofluor white staining was found to have more validity (sensitivity and specificity) than other staining methods such as Geimsa , Modified Geimsa and Toluidine blue O (TBO) .The study showed the CWS to be more valid , faster and easier to perform for detecting of *P. carinii* rganism.

Key words: *Pneumocystis carinii* , Stanining , Calcofluor white , Rat .

Introduction

Pneumocystis carinii is an opportunist pathogen organism causing *P.carinii* Pneumonia (PCP). PCP is a major cause of morbidity and mortality in immunocompromized people and some of the mammalians hosts (4, 10).

Diagnosis of PCP is currently depends on the accurate morphological identification of the organism from broncho-alveolar lavage (BAL) fluid and / or lung tissue obtained by either transbronchial or open lung biopsy (2). A number of staining methods are used for detecting of *P.carinii* but these procedures are more laborious, intensive and time consuming (6, 9).

In the present study , calcofluor white M2R staining was compared with Giemsa , Modified Geimsa , Toluidine blue O (TBO) and Gomeri Methenamine Silver (GMS) staining methods in order to finding a rapid detection of *P.carinii* in rats whom are immunosupressed by dexamethasone drug. Because rat is a sensitive animal against *P.carinii (*6), thus, this animal was selected as an appropriate model for human infection.

Materials and Methods

Rats were divided into two groups. Group 1 received increasing doses (from 0.6 to 1.2 mg) Dexamethasone (manufactured by Ghostaresh drug's company, Iran) subcutaneously, twice a week for 8-10 weeks (5). Group 2 did not receive any immunosuppressive drugs and selected as control group. After completion of immunosuppressant, all of the rats were killed and necropsy was performed. The lungs were removed aseptically and then impression smears and broncho- alveolar lavage (BAL) specimens were prepared and stained by Giemsa, modified Giemsa , Toluidin blue O and Gomeri methenamine silver according to standard procedures (1,2,8) . Besides, the wet slides were stained by calcofluor white M2R stain (8). At first , one drop of calcofluor white M2R dye , containing calcofluor white M2R (SIGMA 0.01 g) , Evans blue (SIGMA , 0.05 g) and distilled water (100ml), was added to one drop of lung cells, after 1-2 min one drop of potassium hydroxide 10% was added. The slides were examined immediately and viewed with a fluorescence microscope (Nikon optiphots.2 lab photo- 2A2) and a 510 –nm dichroic mirror, 520 barrier filters and 420 - 490 exciter filters. The characteristic morphology of *P.carinii* was used in the interpretation of the calcofluor white – stained slides.

Results

Totally 20 immunosupressed rats, showed infection with *P.carinii* after 10 weeks, whilst none of the control group was affected.

The study showed that Geimsa , could stain the sporozoites and nuclei of the trophozoite, violet , whereas the cytoplasm of trophozoite may appear blue . The cyst wall was not stained but could often be delineated against a stained mucoid back ground. Modified Geimsa stain may also be employed. In this method, the cyst wall stained lavender against a greenish gray back ground.

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Table 1 : Results of different staining methods in order to detecting *P.carinii* in respiratory specimens in rats .

Staining methods	Interventional population				Control population			
	Positive		Negative		Positive		Negative	
	No	%	No	%	No	%	No	%
Geimsa	14	70.00	6	30.00	0	0	15	10.00
Modified Geimsa	16	80.00	4	20.00	1	6.6	14	93.3
Toluidin Blue	18	90.0	2	10.0	0	0	15	100.0
Gomeri Methenamin silver	20	100.0	0	0	0	0	15	100.0
Calcofluor white MR	20	100.0	0	0	0	0	15	100.0

The GMS and TBO stain could stain the cyst walls black and blue and show the thickened eccentric part of the cyst wall either as an edge-ondisc or pared comma-shaped central structures. In CWS, the cyst wall stains green-white fluorescence with thickened eccentric part of the cyst wall or pared comma- shaped central structures (Fig.1).
Evaluation of CWS has been summarized in tablets 1, 2, 3 which demonstrate the comparative results with different stained methods.

Table 2 : Results of evaluation of different staining methods for detecting *P.carinii* in respiratory specimens of the rats .

Staining methods	Sensitivity	Specificity	Positive predicative value	Negative predictive value	Validity	Efficiency	Reliability
Geimsa	65	100	100	68.2	82.5	80	0.68
Modified Geimsa	75	96.7	96.8	74.3	85.8	84.3	0.70
Toluidin Blue	90	100	100	88.2	89.1	94.3	0.88
Gomeri Methenamin silver	100	100	100	100	100	100	1
Calcofluor white MR	100	100	100	100	100	100	1

Table 3 : Total number of *P.carinii* was overved during 5 minutes by different staining procedures(X 1000)

Staining methods	a/n*	Oberved form of P.carinii
Geimsa	5/10	Trophosoite +cyst
Modified Geimsa	7/10	Cyst
Toluidin Blue	8/10	Cyst
Gomeri Methenamin silver	10/10	Cyst
Calcofluor white MR	10/10	cyst

* a = number of Positive *P.carinii* slides during 5 min micosope axamination (X 1000).

* n= Total number of Positive P.carinii studued (X 1000).

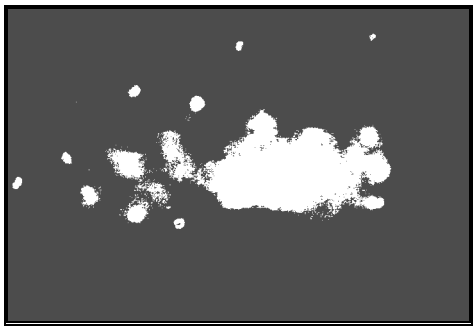

Fig.1: Calcofuor white M2R staining for the diagnosis of *P.carinii* cyst (X1000).

Discussion

Pneumocystis carinii Pneumonia is a frequent and life–threatening complication of the Acquired Immunodeficiency Syndrome (AIDS), and it also occurs in other immunocompromised patients (11). Since the clinical signs are not specific for the diagnosis of the disease, laboratory methods for detecting the organism are very important (2, 6). There are three types of staining methods for the diagnosis of *P.carinii* . The first type is able to stain the sporozoites or trophozoites of *P.carinii* included in Geimsa that is more specific but less sensitive for the detection of the organism and often sporozoite – containing cyst are extremely difficult to find, this stain is suitable for detail morphological studies of *P.carinii* and are not suitable for the rapid diagnosis of the organism. The second type is often unable to stain *P. carinii,* however , could efficiently stain the host cell and is therefore not suitable for diagnosing but is only useful for pathological studies .Of course, these staining methods have not implicated in the present study . The third type, which includes Modified Geimsa , TBO and GMS can stain the cyst wall of *P.carinii* and have a high sensitivity for the diagnosis of *it* , but these staining methods are more laborious, intensive and take too much time to perform . Calcofluor white M2R is a non-specific fluorochrome that binds to cellulose and chitin contained in the cell walls of fungi and other cellulose- containing organisms including *P.carinii* , *Microsporidium* , *Acanthamoeba,* N*aegleria* and *Balamuthia* (3, 7, 12) .

The calcofluor stain could serve as a rapid, inexpensive tool for detecting of *P.carinii* in respiratory specimens because the distinctive cyst morphology with the parenthesis like structure could be revealed.

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