RESEARCH PAPER | Medical Science | Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

CFW (Calcofluor White Stain): A Rapid Detection System for Nosocomial Fungal infection

KEYWORDS

Dr. Shoeb Akhtar Khan	Dr. Immaculata Xess	Dr Amardeep Rai
Assistant Professor Department of Microbiology Bundelkhand Medical College Sagar	Associate Professor Department of Microbiology All India Institute of Medical Sciences New Delhi-29	Professor & Head Department of Microbiology Bundelkhand Medical College Sagar

ABSTRACT *Fungal infections in critically ill patients are an increasing prevalent problem. Candida spp cause the majority of these infections in ICU. They occur most commonly in-patients with severe underlying illness, multiple courses of antibiotics and patients with intravascular catheters.*

Clinical diagnosis of fungal infections in these patients are difficult due to non-specific symptoms.

Therefore we compare the detection of fungal elements in nonbrochoscopic brochoalveolar lavage sample using Calcofluor white stain (CFW) and conventional KOH stain method with culture.

Total 432 no BAL samples were collected from total of 80 patients on ventilator for more than 48 hours developing signs and symptoms of nosocomial pneumonia. The sensitivity and specificity of KOH conventional staining technique were 41% and 100% respectively, where as Calcofluor white stain showed sensitivity of 75.2% and specificity of 100% for direct demonstration of fungal elements. All direct positive samples by both techniques were positive by culture 85 (19.6%). The Sensitivity and Specificity of CFW staining for direct demonstration of fungi in clinical specimen superior than the conventional technique.

Introduction

Technology has developed very rapidly in last few years in the field of medical sciences. Newer medicines, critical care and life support system has increased the life expectancy. Simultaneously increases the population of more susceptible elderly patients, along with this increased use of broad spectrum antibiotics, immunosuppressive drugs and frequent use of invasive procedures and diseases like AIDS increase the incidence of fungal infections many folds in last few years, which are more difficult to treat and carries high mortality. Containment of these infections depends on early recognition and treatment of suspicious agent by physician and obtaining an accurate and quick laboratory diagnosis.

The conventional method commonly used for direct demonstration is KOH mount preparation. It has poor sensitivity and lack of contrast. Where as culture is time consuming. Calcofluor white is a nonspecific fluorochrome, which have a special affinity for cellulose and chitin, essential components of fungal cell wall. It delineates sharply any fungal element in clinical specimen when exposed to UV light (1). This study investigate the efficacy of calcofluor white in detection of yeast in bronchoalveolar lavage sample from ventilated patients in ICU and compare it with KOH mount preparation and culture.

Materials and Method

Inclusion criteria

Patients on ventilator for more than 48 hours developing signs and symptoms of nosocomial pneumonia and other infections were considered.

Total 432 bronchoalveolar lavage samples collected and processed during study for fungal investigations.

BAL refers to sequential instillation and aspiration of sterile physiologic saline to lung subsegments. It is performed by invasive method of bronchoscopic bronchoalveolar lavage (BBAL) or noninvasive technique as non-bronchoscopic bronchoalveolar lavage (NBBAL). The later technique is a double catheter technique. In this technique a catheter is blindly introduced into bronchus, then lavage is performed through a second catheter passed through it into distal airway. It minimizes the risk of contamination of specimen by upper respiratory normal flora. Samples were obtained in the study by NBBAL.

Processing of sample

Ten to fifteen ml of sample homogenized with sterile glass beads. It was then transferred in a test tube and centrifuged at 2500 rpm for 20 minutes. Supernatant was discarded and pellet was used for microscopy and culture.

Direct Microscopy

Samples were examined by conventional method KOH mount preparation and Rapid calcofluor white stain method.

Calcofluor staining: One drop each of Calcofluor white (0.1%) and KOH (10%) were added on a clean glass slide, one drop of deposit of specimen was added to it and mixed. Covered with a clean cover slip and examined under fluorescent microscope, equipped with epifluorescent filter. The preparation was examined by exposure to ultraviolet light of 345-365 mm wavelength under 40 X objective. Fungal elements give chalky white fluorescence with deep blue background.

Isolation - The remaining half of the samples were cultured on standard medium.

Results

Total 64 samples were positive by direct microscopy from 35 patients. All of these were culture positive. Out of 64 samples 35 samples from 21 patients were reported

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

as positive by conventional method, KOH stain . By the calcofluor white staining technique additional 29 samples from other 14 patients were detected as positive. Out of 432 samples 85 were positive by culture scattered among 41 patients (Table1).

The results of conventional microscopy techniques of KOH was compared with culture results, which was considered as gold standard. KOH showed only 35 as positive and 397 as negative, showing sensitivity of 41.1% but specificity was 100% as no false positive was detected. The results of calcofluor white staining were compared with culture it was able to detect 64 samples as positive and 368 as negative showing sensitivity of 75.2 and specificity of 100% as no false positive was detected (Table1, 2).

When the results of two techniques of microscopy (KOH and Calcofluor White) were compared it was found that conventional method KOH stain detect 35 samples as positive, the rapid technique calcofluor white detect additional 29 samples as positive increasing total positivity by microscopy to 64 samples (Table 3).

Discussion

The intensive care units have become indispensable for most of hospitals. It has been reported that approximately 95% of hospitals have at least one intensive care unit. 10-15% of hospital beds was dedicated to ICUs (2).

Sporadic infections and outbreaks are very common in intensive care units because most of patients in ICUs are critically ill and susceptible to infection. In a study on prevalence of nosocomial infections in ICUs in Europe involving 10,038 patients (3). They reported over all infection rate in ICU was as high as 20.6 % in comparison to community infection rate (13 7%). The various type of infections reported were the pneumonia (46.9%), as highest followed by urinary tract infection (17.6%), and blood stream infection (12%). The surveillance of infections conducted in ICUs, involving 178 patients (4). They reported pneumonia as most common ICU acquired infection (65%) followed by UTI (30%) and blood stream infection (19%). Ventilator associated pneumonia (VAP) have been reported as most common, device associated nosocomial infection. The incidence of VAP varies from 18 to 58% (46,47). It has reported that VAP account for approximately 90% of all ICU infection in-patients requiring mechanical ventilation (5). The fungal infections also contribute significantly to ICU infections. The incidence of fungal infections have increased significantly in last 30-40 years due to development in medical sciences, newer life saving medicines and life support system and increase in life expectancy (6).

For collection of lower respiratory tract sample several techniques have been described in literature. Non bronchoscopic bronchoalveolar lavage (NBBAL) using a double catheter system overcomes the problems of repeated bronchoscopy in critically ill patients and avoids risk of contamination from upper respiratory tract and oral cavity (7).

Pugin et al in1991 compare culture result of bronchoscopic bronchoalveolar lavage with NBBAL in 15 trauma patients with clinical pneumonia (8). They reported 100% correlation for type of infection and 67% correlation for number of organism in quantitative culture even when BAL fluid came from different lung segment.

In this study the technique of NBBAL of Rouby et al was adopted. Which is easy to perform, non-expensive and well tolerated in critically ill patients.

For diagnosis of fungal infections, KOH (10%) preparations and Gram's staining have been widely used. KOH preparation was easy to perform, non-expensive and have high specificity but poor sensitivity especially in early stage of the disease. The disadvantages of KOH mounts were artifacts on proteinacious material, lack of contrast between fungi and cellular debris and formation of crystals in hot climate (1).

Calcofluor white (CFW) is a fluorochrome previously used in textile and paper industry. It has special affinity for cellulose and chitin, essential parts of fungal cell wall. It gives fluorescence when exposed to long wave length ultra violate light of 345-365 nm (9).

In India there were few studies on comparison and efficacy of CFW and KOH in direct demonstration of fungal element (10). In a study on corneal ulcer specimen, they found CFW was superior showing 95% positivity as compared to KOH with 75%. This study reveals the utility of calcofluor white staining method of direct demonstration of fungi in scanty sample for rapid diagnosis of fungal infections.

In a present study sensitivity of CFW (75.2%) was higher than the conventional method (41.1%). The high affinity of CFW to cell wall components of fungi (chitin and cellulose) and good contrast due to deep blue background provided by trypan blue were the probable factors for increased sensitivity of calcofluor white over other two techniques of direct demonstration. All the samples positive by either method were subsequently proved by culture. There was no false positive by both methods showing specificity of 100%.

CFW also has its own limitations as seen in 21 specimen reports, but were positive by culture. This may be due to the early ineffective binding of CFW, insufficient number of cells present in the sample or may be presence of immature cell wall especially in early stage of infections.

By CFW we could detect 29 (34.2%) additional samples as positive from 14 patients, which were negative by KOH staining. This reveals the advantage of CFW as a tool for direct and rapid detection of fungi in clinical specimen over other techniques.

Fungi are opportunistic pathogens, normally present on body surfaces as normal flora not causing disease in immunocompetent individuals. However fungal infections were associated with some risk factors. The conventional techniques of direct demonstration has poor sensitivity, while methods of isolation and identification from the culture method is time consuming; takes at least one to two weeks. Which may delay antifungal treatment. Any method for rapid and reliable diagnosis by direct demonstration of fungi in the clinical specimen from the patient with association of risk factors would justify starting antifungal therapy.

Conclusion

Sensitivity and specificity of CFW staining for direct demonstration of fungi in clinical specimen as proved in this study justify its use as method of choice. This method is rapid than the conventional method. If facility of fluorescent microscopy is available for early detection it should

Volume : 5 | Issue : 7 | July 2015 | ISSN - 2249-555X

be used as a routine diagnostic procedure for direct demonstration of fungi in clinical specimen.

Table 1: Comparison of KOH with culture

KOH/GRAMS	CULTURE		TOTAL
	negative	positive	
NEGATIVE	347	50	397
POSITIVE	00	35	35
TOTAL	347	85	432

Specificity = 347/347x100 =100%
Sensitivity = 35/85x100 = 41.1%

Table 2: Comparison of Calcofluor White staining with culture

Calcofluor	Culture		Total
	Negative	Positive	
Negative	347	21	368
Positive	00	64	64
Total	347	85	432

Specificity =100% Sensitivity = 75.2%

Table 3: Comparison of Calcofluor with KOH/ Gram's Stain

Calcofluor	KOH/Grams		Total
	Negative	positive	
Negative	368	00	368
Positive	29	35	64
Total	397	35	432

AGREMENT= 368+35/432 = 93.5%

REFERENCE 1. Hαγεαγε Γϑ, Hαρρινγτον Βϑ. Υσε οφ χαλχοφλυορ ιν χλινιχαλ μψχολογψ. Λαβ Μεδ 1984; 15(2): 111–12. | 2. Πονχε ΔΡΣ, Ραμοσ ΦΜ, Χηεριτ ΓΔ, Φραυστο ΜΣΡ, Ραμοσ ζΓς. Πρεπαλενχε οφ ινφεχτιονσ ιν ιντενσιπε χαρε υνιτ ιν Μεξιχο: Α μυλτιχεντερ στυδψ: Χριτ. Χαρε Μεδ 2000; 28(5): 1316–21. | 3. ςινχεντ ϑΑ, Βιηαρι Δϑ, Συτερ ΠΜ, Βρυινινγ ΗΑ. Τηε πρεπαλενχε οφ νοσοχομιαλ ινφεχτιον ιν ιντενσιπε χαρε υνιτ ιν Ευροπε. ϑΑΜΑ 1995; 274 (8): 639–644. | 4. Υρλι Τ, Περονε Γ, Αχθριασολο Α, Ζππα Σ, Αντομινι Β, Χιανι Α. Συρπειλλανχε οφ ινφεχτιονσ αχθυιρεδ ιν ιντενσιπε χαρε: υσεφυλνεσσ ιν χλινιχαλ πραχτιχε. ϑ Ηοσπ Ινφεχτ 2002; 52: 130–135. | 5. Χοοκ Δϑ, Στεπηεν Γ, Ωαλτερ Ε. Ινχιδενχε οφ ρισκ φαχτορ φορ πεντιλατορ ασσοχιατεδ πνευμονια ιν χριτιχαλλψ ιλλ πατιεντσ. Ανν Ιντερν Μεδ 1998; 129 (6): 433–40. | 6. Φλαναγαν ΠΓ, Βαρνεσ ΡΑ. Φυνγαλ ινφεχτιονσ ιν ιντενσιπε χαρε υνιτ ϑ. Ηοσπ Ινφεχτ 1998; 38: 163–77. | 7. Ρουβψ ϑϑ, Ροσσινγνον ΜΔ, Νιχολυσ ΜΗ. Α προσπεχτιπε στυδψ οφ προτεχτεδ ΒΑΛ ιν διαγνοσισ οφ νοσοχομιαλ πνευμονια. Αναεστηεσιολ 1989; 71(5): 679–685. | | | 8. Πυγιν ϑ, Αυχκεντηαλερ Ρ, Μιλι Ν, Θανσσενσ ϑΠ, Λεω ΠΔ, Συτερ ΠΜ. Διαγνοσισ οφ πεντιλατορ ασσοχιατεδ πνευμονια βψ βαχτεριολογιχαλ αναλψσισ οφ βρονχηοσχοπιχ ανδ νονβρονχηοσχοπιχ ∀βλινδ∀ βρονχηοαλπεολαρ λαπαγε. Αμ Ρεπ Ρεσπιρ Δισ 1991; 143: 1121–29. | 9. Μονηειτ ϑΕ, Χοωεν ΔΕ, Μοορε Δϑ. Ραπιδ δετεχτιον οφ φυνγι ιν τισσυε υσινγ χαλχοφλυορ ωηιτε ανδ φλυορεσχεντ μιχροσχοπψ. Αρχη Πατη Λαβ Μεδ 1984; 108: 616–18. | 10. Χηακραβαρτι Α, Χηανδερ ϑ, Σηαρμα Α, Σαινι ΑΣ. Πανιγραηι Δ. Επαλυατιον οφ χαλχοφλυορ ωηιτε ιν διαγνοσισ οφ φυνγαλ χορνεαλ υλχερ. Μψχοσεσ 1993; 36 (7–8): 243–45. |