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Original Research Article

Comparison of Giemsa Staining and Direct Immunofluorescent Staining For Detecting Pneumocystis Jirovecii in Induced Sputum from Patients with HIV Infection

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Abstract

Introduction: Pulmonary infection is one of the most prominent Human Immunodeficiency Virus (HIV)-related diseases and Pneumocystis carinii pneumonia is one of the most important infections that can occur when the CD4 value falls below the level of 200.

Objective: To detect Pneumocystis jirovecii by Giemsa staining and Direct immunofluorescent staining technique in PLHIV patients and evaluate the performance of Giemsa staining in identification of Pneumocystis jirovecii.

Methods: The study was conducted in Government Hospital of Thoracic Medicine. Thorough clinical examination including general and systemic examination and chest radiography were done. Induced sputum samples were collected and subjected to Giemsa staining and Merifluor DFA immunofluorescence testing.

Results: Out of 178 HIV Seropositive patients 70(39.3%) were positive for Pneumocystis by Direct Immunofluorescent staining and 48 (27%) by Giemsa staining. The positivity was higher in the age group 40 years & above in both the staining. Gender wise positivity was higher in male (77%) in both the staining. The CD4 count was less than 200 in all positive cases. The Sensitivity was 79.1%, Specificity was 100 %. Positive predictive value and Negative predictive value were 100% & 89.1%, respectively, for Giemsa staining compared to DFA as the gold standard.

Conclusion: Induced sputum analysis is a rapid, low-cost diagnostic technique for PCP. Giemsa staining is a cost-effective method with high specificity, suitable for routine use in resource-limited laboratories.

Keywords: Pneumocystis Jirovecii, Giemsa staining, DFA immunofluorescence testing.

Introduction

Pulmonary infection is one of the most prominent human immunodeficiency virus (HIV)-related diseases (Wallace JM et al 1997) and Pneumocystis carinii pneumonia is one of the most important infections that can occur when the CD4 value falls below the level of 200 cells/µL (Selik RMet al 1995). The organism Pneumocystis Jirovecii previously known as Pneumocystis carinii, is the causative agent of Pneumocystis Jirovecii pneumonia (Muhlethaler et al., 2012). In developed countries, the rate of Pneumocystis Jirovecii pneumonia co-infection with HIV has reduced while controlling the prevalence of co-infection in developing countries remains challenging (Ravinder et al., 2015). The prevalence of PCP had reached 80% in this population prior to the introduction of prophylactic treatment against it. Many factors can make PCP diagnosis tricky such as nonspecific symptoms, coexistence of other infections, and difficulty in establishing a reliable culturing system of this pathogen (Kelly et al., 2018). Rapid and accurate identification of infections and suitable recommended treatment, which is based on accurate microbiological results, is still required; however, the current standard clinical tests lack high sensitivity and flexibility (Hirama, 2016). Staining methods to detect P. jirovecii can be Giemsa, Gomorimethenamine silver, and toluidine blue O and Immunofluorescence (IF) staining, which includes direct fluorescent antibody (DFA) and use of a monoclonal antibody

Although various nucleic acid amplification assays, including real-time PCR, have been developed for detecting P. jirovecii, they are not widely available in clinical microbiology laboratories. Therefore, direct visual examination of clinical specimens using staining methods remains the primary laboratory approach for detecting P. jirovecii, an organism that cannot be cultured using standard methods. (Takahashi, T 2002).

Materials and Method

The study was conducted in Government Hospital of Thoracic Medicine from March 2021 to February 2022.Thorough clinical examination including general and systemic examination was done meticulously with vital signs monitoring and chest radiography. Induced sputum samples were collected and subjected to Giemsa staining and Merifluor DFA immunofluorescence testing.

Inclusion Criteria:

Patients clinically diagnosed to have PCP based on:

1. Clinical characteristics: fever, dyspnea, or hypoxemia
2. Radiographic findings: bilateral, diffuse ground-glass opacities on chest X-ray or computed tomography
3. No evidence of bacterial infection

Exclusion Criteria:

- PLHIV with tuberculosis.
- Duplicate of the sample from the same patient

Patients were explained about the nature of the study and informed consent obtained.Clinical diagnosis of PCP was done based on the presence of consistent signs, symptoms and radiology. Sputum of about 3 to 5 ml collected in sterile container after hypertonic (3%) saline nebulization using a nebulizer for five minutes (Cruciani M, Marcati et ai 2002). This procedure was done in a separate room. The sputum was subjected to smear and air-dried, fixed in acetone for 30 s, and stained using the modified Giemsa method(Procop GW et al 2004) Haddad et al)by dipping the slide five times in each of the staining solutions (Eosin and Giemsa). Stained slides were examined using a light microscope and searched for Pneumocystis organisms using a low-power (×10) objective, and confirmed using a high-power (×100) objective. Using this technique, we were able to demonstrate aggregates of Pneumocystis discrete trophic forms (fig_1).We scored as positive if clusters of free trophozoites or precysts were seen ; if no parasites were seen during 30 min of examination, slides were scored as negative. The reference diagnostic test was Immunofluorescent staining (Mohammed Alfaifi et al , 2020) (IFAT) using Merifluor DFA immunofluorescence test, according to the manufacturer’s instructions (Meridian Bioscience, Inc., Cincinnati, Ohio.(fig 2)

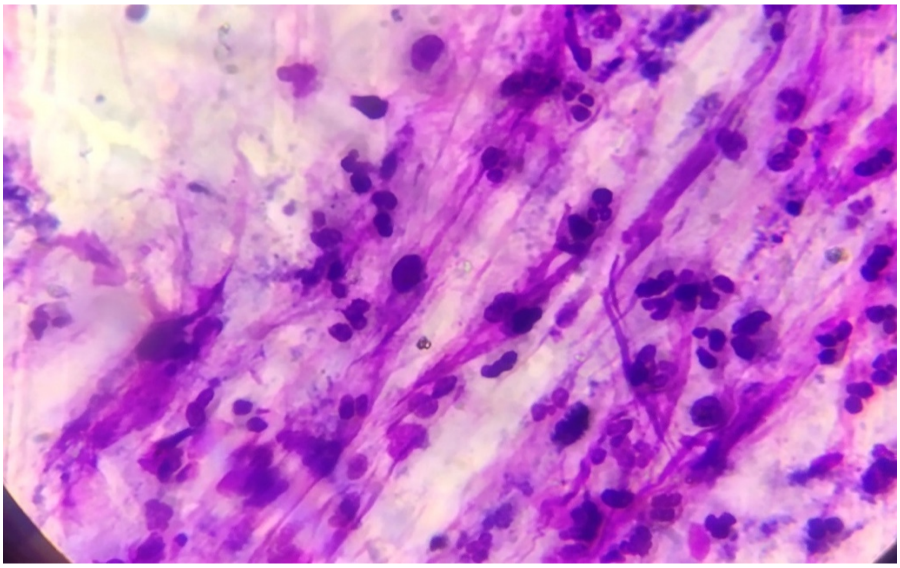

Figure 1: Giemsa method showing trophozoites and precysts

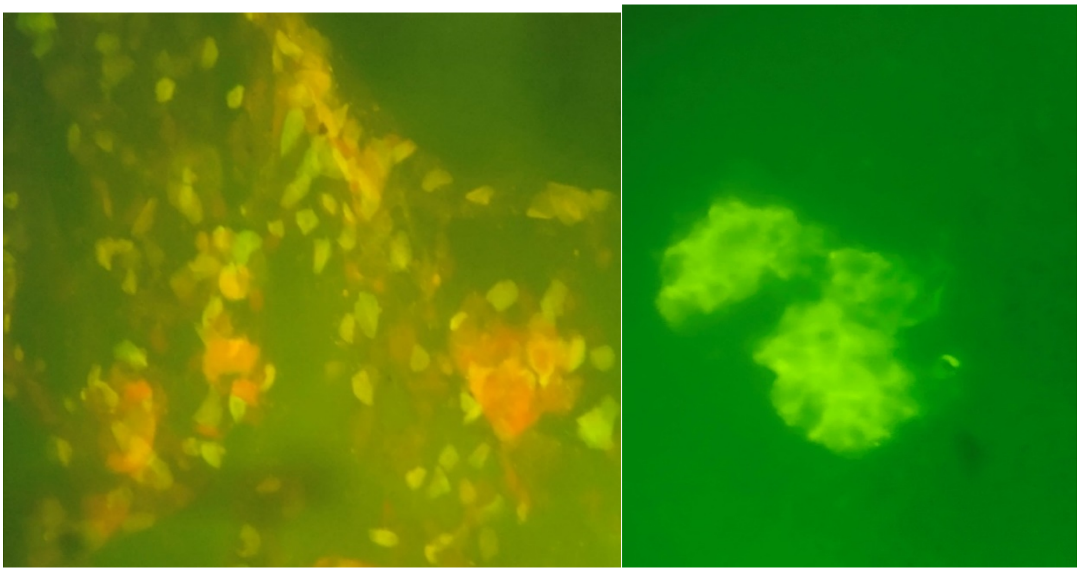

Figure 2: DFA immunofluorescence staining showing P. jiroveci cyst

Result

Out of 178 HIV Seropositive patients 70(39.3%) were positive for Pneumocystis by Direct Immunofluorescent staining and 48 (27%) by Giemsa staining.

Positivity is higher in the age group 40 years & above in both the staining (Table 1, 2). The Gender wise positivity was higher in Male (around 77%) in both the staining (Table 3).The CD4 count was less than 200 in all positive cases (Chart 1, 2).The Sensitivity was 79.1%, Specificity was 100 %. Positive predictive value and Negative predictive value were 100 % & 89.1%, respectively, for Giemsa staining compared to DFA as the gold standard. (Table 4).

Table 1: Age wise distribution of Giemsa staining

			Age Cat				Total
			<=30	31-40	41-50	>50	
GIEMSA	Negative	Count	20	26	48	36	130
		% within Age Cat	71.4%	63.4%	73.8%	81.8%	73.0%
		% of Total	11.2%	14.6%	27.0%	20.2%	73.0%
	Positive	Count	8	15	17	8	48
		% within Age Cat	28.6%	36.6%	26.2%	18.2%	27.0%
		% of Total	4.5%	8.4%	9.6%	4.5%	27.0%
Total		Count	28	41	65	44	178
		% within Age Cat	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	15.7%	23.0%	36.5%	24.7%	100.0%

Table 2: Age wise distribution of Immunofluorescent staining (IFAT)

			Age Cat				Total
			<=30	31-40	41-50	>50	
IF	Negative	Count	16	18	41	33	108
		% within Age Cat	57.1%	43.9%	63.1%	75.0%	60.7%
		% of Total	9.0%	10.1%	23.0%	18.5%	60.7%
	Positive	Count	12	23	24	11	70
		% within Age Cat	42.9%	56.1%	36.9%	25.0%	39.3%
		% of Total	6.7%	12.9%	13.5%	6.2%	39.3%
Total		Count	28	41	65	44	178
		% within Age Cat	100.0%	100.0%	100.0%	100.0%	100.0%
		% of Total	15.7%	23.0%	36.5%	24.7%	100.0%

Table 3: Gender wise distribution of Positivity in both staining

	Giemsa staining(N=48)	Direct Immunofluorescent staining(N=70)
Male	37(77%)	54(77 %)
Female	11 (23 %)	16 (23%)

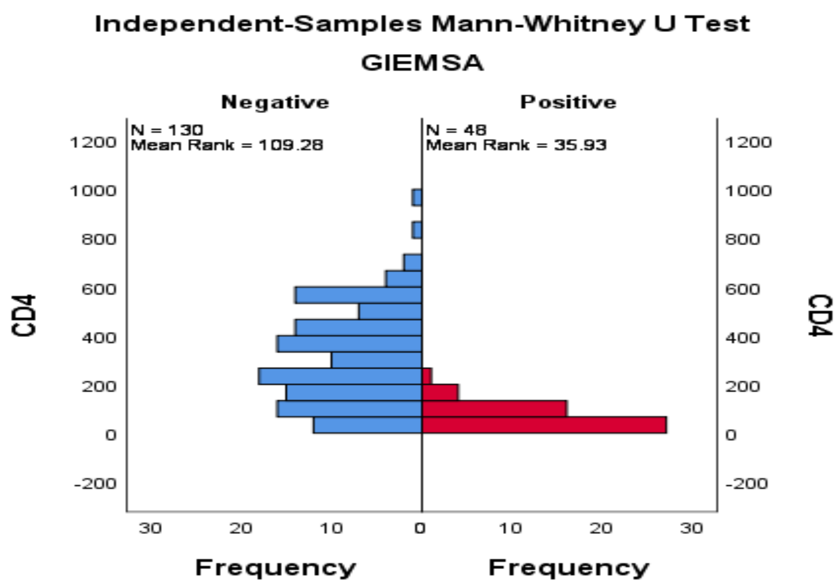

Chart 1: Frequency of Cd4 Count in Giemsa Staining

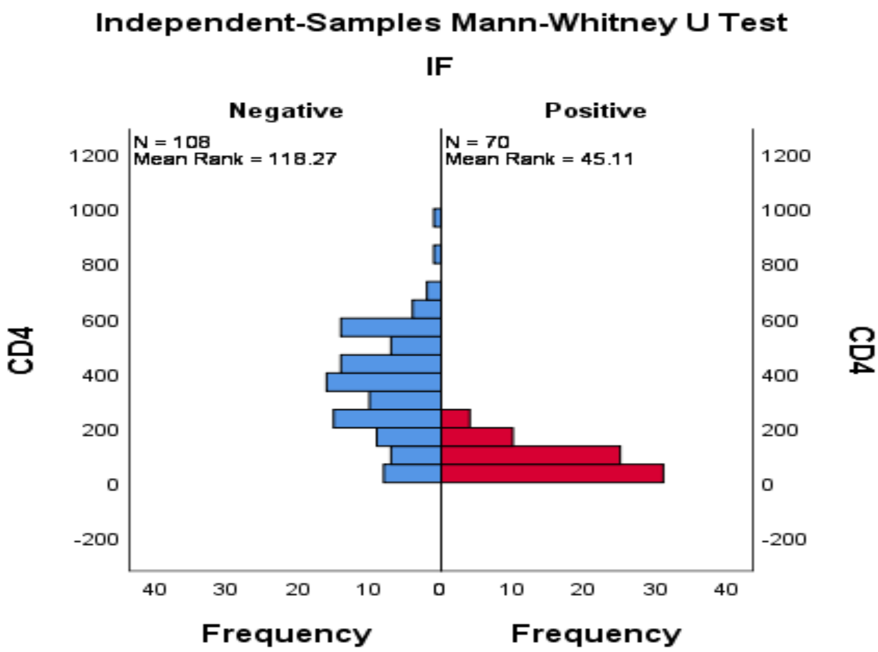

Chart 2: Frequency of Cd4 Count in Immunofluorescent Staining

Table 4: Sensitivity and Specificity of Giemsa staining

Giemsa				Total
IF	Positive		Negative	
POS	48		22	70
NEG	0		108	108
Total	48		130	178
Sensitivity	Specificity	PPV	NPV	Accuracy
79.1%	100%	100%	95.58%	89.1%

Discussion

Pneumocystis jirovecii, previously classified as a protozoan, is now recognized as an ascomycete fungus (Ravinder et al., 2015). This organism has two primary developmental stages. (Ravinder et al., 2015): the pleomorphic trophozoite form and the cystic stage. Notably, P. jirovecii colonization has been observed in adults with chronic obstructive airway disease, potentially serving as a reservoir for infection (Helweg-Larsen et al., 2002).

The clinical presentation of PCP was characterized by symptoms such as cough (100%), fever (65%), and dyspnea (90%), consistent with findings from previous studies in the United States (Stansell et al., 1997). Radiographic findings for all PCP positive patient had typically included interstitial pulmonary infiltrates similar to the study which reported that pulmonary infiltrates were present in 17 out of 19 (89.5%) patients (Doyle et al., 2017).

In our study of 178 PLHIV, P. jirovecii positivity was 39.3% (70/178) using direct immunofluorescent staining (DFA) and 27% (48/178) using Giemsa staining. These findings vary comparable to other studies, such as (Mohammad Y. Alshahrani et al 2020). (100% positivity by DFA) and (W.Wootta et al 2014). (44% positivity).Positivity was higher in the 40-50 age group, consistent with (Mohammad Y. Alshahrani et al,2020)'s findings (mean age 52 years). Males had a higher positivity rate (77%) compared to females (23%), aligning with studies by (Mohammad Y. Alshahrani et al,2020 and Brian P. Epling et al.2025). All positive cases had CD4 counts below 200 cells/µL, highlighting the vulnerability of immunocompromised patients. This finding is consistent with studies by (Brian P. Epling et al2025. and G. Allwyn Vijay et al 2019). Giemsa staining demonstrated a sensitivity of 79.1% and specificity of 100% compared to immunofluorescent staining as the gold standard. These findings are comparable to other studies by (Ana Luísa Tomás et al 2018.) sensitivity 79%, specificity 99%, (Mohamad Mohsen Homayouni et al 2016) 100% sensitivity and specificity and (P. Cregan et al 1990).: sensitivity 92%.These findings emphasize the importance of accurate diagnosis and understanding the characteristics of P. jirovecii infection in immunocompromised patients.

Conclusion

This study concludes that induced sputum analysis is a rapid and low-cost technique for diagnosing PCP. Giemsa staining emerged as a suitable primary method for routine use in clinical laboratories with limited facilities due to its cost-effectiveness, speed, and high specificity. We suggest using Giemsa staining as the initial staining method for efficient evaluation, followed by confirmation with DFA staining for suspicious samples to enhance sensitivity and specificity.

Limitation: One limitation of this study is that PCR (Polymerase Chain Reaction) was not performed, which could have potentially enhanced the diagnostic accuracy for Pneumocystis jirovecii detection.

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