บทความวิจัย

Research article

การศึกษาประโยชน์ของการย้อม Ziehl-Neelsen เพิ่มเติม ในการค้นหาเชื้อวัณโรคในเนื้อเยื่อ

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บทคัดย่อ

วัตถุประสงค์: 1) วัตถุประสงค์หลัก เพื่อการย้อม Ziehl-Neelsen (ZN) เพิ่มเติม จะช่วยทำให้ตรวจพบเชื้อวัณโรค (ผลบวก) เพิ่มขึ้นหรือไม่ และ 2) วัตถุประสงค์รอง ศึกษาปัจจัยลักษณะทางพยาธิวิทยา เช่น necrosis มีความสัมพันธ์กับการตรวจพบเชื้อวัณโรค โดยการย้อม Ziehl-Neelsen (ZN)

วิธีการดำเนินการวิจัย: รูปแบบการวิจัยเป็นการวิจัยแบบพรรณนา (descriptive study) ศึกษาย้อนหลังโดยนำบล็อกชิ้นเนื้อที่เก็บไว้มาย้อมเชื้อวัณโรค โดยวิธี Ziehl-Neelsen (ZN) เพิ่มเติมนำชิ้นเนื้อพาราฟินที่ได้รับการตรวจหาเชื้อวัณโรคเป็นบวกด้วยเทคนิคพีซีอาร์ ในโรงพยาบาลจุฬาลงกรณ์ ตั้งแต่ปี พ.ศ. 2559 - 2561 มาตัดย้อม ZN เพิ่มอีก 1 แผ่น จากนั้น ทำการทบทวนผลการย้อม ZN เดิม และตรวจดูผล ZN ที่ย้อมเพิ่มเติม

ผลการวิจัย: จากตัวอย่างทั้งหมด 78 ตัวอย่าง ที่มีผลการตรวจเชื้อวัณโรคเป็นบวกด้วยเทคนิคพีซีอาร์ พบว่าเมื่อนำมาตัดย้อม ZN เพิ่มอีก 1 แผ่น จะทำให้มีผลการย้อมเป็นบวกเพิ่มขึ้นจากเดิม 15 ตัวอย่าง (จากการย้อมครั้งแรก) กลายเป็น 19 ตัวอย่าง (ความไวของการตรวจเพิ่มขึ้นจาก ร้อยละ 19.23 เป็น 24.36)

สรุป: การตัดแผ่นชิ้นเนื้อย้อม ZN เพิ่มอีก 1 แผ่น สามารถเพิ่มความไวในการตรวจหาเชื้อวัณโรคได้ ร้อยละ 5.13

คำสำคัญ: ชิ้นเนื้อพาราฟิน การตรวจหาเชื้อวัณโรค Ziehl-Neelsen stain ความไวของการตรวจ

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Research article

The study of benefit of an additional Ziehl-Neelsen stain for detection of acid-fast bacilli in tissue sections

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Abstract

Objectives: This research were 1) to determine whether an additional Ziehl-Neelsen (ZN) stain section could increase the positive rate and 2) to correlate pathological findings with the Ziehl-Neelsen stain results.

Materials and Methods: The research design of this study was descriptive study. Formalin-fixed paraffin-embedded (FFPE) tissue with positive polymerase chain reaction (PCR)-based detection of M. tuberculosis were retrieved from the Pathology File at King Chulalongkorn Memorial Hospital during 2016 to 2018. The original ZN stain was reviewed, with an additional ZN stain performed.

Results: Of 78 cases with positive PCR, 15 (19.23%) were positive for acid-fast bacilli (AFB) in the original ZN section. Additional 4 cases (19 positive cases in total) were shown to contain AFB in the additional ZN stain section (sensitivity increased from 19.23% to 24.36%).

Conclusions: An additional ZN stain section increased sensitivity (5.13%) for detection of AFB in tissue sections.

Keywords: formalin-fixed paraffin-embedded tissue, PCR for M Tuberculosis, Ziehl-Neelsen stain, acid-fast bacilli sensitivity

Introduction

Tuberculosis (TB) is still one of the important healthcare problems in many developing countries. Based on the Bureau of TB, the total number of patients in Thailand who registered for TB treatment, including new and recurrent cases, was increased from 62,135 cases in year 2015 to 70,114 cases in year 2016.[1]

Accurate diagnosis of tuberculous infection is crucial for the patients to obtain proper treatment. While the Ziehl-Neelsen (ZN) stain, generally known as "AFB" stain, remains the first method for investigation in most pathology laboratories, polymerase chain reaction (PCR) has increasingly been used to diagnose M. Tuberculosis complex in formalin-fixed paraffin-embedded (FFPE) samples. The sensitivity and specificity of PCR method were 70 - 82% and 99%, respectively.[2] While ZN stain is much less expensive, it is known to have sensitivity problem (27% - 37%).[3,4,5,6] The specificity of both methods was comparable, up to 98%.[5,6]

Since ZN is wildly available and diagnosis is based on direct visualization, we hypothesized that an additional ZN section would increase the diagnostic yield. The study was, therefore, conducted to determine the benefit of the addition ZN-stained section. The second objective was to describe the histopathological features of M. Tuberculosis complex in our patient cohort.

Method

The research design of this study was descriptive study. 78 formalin-fixed paraffin-embedded (FFPE) samples, with positive PCR-based detection of M. Tuberculosis complex and available ZN result, were retrieved from the Pathology File of the Department of Pathology, King Chulalongkorn Memorial Hospital during 2016 to 2018. In that period, PCR was performed in 624 samples by method previously described.[7] Using the positive PCR result as the gold standard, an additional ZN stain was performed by the standard protocol.[8] Histopathological features were correlated with the ZN result. The study was approved by the intuitional review board at Faculty of Medicine, Chulalongkorn University (IRB#312/62)

DNA Extraction from FFPE Samples

Sections (5 μm thick) were cut from each paraffin block. Five to eight sections were taken from the specimens. These sections were used for PCRs. We extracted DNA from each block by standard proteinase K digestion followed by using QIAamp DNA FFPE Tissue Kit (Qiagen, Germany) (Figure 1) for extraction

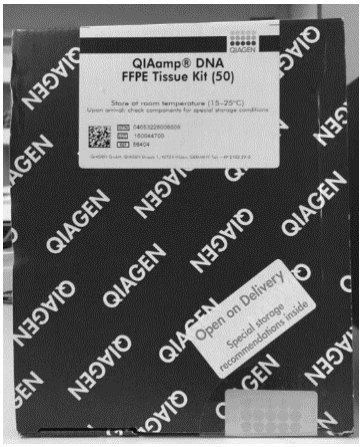

Figure 1: QIAamp DNA FFPE Tissue Kit

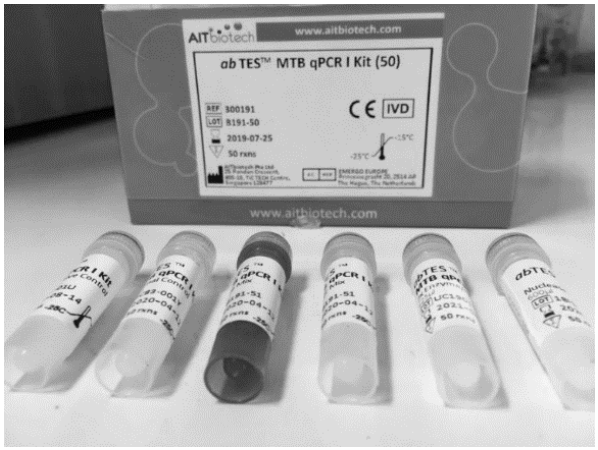

Figure 3: ab TES™ MTB qPCR I KIT

Real-Time PCR for M. Tuberculosis complex

The PCR was processed in real time by means of an ABI Prism 7500 Sequence Detection System (Applied Biosystems) (Figure 2) using the abTES™ MTB qPCR I KIT (Figure 3) for the detection of MTB complex (M.tuberculosis, M. africanum, M.bovis, M.bovis BCG, M.microtic, and M. pinnipedii). The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of the various members of MTB complex using highly specific primer pairs and double-dye hydrolysis probe.

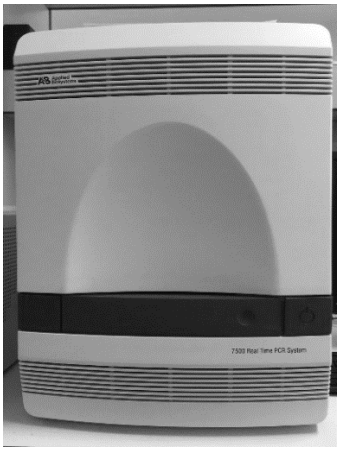

Figure 2: ABI Prism 7500 Sequence Detection System (Applied Biosystems)

Ziehl-Neelsen Staining

1. Dye Acid fast bacilli on the slide thick meat 2 μm
2. Deparaffinize slde into boiled water
3. Dip slide in Modifled Ziehl - Neelsen carbon - fuchsin solution for 5 minutes
4. Rinse with tap water for 3 minutes
5. Wash the color out with 1% acid alcohol, alternating with tap water until get slide in clear version
6. Wash it with tap water for 3 minutes
7. Counterstain with Methylene blue solution for 5 minutes
8. Wash it with tap water for 3 minutes
9. Dehydrate, clear and then mount with permount and examine with microscope.

Result

Most of the samples with positive PCR showed necrotizing granulomatous inflammation (43/78, 55.13%), and this type of pathology showed the highest percentage of positive ZN stain

(Table 1). Based on the original ZN stain, 15/78 PCR-positive cases were shown to have positive result (19.23% sensitivity). With an additional ZN stain, 4 more positive cases were noted (19 positive cases in total, 24.36% sensitivity). Therefore, an extra ZN stain section could increase the sensitivity for detecting acid fast bacilli by 5.13%. Of the 4 additional positive ZN cases, there were 2 cases of necrotizing granulomatous inflammation, 1 granulomatous inflammation, and 1 non-specific inflammation. Of the total 19 cases with positive ZN stain, 3 patients were immunocompromised (2 with acquired immunodeficiency syndrome and 1 with T cell acute leukemia), and specimens from all these 3 cases showed necrotizing granulomatous inflammation.

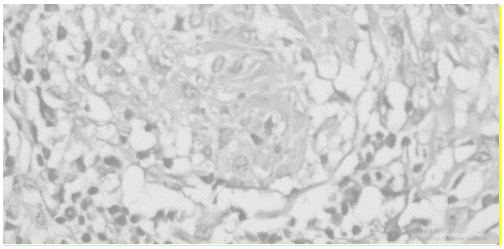

Figure 4: Acid fast bacillus in additional Ziehl-Neelsen (ZN) stain (arrow)

Table 1: Histopathological features with results of the original and additional ZN stains

		Histopathological Features			
		Necrotizing granulomatous inflammation	**Granulomatous inflammation**	**Inflammation without definite evidence of granulomatous inflammation**	**Total**
The original ZN stain results (N)	Positive	11	2	2	15
	Negative	32	15	16	63
The additional ZN stain (N)	Positive	13	3	3	19
	Negative	30	14	15	59
No. of cases		43	17	18	78

Discussion

Accurate and timely diagnosis of tuberculous infection is crucial, especially in countries, including Thailand, where the infection is still common.[1] Granulomatous inflammation with caseous necrosis is the well-known pathology of tuberculosis although sometimes it is not straightforward to determine whether the necrosis is caseous or non-caseous necrosis.[9] Therefore, diagnosis of tuberculous infection cannot be made reliably in all

cases by morphological examination alone. While PCR-based method has increasingly been applied with FFPE samples to diagnose tuberculosis,[2] ZN stain remains the first method in many pathology laboratories. The ZN is simple, cheap, and wildly available but its sensitivity is limited.[3,4,5]

In this study, we were able to increase the sensitivity of ZN stain (from 19.23% to 24.36%) simply by performing an additional ZN stain section. Although the sensitivity increased (5.13%) is not so impressive, it would be cost-effective in term of laboratory workload and cost. The cost of PCR is 2,000 Baht but the cost of ZN stain is 50 Baht. However, examination of ZN stain is time-consuming and requires skillful pathologists. Future development of artificial intelligence technology may resolve this obstacle and may increase sensitivity of detection.

In conclusion, we were able to increase in the sensitivity of ZN stain simply by performing an additional ZN-stained sections. Although the sensitivity increased (5.13%) is not so impressive, it would be cost-effective in term of laboratory workload and cost.

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