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Cutaneous TB: Different Clinical Types and Comparing the Values of its Diagnostic Tests

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Cutaneous tuberculosis (TB) can present with a wide range of clinical presentations depending on the route of infection, immune status of the patient and whether or not there has been previous sensitization with TB. The occurrences of different forms of cutaneous TB vary globally. Depending on the types of cutaneous TB, the efficacies of different diagnostic tests are varying and there is no single perfect tool. In this study, the available diagnostic tests for cutaneous TB such as tuberculin test, smear for acid-fast bacilli (AFB), histopathologic examination, TB culture and polymerase chain reaction (PCR) for *M. tuberculosis* DNA from skin biopsy specimen were done in 25 clinically diagnosed cutaneous TB cases attending the Dermatology Ward, YGH from June 2014 to August 2015. The positivity of diagnostic test results were compared according to the types of cutaneous TB. Among different clinical types recorded, lupus vulgaris was the most prevalent one (13 cases, 52%) and the least was tuberculids (4 cases, 16%). When comparing the positivity of different diagnostic tests, PCR was positive in 13 cases (52%) which was the most and there was no culture positive nor AFB smear positive cases. These results showed that careful clinical examination is still essential for the evaluation and treatment of cutaneous TB while also highlighting the TB PCR as a sensitive test for the confirmation of cutaneous TB.

Key words: Cutaneous TB, PCR, Diagnosis

INTRODUCTION

The resurgence of tuberculosis (TB), coincided with human immunodeficiency virus (HIV) epidemics, has been documented in both developed and developing nations, in last 20 years.[1] Similarly, the incidence of cutaneous TB is increasing globally including Europe.[2] The occurrence of different forms of cutaneous TB varies globally. Scrofuloderma was the commonest form of cutaneous TB in UK,[3] lupus vulgaris in South Africa and Spain,[2, 4] the tuberculids in Japan[5] and scrofuloderma and lichen scrofulosorum were the most frequently found forms in childhood, whereas lupus vulgaris was the commonest form in adults in India and Pakistan.[6-8] In addition, serial reviews from Hong Kong have shown a change in the commonest form of skin tuberculosis in recent years from tuberculosis verrucosa cutis in 1968[9] to the tuberculid, erythema induratum in 1995[10] and 2006.[11] Despite being considered a benign form of TB, cutaneous TB can be accompanied by TB in internal organs especially lungs, and severe complications can occur, such as the development of squamous cell carcinoma in long-lasting lesions.[2]

The diagnosis of cutaneous TB is usually confirmed by the conventional methods such as smear examination, conventional (LJ media culture) and rapid BACTEC culture and histopathological examination

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for mycobacteria as well as TB PCR nowadays.[12] Although they are confirmatory, a negative smear for acid-fast bacilli, lack of granulomas on histopathology and failure to culture *Mycobacterium tuberculosis* do not exclude the diagnosis of cutaneous TB.[1] Likely, TB PCR has a definite role in rapid diagnosis of cutaneous TB, where paucibacillary tuberculosis is suspected, clinical decision should not be based on PCR results alone.[13] In an Indian study, PCR test showed the maximum positivity of 79.4% followed by histopathology 73.5%, BACTEC culture 47.5%, LJ media culture 29.4% and smear examination 5.8%.[12] These results showed that TB PCR is a rapid and sensitive test for diagnosis of cutaneous TB using skin biopsy samples[12] but it is accepted that careful clinical examination is still the gold standard for the evaluation and treatment of cutaneous TB.[14]

In Myanmar, only scanty data on cutaneous TB existed. Concerning PCR, there were some studies of PCR usage in identification of *M. tuberculosis* in TB meningitis,[15] in smear-negative pulmonary tuberculosis[16] and identification of *M. leprae* from nasal swab and skin biopsy[17-19] but not in cutaneous TB diagnosis. Combining these with the fact that cutaneous TB cases were increasingly seen in daily clinical practice, from only a few cases per year before 2010 to nearly 20 cases in 2013, it is thought that it would be beneficial to get the knowledge of prevailing forms of cutaneous TB, the association of cutaneous TB and pulmonary TB, and the efficacies of different diagnostic tools in cutaneous TB including that of PCR, in Myanmar. In this study, apart from PCR that could not do as a routine clinical investigation for cutaneous TB, it is planned to find out these facts by using clinically available diagnostic tests, which would be done as usual clinical laboratory investigations, in order to observe the findings that might be directly usable in daily clinical practice.

MATERIALS AND METHODS

All clinically diagnosed cutaneous TB cases attending Dermatology Ward, Yangon General Hospital, from June 2014 to August 2015, who gave informed consent were included. The available diagnostic tests for cutaneous TB such as tuberculin test, smear for AFB, histopathologic examination, TB culture and PCR for *M. tuberculosis* DNA from skin biopsy specimen were done in all 25 cases. Skin tuberculin test was done at Parami Laboratory, smear for AFB from skin biopsy specimen and histopathologic examination were done at YGH Clinical Pathology Laboratory, TB culture was done at Aung San TB Hospital Laboratory, and PCR for *M. tuberculosis* was done at Bacteriology Research Division, Department of Medical Research.

To detect the associated pulmonary TB infection, chest X-ray (PA) (YGH Radiology Department), blood for CP and ESR (YGH Clinical Pathology Laboratory) were done also. All the specimens and patients for the tests, except for PCR, were sent as routine clinical samples and patients with relevant clinical data but without the prior notice to the respective laboratories as they were sent for this study. The detail methods for those investigations were according to the usual practice of respective laboratories and only the detail method of TB PCR was noted here.

DNA extraction

Skin specimens (skin biopsy) were stored at -20ºC till DNA extraction. Before DNA extraction the skin specimen was cut into small pieces and crushed in 2 ml of normal saline using sterile glass homogenizer and concentrated again by centrifugation at 12000 rpm for 10 minutes. The supernatant was removed while leaving 1 ml. DNA was extracted by in-house method using 10 x digestion buffer which is composed of proteinase K, Tween 20 and 1 M Tris HCl followed by boiling.

IS6110 Polymerase Chain Reaction assay

Insertion segment, IS6110, which is a specific gene segment found in *Mycobacterium tuberculosis* is detected by PCR assay. Each PCR mixture was prepared in a volume of 50 µl containing 20 ng of DNA, 0.2 U of Taq polymerase, a 0.1 mM concentration of each deoxynucleoside triphosphate (dNTP), and 0.2 µM (each) primer. The amplification cycle was 3 minutes at 94ºC; followed by 40 cycles of 1.5 minutes at 94ºC, 2 minutes at 65ºC, and 3 minutes at 72ºC; with a final step for 10 minutes at 72ºC. PCR products were electrophoresed on 0.8% agarose gels and was checked the presence of IS6110 sequence (245 bp).

Data entry and analysis

Microsoft excel software was used for data entry. Consistency checks were done.

Ethical consideration

This study was carried out with the approval of the Ethical Review Committee on Medical Research Involving Human Subjects, Department of Medical Research.

RESULTS

It was found that 15 out of 25 cutaneous TB cases (60%) were between the age of 20 to 50 years (the youngest was 8 and the oldest was 77 years old) and majority of cases were females (17 cases, 68%) in this study. The most affected site were extremities (17 cases, 68%). Duration of the disease revealed a wide range, from 2 months to more than 50 years (Table 1). It was also noted that most of the tuberculid cases affected more than one site of body parts, lesions on both extremities and trunk. In this study, only 3 types of cutaneous TB, lupus vulgaris (LV), tuberculosis verrucosa cutis (TVC) and papulonecrotic tuberculid of 13 cases (52%), 8 cases (32%) and 4 cases (16%), respectively, were diagnosed clinically. ESR changes were not consistent and pulmonary TB was found in only 2 cases by CXR.

Table 1. Patient characteristics, clinical diagnosis and ESR and chest X-ray changes of 25 patients with cutaneous TB

Age (year)	Sex	Site	Duration (year)	Clinical Dx	ESR	CXR (PA)
50	M	Rt knee	3	TVC	20	NAD
12	M	Lt elbow	1	LV	24	NAD
77	F	Chest	1	LV	16	NAD
14	F	Face	4	LV	60	NAD
60	F	Face	2	LV	41	NAD
36	F	Rt ankle	0.5	TVC	30	NAD
65	F	Face, neck	50	LV	60	NAD
37	M	Finger	2	TVC	10	NAD
45	F	Extremities and trunk	0.25	Tuberculid	40	NAD
27	M	Rt F arm	0.25	TVC	15	NAD
23	M	Rt knee	10	TVC	10	Chest Inf
40	F	Extremities and trunk	1	Tuberculid	37	NAD
22	F	Extremities and trunk	0.16	Tuberculid	22	NAD
36	F	Extremities	0.5	Tuberculid	12	NAD
47	F	Face	3	LV	15	NAD
66	F	Lt arm	1	LV	40	NAD
41	F	Rt thigh	3	LV	10	NAD
8	M	Lt knee	2	LV	10	NAD
23	F	Lt knee	2.5	TVC	10	NAD
28	F	Face	5	LV	40	NAD
76	F	Face	5	LV	35	Cardio-megaly
19	M	Face	1	TVC	45	NAD
12	M	Rt knee	11	TVC	10	Pul TB
29	F	Lt arm	0.75	LV	12	NAD
42	F	Lt F arm	16	LV	15	Pul TB

F arm=Fore arm, Inf=Infection, Lt=Left, Rt=Right, Pul=Pulmonary, M=Male, F=Female, TVC=Tuberculosis verrucosa cutis, LV=Lupus vulgaris

The clinical appearance of cutaneous TB might vary not only according to different clinical types (Fig. 1) but also in cases of a single variant and it was recorded that tattooing might cause cutaneous TB in our study. Concerning the diagnostic tests, TB PCR was positive in 13 cases (52%), T test in 10 cases (40%), histopathology (chronic granulomatous inflammation, CGI) 7 cases (28%), LV 6 cases (24%), tuberculid 1 case (4%) and nonspecific dermatitis (ND) 11 cases (44%)) (Table 2). For the positive results of PCR among different clinical types, 7 cases (53.8%) of 13 LV patients, 5 cases (62.5%) of 8 TVC patients and 1 case (25%) of 4 tuberculid patients were positive. Concerning T test, 4 cases (31%) of LV, 3 cases (37.5%) of TVC and 3 cases (75%) of tuberculid were positive. In histopathology results, among LV cases 2 cases (15%) were CGI, 6 cases (46%) were LV and 5 cases (39%) were ND; among TVC cases 5 cases

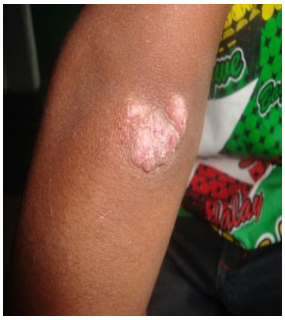

Fig. 1(a). Lupus vulgaris on Lt elbow

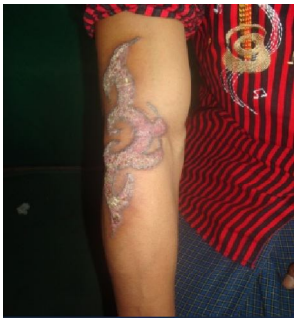

Fig. 1(b). Tuberculosis verrucosa cutis in a tattoo

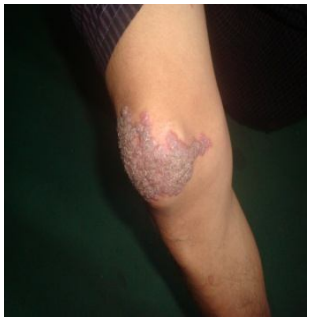

Fig. 1(c). Tuberculosis verrucosa cutis on Rt knee

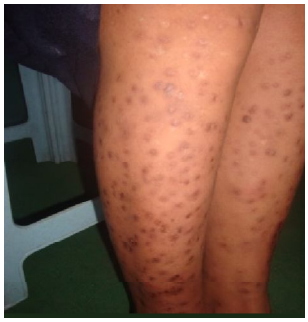

Fig. 1(d). Tuberculid on both lower extremities

Fig. 1. Different clinical types of cutaneous TB

Table 2. Diagnostic tests' positive results according to different clinical types of cutaneous TB.

Morphological/ Clinical types	PCR (n=25)	T test (n=25)	Histopathology (n=25)			
			CGI	LV	Tuberculid	ND
LV (n=13)	7	4	2	6	0	5
TVC (n=8)	5	3	5	0	0	3
Tuberculid (n=4)	1	3	0	0	1	3
Total	13 (52%)	10 (40%)	7 (28%)	6 (24%)	1 (4%)	11 (44%)

LV=Lupus vulgaris, TVC=Tuberculosis verrucosa-cutis, CGI=Chronic granulomatous inflammation, ND=Nonspecific dermatitis

(62.5%) were CGI, and 3 cases (37.5%) were ND; among tuberculid cases 1 case (25%) was tuberculid, and 3 cases (75%) were ND. Smear for AFB and TB culture from skin biopsy specimen were negative in all 25 cases.

DISCUSSION

Cutaneous TB can be classified into many different clinical types and their presentation is quite variable. Although only 3 different categories of cutaneous TB in this study were noted, there would be other varieties also occurred but they were not included because study area and study population were very limited. Also, scrofuloderma cases might common in patients with HIV and TB co-infection, but the result might not reflect the true prevalence of cutaneous TB.

Regarding positive histopathology results, even the CGI is not specific result for cutaneous TB as it might also be found in other chronic granulomatous cutaneous conditions such as deep mycosis or sarcoidosis. Another fact to note is that the biopsy specimen were sent as routine clinical samples without the special notice of this research purpose but with relevant clinical data and there might be possibility of interpersonal variation in interpretation of the histology slides and consequently the diagnosis. Concerning T test, the positive or negative result of only this could not be a confirmatory one. The gold standard confirmation test of TB culture was not positive in this study but that could not totally rule out cutaneous TB.[1] In this study, the results showed that TB PCR was the most specific and sensitive diagnostic test but the positive rate was lower than that found in the mentioned Indian study.[12] This might be due to the different method of DNA extraction and the method employed should be optimized and further large scale studies are still needed. Here, it was highlighted that different type of cutaneous TB, whether multi-bacillary or pauci-bacillary might also affect the result of PCR or other diagnostic tests.

Conclusion

Though further studies of wide study areas and population based are still necessary, it is shown in this study that the careful clinical examination and clinical acumen is still essential for the evaluation and treatment of cutaneous TB cases, especially in resource limited setting, while also highlighting the TB PCR as a sensitive test for the confirmation of cutaneous TB.

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