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CULTURE VS ACID FAST BACILLI SMEAR FOR THE DIAGNOSIS OF EXTRA-PULMONARY TUBERCULOSIS-A STUDY DONE AT PAKISTAN

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Abstract:

Objective: *The objective of the study was to compare the Acid-Fast Bacilli (AFB) staining and Culture for the diagnosis of extra-pulmonary tuberculosis and to find out extra-pulmonary tuberculosis burden from various sites of the body in suspected tuberculosis patients.*

Methods: *The study was conducted in Bahawal Victoria Hospital, Bahawalpur, Pakistan from June 2019 to November 2020. Standard method for Acid Fast Bacilli (AFB) staining and culture was followed. The standard Ziehl-Neelsen Technique was done for AFB staining and culture was done in Ogawa Medium.*

Results: *Out of 1058 extra-pulmonary samples cultured 20 (1.9%) were positive while out of 292 extra-pulmonary samples tested by AFB smear a mere 0.7% (2) were positive. The isolation rate for extra-pulmonary samples was three-fold higher in culture in comparison to AFB Smear. The confirmation rate of extra-pulmonary tuberculosis was approximately 1/ 8th of the pulmonary tuberculosis by conventional bacteriological diagnostic methods. Of the extra-pulmonary tuberculosis renal, endometrial or pelvic and cold abscess were common in this study. Tuberculosis was also isolated from peritoneal fluid, pericardial fluid, Synovial fluid, lymph node and cerebrospinal fluids only by culture.*

Conclusion: *The bacteriological conventional technique could diagnose a very low number of extra-pulmonary tuberculosis while the standard AFB culture has a much better role for diagnosis of extra-pulmonary tuberculosis. Novel techniques of diagnosis should be considered to confirm more extra-pulmonary tuberculosis.*

Keywords: *AFB Smear, Extra-pulmonary tuberculosis, Culture, Diagnosis.*

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INTRODUCTION:

Tuberculosis is a pretty common disease with 8 million people getting infected each year. The disease is more prevalent in developing and third world countries. According to an estimate 1.7 billion individuals are infected with Mycobacterium tuberculosis. Ninety-five percent of these cases are in developing countries and approximately 3 million patients die yearly [1]. South Asian Association for Regional cooperation (SAARC) region bears 22% of the global population and 29% of the global burden of tuberculosis. Another estimate shows that SAARC region has 2.5 million new cases and 0.6 million deaths per year [2]. Pakistan is among the developing countries in SAARC region with population of 212 million. According to WHO TB report 2019, in 2018, 562000 people got infected with tuberculosis in Pakistan and 44000 died because of it [3].

Extra-pulmonary tuberculosis is often difficult to diagnose using standard methods, although a history of previous exposure to tuberculosis and X-ray changes consistent to old pulmonary diseases were found in many cases [4]. The developing countries currently rely on AFB staining and culture of sputum in conjunction with assessment of clinical symptom and radiographic evidence to diagnose tuberculosis. Detection by AFB staining and culture lack sensitivity particularly in case of sputum negative diseases. Extra-pulmonary tuberculosis presents even more problems as the sputum samples are often not available and obtaining specimen from the suspected sites of infection often involve highly and expensive procedures [5]. The Study done in University Hospital in Spain revealed that 20% of all bacteriologically confirmed cases were extra-pulmonary and the most frequent site was urinary tract [6]. Extra-pulmonary tuberculosis was generally observed most often in immunosuppressed patients but 34% of cases were observed in people without any underlying diseases or risk factors. Delay to diagnosis was especially long in non-immunosuppressed patients [7]. The women, non-Hispanic black and HIV positive persons have been found to be higher risk for extra-pulmonary tuberculosis [8].

MATERIALS AND METHODS:

After taking consent from the ethical review committee, the study was conducted in Bahawal Victoria Hospital, Bahawalpur, Pakistan from June 2019 to November 2020. Standard method for Acid Fast Bacilli (AFB) staining and culture was followed[9]. For sputum samples modified Petroff methods was used and sputum culture was done only clinically suspected and AFB smear negative samples. All the suspected patients of pulmonary and extra-pulmonary tuberculosis from any site of the body were included in this study. Samples from the sterile site such as endometrium, peritoneal fluid, pleural fluid, synovial fluid, lymph node aspirate and bone marrow were centrifuged at 3000xg for 15 minutes, and then supernatant was discarded and deposit was inoculated in 2 tubes of 3% Ogawa medium. Lymph node, endometrial tissue or aspirate and other surgically resected tissue were cut into small piece with sterile scalpel or scissors. The specimen was homogenized in a sterile mortar using 0.5 to 1 ml sterile saline and a small quantity of sterilized sand. The suspension was mixed with 5ml of sterile saline, mixed well and centrifuged at 3000xg for 15 minutes, then supernatant was discarded and 0.1 ml of sediment was inoculated in each 3% Ogawa medium in duplicate. Whenever doubt existed about the contamination of the specimen, an untreated portion of the specimen was inoculated into a nonselective bacteriological medium e.g. nutrient agar and incubated for 24 hours to check for the presence of fast growing non mycobacterial organisms. The remaining portion of the specimen was kept untreated in refrigerator until the absence of contamination was confirmed. Those samples having grown contaminants were processed like urine sample. Patients were instructed to collect 24 hours' urine sample in a sterile container. The urine was kept at refrigerator for over-night for sedimentation purpose. Then the sediment was centrifuged at 3000x g for 15 minutes. The supernatant was discarded, then equal volume of 4% sulphuric acid was added on sediment, mixed and kept at room temperature for 15 minutes, then 15 ml of sterile saline was added in it and centrifuged at 3000xg for 15 minutes. The supernatant was discarded and sediment was neutralized with 4% NaOH containing a phenol red indicator. Then 0.1 ml each of sediment was inoculated in two tubes of 3% Ogawa medium and incubated at 37^0C. Cultures were observed every week for 8 weeks. The culture was discarded when there was no growth observed until 8 weeks of incubation.

RESULTS:

Of the 1413 samples, 1121 (79.3%) from the pulmonary and 292(20.7%) from the extra-pulmonary were subjected for smear microscopy. Similarly, out of 1521 samples cultured for M. tuberculosis, 463(30.4%) of pulmonary and 1058 (69.6%) samples were from the extra-pulmonary sources. Out of 1121 pulmonary samples for AFB smear 182(16.2%) smears were positive. Out of 292 extra-pulmonary smears only 2(0.7%) smears were positive. Of the 463 samples cultured, only 22(4.8%) were culture positive. Similarly, out of 1058 samples cultured from extra-pulmonary sources 20(1.9%) were culture positive. Of the 20 isolates obtained from the culture 7 from urine, 4 from endometrial tissue, 4 from cold abscess, one each from peritoneal, pericardial, Synovial fluid and lymph node aspiration. In comparison to 16.2% and 4.8%

of pulmonary tuberculosis isolated from the AFB staining and culture of the pulmonary sources, only 0.7% and 1.9% tuberculosis were confirmed by AFB staining and culture from the extra-pulmonary sources (Table I).

Table 1: Bacterial investigations.

Origin of samples		A.F.B Smear			Culture		
		No. Smear	Smear Positive	%	No. Culture	Culture positive	%
1.	**Pulmonary:**						
a.	Sputum	1099	182	16.5	368	20	5.4
b.	Bronchial washing	22	0	0	95	2	2.1
Subtotal		**1121**	**182**	**16.2**	**463**	**22**	**4.8**
2.	**Extra-pulmonary:**						
a.	Endometrial asp.	140	0	0	650	4	0.6
b.	Peritoneal fluid	10	0	0	22	1	4.5
c.	Pericardial fluid	5	0	0	16	1	6.3
d.	Urine	40	1	2.5	130	7	5.3
e.	Pus (Cold abscess)	24	1	4.2	68	4	5.8
f.	Synovial fluid	1	0	0	8	1	12.5
g.	Lymph node asp.	2	0	0	25	1	4.0
h.	C.S.F	30	0	0	69	1	1.4
i.	Bone marrow	2	0	0	11	0	0
j.	Pleural fluid	38	0	0	59	0	0
Subtotal		**292**	**2**	**0.7**	**1058**	**20**	**1.9**
Total		**1413**	**184**	**13.0**	**1521**	**42**	**2.7**

DISCUSSION:

The percentage of isolation among the suspects of extra-pulmonary tuberculosis was very low in comparison to pulmonary suspects in this study. The isolation rate of extra-pulmonary tuberculosis was approximately 1/8th of the pulmonary tuberculosis. Similar to present study extra-pulmonary tuberculosis was found 20% of the all the bacteriologically confirmed tuberculosis in Spain [6]. Many laboratories of the resource poor countries have to still relay on smear examination and AFB culture, although more sensitive methods are available for diagnosis of extra-pulmonary tuberculosis [5,9]. Similar to present study significantly higher number of suspected cervical tuberculosis was confirmed by culture in comparison to AFB smear staining. Polymerase chain reaction (PCR) was highly sensitive than culture but specificity was lower in PCR in comparison to culture [10].

Out of the investigated samples from the various extra-pulmonary sites most of the isolates were obtained from urinary, pelvic and cold abscess in this study. The incidence of urogenital tuberculosis has significantly decreased over the years due to unknown reason and the incidence of other extra-pulmonary tuberculosis remained same [11]. In contrast to present study the most commonly observed site was pleura in a region of high prevalence of tuberculosis and low prevalence of HIV [12]. The peripheral lymph node and abdominal tuberculosis were the most commonly observed in Denis clinic [13]. The diagnosis of tuberculosis peritonitis was less than 3% of cases by using acid fast staining of ascitic fluid and culure of M. tuberculosis could detect up to 20% of cases in several trials [14]. The present study was able to isolate M tuberculosis from 4.5% of the investigated samples which were negative in AFB smear. Only 4(0.6%) of the endometrial or pelvic samples were positive for M tuberculosis in present study. The isolation rate of Mycobacterial culture from endometrial aspirates of this study was comparable to Indian studies [15]. In contrast to present and Indian studies, high percentage of endometrial tuberculosis was detected by BACTEC culture and histopathological examination in Rawal Pindi, Pakistan [16]. About 10% of patients of pulmonary develop genital tuberculosis although less than 1% of salpingitis can be attributed to Mycobacterium tuberculosis [17].

Urogenital tuberculosis was the most commonly found exta pulmonary tuberculosis in this study. Urogenital tuberculosis occurs with the haematogeneous spread of tubercle bacilli to the glomeruli. The infection spreads to involve renal pelvis, ureter, bladder, seminal vesicles, epididymis and testes [18]. The urogenital tuberculosis comprises about 15 to 20 % of extra-pulmonary tuberculosis in developing countries but it was

found 6% of the extra-pulmonary tuberculosis in United States [19]. Similar types of result were found in Spain whereas lymph node and bone tuberculosis was more common in Shanghai [3,20].

CONCLUSION:

To conclude, the isolation rate of M.Tuberculosis by AFB smear examination and culture was low in present study and it is agreed that there is a urgent need for improving the diagnosis of extra-pulmonary tuberculosis. The new methods of diagnosis should be considered on the basis of sensitivity, specificity, the cost per test, and time taken to perform the test. In addition, it should also address the demographic factors, HIV status, acceptability of the new technique.

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