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THE VALUE OF HISTOLOGY, CULTURE AND GUINEA PIG INOCULATION EXAMINATION IN OSTEO-ARTICULAR TUBERCULOSIS

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Tuberculosis is on the decline in affluent countries, but in developing countries it remains one of the major public health problems. Osteo-articular tuberculosis in economically underdeveloped countries of the world remains one of the main conditions to be dealt with in any orthopaedic centre. About 7.5 per cent of the total cases attending the outpatient services of the Orthopaedic Department of the Institute of Medical Sciences, Banaras Hindu University, were those of osteo-articular tuberculosis (Tuli et al. 1967).

Availability of effective anti-tubercular drugs has made the treatment of this condition relatively simple and safe provided the diagnosis is accurately made. Clinical and radiological methods which are adopted to diagnose this condition are not always accurate, as many conditions may mimic tuberculosis clinically and radiologically. At the same time osteo-articular tuberculosis does not always present a typical clinical and radiological picture; thus some lesions can be missed. The diagnosis can be accurately established by submitting the material from the suspected lesions for histological examination and/or culture and/or guinea pig inoculation. However, histology alone does not confirm the diagnosis in all cases as the anti-tubercular drugs are now known to change the histological pattern of tuberculous tissue (Wilkinson & Notley 1953). To prove the diagnosis of tuberculosis, ideally speaking, the causative organism must be recovered from the lesion.

In the present work an attempt has been made to correlate clinical and radiological observations with those of histological findings and microbiological findings for *Mycobacterium tuberculosis*.

METHODS AND MATERIAL

To establish the diagnosis of osteo-articular tuberculosis we studied the material of 105 cases who attended the Orthopaedic Department of the Institute of Medical Sciences, Banaras Hindu University, during the period from May 1969 to July 1970. Only those cases were included in this study who could provide material, such as pus, granulation tissue or curettings from sinus walls or abscess cavities or material during surgical excisions of diseased tissue for investigation. The material available was subjected to histological examination and/or culture and/or guinea pig inoculation. Of the 105 cases studied, 43 patients gave history of treatment by antitubercular drugs, mostly irregularly, for a period varying between three months and one year. Lowenstein Jensen medium was used for culture.

Material subjected to culture

All the 105 samples from 105 lesions were subjected to culture. In 36 cases where both pus and granulation tissue were available, granulation tissue was subjected to histological examination, whereas pus was used for cultural and guinea pig inoculation.

OBSERVATIONS

Table 1. Analysis of results of culture.

Total no. subjected	Positive	Negative	Contaminated
105	52	31	22
100	49.53 %	29.5 %	20.95 %

Culture

At the end of 20 weeks cultures were positive in 52 (49.52 per cent) cases; 31 (29.5 per cent) cultures were negative; 22 (20.95 per cent) became contaminated. The conventional period for growing acid fast bacilli is eight weeks at the most. However, observations were made for 20 weeks in the present series. Visible colonies appeared in 27 per cent of the positive cultures on incubation beyond 8 weeks. Findings are summarized in Table 1.

Histopathological observation

Fifty-five samples (from 105 lesions) were subjected to histological examination, 40 (72.72 per cent) of which were diagnosed as tuberculous; 11 (20 per cent) as of chronic non-specific inflammation and one (1.81 per cent) as chronic eosinophilic granuloma (Table 2). Three (5.45 per cent) cases were subjected to histological examination twice.

Table 2. Analysis of histological findings in 55 cases.

Total no. subjected	Diagnosis tuberculosis	Diagnosis non-specific chronic inflammation	First diagnosis tuberculosis. 2nd diagnosis chronic inflammation	Other diagnosis chr. eosinophil. granuloma
55	40	11	3	1
100 %	72.77 %	20 %	5.45 %	1 %

The first diagnosis was tuberculosis. Repeat samples, when examined after eight months of anti-tubercular therapy, were found to have chronic inflammatory change with no evidence of tuberculosis.

Table 3. Analysis of results of guinea pig inoculation in 56 samples.

Total no. subjected	Positive	Negative	Died of intercurrent infection
56	40	10	6
100 %	71.43 %	17.86 %	10.71 %

Guinea pig inoculation

As summarised in Table 3, guinea pig inoculation was done in 56 cases. The result was positive for tuberculosis in 40 (71.43 per cent); 10 (17.86 per cent) were negative and six (10.71 per cent) died of intercurrent infections.

Proof of tuberculosis

Diagnosis of tuberculous infection was considered proven if there was positive evidence of tuberculosis in histopathology or on culture or on guinea pig inoculation. As shown in Table 4, the diagnosis of tuberculosis was proven in the present series in 84 (80 per cent) out of 105 cases who were suspected to have tuberculosis on a clinical and radiological basis. Histology and culture and guinea pig inoculation were positive for tuberculosis in 11 cases only. Positive histology and culture were found in 8 cases only. Culture and guinea pig inoculation were found positive in 9 cases. In another 9 cases diagnosis was proven by guinea pig inoculation and histological examination. Histological proof alone was positive in 12 cases; only culture was positive in 24

Table 4. Analysis of material showing incidence of proof of tuberculosis in 105 lesions.

Sl. No.	Diagnosis proved by	No. of cases	Total	Total percentage
1	Positive; histology + culture	8		
2	Positive; histology + culture + guinea pig inoculation	11		
3	Positive; culture + guinea pig inoculation	9		
4	Positive; histology + guinea pig inoculation	9	84	80 %
5	Positive; histology only	12		
6	Positive culture only	24		
7	Positive; guinea pig inoculation only	11		
8	Negative; by culture and/or by histology and/or guinea pig inoculation	21	21	20 %

cases, and diagnosis was solely made by guinea pig inoculation in 11 cases. In 21 cases the diagnosis of tuberculosis was only on the basis of clinical and radiological findings; histology, culture and guinea pig inoculation results were nonconfirmatory.

DISCUSSION

Cultures

Dobson (1951), Dahl (1951), Wilkinson & Notley (1953) and Hald, Jr. (1964) got higher percentages (approximately 70–80) of positive cultures because their patients did not have antitubercular drugs before investigations were done. In the present work tuberculosis was proven in 49.52 per cent of 105 cases subjected to culture. If contaminated cultures were excluded, the percentage of positive cultures in the present series was 62.65 per cent.

Use of anti-tubercular drugs before submitting material for culture may be responsible for lower incidence of positive culture in the present study.

Value of histology

Even before the availability of anti-tubercular drugs the histological proof of tuberculosis was not forthcoming in all cases. Average histological proof of tuberculosis in skeletal lesions in the pre-antitubercular era has been reported to be 76.56 per cent (Swift 1936, Barr 1936,

Brodin 1951, Yu 1951). The percentage of histological positivity does not seem to have altered appreciably in the era of specific antitubercular drugs. The average reported in the post antitubercular era is 74.37 per cent (Wilkinson 1955, Paus 1964, Hald, Jr. 1964). In the present study 55 cases were subjected to histological examination and proof of tuberculosis was available in 72.73 per cent of cases.

Guinea pig inoculation

In the present work guinea pig inoculation was positive in 71.53 per cent of cases submitted for this investigation. In general our findings of guinea pig inoculation are in agreement with the observations of many other workers such as Swift (1936), Guri (1947) and Yu (1951).

Histology and culture

Kondo (1957), Paus (1964), and Hald, Jr. (1964) reported proof of skeletal tuberculosis in about 73 to 80 per cent of cases by combining histology and culture. In the present work the proof of tuberculosis was available in 89.09 per cent of 55 cases submitted for combined investigations. Thus if material from cases of osteo-articular tuberculosis is subjected simultaneously to culture and histological study, diagnosis can be proven in about 74 to 89 per cent of the cases.

Culture and guinea pig inoculation

We failed to get any literature on culture and guinea pig inoculation study in osteo-articular tuberculosis. In our study tuberculous nature of the lesion could be proven in about 89.29 per cent cases when material was cultured and inoculated into the guinea pigs. Culture and guinea pig inoculation together gave almost similar percentages of positively as that obtained by culture and histology.

Histology and culture and guinea pig inoculation

Material from 28 cases was simultaneously submitted for histological examination, culture and guinea pig inoculation. The diagnosis of tuberculosis by combining the results of these investigations was proven in 100 per cent of the cases. It seems that if material and facilities are available to conduct histological investigations, culture and guinea pig inoculation in a particular case, there is a 100 per cent chance of getting the proof of tuberculosis.

Proof of tuberculosis

Cleveland (1955), Ahlberg (1948), Brodin (1951), Weinberg (1957) and McNeur (1955) reported proof of tuberculosis in about 77 to 92 per cent of the cases by histology and/or culture and/or by guinea pig inoculation. In the present series proof of skeletal tuberculosis was available in 80 per cent of the cases by histology and/or by culture and/or by guinea pig inoculation. Thus, in conclusion when material was subjected to histology and/or culture and/or guinea pig inoculation, diagnosis in skeletal tuberculosis could be proven in 76 to 91 per cent cases.

Best investigation to prove diagnosis

Histological proof of tuberculosis was available in 72.72 per cent of the cases in the submitted series, whereas disease was proven by culture in 49.52 per cent. Obviously disease was proven histologically in a greater number of cases than by culture. But in 50 per cent of the cases found histologically negative, cultures were positive for *Mycobacterium tuberculosis*. It seems that in histologically negative cases, culture may be positive in an appreciable percentage. In comparison to culture, guinea pig inoculation yielded better results. Thus it may be concluded that guinea pig inoculation as a single investigation was the best method for establishing diagnosis of osteo-articular tuberculosis, if material is not available for a combination of histological and microbiological investigations and guinea pig inoculation simultaneously.

SUMMARY AND CONCLUSION

Proof of tuberculosis was available by positive culture in 62.55 per cent of the cases, after excluding contaminated cultures. Of the cases subjected to histological examination: 72.72 per cent were reported as tuberculous, 11 cases had evidence of chronic inflammatory change, but 50 per cent of these had positive culture for *Mycobacterium tuberculosis*. One case reported as eosinophilic granuloma histologically was proven to be tuberculous by culture.

Guinea pig inoculation yielded the proof of tuberculosis in 71.43 per cent of cases; when material was subjected simultaneously to histological examination and culture and guinea pig inoculation the proof of tuberculosis was obtained in 100 per cent of the cases.

It is suggested that all infective lesions and lesions of doubtful

etiology in the osteo-articular system should be submitted for microbiological examination (for acid-fast bacilli and pyogenic organism), guinea pig inoculation and for histopathology for arriving at the final diagnosis.

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