



CYTODIAGNOSIS OF TUBERCULOSIS USING CONVENTIONAL ZIEHL-NEELSON STAINING METHOD AND MODIFIED BLEACH METHOD ON LYMPH NODE ASPIRATES ALONG WITH CLINICAL CORRELATION IN TERTIARY CARE CENTRE.

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ABSTRACT Tuberculosis (TB) is a common public health problem and second leading cause of communicable disease world wide. Tuberculosis is one of the top ten causes of death and the leading cause from a single infectious agent (above HIV/AIDS). India accounting for almost one fifth (21%) of global incidence. Lymphadenopathy accounts for the most common form of Extra-Pulmonary Tuberculosis (EPTB) 3. A prospective study was carried on 100 cases of suspected tubercular lymphadenopathy patients over a period of 18 months at Gajra Raja Medical College and JAH Group of Hospitals, Gwalior from Jan 2020 to Jun 2021. All cases of clinically suspected tubercular lymphadenopathy reporting for Fine Needle Aspiration Cytology (FNAC) examination in Department of Pathology, Gajra Raja Medical College and Jay Arogya Group of Hospitals (J.A.H.), Gwalior, Madhya Pradesh (M.P.) with proper marking for affected lymph nodes and documentation. Aim was to evaluate the efficacy of bleach method with cytological diagnosis and the conventional method. Patients with tubercular lymphadenitis were evaluated. The acid-fast positivity by routine staining was compared with modified bleach method of AFB staining. Among 100 patients, 23(23%) were positive for AFB on conventional ZN method and the smear positivity was increased to 33 (33%) by bleach method. The bleach method is simple and inexpensive. It clearly improves microscopic detection of AFB.

KEYWORDS : Modified bleach, tubercular lymphadenitis, Ziehl- Neelsen method.

INTRODUCTION:-

Tuberculosis (TB) is a common public health problem with second leading cause of communicable disease worldwide. Tuberculosis is one of the top ten causes of death and the leading cause from a single infectious agent {above Human Immunodeficiency Virus (HIV) Infection/Acquired Immune Deficiency Syndrome (AIDS)}. Millions of people were affected by it in each and every year in world. In 2017, TB leads to an estimated 1.3 million deaths in HIV-negative people and there were an additional 3 lakh deaths from TB among HIV-positive people. Globally 10.0 million people developed TB disease in 2017¹.

In India it is also the most common infectious disease accounting for almost one fifth (21%) of global incidence². It is the developing countries that suffer a major global burden of the disease. TB was declared as a global emergency in 1993 by the World Health Organization (WHO). Lymphadenopathy accounts for the most common form of extra-pulmonary tuberculosis (EPTB)³.

Tubercular lymphadenitis (TBLN) is the most common form of extrapulmonary tuberculosis it accounts for almost 40% of extrapulmonary tuberculosis⁴. TB lymphadenitis was affected females and younger age groups peoples more commonly than males and the older age groups people which are more affected in pulmonary TB. Its peak age of onset is 20-40 years⁵.

The diagnosis of TB primarily involves clinical examination, FNAC with subsequent laboratory confirmation by bacteriological examination for Mycobacterium Tuberculosis bacilli by conventional Ziehl-Neelsen (Z-N) staining and Modified Bleach Method³.

However Culture method is believed to be gold standard for the diagnosis of tuberculosis but still nowadays FNAC is more convenient procedure because it is rapid, simple and cost effective diagnostic technique with fast results, high accuracy and minimum invasion⁶.

Many studies in last decade had suggested that the performance of conventional Z-N smear microscopy can be improved more if the aspirate is liquefied with one or other chemical reagents like bleach solution and then concentrated by centrifugation of sediment before staining for identification of tubercle bacilli⁷.

MATERIAL AND METHOD-

100 cases of suspected tubercular lymphadenopathy patients over a

period of 18 months at Gajra Raja medical college and JAH Group of hospital Gwalior from Jan 2020 to Jun 2021 were studied. All cases were clinically suspected for tubercular lymphadenopathy came for FNAC examination in Department of Pathology, Gajra Raja Medical College and J.A. Group of Hospitals, Gwalior (M.P.) with proper marking for affected lymph nodes and documentation, except those who have taken treatment for TB within previous 3 months or at initiation of TB treatment before sampling. Detailed clinical history and relevant clinical examination were taken.³

FNAC procedure was explained to the patient. FNAC was performed by using 22-24 gauge disposable needle and 20 ml syringe under strict aseptic precautions after taking informed written consent. The gross appearance of aspirate was noted in each case, which was divided grossly as blood mixed, cheesy and purulent¹, 20 cases slides were fixed in 95% ethyl alcohol for a Pap Stain, and all 100 cases slides were air dried for May-Grunwald-Giemsa (MGG) and Z-N stain. 5ml sterile screw capped tube was taken. The remained aspirate in needle was irrigated with 1 ml of 5% sodium hypochlorite solution (NaOCl/bleach). After thorough mixing, the sample is incubated for 15 min at room temperature with frequent mixing at intervals. An equal volume of distilled water was added and mixed thoroughly and then centrifuged at 3000 rpm for 15 min. The supernatant was discarded, and 1-2 smears be prepared using one drop of the sediment, the area was marked with marking pencil, air dried, heat fixed and was stained by Ziehl-Neelsen staining technique.

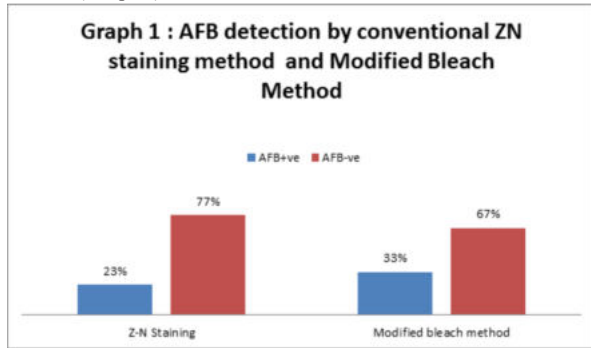
Direct Microscopy of MGG and PAP stained smear was done and detailed cytomorphological features were noted like epithelioid granuloma without necrosis (non caseating type), epithelioid granuloma with necrosis (caseating) and necrosis only³.

After that the conventional ZN-stained smears and Modified Bleach Concentration method smears were examined under oil immersion ($\times 1000$) for the presence of AFB and was compared. The data was processed using test of association (Chi-square test)³.

RESULTS

Out of 100 clinically suspected patients of tuberculous lymphadenitis, 92 presented with cervical lymphadenopathy, 7 with axillary lymphadenopathy, 1 with inguinal lymphadenopathy of variable duration. Out of 100, 23% cases were AFB positive by conventional

ZN method and 33% cases were AFB positive by Modified Bleach Method (Graph 1)

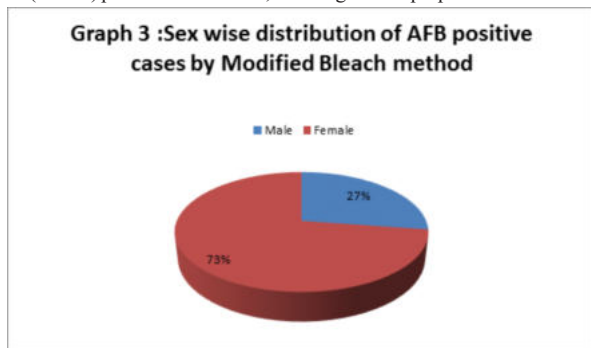


Out 100 cases, Maximum case were between 16 – 30 years 15 (45.4%) ,10 (29.4%) were between 31 – 45 years, 04(12.1%) patients were below 15 years & 04(12.1%) above 45 years. (Table-1)

Table 1: Distribution Of Cases According To Age And Sex Among Positive AFB By Bleach Method

Age (yrs)	Male		Female		Total		Chi square test	P value
	No.	%	No.	%	No.	%		
≤15	2	22.2	2	8.33	4	12.1	2.62 (NS)	0.758
16-30	2	22.2	13	54.2	15	45.4		
31-45	3	33.3	7	29.2	10	29.4		
>45	2	22.2	2	8.33	4	12.1		
Total	9	100	24	100	33	100		

Out of 33 AFB positive cases, 24(72.7%) patients were female and 09(27.3%) patients were males, showing female preponderance.



Out of 100 cases, 21 (21%) patients were presented with history of fever and weight loss. Out of these 21 ; 7 (33.3%) patient were detected AFB positive and 14 (66.7%) were detected AFB negative by conventional ZN staining method, It was not statistically significant correlation (P = 0.206). Out of above 21 ; 11(52.4%) patient were detected AFB positive by modified bleach method & (47.6 %) AFB negative. It was a statistically significant correlation (P = 0.034).(Table-4)

Table 4 : Comparison Of AFB Detection By Conventional ZN Staining Method And Modified Bleach Method

History of fever and weight loss	Conventional Method				Modified Bleach Method			
	AFB Positive		AFB Negative		AFB Positive		AFB Negative	
	No.	%	No.	%	No.	%	No.	%
Yes	7	33.34	14	66.66	11	52.4	10	47.6
No	16	20.30	63	79.70	22	25.30	57	74.70
Chi square test	1.603				4.516			
P value	0.206 (NS)				0.034 (S)			

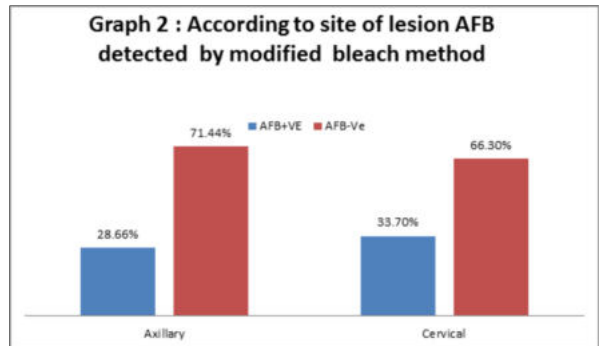
By conventional ZN staining method 01(14.3%) axillary and 22(23.9%) cervical AFB positive cases were detected and AFB detection was increased to 2(28.6%) axillary and 31(33.7%) cervical by modified bleach method (Table-5)

Table 5 : AFB Detection By Conventional ZN Staining Method

Site of lymph node	Conventional ZN method				Modified Bleach Method			
	AFB Positive		AFB Negative		AFB Positive		AFB Negative	
	No.	%	No.	%	No.	%	No.	%
Axillary	01	14.3	06	85.7	2	28.6	5	71.4
Cervical	22	23.9	70	76.1	31	33.7	61	66.3
Inguinal	0	0	1	100	0	0	1	100
Chi square test	0.642				0.575			
P value	0.725 (NS)				0.750 (NS)			

Out of 100 cases, 92 patients with cervical lymphadenopathy, among them 22(23.9%) patient were detected AFB positive by conventional ZN staining method.

7 patients with axillary lymphadenopathy, among them 01(14.3%) patient were detected AFB positive by conventional ZN staining (Graph-2)



DISCUSSION:-

FNAC has high accuracy in the early diagnosis of tubercular lymphadenopathy. The ZN method for AFB confirms the diagnosis. Methods used to increase the sensitivity of ZN method was the use of NaOCl (bleach) as a mucolytic for sputum samples. Bleach is cheap, readily available, acts as effective disinfectant, and gives a higher density of bacilli per microscopic field and reduction of debris resulting in a clear background. Out of 100 patients, 23% cases were AFB positive by conventional ZN method and 33 cases were AFB positive by modified bleach method.

According to Park⁶ In India tuberculosis is more prevalent in adults than in children. It affects adults in the most productive age group (15-54 years). In the present study 74.8% cases were in the major age group was also 16-45 years which correlates with the study of Singh et al³⁹ who reported major age group was 30-39 years and Gunja Dwivedi et al¹ who reported 30% cases in 21-30 years major age group.

According to Park, TB primarily affects people in their most productive years of life. While two-thirds of the cases are male, TB takes disproportionately larger toll among young females, with more than 50 per cent of female cases occurring before the age of 34 years. In present study females contributed 72.7% similar to the study by Singh et al⁷ who reported females contributed 54.0%.

In present study, out of 21 cases, 11 (52.4%) had lymphadenopathy along with fever and weight loss. And, out of 79 cases, 22 (27.8%) cases presented without any other associated symptoms except lymphadenopathy. Gunja Dwivedi et al¹, reported that out of the 200 cases 116 (58%) had lymphadenopathy along with mild fever. Also, 50 (25%) cases presented without any other associated symptoms except lymphadenopathy.

In present study, the cervical group of lymph nodes was most predominantly affected in (23 %). Similar result was observed by Singh et al⁷ who also reported most common site was cervical. Gunja Dwivedi et al¹ also reported that the cervical group of lymph nodes was most predominantly affected in 65.5% (n=131) cases.

In the present study of 100 cases, 92 (92 %) cases were suggestive of TB on cytomorphological findings, 23/100(23%) were positive for AFB by conventional ZN staining and 33/100 (33%) for AFB by bleach method. Singh, et al reported that 575 cases, 310 (53.9 %) cases were suggestive of TB. On cytology 218/310 (70.3%) were positive for AFB

by conventional ZN staining and 277/310 (89.3%) for AFB by bleach method. The reason behind this can be explained by the fact that due to their lipid coat, Mycobacteria remain buoyant during centrifugation. Bleach method allows the deposition of bacilli at the bottom of the test tube after centrifugation. Annam *et al*⁸ explained it due to changes in the surface properties of the bacilli (i.e., charge and hydrophobicity) and denaturation of the specimen leading to flocculation and subsequently increased sedimentation rate of the AFB. As bleach kills bacteria, good results are possible only when the method is to be used within 1 hour of a collection of specimen.⁹

Among 7/100 (7%) specimen diagnosed as suppurative lymphadenitis, 1/7(14.3%) cases were positive by conventional ZN method while bleach method yielded positivity in 5/7 cases (71.4%) for AFB. Similar result was observed by Singh, et al who reported that among 71/575 (12.3%) specimen diagnosed as suppurative lymphadenitis, 39/71 (54.3%) cases were positive by conventional ZN method while bleach method yielded positivity in 63/71 (88.7%) cases, the reason may be loss of the bacilli among the debris.

Thus, the primary outcome of the study is that bleach method is more efficient in demonstrating the presence of AFB on cytology smears in reactive, tubercular and suppurative lymphadenitis. It is also concluded that there are least chances of detection of AFB in suppurative lymphadenitis by conventional AFB technique. Thus, if such cases of suppurative lymphadenitis are not responding to the routine course of antibiotics than they should definitely be screened for AFB by bleach method.

CONCLUSION-

The bleach method is simple and inexpensive. It clearly improves microscopic detection of AFB. Early and definite diagnosis of TB is essential to treat patients to reduce the spread of disease. Combined method of FNAC and AFB detection by bleach modification increased the diagnostic value of ZN method. This is also very effective in detecting AFB in cases having suppurative lymphadenitis.

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