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Research Article

Anti-tuberculosis drugs sensitivity of BCG pasture strain isolated from lymphadenitis of children after vaccination by BCG vaccine

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ABSTRACT:
The common disadvantages of BCG vaccine is topical lymphadenitis, this patient must be treatment by anti-tuberculosis drugs. Systematic form of lymphadenitis has high mortality. This study aimed to evaluate anti tuberculosis drugs sensitivity of BCG pasture strain isolated from children with lymphadenitis after BCG vaccination to compare with BCG pasture strain. The samples from children with lymphadenitis were collected and cultured on glycerol free-middle brook 7h11 and lowenstien Jensen. The positive culture was confirmed by real time PCR and MIC of Ethionamide, Rifampicin and. For BCG pasture strain vaccination and isolation from adults was determined. In the all samples the 30 cases (37.5%) had positive culture, in 2 cases of which, because of patient`s underlying immunodeficiency, the co-bacterial infection was observed. The MIC of Isoniazid, Rifampicin, Streptomycin, Ethambutol, Clarithromycin, Amikacin and Ethionamide in pasture strain of BCG vaccine and adenitis almost were similar. In order to similarity of anti-tuberculosis drugs, sensitivity of vaccine strain and adenitis in systemic cases, the first line drug could use.

Key words: Mycobacterium Bovis-BCG, lymphadenitis, anti-tuberculosis drugs.

1. INTRODUCTION:

The Tuberculosis is the most common cause of death in single-factor infectious diseases in the world, and it has 10th rate in the global level of disease. It is estimated that it remains in that place until 2020 or rises to 7thrate. However, according to priority of TB-controlling plans, WHO (World Health Organization) has specified some goals to control TB; and the most important of them are that the prevalence of tuberculosis in 2015 must be half of that in 1990, and it decreases to 1 in 1 million death due to active tuberculosis until 2050 (Fernandes PM., 2014; Havasian MR., 2015). In 1845 the tuberculosis Bovis was diagnosed by Anteinal villemin and the mycobacterium was dissociated from tuberculosis by Robert Roch and the human BCG vaccine was produced in 1921for first time by Albert Caletc and Camille Gueviu (Luca S et al,. 2013). Also, there are different reports from different species in line with

vaccine production according to the variety of produced species of BCG (Dubos RJ et al., 1956; Horwitz MA et al., 2006; Montagnani C et al., 2014). The most important sub-strain produced of BCG is BCG Tice that it used to treat bladder cancer (De Jager R et al., 1991; Crispen R et al., 1989). The MPB64 gene was reported for the first time in sub-strains of BCG, that it existed in virulence M. Bovis and the MPB51 was detected in all sub-strains of BCG (Li H et al., 1993; Ohara N et al., 1997). The BCG vaccine has significant effects on children in decreasing the tuberculosis meningitides and miliary pulmonary tuberculosis and variable effects on pulmonary tuberculosis. The variety in effectiveness of this vaccine was reported in many clinical trials; so that the effectiveness of that was 60% in United Kingdom and 25% in others countries (Colditz GA et al., 1994; Fine PE et al., 1995). In fact the tuberculosis vaccine is not only a control panel because it cannot prevent the pulmonary mycobacterial infection, but it also has the most effect in birth injection for prophylaxis of miliary pulmonary tuberculosis and meningitides in children (Rodrigues LC et al., 1993). Also, the BCG vaccine has the prophylactic role in leprosy patients, Buruli ulcer, cancer chemotherapy and diabetes type I (Setia MS et al., 2006; Tanghe A et al., 2001; Lamm DL et al., 1991). The reactions of BCG injection are scar, pain, occasionally big colloids. Generally, the term BCG- induced Iymphadenitis is used when the lymph node is big and touchable (Milstien JB et al., 1990). However, these reactions are seen in 2 weeks after injection, but almost they are seen after 24 months (Ustvedt HJ et al., 1950; Chaves-Carballo E et al., 1972), so that in 95% of cases axillary lymph is touchable in the same side; sometimes the supraclavicular or neck node lymph appears with the same side axillary node lymph (Snider JR et al., 1988; Çaglayan S et al., 1987). The systemic BCG occurs in individuals with inherent or acquired immune deficiency (Reichenbach J et al., 2001; Gonzalez BE et al., 1989; Casanova JL et al., 1995). Another reaction of BCG vaccine is Osteitis that it is in epiphysis or metaphysis of long bones, which appears from 2 months to 5 years after injection (Bergdahl S et al., 1976; Berges O et al., 1981; Gormsen H et al., 1956). This study aim to determine the anti-tuberculosis drugs sensitivity M.Bovis-BCG in lymphadenitis after BCG vaccine and BCG pasture strain.

2. MATERIAL & METHOD:

This present study is a cohort that was done on 80 infants and children who suffered from suppurative lymphadenitis caused by BCG vaccine injection. The exclusion criteria of the was take antibiotics that have effect on mycobacterium growth such as Rifampin, Ethambutol, Aminoglycosides, Isoniazid, Fluroquinolone, Pyrazinamide at least 72 hours.

Sampling:

The skin on lymph node was sterilized by alcohol, the local anesthesia was done by xylocaine and the purulent of lymph node was aspirated by syringe 20 cc; and it was transported to Alborz Clinical Microbiology Research Center. Also the age of child, start time of lymphadenopathy, the lymphadenopathy of front side or same side of injection place, the time of changing lymphadenopathy to lymphadenitis, the history of immunodeficiency in the child, the family history of immunodeficiency, clinical symptoms such as hepatomegaly, facial and pulmonary lesions and lymphadenopathy of the others organs were recorded.

Measurement of MIC:

For every sample after transport to laboratory, acid fast stain was done and cultured on Middle-brook-7H10 (Fluka Chemie Ag,cat NO.M0428) and Lowenstein – Jensen was done. The Lyophilized of BCG Pasteur vaccine with N. Batch 8822 that was dissolved in 1cc distilled water was used. After harvesting, distilled water was added to every sample and then vortex was used again for 10 seconds. Every one of solutions that had aerosol bacillus in 10 cc of Middle-brook 7H9 broth; enriched with albumin, dextrose and catalase was transported and the density was set in 530 nm. The culture tubes were incubated in 37c° degrees centigrade and checked for OD every day after mixing. When OD=0.2 the solution was used as initial bacterial solution. In order to make solutions

with 1/100 density, the 100 micro liter of initial solution was added to 10cc of sterile distilled water. The 0.01 µ/l of initial solution was added to Middle-brook 7H11 agar plate with exact density of anti-tuberculosis drugs.

Final concentration of Ethionamide: 0.5, 1, 2, 4, 8 and 16 µg/µl

Final concentration of Rifampin: 0.06, 0.125, 0.5, 1 and 2 µg/µl

Final concentration of Amikacin: 0.25, 0.5, 1, 2, 4, 8 and 16 µg/µl

Final concentration of Isoniazid: 0.06, 0.125, 0.25, 0.5, 1 and 2 µg/µl

Final concentration of Ethambutol: 0.25, 0.5, 1, 2, 4, 8, 16 and 32 µg/µl

Final concentration of Streptomycin: 0.25, 0.5, 1, 2, 4, 8 and 16 µg/µl

Final concentration of Clarithromycin: 0.125, 0.25, 1, 2, 4, 8 and 16 µg/µl

Final concentration of Flucloxacillin: 0.25, 0.5, 1, 2, 4, 8 and 16 µg/µl

In order to make control medium, the 0.01 and 0.0001 concentration from initial solution was used on Middle-brook 7H11 without antibiotic. When the control culture suspension 1:100 showed enough growth (more than 100 visible colonies), the MIC[1] was measured comparing with control solution with 0.0001 concentration and colonies count in medium containing antibiotic.

DNA Extract:

The bacilli were cultured on Middle-brook 7H11 for 2-4 weeks at 37 degrees. In order to extract the DNA, a loop full of bacteria was suspended in 500 µl of DNA was boiled for 10 minutes. Then c centrifuged in 11000 rpm for 2 minutes and supernatant was used for PCR.

Multiple real-time PCR:

Every 25 µl of PCR reaction contain 2.5 µl of every primer of 1X Syber Green PCR Master mix (Applied Biosystem US) and 5 µl of DNA template. The reaction I has a primer for detecting RD19 (RD19 Present forward and RD19 Present reverse) and not of detecting RDI (RDI Deleted forward and RDI Deleted reveres). Also this reaction has a primer for detecting the gene region of 16 S rRNA that is common in all mycobacterium. The reaction II has a primer for either detecting or not detecting RD4 (RD4 common forward, RD4 present reveres and RD4 deleted reveres RD4). This reaction was done in Real Time 7500 (Applied Biosystem, US) system and with green syber fluorescence in the Real-Time. After denaturing in 95 degrees for 5 minutes, the reaction process contained 40 cycles at 95 degrees for 15 seconds, in 60 degrees for 30 seconds and 72 degrees for 30 seconds. The final step was done in 60-90 degrees for 0.2 seconds for making Melting curve (Table 1).

1 . minimum inhibitory concentration

3. RESULTS:

In the present study, the 80 samples were evaluated of whom 62.5% were males and 37.5% were females. The average age of the sample group was 11.8 months and there were 16 male-positive and 14 female-positive aspect culture (p=0.05). Generally, the 37.5% of all samples were positive (Figure 1). The results showed that the age range of child-positive were 2-15 months, and their average age was 6 months (p=0.00). The average age of starting lymphadenopathy was 11.8 months among all 80 samples. The average age of positive culture was 2.62 months and for negative culture was 3.91 months (p=0.007). Also the timetable for 30 positive samples was 3.88 months and for 10 negative samples was 11.8 months (p=0.00). From purulent lymphadenitis samples, the 42.5% had the acid fast strain positive results (Figure 2). The acid fast strain was negative in 8 cases of positive culture. From vaccine lymphadenitis samples, the 26 cases of positive lymphadenitis had positive culture results, and there were 4 positive cases in negative culture results. In the samples which had positive result in initial acid fast strain, there was strong possibility for getting positive culture and with negative results of strain, the possibility of negative culture got stronger (p=0.00). The results of lymphadenopathy in comparison with place of BCG vaccine injection showed that the 96.3% of cases were in ipsilateral, 5 cases were in contralateral and 2 cases were in generalizet. Also the 28 cases of ipsilateral had positive and

49 cases had negative culture results. So, in the state and type of ipsilateral lymphadenitis, the possibility of negative culture got strong (p=0.558). Among contralateral cases, the 4 cases had positive culture results and 1 case had negative (p=0.06). So, the possibility of getting positive in contralateral lymphadenitis gets stronger and possibility of getting negative gets weaker. The results of acid fast strain showed that the 40% of ipsilateral had positive and 56% had negative results (p=0.395). The results showed that the time length of pus node had a relationship with possibility of negative acid fast (p=0.00). The evaluation of immunodeficiency showed that there are 5 disseminated BCG cases, 2 immunodeficiency cases according to flow cytometry examination, 2 chronic granulomatous diseases (CGD) according to DHR examination and 1 severe combined immunodeficiency case. All of cases were sensitive to Rifampin, Isoniazid, Streptomycin, Ethambutol, so that the MIC of Isoniazid (0.06 µg/dl) in all 24 samples was equal with the MIC of BCG vaccine. The MIC of Rifampin in 21 cases was equal with MIC of BCG vaccine (0.06) and only in 3 cases was 0.125µg/dl. The MIC of streptomycin was equal with MIC of BCG vaccine (0.25µg/dl) in 21 cases; and in 3 cases it was 0.5µg/dl. The MIC of Ethambutol was 1µg/dl in 8 cases which was equal with MIC of BCG vaccine; it was 0.5µg/dl in 12 cases; 2µg/dl in 2 cases; and less than 0.25 in 2 cases. All disseminated BCG cases were sensitive to Isoniazid.

4. DISCUSSION:

This cohort study was performed on infants and children who suffered from suppurative lymphadenitis caused by BCG vaccine injection. In all 80 individuals, the start time of lymphadenitis was 2-4 months and had a peak in 2nd months. There were 50 male cases (62.5%) and 30 female cases (37.5%). It seems that the prevalence of this disease was more in males but there was not any significant relationship between sex and culture results and acid fast smears; whereas it has been claimed that the incidence of this disease was more in males (Hengster P et al., 1997; Milstien JB et al., 1997). In general, there was not any significant relationship between sex and the incidence of suppurative lymphadenitis (Milstien JB et al., 1997). That was in line with our results. In the present study, there was 6% non-regional lymphadenopathy that was appeared in immunodeficiency base as BCG-Osis. The regional lymphadenitis occurred in the normal course of action in immune system of the body. In the bacteriologic evaluation of this study, it was found that out of the 80 cases, 30 cases (37.5%) had positive culture, in 2 cases of which, because of patient`s underlying immunodeficiency, the co-bacterial infection was observed. Another case might have occurred during sampling or during the incubation process (There was a possibility of carry over pollution in length of sampling or incubation period).There were 4 positive cultures and 1 negative culture in all 5 contralateral lymphadenitis. There was a possibility for this positive culture to be related to underlying immunodeficiency and higher reproduction of bacilli. In fact, the negative culture showed another etiology than vaccine. The acid fast evaluation of initial pus samples of lymphadenitis was positive in some lymphadenitis samples that had positive culture, and it could show the high load of bacillus in exodus (purulent secretion). In 4 positive culture adenitis samples, the acid fast result was negative and probably it was related to low bacillus count in pus samples. 8 positive culture cases had negative acid fast results that were related to low load of bacillus in acid fast smears. There was a significant relation between time length of pus lymphatic node and culture results. In other words, if this time got longer the chance of positive culture and acid fast results were lower. This was related to Dormancy phenomenon and producing the L-forms in B. bovis that was in line with results of Easton et al; that was done in vivo state (Easton PA et al., 1984). Most of the studies were about immune-deficient patients (mostly HIV) with history of BCG vaccine. In studies performed in order to diagnose Golden Standard, lymphatic biopsy in AFB culture was used, the half of samples were positive (Lee H et al., 2012), which might be

related to Delayed hypersensitivity lymphadenitis (Galal N et al., 2012). There are many other factors for accelerating the bacillus reproduction and increasing the load of bacteria such as enzymatic and inhibitor materials like interleukins and lymphokines present in purulent secretions, vaccine strain, viable unities present in vaccine dose, subcutaneous injection instead of intradermal or initial immunodeficiency state. The above mentioned items can be effective on the incidence of lymphadenitis and culture results (Nyerges G et al., 1985). It is possible that individual features of the society that got the vaccine were effective on the incidence of lymphadenitis and microbiologic results. This is because of the incidence of lymphadenitis in a normal population and uniform conditions, in different statistical data of adenitis or culture results (Behr MA et al., 1999). The results of this study showed the sensitivity of BCG Pasteur species against first line anti-TB drugs such as Isoniazid, Streptomycin, Rifampicin and Ethambutol and second line drugs like Clarithromycin, Amikacin, Ethionamide, Flucloxacillin and the resistance against Pyrazinamide. In a study that was performed in order to determine the sensitivity of species like Medac, Japan and Bulgaria, all of them were sensitive against Pyrazinamide, but the Denemark and Connaught showed Low-level resistance against Isoniazid and Ethionamide (Ritz N et al., 2009). The antibiogram results of lymphadenitis positive culture BCG-induced vaccine and systemic cases of BCG-osis showed the sensitivity of all first and the second line of TB drugs except Pyrazinamide. This confirmed that genetic pattern of species did not change in normal body condition has not undergone mutation, while in some studies the genetic mutation and subsequently drug-resistance has been reported (Close GG et al., 1983; Mohamadi J et al., 2014; Tam PK et al., 1982).

5. CONCLUSION:

The drug-sensitivity evaluation of BCG vaccine showed that it was sensitive to all anti-TB drugs except PZA; so in BCG Pasteur species of vaccine, that is common in Iran, no mutation has been occurred in spite of several passages. Also the MIC results obtained from microorganisms isolated from BCG-induced adenitis had the same pattern as BCG vaccine and in disseminated cases were sensitive to INH and other drugs. The results showed that it was not necessary to use multiple-drug treatment in disseminated cases and the first line treatment drugs (INH, RMP and ETB) instead of multiple-drug treatment was sufficient in systemic form.

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REFERENCES:

1. Fernandes PM (2014). Benefits of BCG vaccination other than protection against Mycobacterium tuberculosis. BMJ, 349, 543-548.
2. Havasin MR, Tajbakhsh P, Panahi J (2015). Evaluation of Macrolide Resistance and Distribution of Resistant Gene in Staphylococcus Aureus Between, 2010–2013; A Systematic Review. Mintage Journal of Pharmaceutical and Medical Sciences; 1, 19-21.
3. Luca S, Mihaescu T (2013). History of BCG vaccine. *Maedica (Buchar)*, *8*, 53-58.
4. Dubos RJ, Pierce CH (1956). Differential characteristics in vitro and in vivo of several substrains of BCG. IV. Immunizing effectiveness. *American review of tuberculosis*, *74*, 699-717.
5. Horwitz MA, Harth G, Dillon BJ, et al (2006). A novel live recombinant mycobacterial vaccine against bovine tuberculosis more potent than BCG. *Vaccine*, *24*, 1593-1600.
6. Montagnani C, Chiappini E, Galli L, et al (2014). Vaccine against tuberculosis: what's new?. *BMC infectious diseases*, *14*, 1-2.
7. De Jager R, Guinan E, Lamm D, et al (1991). Long-term complete remission inbladder carcinoma in situ with intravesical tice bacillus Calmette guerin: Overview analysis of six phase II clinical trials. Urology, 38, 507-13.

8. Crispen R (1989). History of BCG and its substrains. Progress in clinical and biological research, 310, 35-50.
9. Li H, Ulstrup JC, Jonassen TO, et al (1993). Evidence for absence of the MPB64 gene in some substrains of Mycobacterium bovis BCG. Infection and immunity, 61, 1730-34.
10. Ohara N, Nishiyama T, Ohara-Wada N, et al (1997). Characterization of the transcriptional initiation regions of genes for the major secreted protein antigens 85C and MPB51 ofMycobacterium bovisBCG. Microbial pathogenesis, 23, 303-10.
11. Colditz GA, Brewer TF, Berkey CS, et al (1994). Efficacy of BCG vaccine in the prevention of tuberculosis: meta-analysis of the published literature. *Jama*, *271*, 698-702.
12. Fine PE (1995). Variation in protection by BCG: implications of and for heterologous immunity. *The Lancet*, *346*, 1339-1345.
13. Rodrigues LC, Diwan VK, Wheeler JG (1993). Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *International journal of epidemiology*, *22*, 1154-58.
14. Setia MS, Steinmaus C, Ho CS, et al (2006). The role of BCG in prevention of leprosy: a meta-analysis. *The Lancet infectious diseases*, *6*, 162-70.
15. Tanghe A, Van Vooren JP, Portaels F, et al (2001). Protective efficacy of a DNA vaccine encoding antigen 85A from Mycobacterium bovis BCG against Buruli ulcer. *Infection and immunity*, *69*, 5403-11.
16. Lamm DL, Blumenstein BA, Crawford ED et al (1991). A randomized trial of intravesical doxorubicin and immunotherapy with bacille Calmette–Guerin for transitional-cell carcinoma of the bladder. *New England Journal of Medicine*, *325*, 1205-09.
17. Milstien JB, Gibson JJ (1990). Quality control of BCG vaccine by WHO: a review of factors that may influence vaccine effectiveness and safety. *Bulletin of the World Health Organization*, *68*, 93-108.
18. Ustvedt HJ (1950). Local reaction in BCG vaccination. *Bulletin of the World Health Organization*, *2*, 441-68.
19. Chaves-Carballo E, Sanchez GA (1972). Regional Lymphadenitis Following BCG Vaccination (BCGitis) Clinical Comments Based Upon 25 Instances Among 1295 Childhood Vaccinees. *Clinical pediatrics*, *11*, 693-97.
20. Snider JR, Rieder HL, Combs D, et al (1988). Tuberculosis in children. *The Pediatric infectious disease journal*, *7*, 271-78.
21. Çaglayan S, Yegin O, Kayran K, et al (1987). Is medical therapy effective for regional lymphadenitis following BCG vaccination?. *American Journal of Diseases of Children*, *141*, 1213-14.
22. Reichenbach J, Rosenzweig S, Döffinger R, et al (2001). Mycobacterial diseases in primary immunodeficiencies. *Current opinion in allergy and clinical immunology*, *1*, 503-11.
23. Gonzalez BE, Moreno SAB, Burdach RO, et al (1989). Clinical presentation of Bacillus Calmette-Guerin infections in patients with immunodeficiency syndromes. *The Pediatric infectious disease journal*, *8*, 201-06.
24. Casanova JL, Jouanguy E, Lamhamedi S, et al (1995). Immunological conditions of children with BCG disseminated infection. *The Lancet*, *346*, 581-84.
25. Bergdahl S, Fellander M, Robertson B (1976). BCG osteomyelitis: experience in the Stockholm region over the years 1961-1974. Bone & Joint Journal, 58, 212-16.
26. Berges O, Boccon-Gibod L, Berger JP, et al (1981). Case report 165. Skeletal radiology, 7, 75-7.
27. Gormsen H (1956). On the Occurrence of Epithelioid Cell Granulomas in the Organs of BCG-Vaccinated Human Beings. Acta Pathologica Microbiologica Scandinavica, 38, 117-20.
28. Easton PA, Hershfield ES (1984). Lymphadenitis as a late complication of BCG vaccination. *Tubercle*, *65*, 205-08.
29. Lee H, Dockrell HM, Kim DR, et al (2012). The current status of BCG vaccination in young children in South Korea. Tuberculosis and respiratory diseases, 72, 374-80.
30. Galal N, Boutros J, Marsafy A, et al (2012). Mendelian susceptibility to mycobacterial disease in egyptian children. Mediterranean journal of hematology and infectious diseases, 4, 201-209.
31. Nyerges G, Drinoczy M (1985). Significance of the number of viable units in BCG vaccines. *Developments in biological standardization*, *58*, 331-336.
32. Behr MA, Small PM (1999). A historical and molecular phylogeny of BCG strains. Vaccine, 17, 915-22.
33. Ritz N, Tebruegge M, Connell TG, et al (2009). Susceptibility of Mycobacterium bovis BCG vaccine strains to antituberculous antibiotics. *Antimicrobial agents and chemotherapy*, *53*, 316-18.
34. Close GG, Omar M (1983). Caseating regional lymphadenitis complicating BCG

vaccination. *Archives of disease in childhood*, *58*, 939-40.

35. Mohamadi J, Motaghi M, Havasian MR, et al (2014). Anti-fungal resistance in candida isolated from oral and diaper rash candidiasis in neonates. *Bioinformation*. 10, 667-70.
36. Tam PK, Stroebel AB, Saing H, et al (1982). Caseating regional lymphadenitis complicating BCG vaccination: a report of 6 cases. *Archives of disease in childhood*, *57*, 952-54.

Description of target	Product length (bp)	Product T_m (C)°	Primer names	Primer sequences
RD9 present	51	76.3 ± 0.1	RD9 Present Forward RD9 Present Reverse	TTTCGAGCCGTAAATTACTGTG GAGCATTCTCGCTCCGAAT
RD1 deleted	226	86.2 ± 0.6	RD1 Deleted Forward RD1 Deleted Reverse	GGATTTGACGTCGTGCTTCT TTCAACGGGTTACTGCGAAT
RD4 present	55	77.8 ± 0.2	RD4 Common Forward RD4 Present Reverse	AGAAGCGCAACACTCTTGGA CATGCGCCCTATTTGATCTC
RD4 deleted	94	83 ± 0.2	RD4 common Forward RD4 Deleted Reverse	AGAAGCGCAACACTCTTGGA TTGCTGAAAAATGGCTATTGA
Mycobacterial 16S rRNA	78	79.3 ± 0.1	Genus Control Forward Genus Control Reverse	CAACGCGAAGAACCTTACCT TGCACACAGGCCACAAGGGA

Table 1: Primers used in this study

Figure 1: Results of culture 80 cases with post BCG lymphadenitis

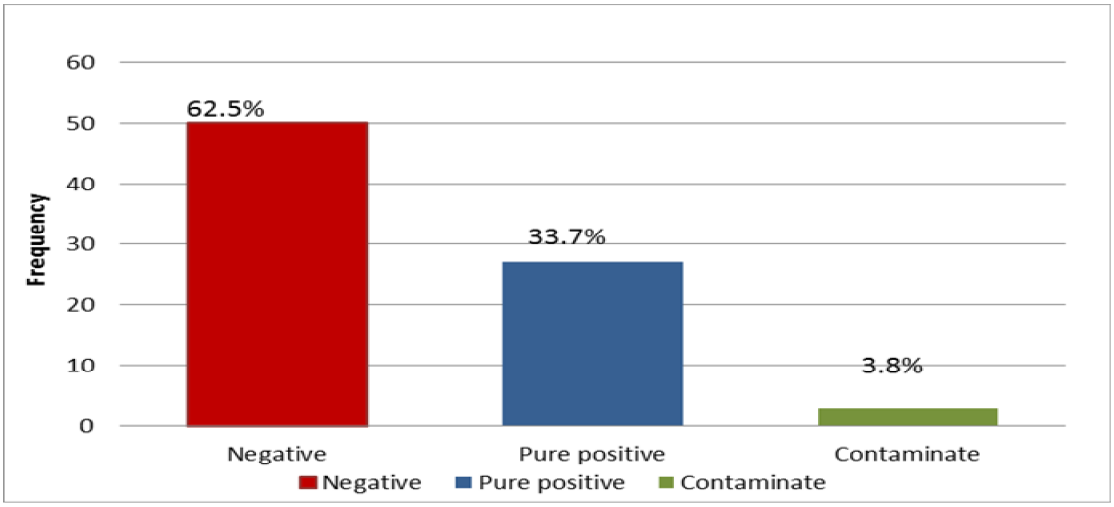

Figure 2: Results of acid fast purulent lymphadenitis