An Evolutionary Bone Marrow Examination for L.D. Bodies Detection in Nepal

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Abstract

Introduction For differential diagnosis of cellular malignancy of blood cells including the detection of the L.D. bodies, the examination of bone marrow is being done. L.D. Bodies for Kala-azar are directly demonstrated under microscope.

Objectives The aim of this study is to detect the L.D. Bodies for Kala-azar diagnosis in the bone marrow sample and to promote the application of this method for health laboratory technicians.

Methods Thirty six bone marrow samples were aspirated by para-medical laboratory personnel from suspected Kala-azar patients at Mallik Pathological Lab, Janakpurdham, Nepal. Those samples were sent to the Department of Proto-zoology, Nagasaki University, Japan. Culture and microscopic examination methods applied in sending samples and detected the *Leishmania donovani* in Japan. Only microscopically bone marrow slides also examined in Janakpurdham.

Results *Leishmania donovani* had culturally grown in 13 samples and microscopically detected in 25 samples in the Japanese Laboratory while microscopically L.D. Bodies detected in 25 samples in the Mallik Laboratory at Janakpurdham. Microscopically, 27 samples would have similar results and 8 samples would have variation from each other. In which, one sample was missed. Clinically, 27 patients had markable splenomegaly with fever and suspected as Kala-azar disease. The findings of study suggested that the quality microscopic examination of bone marrow test in the Mallik laboratory at Janakpurdham for detecting L.D. Bodies is similar with respect of highly equipped laboratory of Japan.

Conclusion The quality microscopic bone marrow examination of L.D. bodies done in Mallik Pathological Laboratory of Janakpurdham is good at its sensitivity perspective. All para-medical laboratory personnel have benefited with described methodology contrary of traditional medical personnel.

Key words L.D. Bodies, Bone Marrow, Kala-azar

Introduction

Bone Marrow Examinations are being done as cytological examination for differential diagnosis of cellular malignancy of blood cells and anaemia as well as detecting the L.D. Bodies as a golden standard test[1], which are the causative organisms of Kala-azar disease. Kala-azar disease becomes a burning health problem of thirteen eastern districts of Nepal. These districts are endemic from Kala-azar disease. Mainly, poorest people are suffering from this type of disease. Near about 88 percent Kala-azar patients could not be able to afford better treatment in the better place. Most of them are belonging from low-income group of their society[2]. In other hand cheep and simple tests do not confirm the disease. Some recent immunological tests are very costly, but that could not be able to confirm the true disease. Spleen Puncture and Bone Marrow Aspiration are the best test to be confirmed the disease because causative organisms of Kala-azar disease, L.D. Bodies are directly demonstrated under microscope. Spleen Puncture may create problem during the proceeding process like severe bleeding. Bone Marrow Aspiration is only the safe test to be confirmed the Kala-azar disease. Traditionally, Bone Marrow Aspiration is a surgical procedure, only a medical doctor and pathologist can be able to do the procedure but such medical personnel's are not available in all types of hospital of the country. Therefore, in July 16-19, 2000 AD, Bone Marrow Aspiration and Cytology for Kala-azar diagnosis training program was organized by

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HMG/MOH/USAID/EHP in B. P. Koirala Institute of Health Sciences, Dharan (Nepal)[3]. There were para-medical laboratory personnel's from thirteen districts and two medical doctors had got such training.

Kala-azar disease bricks in the rural and urban village area of southern-eastern districts of Terai Region of Nepal. A total 19868 number of patients had been suffering from Kala-azar disease during 1980-2001 A. D. in the Terai Region of Nepal[4]. After getting the training; every participant could be able to be doing the examination of bone marrow aspiration for detection of L.D. Bodies except cytological studies of cells. Regarding such training, near about 1000 of Kala-azar suspected patients were benefited in the Janakpur Zonal Hospital and nearly 2000 such types of patients were examined their bone marrow aspiration in the Private Lab (Mallik Pathological Laboratory), Janakpurdhm by para-medical laboratory personnel. An evolutionary development of proceeding process has established for bone marrow aspiration with 18-gauge needle and 5 ml syringe under aseptic condition. Traditionally, using procedure has needed bone marrow aspiration needles and anaesthetic condition during aspiration of bone marrow. Developed proceeding process may not be highly risked of the surgical procedure and any trained para-medical laboratory personnel can use the procedure. Such procedure has also a scientific background previously based on the negative pressure proceeding process such as Fine Needle Aspiration Cytology (FNAC) technique[5]. There are not any authorized organization in Nepal who has to check the quality control of microscopic examined bone marrow slides of any lab. This study may suggest to be checked the quality control of microscopically examination of bone marrow slides of Janakpur. Regarding this, physicians sent patients of Kala-azar suspected disease to Mallik Pathological Lab for detecting the L.D. Bodies in bone marrow had aspirated and such samples sent to Japan. During the months of July, August and September 2003, 36 bone marrow aspirated samples put into NNN Culture Media Tube for growth of *Leishmania donovani* and prepared 7 slides. Culture tube and one slide had sent to the lab, the Department of Protozoology, Nagasaki University, Japan and 3 slides stained with *Leishman Stain* in the Lab of Janakpur. Stained slides examined under Olympus Binocular Microscope for detecting the L.D. Bodies and recorded the results then issued the report to related patient. When Japanese examined the sending slide and checked the growth of *Leishmania donovani*, then the results came back to Janakpur by email. Results of microscopic examination of Janakpur correlated with culture result of Japan and clinically suspected cases correlated with microscopic results of Janakpur. This study suggests to related government organizations to be making a policy for the rules and regulations about this field on the capability of trained para-medical laboratory manpower.

Methodology

The Kala-azar disease suspected patients were sent from many consultant physicians to the Mallik Pathological Lab in Janakpurdham, Nepal. According to their sings and symptoms, patients were screened for the aspiration of bone marrow sample and recorded the history of fever, size of spleen and taking of treatment on the forms. Then patients were prepared for aspirating the bone marrow sample. Under aseptic condition, the bone marrow samples were aspirated from iliac crest bone of the patients by simple 18 gauge needles and 5ml disposable syringes.

Collection and Methodology of Bone Marrow Aspiration:

1. Patient lay down on a bed in the posterior position and identified the iliac crest bone of hip.
2. Then wear the gloves in hand, swab with sprite first, secondly with beta dine and lastly with sprite center to outer of the identified iliac crest bone of hip.
3. Put 18 gauge needles with 5 ml syringe and puncture the identified iliac crest bone under upward position with some pressure then aspirate the marrow from the bone under negative pressure as FNAC technique.
4. When some drops of marrow have drawn in syringe then put off the needle from bone.
5. Put one-drop marrow in the NNN culture media tube and make 6-7 slides.
6. Record the code or lab number on the slides as well as culture tube.
7. Three slides stained with Leishman Staining Technique for detecting the L.D. Bodies under oil immersion lens of Olympus binocular microscope at Janakpurdham.
8. Pack one or two slides and culture tube for sending to Japan.
9. Record the results in register and issue the report to patient.
10. Giemsa stained using for detecting the L.D. Bodies under oil immersion lens of Olympus binocular microscope in Japan also.
11. Size of samples: In the July 2003, only six samples were sent and thirty samples were sent in Aug-September 2003 to Japan.
12. 36 samples of bone marrow of suspected patients of Kala-azar had performed microscopic examination and culture test in the Japanese lab.
13. Microscopically, para-medical laboratory personnel and medical laboratory personnels of Dept. of Protozoology, Nagasaki University, Japan respectively examined the sending slides.
14. Para-medical laboratory personnel of Mallik Pathological Lab, Janakpurdham also examined previously such sending slides.
15. Results of culture and slides were received by email. After receiving the results of culture and microscopic of bone marrow from Japan, correlate the results of Janakpur.

Results

Thirty-six samples were taken as this purpose in July, August and September 2003. Total positive numbers were 25 and total negative numbers were 10 in both laboratories and one of slides is missing. Clinically, 27 numbers of cases were highly suspected for the disease and 4 cases were differing between each other.

Comparative result of both Labs (Microscopic)

Total numbers of Cases	No. Positive in both Laboratories	No. Negative in both Lab.	No.Variation	No. Missing
36	21	6	8	1

After combination, the results of both labs are shown in the following chart.

Distribution of Cases

	Clinical Sing						Results of Nagasaki		Results of Janakpur	
S.No.	**Sample No.**	**Age**	**Sex**	**Fever**	**Spleen**	**R/treatment**	**Microscopy**	**Culture**	**Microscopy**	**Remarks**
1	050603-1	30	M	Yes	4	No	Positive	Positive	Positive	Same Positive
2	050603-2	30	F	Yes	3	No	Positive	Negative	Positive	Same Positive
3	070603-1	45	M	Yes	4	No	Positive	Positive	Positive	Same Positive
4	070603-2	18	M	Yes	palpable	No	Negative	Negative	Negative	Same Negative
5	080603-1	6.5	F	Yes	2	No	Positive	Positive	Positive	Same Positive
6	080603-2	35	M	Yes	2	No	Positive	Positive	Positive	Same Positive
7	200803-1	22	F	Yes	palpable	No	Positive	Negative	Negative	Variation
8	200803-2	16	F	Yes	palpable	No	Negative	Negative	Negative	Same Negative
9	200803-3	28	M	Yes	1	No	Positive	Negative	Positive	Same Positive
10	210803-1	15	M	Yes	4	No	Positive	Positive	Positive	Same Positive
11	230803-1	10	M	Yes	4	No	Negative	Negative	Positive	Variation
12	240803-1	10	M	Yes	4	No	Positive	Positive	Positive	Same Positive
13	240803-2	7	F	Yes	2	No	Negative	Negative	Positive	Variation
14	240803-3	17	M	Yes	1	No	Negative	Negative	Negative	Same Negative
15	270803-1	26	M	Yes	palpable	No	Positive	Negative	Negative	Variation
16	280803-1	6	M	Yes	4	No	Positive	Negative	Positive	Same Positive
17	290803-1	2	M	Yes	3	No	Positive	Positive	Positive	Same Positive
18	290803-2	34	M	Yes	palpable	No	Positive	Negative	Negative	Variation
19	290803-3	24	F	Yes	3	No	Positive	Negative	Positive	Same Positive
20	300803-1	30	F	Yes	3	No	Positive	Positive	Positive	Same Positive
21	300803-2	6	F	Yes	3	No	Positive	Negative	Positive	Same Positive
22	010903-1	30	F	Yes	palpable	No	Negative	Negative	Negative	Same Negative
23	020903-1	36	M	Yes	palpable	No	Negative	Negative	Negative	Same Negative
24	030903-1	55	M	Yes	4	No	Positive	Negative	Positive	Same Positive
25	060903-1	12	F	Yes	4	No	Positive	Positive	Positive	Same Positive
26	070903-1	27	M	Yes	palpable	No	Positive	Negative	Negative	Variation
27	070903-2	8	F	Yes	3	No	Positive	Positive	Positive	Same Positive
28	090903-1	35	F	Yes	4	No	Positive	Negative	Positive	Same Positive
29	100903-1	24	F	Yes	4	No	Positive	Positive	Positive	Same Positive
30	150903-1	40	M	Yes	1	No	Positive	Negative	Positive	Same Positive
31	240903-1	28	F	Yes	2	No	Negative	Negative	Positive	Variation
32	240903-2	17	F	Yes	palpable	No	No slide	No culture	Negative	Missing
33	280903-1	19	M	Yes	4	No	Positive	Positive	Positive	Same Positive
34	280903-2	35	F	Yes	1	No	Negative	Negative	Negative	Same Negative
35	300903-1	26	F	Yes	3	No	Negative	Negative	Positive	Variation
36	300903-2	25	M	Yes	4	No	Positive	Positive	positive	Same Positive

The microscopic study of Bone Marrow Slides of Janakpur Lab correlated with Bone Marrow Culture Test (As Golden Standard Test).

	No. Culture Positive	No. Culture Negative	
No. Microscopic slide Positive in Janakpur	13	12	25
No. Microscopic slide Negative in Janakpur	0	10	10
	13	22	35

- Sensitivity of Bone Marrow Test for Detection of L.D. Bodies = 13/13 = 100 percentage (If true disease in the community, 100 percent suspected people must be detecting of L.D. Bodies in the Bone Marrow Test)
- Specificity of Bone Marrow Test for Detection of L.D. Bodies = 10/22 = 45 percentage (If no true disease in the community, 45 percent people could have chances to be the detecting of L.D. Bodies in the Bone Marrow Test.)
- PPV (Positive Predictive Value) = 13/25 = 52 percentage (52% suspected people of Kala-azar disease could have chance to be detecting of L.D. Bodies by Bone Marrow Test in such laboratory.)
- NPV (Negative Predictive Value) = 10/10 = 100 percentage (100% unsuspected people of Kala-azar disease could not have any chance to be detecting of L.D. Bodies by Bone Marrow Test in such laboratory.)

A correlation between The Clinical examination and Bone Marrow examination of Kala-azar suspected Patients of Janakpur.

	No. Bone Marrow Positive	No. Bone Marrow Negative	
No. Suspected Patient(Fever with enlarge Spleen)	26	1	27
No. Unsuspected Patient (No fever with enlarge Spleen)	0	9	9
	26	10	36

1. Sensitivity of the clinical suspected patients for Kala-azar disease = 26/26 = 100 percentage (If true disease of Kala-azar in the community, 100% suspected people must have fever with enlarge spleen.)
2. Specificity of the clinical suspected patient for Kala-azar disease = 9/10 = 90 percentage (If no true disease of Kala-azar in the community, 90% suspected people could have chance of fever with enlarge spleen.)
3. PPV (Positive Predictive Value) = 26/27 = 96 percentage (96 % suspected patients of Kala-azar must have fever with enlarge spleen under the clinical examination)
4. NPV (Negative Predictive Value) = 9/9 = 100 percentage (100% unsuspected people of Kala-azar could not any chance to have fever with enlarge spleen.)

Discussion

Bone marrow examination is a challenging task for the para-medical laboratory personnels. Medical laboratory personnel could not reach to the rural and urban area of Nepal where would have needed the bone marrow examination for the diagnosis of Kala-azar disease (*Visceral Leishmaniasis*). A rising number of cases had reported from southern plain of Eastern and Central region of border area of Nepal or 13 districts of eastern and central Tarai of Nepal since 1980. The Case Fatality Rate (CFR) varied from 0.23 to 13.6 percentage during 1980 to 2001 A. D. because Kala-azar disease would be having 100 percentage mortality rate for untreated cases therefore, disease means a deadly disease[6]. . Medical Laboratory Personnel, who can be easily, performed the bone marrow examination, is least in the number in our country. HMG/MOH/USAID/EHP deigned a training program for para-medical laboratory personnels of epidemic districts and trained them. The study would be giving the idea to be managing the package programme of training and rules for para- medical laboratory personnel. If causative organism *Leishmania donovani* would be demonstrating under microscope certainly that examination is true as a diagnostic tool. Contrary, causative organism of tuberculosis would be demonstrating under microscope in the sputum sample, that test is called a golden standard test for pulmonary tuberculosis. There are so many diagnostic tools to be confirming the Kala-azar disease but bone marrow, like sputum for tubercle bacilli is a best sample for *Leishmania donovani*. Collection of bone marrow is complicated than sputum. Certainly, the collection of bone marrow is painful and having highly risk of infection in the bone. Describing methodology would help to be easily reducing the risks of pain and infection. After orientation of collecting procedure, any para-medical or medical personal could collect the bone marrow sample from the patient. Aseptic condition should be maintained during collection of bone marrow sample.

Quality Assurances would be most important for any laboratory tests. Unfortunately, nobody manage the quality control set-up whom could be checked the bone marrow slide for this purpose. Every body challenges their quality control measures. But nobody has any black and white paper to be supporting his or her challenge. This study will help to

those people for establishing the quality control set-up for bone marrow examination. We would have an attention on statistical value of finding results of bone marrow for L.D. Bodies of suspected Kala-azar patient. Results would be very satisfactory to all. The microscopic study of Bone Marrow Slides of Janakpur Lab correlated with Bone Marrow Culture Test (As Gold Standard Test). Results of Bone Marrow Test of Janakpur are having 100 percent sensitivity rate that means incase of true disease having a patient has no chance to be missing the diagnosis of Kala-azar disease. Specificity rate of Janakpur is also 45 percent that means incase of untrue disease having other disease would have a chance for false positive.

Twenty seven samples have same results in laboratories, which cover the 75 percent reliability of a rural laboratory of Janakpurdham. 9 cases have differs in result. In a contrast, both laboratories have same numbers of positive cases that is very good sign for quality assurance. 27 cases have clinically suspected for Kala-azar and twenty-five are positive for disease that is best evidence for quality assurance for the bone marrow examination for the detection of L. D. Bodies in Kala-azar patients.

Conclusion

The para-medical laboratory personnel can do the bone marrow aspiration for detecting the L.D. Bodies to be confirmed the Kala-azar disease after the orientation of a simple training program. The quality assurance of Mallik Pathological Lab of Janakpurdham would be assured the presence of L.D. Bodies in the bone marrow aspirated sample because of the high rate of sensitivity and low rate of specificity. Comparative microscopic study may help to the planning for training program of laboratory health workers where Kala-azar disease is endemic. The study supports to facilitate the centre for detecting of L.D. Bodies in the bone marrow aspirated sample and suppress the monopoly of those people who would be thought that bone marrow aspiration examination for detecting L.D. Bodies might be their right. It is true that cytological examination of bone marrow is really difficult work for the para-medical laboratory personal to be differentiating the abnormal structure of the cellular components of bone marrow. The study should be encouraged the para-medical lab personals to build up their skill in this field. The result of clinically suspected patient of Kala-azar disease which medical doctor sends has high sensitivity rate and also high specificity rate. Therefore, bone marrow aspiration should be start for detecting the L.D. Bodies as golden standard test like the sputum for detecting AFB to be confirmed tuberculosis disease.

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