International Journal of Multidisciplinary and Current Research ISSN: 2321-3124
Research Article Available at: http://ijmcr.com

Detection of the Dapsone gene resistance in Sudanese Leprosy patients diagnosed by PCR

Mervat Samaan[1], Hassan A/ Musa[2], Yasir Hassan [3]and Mouna Samaan[4]

[1]Faculty of Laboratory Sciences Alzaiem Alazhari University, Sudan
[2]The medical research centre, the Ribat National University, Sudan
[3]Faculty of medicine, ElRazi University, Sudan
[4]The Ribat National University, Sudan

Accepted 10 April 2017, Available online 20 April 2017, **Vol.5 (March/April 2017 issue)**

Abstract

Leprosy is a chronic disfiguring infection. The conventional methods of diagnosis are not conclusive and most of the physicians rely on the clinical presentation of the patients. Long duration treatment and compliance of the patients is required to clear out the infection. Emergence of resistance in the antileprosy drugs is an extra problem. In this study we tried to compare the conventional methods of diagnosis (ZN stain) with the molecular method (PCR) and to look for the presence of resistance in the recovered strains in Sudanese Leprosy patients. Ninety one suspected leprosy patients were included in the study. ZN stained Slit skin smears and PCR using the primers S13 (CTCCACCTGGACCGGCGAT) and S62 (GACTAGCCTGCCAAGTCG) was done for all specimens. All the positive specimens by PCR were further tested for the presence of the folpi resistant gene using the 2 other primers 5' GCTTCTCGTGCCGA–AGCGCTCG - 3'and 5'AGCCGACATCAGTCGCCAGTGC 3' to detect the resistance to dapsone. Out of the 91 suspected leprosy patients 32 (35.2%) were positive and 59 (64.8%) were negative by ZN stain. The PCR detected 50 (54.9%) as positive and 41 (45.1%) as negative. All the positive by the ZN stain were positive by the PCR. The resistance rate within the 50 positive samples by PCR was 10% (5 out of 50). Our results showed that The PCR method has better sensitivity in the diagnosis of the disease .The detection of the resistance gene to dapsone is emerging as a burden added to the difficulty of the treatment of the infected patients.

Keywords: *Leprosy, polymerase chain reaction, dapsone resistance, Sudanese patients.*

Introduction

Leprosy is a chronic infectious disease caused by *M.leprae,* was identified by the Norwegian physician G.H.A. Hansen in 1873 [(1).]. The different clinical presentations of the disease are determined by the quality of the host immune response. In 1991 the Word Health Organization (WHO) committed itself to eliminate leprosy by the year 2000 . At the deadline of the program, 597,232 leprosy cases were registered for treatment and719, 3030 new cases were reprted [(2).]. Gene probes have been developed for the demonstration of *M.leprae* in various specimens. Very low bacterial loads(lessthan 10 bacilli) can be detected . As a result PCR was used to monitor diagnosis,treatment and relapses [(3).]. Sensitivity and utility of PCR to detect *M.leprae* in comparsion with other conventional methods for the diagnosis of leprosy was evaluated by Bang PD and his collegues. They enrolled Thirty seven multibacillary patients(MB) with positive bacteriological index, 32 paucibacillary (PB) patients with negative bacteriological index and thirty psoriasis patients as controls. The sensitivity of PCR in the MB and the PB was 100% and 50% respectively and the specificity was 100% [(4).] . Multidrug therapy (MDT) was used for the treatment of leprosy to minimize the development of drug resistance in *M.leprae*. However isolates with resistance to one or more antibiotics have been detected in many areas [(5,6,7,8,9,10,11)].

In this study the frequency of *M.leprae* detection by the conventional and molecular methods was evaluated and the frequency of drug resistance to dapsone using folP 1 gene was examined in a group of 91 slit skin smears from Sudanese leprosy patients newly diagnosed, relapsed and defaulters.

Material & Methods

This is a qualitative cross sectional descriptive study. A group of 91 slit skin smears were collected from leprosy patients including newly diagnosed, relapses and

defaulters. The specimens were collected from the clinic of the national program of leprosy control Khartoum, Dar Elsalam leprosy clinic in Omdurman state and the dermatology hospital at Khartoum state. The skin slit smears were collected using sterile scalpel blade after disinfecting the skin with cotton wool swab soaked in 70% alcohol. The bottom of the slit was scraped using the blunt edge of the blade and the smear was made on clean new slide. The remaining tissues on the tip of the scalpel was placed in 1.5 ml of 70% alcohol for further processing and DNA extraction. The smears were stained by ZN stain and examined microscopically reporting the bacterial index as well as morphological index [12].

DNA extraction

The tissues samples were centrifuged at 12000 rpm/ 2 minutes to sediment the cells. Ethanol was pipetted and evaporated from the samples. A 250 µl of bufferTL and 20 µl of proteinase K were added to each sample. Twelve µl of lysis enhancer was added and mixed immediately and incubated at 65 ˚c for 1-2 hours in a shaking water bath. A 500 µl of buffer TB was added and mixed thoroughly until a homogenous solution was obtained and incubated for 10 minutes at 65 ˚c. A 200µl of absolute ethanol was added and mixed thoroughly. Then approximately 600 µl of sample was centrifuged at 8000 rpm for one minute and washed with750 µl wash buffer and finally centrifuged at 12000 rpm for 1 minute to remove all traces of ethanol. The loaded column was placed into a micrcentrifuged tube. A 100 µl of elusion buffer was added and left at room temperature for 2minutes and centrifuged at 8000 rpm for 1 minute to elute the DNA. The DNA concentration, protein ratio and purity were measured using DNA spectrophotometer at wavelength of 260 nm . The samples were stored at-20˚c. The primers for the detection of *M.leprae* were the oligonucleotides S13 and S62 (table1). The PCR was performed in a PCR processor according to the manufactures instruction and then loaded onto 2% agarose gel for electrophoresis [13].

Detection of dapsone gene resistance

On the basis of folpi sequence, 2 other primers were designed (table 1) to amplify a 450 bp fragment to determine the sulphone resistance region. The PCR reaction was carried out according to the manufacturer instructions [14].

Table 1: The oligonucleotides primers for M.leprae and the folP 1 gene

Primers For M.leprae	Primers for the Folpi sequences
1- S13 (CTCCACCTGGACCGGCGAT)	5' GCTTCTCGTGCCGA – AGCGCTCG -3'
2- S62 (GACTAGCCTGCCAAGTCG)	5'AGCCGACATCAGTCGCCAGTGC 3'

Table 2 The leprosy patients included in the study

		diagnosis 1		Total
		Multibacilary	Paucibacillary	
diagnosis 2	complete treatment	10	1	11
	defaulter	6	1	7
	newly	45	16	61
	relapses	1	0	1
	resistance	1	0	1
	suspected	2	8	10
Total		65	26	91

Results

A group of 91slit skin smears were collected from the leprosy patients including the newly diagnosed, relapses and defaulters (table 2). They were processed using conventional and genetic methods. Ziehl Neelsen 's stain was used as the conventional method. By the use of ZN stain, thirty two (35.2%) were positive and 59 (64.8%) were negative. By the molecular method, using the PCR,50 (54.9%) were positive and 41 (45.1%) were negative (Fig 1) . All those positive with the ZN stain were positive by the PCR. Eighteen out of the negative specimens by ZN were found to be positive by the PCR. All the PCR positive specimens were tested for the dapsone gene resistance. Out of the fifty samples five (10%) were found to carry the resistant gene (fig 2,3).

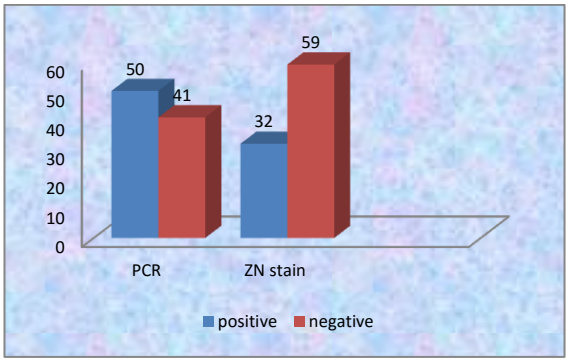

Fig.1: The positive and negative results by ZN and PCR

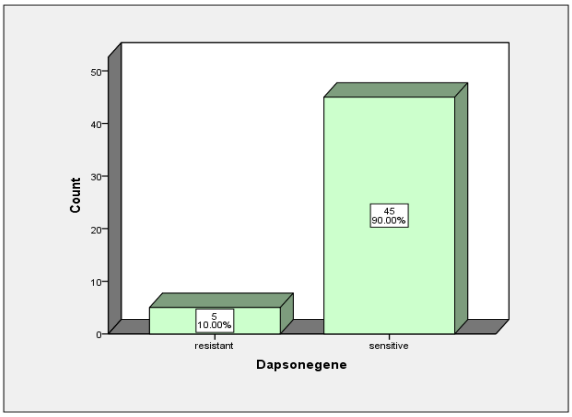

Fig.2: Carriage of Dapsone resistance gene in tested specimens

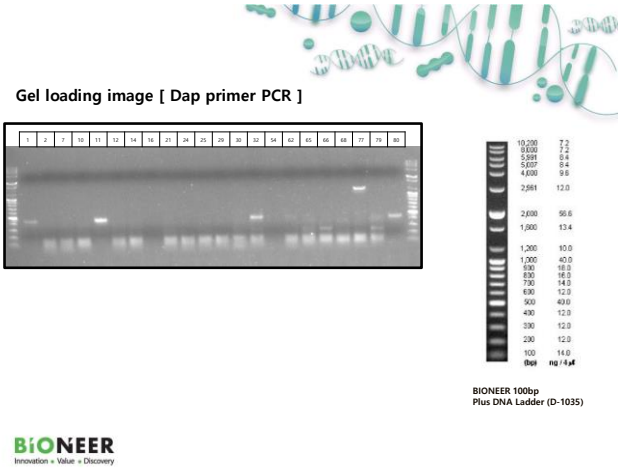

Fig.3: Detection of dapsone resistance gene's bands

Discussion

Diagnosis of Leprosy is one of the public health problems. The outcome of *M.leprae* infection depends on the immune status, host genetic background and load of the infecting mycobacterium. The gold standard method for the diagnosis of leprosy is the demonstration of acid fast bacilli in the slit skin smears. This method suffers from a limited sensitivity when the patient is presenting with tuberculoid leprosy with paucibacillary mycobacterial load. In certain cases clinicians rely on the clinical presentation of the patient to start treatment. Confirmation of the infection of the suspected leprosy patients is vital for the proper management of the patients and control of the spread of infection.

In our study among the 91 slit skin smears from leprosy patients, 32 were positive by ZN stain and PCR. However ZN showed 59 negative while the PCR showed 41 negative and 50 positive. Eighteen out of the 59 ZN negative were positive by PCR. The results indicates that PCR has a clear advantage over the microscopic examination. This is similar to the results of a study carried out in Thailand where the PCR was more sensitive than the conventional methods[(15)]. . Also the PCR results were found more sensitive when compared by the conventional methods in a study done by Bang et al in 2009 [(4)] . The PCR appears to meet the criteria of specificity and sensitivity required as a useful tool in epidemiology and the control of leprosy . In this study all the positive samples by PCR were tested for the dapsone gene resistance. Out of the 50 samples five (10%) were found to carry the resistant gene. In a study done by Cambaue et al *M.leprae* strains were tested for the presence of FOLPI GENE. Ten of them were found to have mutation at that gene and were dapsone resistant whereas the 22 dapsone susceptible strains did not harbor the mutation[(11)]. In Venzeuela and three other American countries leprosy patients were surveyed by molecular method. Non of the newly diagnosed leprosy cases exhibited drug resistance associated mutations. However 2 of 3 strains from relapsed cases contained dapsone resistance mutation[(9)]. This emphasis the fact that relapsed cases should be tested for the presence of the resistance genes.

In our result, the appearance of 10% resistance to dapsone should be considered as an emerging problem adding to the burden of the difficulty of treating the leprosy patients.

Conclusion & Recommendation

Although the PCR technique is not easy to be applied as a routine technique but it is definitely a sensitive method to clear out any ambiguous clinical presentation. Search for the resistant strains should be carried out specially in patients not responding to treatment or having relapses to prevent the repetition of treatment and its undesirable side effects.

Acknowledgment

I would like to acknowledge those who helped us in the laboratory work Mr. Salih and Dr. Waleed Mohamed Elamin for their kind assistance. This work was financed by the National Ribat University.

References

[1]. Henk Buddingh, Gwen Idle, Emile Nagib Tanyous. Leprosy. Manual for general health workers in sudan. 1st edition. Khartoum press 2000: 3 - 11
[2]. Norihisa Ishu, M.D., PHD. Recent advances in the treatment of leprosy. Dermatology online Journal. 2005, 9 (2): 5
[3]. Ramaratnam Sridharan M.D. Neurological infections leprosy. Medicine journal. 2001, 2 (12): 8- 34.
[4]. Bang PD, Suzukik, Phuong Le T, Chu TM, IShu N, Khang TH. Evaluation of Polymerase chain reaction – based detection of Mycobacteruim Leprae for the diagnosis of Leprosy. Dermatology Journal. 2009, 36 (5): 269 -76.
[5]. Ebenezer GI, Norman G, Joseph GA et al. Drug resistant Mycobacteruim leprae- results of mouse footpad studies from a laboratory in south india. Indian J Lepr. 2002: 74, 301 -312.
[6]. Cambau E, Perani E, Guillemin I et al. Multidrug resistance to dapsone, rifampicin and ofloxacin in Mycobacteruim leprae. Lancet.1997:349, 103 -104.
[7]. MaedaS, Matsuoka M, Nakata N et al. Multidrug resistance Mycobacteruim leprae from patients with leprosy. Antimicrob agents Chemothr, 2001, $5: 3615 -3639.
[8]. Matsuoka M, Kashiwabara Y, Namisato M. A Mycobacteruim Leprae isolate resistant to dapsone, rifampin, ofloxacin and sparfloxacin Int J Lepr, 2000, 68 452 -455.
[9]. Cambau E. BonnafousP, Perani E et al Molecular detection of rifampicin and ofloxacin resistance for patients who experience relapse of multibacillary leprosy. Clin infect Dis, 2001:34, 39 -45.
[10]. Matsuoka M, Kashiwabara Y, ZhangL. Et al. A second case of multidrug resistant Mycobacteruim Leprae isolated from japenese patient with relapsed lepromatous leprosy. Int J Lepr 2003,71:240 – 243.

[11]. Cambau E. Carthagena L. Chauffour A et al Dihydropteroate synthase mutations in the FolP 1 gene predict dapsone resistance in relapsed cases of leprosy. Clin infext Dis, 2006: 42, 238 -241.

[12]. Monica Cheesbrough. District Laboratory Practice in tropical countries. Cambridge university Press. 2000. Volume 2: 377-387.

[13]. Rudy A, Hartskeerl , MadeleineY.L., Dewit and Paul A. Klatser. Polymerase chain reaction for the detection of Mycobacteruim Leprae. Journal of general Microbiology. 1989, 135 , 2357 – 2364.

[14]. Emmanuella Cambau, Laetitia Carthageng, Aurelia Chauffour. Dihydropteroate synthase mutations in the FOLPI gene predict Dapsone resistance in relapsed cases of leprosy. Oxford journals. 2006, 42, 238- 241

[15]. Jes dawan, Wichit Wechkarn, Somjit Karnjan, Siriphan Shunta Wuttis etal. Detection of Mycobacteruim Leprae infection by PCR clinical Microbiology journal. 1995, 33 (1): 45 -49.