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Original Article

Evaluation of Myelin Sheath Marker Krox-20 for Detection of Early Disability in Leprosy

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Early diagnosis of nerve damage in leprosy is quite important for preventing the deformities. With an aim to find a good marker we have investigated Krox-20 as early peripheral nerve damage indicator in leprosy. Ambulatory patients of Kediri Leprosy Hospital, Malang, Indonesia have been studied. Degree of disability was measured based on WHO's criteria. Immunohistochemistry was used to study the expression of Krox-20 on Schwann cells on nerve twigs in skin biopsies from leprosy cases. Receiver Operating Characteristic (ROC) curve was used to determine cut off value. Of all 79 leprosy patients studied, 36 patiens had degree of disability 0, and 43 patients had degree of disability 1. Analysis of ROC curve shown that cut off value for Krox-20, was 8 and the cases in whom degree of disability was 0 (zero) had significantly higher mean values than those with degree of disability 1 (mean: 12.56 *vs* 4.24 ($p<0.05$)). Krox-20 appears to be a potentially good biomarker to identify early peripheral nerve damage in leprosy.

Key words : leprosy, degree of disability, Krox-20

Introduction

Leprosy, a chronic infectious disease caused by *Mycobacterium leprae,* is known to have predilection for sites like skin and peripheral nerves, especially Schwann cells (Barker 2006, Scollard 2008, Bhat and Prakash 2012). Large number of people in different countries of the world have suffered from disabilities caused by leprosy in the past, many continue to have deformities even now (Gitte et al 2015, Goncalves et al 2009, Nardi et al 2012, Noor et al 2010, Ramos et al 2011 & 2012, van Brakel et al 2008). Current treatment for leprosy mainly focuses on inhibition/ killing of *M. leprae* but has only partial role directly in influencing the recovery the damaged peripheral nerves (Goncalves et al 2009). Early detection of nerve damage is a challenge and is considered to be very important (Harboe et al 2005, Walker and Lockwood 2006). Several studies about early detection of damaged nerves by *M. leprae* invasion had been done, these studies have employed different tools and

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techniques such as the usage of ultrasound (USG) to detect the damaged peripheral nerves (Jain et al 2009), vasomotor reflexes and sympathetic skin response (Wilder-Smith 1998), the examination of the electrophysiology of the involving nerves (Kar et al 2013), the examination of axonal signs on the skin lesions (Michelin et al 2012) and myelin protein based immunological assays (Singh et al 2015).

According to WHO, there are 3 stages of leprosy disabilities, from asymptomatic to full blown disabilities (WHO 1970). WHO (1988) grading is different. In general, Saddon classified peripheral nerve damage into 3 stages: neuropraxia, axonotmesis, and neurotmesis; while in leprosy case, the nerves are damaged by demyelination of peripheral nerves (Barker 2006, Bhat and Prakash 2012).

Schwann cells have an important function to synthesize and maintain the myelin sheath. If these cells are infected, then demyelination will occur as a result of neuritis. It has been observed that infection of Schwann cells by *M. leprae* results in the demyelination of nerves in leprosy patients (Scollard 2008). The presence of Schwann cells infection by *M. leprae* will cause Schwann cells dysfunction, including decrease in neurotropic substances and also myelin synthesis; which may lead to demyelination. Relationship between triggering of certain signaling mechanism and demyelination has been reported by Rambukkana et al (2006).

Krox20 or Early Growth Response 2 (Egr 2) is a transactivator needed for the myelination proccess of Schwann cells. It is a gene that encode the formation of myelin sheath in Schwann cells (Ghislain and Charnay 2006, LeBlanc et al 2007). The other function of Krox20 is to regulate myelin genes like MPZ, PMP22, Connexin-32, and MAG (myelin associated glycoprotein) (LeBlanc et al 2007). One of the many strategies to detect early peripheral nerve damage would be studying the alterations in various markers associated with myelination or demyelination such as NRG1 (Fricker and Bennett 2011, Liu et al 2011), Krox-20 (Decker et al 2006, Ghislain and Charny 2006, Murphy et al 1996, Parkinson et al 2004), P0 (D'urso et al 1999, Suneetha et al 2003, LeBlanc et al 2007), and PMP22 (D'urso et al 1999, Jones et al 2011). Among these Krox-20 is an attractive target because of its role in myelin maintenance (Decker et al 2006). While leprosy can be diagnosed easily and it does not require advanced technology, early detection of nerve damage is a challenge. The purpose of this study is to determine the usefulness of expression of Krox-20 as a marker of early nerve damage in leprosy patients.

Materials and Methods

All leprosy patients who came to the outpatient clinic in RS Kusta Kediri during August 2014 until December 2014 and who fulfilled the inclusion criteria were included in the study. Criteria for inclusion was - multibacillary (MB) types of leprosy patients with degree of disability 0 and 1, age 14 until 50 years old, and who agred to sign the informed consent of this study. The exclusion criteria were - leprosy patients who were quite sick, had received oral glucocorticoid during the last 7 days, had history of tuberculosis and/or diabetes mellitus, and history of head trauma and any peripheral nerve lesion.

This study evaluated the Krox20 expression on Schwann cells in the skin biopsy of leprosy patients. Krox-20 expression was determined by counting the cells that express Krox-20, characterized by its brown color (exceed the control's color), using light microscope Olympus® (Antunes et al 2003, 2006, check/Cite reference). the samples were chosen using consecutive sampling methods. Diagnosis of leprosy was made using the WHO criteria (WHO 1988) based

on original Ridley and Jopling Classification (Ridley and Jopling 1966). Degree of disability wasalso determined using the WHO criteria (WHO 1970). The number of cells showing Krox 20 expressionwerecompared between two groups by using student T-test. The cut-off point of Krox-20 expression was determined using Receiver Operating Characteristic (ROC) analysis.

Results

This study was conducted on 79 MB leprosy patients: 36 patients with type 0 degree and 43 patients with type 1 degree of disability. The basic characteristics of the study subjectsare summarized in Table 1.

This study evaluated the Krox20 expression on Schwann cells in the skin biopsy of leprosy patients and it was observed that there was a significant difference of Krox20 expression between patients with degree of disability 0 and 1. (T-tailed test shows F=8.881 with p=0.000 (p<0.05)).

Findings summarized in Fig. 1 show a significant decline in Krox-20 expression in degree of disability 1 compared to degree of disability 0 (T-test, F=8.881, p=0.000 (<0.005)).

Table 2 shows that with cut off value 8 the sensitivity and specificity can be 100%. It means that if the Krox20 expression is more than 8,

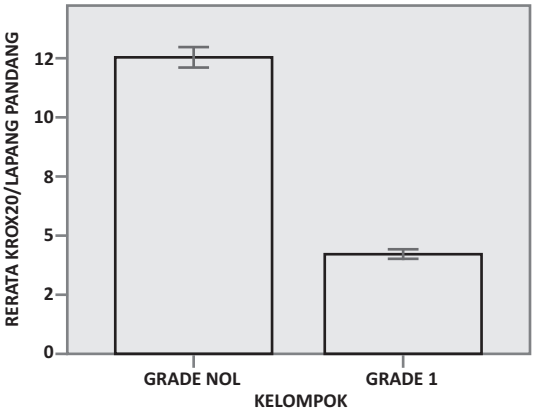

Fig. 1 : The histogram of Krox20 distribution on Schwann cells

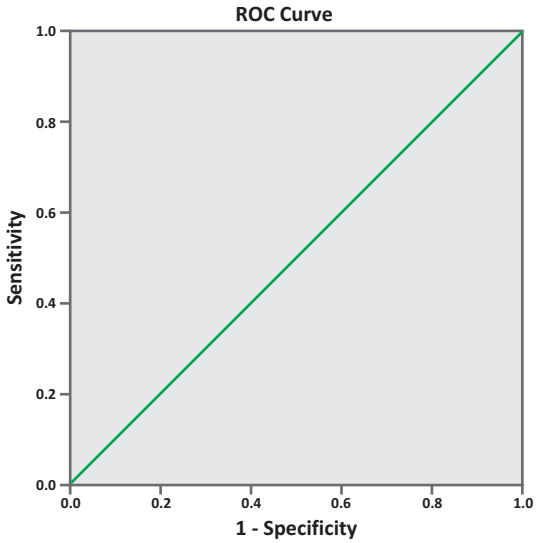

Fig 2 : ROC (Receiver Operating Characteristic) curve of Krox-20.

Table 1 : Age and gender distribution in leprosy patients with degree of disability 0 and 1

Age group	Degree 0		Total (%)	Degree 1		Total (%)
	Male(%)	Female(%)		Male(%)	Female (%)	
14 - 25	4(66)	2(33)	6(17)	3(43)	4(57)	7(16)
26 - 35	7(54)	6(46)	13(36)	5(38)	8(62)	13(30)
36 - 45	6(50)	6(50)	12(33)	8(47)	9(53)	17(40)
46 - 55	3(60)	2(40)	5(14)	3(50)	3(50)	6(14)
Total	20(56)	16(44)	36(100)	19(44)	24(56)	43(100)

Table 2 : Krox20 expression on Schwann cells

KEL	N	Mean	SD
Degree of disability 0	36	12.56	2.39
	43	4.24	1.34
Degree of disability 1			
F	8.88		
P	0.00		

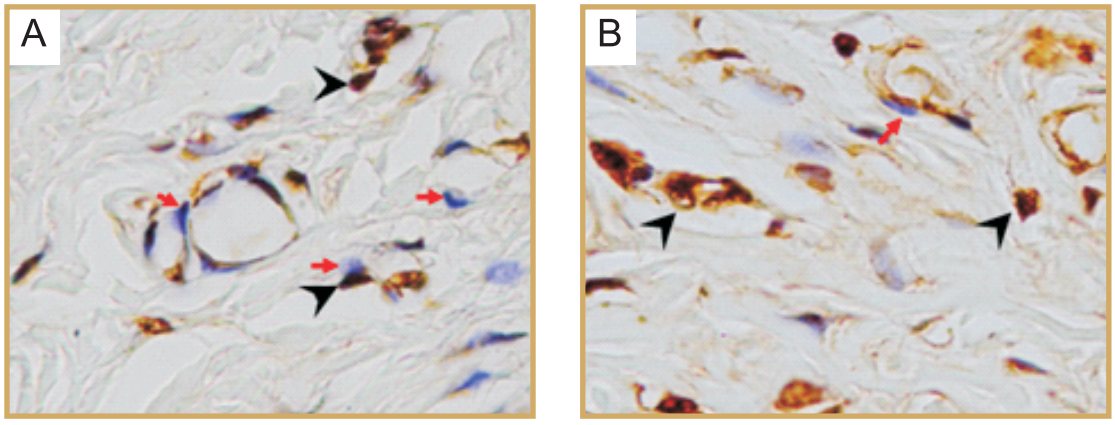

Fig 3 : The expression of Krox20 on Schwann cells

the degree of disability will be 0, and vice versa. From the ROC curve below (Figure 2), the area under the curve is 1.0 (100%) with p=0.000.

From the picture above (Fig. 3), it can be seen that in panel A (degree of disability 0), there were still many Krox2 - expression (red arrow) among the Schwann cells (back arrow). On the other hand in patients with degree of disability 1, it can be seen that the expression of Krox 20 was minimal (red arrow).

Discussion

In this study, the sample population was 36 MB leprosy patients with degree of disability 0 and 43 patients with degree of disability 1. This sample size was chosen because the purpose of study was to detect the presence of peripheral nerve damage in patients with degree of disability 1. Most patients with disability grade 0 were in the age group of 26-35 years old (13 patients, 36%), while a major proportion with disability grade 1 was in the age group 36-45 year old (17 patients, 40%). A large study in Ethiopia with total 839 patients showed that most leprosy patients were more than 35 years old (Ramos et al 2012). This indicated that many leprosy patients are in productive age in which disabilities become an important problem from economic point of view.

Our study included only disability grade 0 and 1, since goal is to find a diagnostic marker for detection of early nerve damage. In the outpatient clinic of RS Kusta Kediri, there were 43.54% patients (from total 79 leprosy patients) who had disability grade 1; the rest had disability grade 0, thus the groups were largely comparable by names, age and gender, however, there were

some differences. Clearly patients with disability grade 0 in this study significantly expressed more Krox-20 than patients with disability grade 1.

This study used immunohistochemistry approach to identify the proteins through Ag-Ab binding using light microscope. The limitation of this study are that it cannot describe the whole proccess and cannot prove the causal relationship because of the study methods. Though there is disadvantage of being an invasive approach in which biopsy is necessary, ithas the advantage of being feasible in the same specimen used for diagnosis and classification. This techniques needs to be compared with other approaches tried earlier or being used currently (Antunes et al 2003 and 2006, Jain et al 2009, Kar et al 2013, Michelin et al 2012, Singh et al 2015, van Brakel et al 2008) for detection and or quantify nerve damage in leprosy. Such comparison should be on significant numbers using proper sampling procedure which is the limitation of our study.

To conclude the present study shows that Krox-20 expression on Schwann cells in skin biopsy specimens is a good useful diagnostic tool to detect early disability in leprosy patients with cut-off value 8 cells/high power field. Further studies are needed to evaluate the role of Krox-20 in PB types of leprosy patients.

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