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The Evaluation of PMP22 and Protein 0, Examinations for Early Disability Detection in Leprosy Patients

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Introduction: Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that has a predilection for peripheral nerves, especially Schwann cells. Leprosy medications may only eradicate the bacteria without preventing or recovering peripheral nerve damage. Previous studies proved that Krox-20 could be a useful diagnostic tool for early peripheral nerve damage detection in leprosy. **Objective:** To analyse and to determine PMP22, and P0 cut-off points as diagnostic tools of early disability in leprosy. **Methods:** We examined ambulatory patients at Kediri Leprosy Hospital, Indonesia. We employed WHO's criteria to assess the degree of disability and measured the study variables using ELISA. We then determine the cut-off value using Receiver Operating Characteristic curve. **Results:** From overall patients (n=79), 36 patients had 0-degree of disability, and 43 patients had 1-degree of disability. The ROC curve analysis revealed cut-off values for PMP22 and P0 at 4,42 pg/mL and 11,39 pg/mL, respectively. The mean value for all variables in patients with 0-degree of disability were higher than that in patients with 1-degree of disability at 12,56 pg/mL vs 4,24 pg/mL (p<0,05) and at 9,85 pg/mL vs 2,86 pg/mL, respectively (p<0,05). **Conclusion:** Leprosy is a chronic infectious disease that brings forth many degrees of disability secondary to peripheral nerve invasion, particularly Schwann cells. Hence, early detection of peripheral nerve damage becomes crucial. The evaluation of PMP22 and P0 examinations is useful to identify early peripheral nerve damage in leprosy.

Keywords: leprosy, degree of disability, PMP22, P0

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* and has a predilection for the skin and peripheral nerves, especially in Schwann cells (1-4). Furthermore, leprosy is also cited as the most frequent aetiology of peripheral neuropathy.4 Approximately 2-3 million people globally have suffered from defects secondary to the disease. A large number of global leprosy incidents in the world is caused, amongst many, by inadequate access to health services and the challenges faced by health workers to reach the patients in isolated areas. Owing to late diagnosis and management common in the countryside, many have become disabled, both due to the past and the newly acquired infections. Additionally, the vaccine for leprosy remains unavailable until now (1), 2

Even now, leprosy treatment is fundamentally aimed only at eradication of bacteria, without being able to prevent or to cure the damage to the peripheral nerve and its components. Early

detection is hence crucial to determine the presence of acid-fast bacteria in Schwann cells (5, 6). *Mycobacterium leprae* causes peripheral nerve damage resulting in disability and deformity. Anti-leprosy medications can indeed eradicate the bacteria albeit unable to restore the defects and the deformities that have occurred, such as recovering the function of a nerve. By understanding the mechanism of nerve damage caused by *Mycobacterium leprae*, we can better prevent nerve damage (7).

The WHO divides the levels of disability in leprosy patients into 3 degrees, ranging from the absence of symptoms to visible damage or apparent disability (8). However, peripheral nervous disorders may already occur without specific leprosy symptoms.

Schwann cells reside within the peripheral nerves. The expression of specific transcription factors governs their differentiation. Immature Schwann cells will increase the expression of

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multiple transcription factors such as NF κ B, Oct-6, and Brn2 after receiving signals from axons, including NRG1.

Mycobacterium leprae enter Schwann cells, and as self-defense, the cells produce and excrete cytokines such as IFN- γ , TNF- α , Erk 1/2 signalling, Ras, Raf and other inflammatory cytokines. Erk 1/2 activate c-Jun, which inhibits myelination through Krox-20 down-regulation. Krox-20 is a gene crucial in myelin sheath synthesis by activating neuregulin (NRG) and Neuron Growth Factor (NGF) (9). The initial signs of myelin sheath formation in peripheral nerve cells are the generation of Myelin Protein Zero (MPZ) or protein 0 (P0), and Peripheral Myelin Protein 22 (PMP22).

Previous research indicated that Krox-20 was valuable as an early diagnostic tool for peripheral nerve defect, (10) and thus, we assumed that PMP22, P0, NGF, and NRG1 might also show positive correlations as early indicators of disability in leprosy patient.

PMP22 is a tetraspan protein required for maintaining myelination stability. Amongst many strategies to determine the presence of early peripheral nerve damage following *Mycobacterium leprae* infection is to assess Schwann cell behaviour by measuring the changes in the myelin sheath synthesis markers produced by Schwann cells. These include P0, and PMP22. However, up to date, there is still no published research that discusses the early detection of disability in leprosy patients.

Early identification remains an impediment in establishing the diagnosis of disability as leprosy sequelae secondary to peripheral nerve cell demyelination. It is impossible to restore the condition once demyelination begins; therefore precautionary endeavours are necessary. An understated approach is an early detection of possible nerve damage that may occur so that the patient can receive immediate preventive managements.

With this research, the authors sought to determine the values of PMP22, and P0 as early diagnostic tools for disability in leprosy patients.

AIMS

The study aimed to investigate the validity as well as to determine the cut-off points of PMP22 and P0 as early diagnostic tools of disability in leprosy patients.

METHODS

We took the samples from ambulatory patients visiting the outpatient polyclinic of Kediri Leprosy Hospital, Indonesia, during the period of August-December 2014. The inclusion criteria comprised

of everyone who showed 0- to 1-degrees of disability, aged between 14 to 50 years old, and were willing to participate in the research.

We establish leprosy based on cardinal signs, i.e., anaesthetic skin disorder, peripheral nerve enlargement with autonomic, sensory and motor function abnormalities, and the detection of acid-fast bacilli on skin scrapings or ear lobes. We based the determination of the degrees of disability on the WHO criteria published in 1970. ELISA examination provided by the Biochemistry-Biomolecular Laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia measured the PMP22, P0, NGF, and NRG1 levels.

We analysed the data obtained using SPSS 17 compatible with Windows® operation system.

RESULTS

We conducted the study on Multibacillary leprosy patients (n=79) with 0- and 1- degrees of disability (n=36 and n=43, respectively). The time span required for the subject collection at Kediri Leprosy Hospital, Indonesia, was five months. We briefed each multibacillary leprosy patient who met the inclusion criteria about the research. After obtaining a signed informed consent, we took a biopsy of the skin lesion. We sampled peripheral blood for PMP22, and P0 level measurements. Two-tailed T-test results showed F = 58.869 with p = 0.000 (p < 0.05). The differences between plasma PMP22 examination results in patients with 0 versus 1-degrees of disability were significant (Figures 1 and 2). The result of 2-tailed T-test in P0 level showed F value = 9.909 with p=0,000 (p<0,05). This showed a significant difference of P0 plasma level in MB leprosy patients grade 0 and grade 1. The cut off value for P0 is 11,40 pg/mL.

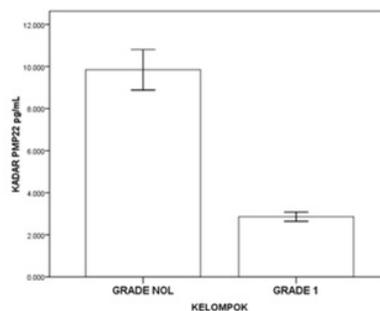


Figure 1. Plasma PMP22 levels in leprosy patients with 0- and 1-degrees of disability

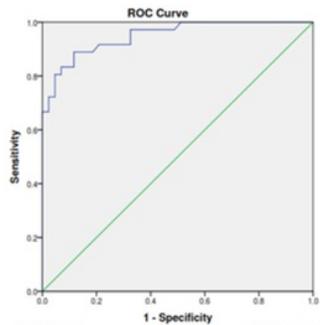


Figure 2. ROC curve of plasma PMP22 levels

Examination of P0 levels in blood revealed significant differences in plasma P0 levels between multibacillary leprosy patients with 0 versus 1-degrees of disability. The two-tailed T-test results further showed $F = 9.909$; $p = 0,000$ ($p < 0.05$) (Figures 3 and 4).

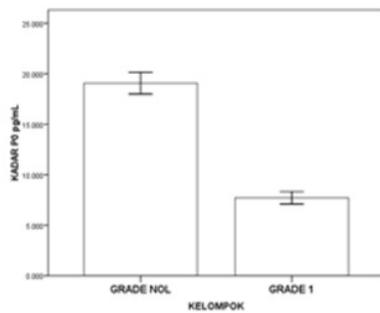


Figure 3. Plasma P0 levels in leprosy patients with 0- and 1-degrees of disability

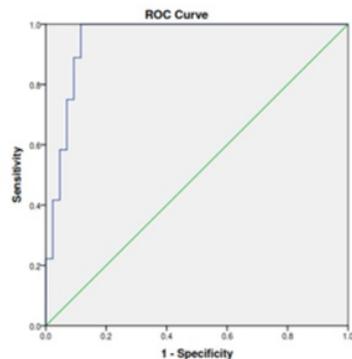


Figure 4. ROC curve of plasma P0 levels

DISCUSSIONS

This research involved samples taken from 36 leprosy patients with 0-degree of disability and 43 leprosy patients with 1-degree of disability. We settled on these group based upon the assumption

that peripheral nerve disorder, which was a myelinated disorder caused by *Mycobacterium leprae* infection, would have begun to develop in leprosy patients with 1-degrees of disability.

The sample collection required a period of between October - December 2014 in the Leprosy Department outpatient clinic at Kediri Leprosy Hospital, Indonesia. The age distribution of leprosy group with the highest degree of disability ranged from 26 to 35 years old ($n=13$; 36%), whilst 1-degree of disability was predominant between 36 to 45 years ($n=17$; 40%). A Brazilian study by Nardi et. al. revealed that of 232 patients who had completed their leprosy treatment, 32% suffer from 1- and 2-degrees of disability as per the WHO criteria ⁽¹¹⁾.

Women comprised the highest gender distribution (56%) amongst the leprosy patients studied with 1-degree of disability. This was in accordance with an earlier study by Van Brakel, et. al. conducted in Indonesia, which revealed that of the overall 1358 patients enrolled, 31.8% are leprosy patients with 1-degree of disability ⁽¹²⁾.

We employed the WHO criteria (see Table 2) to determine the degrees of disability, i.e. 0-, 1-, and 2-degrees of disability. The study examined patients with merely 0- and 1-degrees of disability due to its diagnostic nature. We identified 43.54% people who suffered from 1-degree of disability out of the overall study population ($n=79$) in the Leprosy Department outpatient clinic at Kediri Leprosy Hospital, Indonesia. The rest were those with 0-degree of disability. The definition of 1-degree of disability involved anaesthesia of the hands and feet in the absence of deformity. Visual acuity decrease ought to be no worse than 6/60, or a pass in 60-metre finger count test. A study by Ramos et. al. in 210 leprosy patients on therapy at Ethiopia Leprosy Centre revealed that during 1999-2009, 128 patients (61.5%) had suffered disability. Within the group, 26% experienced 1-degree of disability, whereas 35.6% developed 2-degree of disability. Moreover, 13.5% acquired eye defects, 44.5% were physically handicapped, and 44.7% procured defects of the lower limbs ⁽¹³⁾.

PMP22

Myelin protein expression regulation is an intricate process, reflecting the essential tasks of the myelin sheath in the nervous system. There is a halt in nerve cell proliferation during the development and subsequently following an insult to the peripheral nervous system; the myelin formation ensues thereafter. Myelin protein synthesis correlates tightly with that of myelin formation during the developmental periods in both central- and peripheral nervous system.

Following a peripheral nervous system lesion, myelin protein expression lagged, probably due to transcriptional regulation secondary to axonal contact loss. Hence, myelin protein synthesis progressing on trauma-damaged nerves at a given time is tantamount with the remyelination of a regenerated axon. Thus, the expression of the myelin protein exhibits the same regulatory exemplars both at the developmental stages and during nerve regeneration⁽¹⁴⁾.

PMP22 is a 22-kDa myelin protein expressed by Schwann cells exclusively in the peripheral nervous system and acts as an indispensable element of all myelin sheaths therein. Furthermore, the PMP22 expression corresponds closely with myelin formation during the development of the peripheral nervous system and thus is closely linked to myelin degradation and remyelination processes during the peripheral nerve cell regeneration. The central nervous system does possess PMP22 albeit in significantly minor amounts compared to that within the peripheries⁽¹⁴⁾.

Within our² study, two-tailed T-test revealed $F = 58.869$; $p = 0.000$ ($p < 0.05$), signifying significant differences in plasma PMP22 levels between MB leprosy patients with 0- and 1-degrees of disability. It confirms that emerging nerve deterioration in patients with 1-degree of disability resulted in minute amounts of NGF, in contrast with those of 0-degree of disability. Snipes et. al. confirmed that PMP22 resided exclusively within the peripheral nerve myelin sheaths and was a product of Schwann cell activities.¹⁶ Consequently, PMP22 is requisite in the myelin synthesis in the peripheral nervous system.

P0 Levels⁸

Protein P0 is the principal structural protein of myelin in the peripheral nervous system. It belongs to Type-I glycoprotein with a single immunoglobulin-like domain and has a molecular weight of 30 kDa. The precise mechanism by which the mesaxon membrane transforms into myelin remains obscure, yet it is clear that the membrane insertion with P0 and subsequent homophilic bonds in the cis- and trans-orientation excludes Myelin Associated Glycoprotein (MAG) and brings forth the formation of intact myelin. A single myelinating Schwann cell is capable of generating several square millimetres of surface membrane. The process requires the transcription of the myelin protein gene and an exact measure of correctly translated protein. The duplication of myelin protein genes may occur either naturally or inducibly and subsequently triggers demyelination that often leads to heavier phenotypes compared to

4 null mutations in the same genes. It reflects the importance of having the appropriate dose of myelin protein gene throughout the myelination process⁽¹⁵⁾.

High levels of⁵ P0 are characteristic in the differentiation of myelinating Schwann cells. The expression of P0 mRNA peaks during the period of active peripheral nerve myelination i.e. during the first three weeks postpartum in rodents, and is maintained at lower sedentary levels unto adulthood. P0 gene expression exists during Schwann cell development along with the expressions of other genes that code for specific myelin-forming proteins, such as MBP, PMP-22, and MAG. It is worthy to clarify a common misconception that oligodendrocytes produce P0 in the central nervous system; instead, Schwann cells in the peripheral nervous system execute the task exclusively. Increased P0 biosynthesis manifests early in the myelination process secondary to signals associated with the axonal surface. cAMP potentially enhances these signals for intracellular transduction.

In this study, we found that two-tailed T-test results showed $F = 9.909$; $p = 0.000$ ($p < 0.05$), implying a significant difference of plasma P0 levels between MB leprosy patients with 0- and 1-degrees of disability. Figure 3 displays a significant decrease ($p < 0.05$) of P0 level amongst the 1-degree of disability group compared with the 0-degree of disability group. As per aforementioned, P0 is a transmembrane glycoprotein expressed specifically by Schwann cells that occupy the whole myelin. Besides, P0 is an adhesion molecule that belongs to the IgG gene superfamily and is responsible for maintaining the integrity of the myelin membrane through interactions between the intracellular and extracellular domains. Proteins P0 and PMP22 collectively form a complex on the myelin membrane periphery and in the eukaryotic system in vitro. One may observe mutations of the P0 and PMP22 genes in hereditary peripheral neuropathy diseases e.g. CMT1A or CMT1B⁽¹⁶⁾.

These diseases emerge from heterozygous genetic mutations and express only low levels of sound protein. They exhibit properties in which initially, there is normal myelin formation. However, subsequent loss of self-integrity that leads to eventual demyelination soon follows. It suggests that the aforementioned small number of functional proteins are incapable of keeping the myelin sheath intact. Previous studies support this assumption by revealing that heterozygous transgenic mice with only one single copy of the P0 or PMP22 genes exhibit relatively slow onsets of myelin deficiency.¹¹ Thus, P0 is a myelin-forming protein produced solely by Schwann cells in the

peripheral nervous system and ³ plays a vital role in the myelination process.

CONCLUDING REMARKS

Examination of PMP22, P0 levels is invaluable in recognising the risk of early disability in leprosy patients ahead of clinical symptom substantiations, and hence one may employ a variety of preventive endeavours for irreversible disabilities that morbidly influences numerous aspects of patient life. The former appears to display higher degrees of sensitivity and specificity compared to the latter examination. The exploration of other roles of PMP22, P0, NGF, and NRG1 in leprosy patients relevant to peripheral nerve damage in subclinical and paucibacillary leprosy, amongst many, by examining PGL-1 demands further studies.

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