Resistance to anti-leprosy drugs: A cross-sectional study from a tertiary care hospital in Puducherry

Dear Editor,

This was a cross-sectional study conducted in the department of dermatology, venereology and leprosy and central research laboratory in Sri Manakula Vinayagar Medical College and Hospital, Puducherry. The data was collected over a period of one and a half years after approval from the institutional ethics committee. All new leprosy patients diagnosed on the basis of clinical features and histopathological examination were included in this study. Patients under treatment and those with indeterminate leprosy were excluded from the study.

A part of the skin biopsy sample was subjected to DNA extraction with concentrations varying from 0.1–1 microgram, followed by amplification by polymerase chain reaction (PCR) using primers [Table 1] of the drug-resistant determining regions in the *rpoB, folP1* and *gyrA* genes. The samples in which genes were amplified were subjected to DNA sequencing to detect mutations, following standard protocol for DNA extraction and PCR amplification.[1]

The *rpoB, folP1* and *gyrA* gene PCR products of *Mycobacterium leprae* were sequenced at EurofinsGenomics (Bengaluru, India) using ABIPRISM®BigDye™ Terminator and ABI 3730XL sequencer (Applied Biosystem, USA), using both forward and reverse primers mentioned previously. After sequencing, the sequence chromatogram files were examined for quality and the low-quality ends of sequences were trimmed by using bio-edit version 7.0.9 (Isis Pharmaceuticals). Species identification of the bacteria was achieved by comparing the nucleotide sequence of genes against known sequences available in the Genbank microbial genomes database using the basic local alignment search tool.[2] Mutation analysis of *rpoB, folP1* and *gyrA* genes was done by multiple nucleotide alignment of each gene sequence. Based on the analysis, mutation at the nucleotide level and corresponding amino acid changes at the protein level were documented. The *rpoB, folP1* and *gyrA* genes sequences of the present study bacterial isolates were deposited in the NCBIGenBank database and accession numbers were obtained MW239611–23 and MW245059–61, respectively.[3]

Table 1: Sequence of primers used in this study

Genes	Primer	Sequence (5'-3')
folP	*folP*-F	CTTGATCCTGACGATGCTGT
folP	*folP*-R	CCACCAGACACATCGTTGAC
rpoB	*rpoB*-F	GTCGAGGCGATCACGCCGCA
rpoB	*rpoB*-R	CGACAATGAACCGATCAGAC
gyrA	*gyrA*-F	ATGGTCTCAAACCGGTACATC
gyrA	*gyrA*-R	TACCCGGCGAACCGAAATTG

DNA was extracted from 31 (20 male and 11 female) patients. The age of the patients ranged from 18 to 74 years (mean age 46.5 years). Primary amplification is a technique for cloning specific or targeted parts of a DNA sequence. In our study, it yielded *folP1* 255, *rpoB* 279, *gyrA* 225 base pairs. Of the 31 cases, 21 did not show any mutation. In 19 amplicons, *rpoB* was amplified in 10, *folP1* in six and *gyrA* in three cases and when we attempted sequencing, sequencing errors occurred in three amplicons [Figures 1a to c]. Among 16 amplicons in our study, rifampicin resistance was detected in one. It was detected in a 67-year female patient who was untreated. There was no history of anti-tuberculosis therapy, chest X-ray was normal. Her bacteriological index was one and she had borderline tuberculoid Hansen's disease. Of the eight amplified samples of *rpoB,* one showed a mutation in 412 position coding (Ile→Asp) isoleucine to asparagine [Figure 1a]. This mutation is unique and not reported in India.

According to other previous studies, drug resistance in *Mycobacterium leprae* may be primarily attributed to mutations in genes encoding drug targets. For treatment and containment of drug resistance it is mandatory to have data on drug resistance.[4]

We are reporting rifampicin resistance in a treatment-naïve leprosy patient. The significance of our study is to establish the relevance of a drug resistance monitoring policy and careful post-treatment follow up of cured patients in order to detect relapse earlier. Without doubt, rifampicin in multidrug therapy is the standard regime for leprosy; extended therapy

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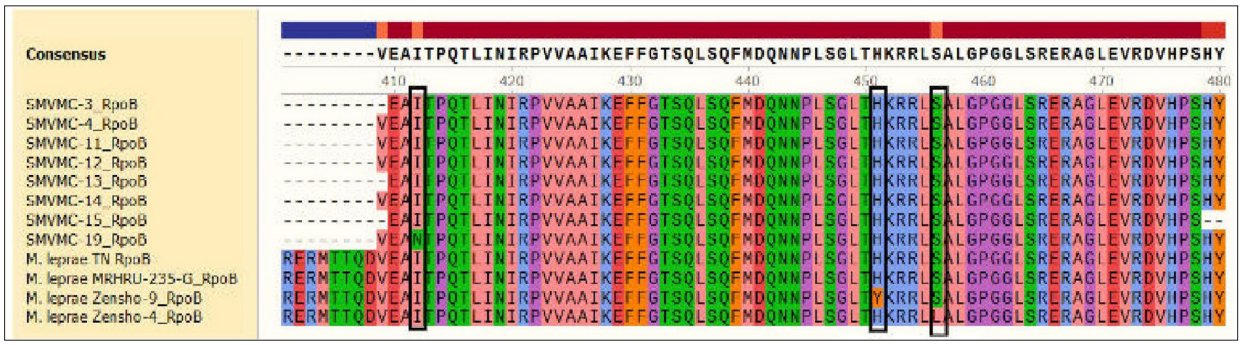

Figure 1a: It was a noval mutation named as SMVMC-19 at 412 position Ile 412 Asn(I412N) ATC-AAC (T1235A).Out of 8 samples of *rpoB* one showed mutation

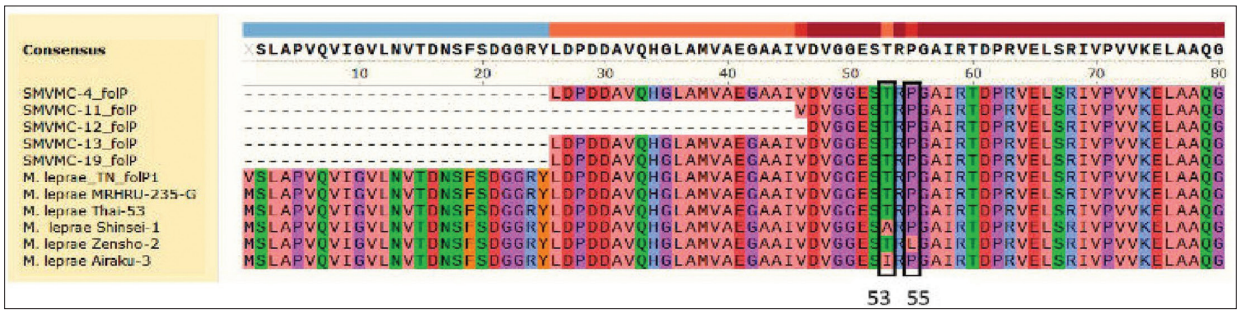

Figure 1b: Of five samples of *folP*no mutation was detected

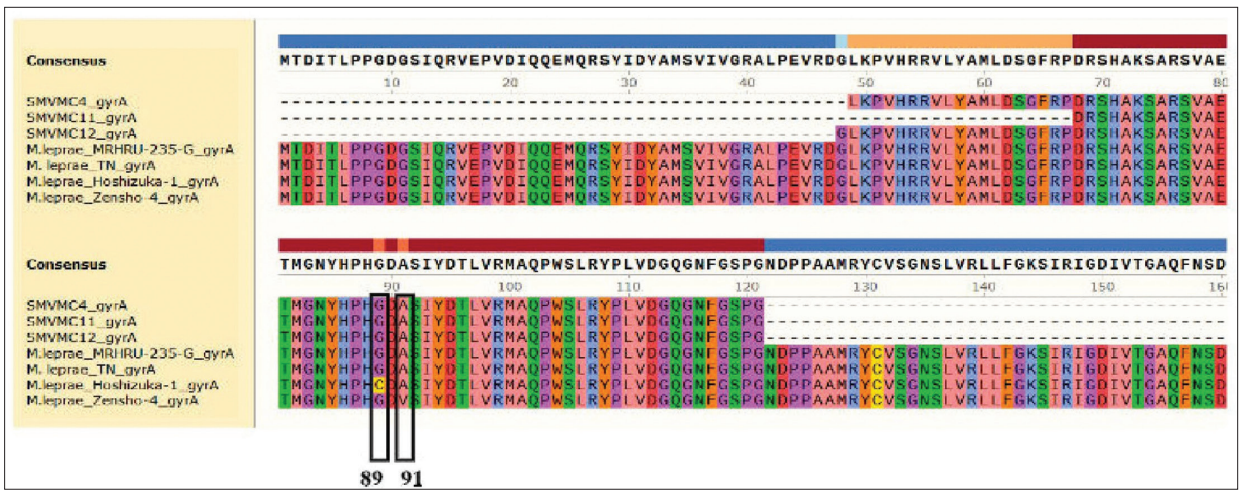

Figure 1c: Of the three samples of *gyrA*no mutation was detected

can be useful in secondary resistance for their inclusion in new drug regimen.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflict of interest

There are no conflicts of interest.

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Concentration of substance P in patients with atopic dermatitis with and without past history of treatment

Dear Editor,

Atopic dermatitis is a chronic, inflammatory skin disease that initially appears in childhood. Itching is the primary symptom in atopic dermatitis patients. It causes sleep disturbances and skin infections.[1] The prevalence rate of atopic dermatitis has proliferated in the last decade, that is, 10–20% cases in infants and children and around 1–3% in adults. Atopic dermatitis was present in about 1.1% of patients aged 13–14 years in 2012.[2] The International Study of Asthma and Allergies in Childhood found that the morbidity rate reached 20% in Asian countries, including South Korea, Taiwan and Japan. In developing countries, an estimated 10–20% of children suffer from atopic dermatitis. Additionally, 60% of atopic dermatitis patients live until adulthood.[3]

The pathogenesis of atopic dermatitis is poorly understood. Skin neuropeptides, particularly substance P, contribute to the pathogenesis of various skin diseases, such as atopic dermatitis. Substance P promotes nerve growth factors from keratinocytes, histamine release, leukotriene or tumour necrosis factor from mast cells. This condition causes the growth of sensory nerve fibres and augmentation of skin inflammation. Therefore, substance P is currently considered a pruritogenic factor.[4] Substance P induces an itching response in humans and mice and is mediated through the activation of the neurokinin 1 receptor on mast cells and keratinocytes. Moreover, it causes an increased inflammatory response and supports substance P's indirect effect in mediating pruritus.[5] Several studies have shown that blocking itching signals through neurokinin 1 receptor reduces itching complaints.[6]

This study aimed to evaluate the relationship between treatment history and substance P levels in atopic dermatitis in children.

This was a cross-sectional study conducted from February to June 2020 at the outpatient Department of Dermatology and Venereology, Division of Pediatric and Adolescent Dermatology at Universitas Sumatera Utara in Medan, Indonesia. Blood samples were analysed at the Integrated Laboratory.

Female and male patients with atopic dermatitis were enrolled in this study based on Hanifin and Rajka's criteria. All participants who were willing to participate had signed informed consents. The exclusion criteria were: participants who had any other skin disease or a systemic disease and patients whose parents were unable to answer the questions.

Blood samples were obtained to measure the substance P levels using an ELISA kit (R&D Systems) according to the manufacturer's instructions. Determination of the optical density of each sample was conducted in 30 min using a microplate reader set at 450 nm. The results were presented in units of pg/mL. This study was conducted in accordance with the Declaration of Helsinki.

A total of 46 participants with atopic dermatitis, 29 males (63%) and 17 females (37%) [Table 1] were enrolled in this study. The mean age of subjects with atopic dermatitis was 10.35 years (standard deviation = 4.01), with the youngest being 1 year and the oldest being 17 years old [Table 1].

Based on treatment history, 17 subjects (37%) had a history of atopic dermatitis treatment, while 29 subjects (63%) had never received or had no history of atopic dermatitis treatment [Table 1]. The mean value of substance P levels from 46 atopic dermatitis patients was 300.88 (standard deviation = 127.55) with the lowest level of 172.4 pg/mL and the highest level of 764.4 pg/mL [Table 1].

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