Use of Real-Time Multiplex PCR for the diagnosis of dermatophytes onychomycosis

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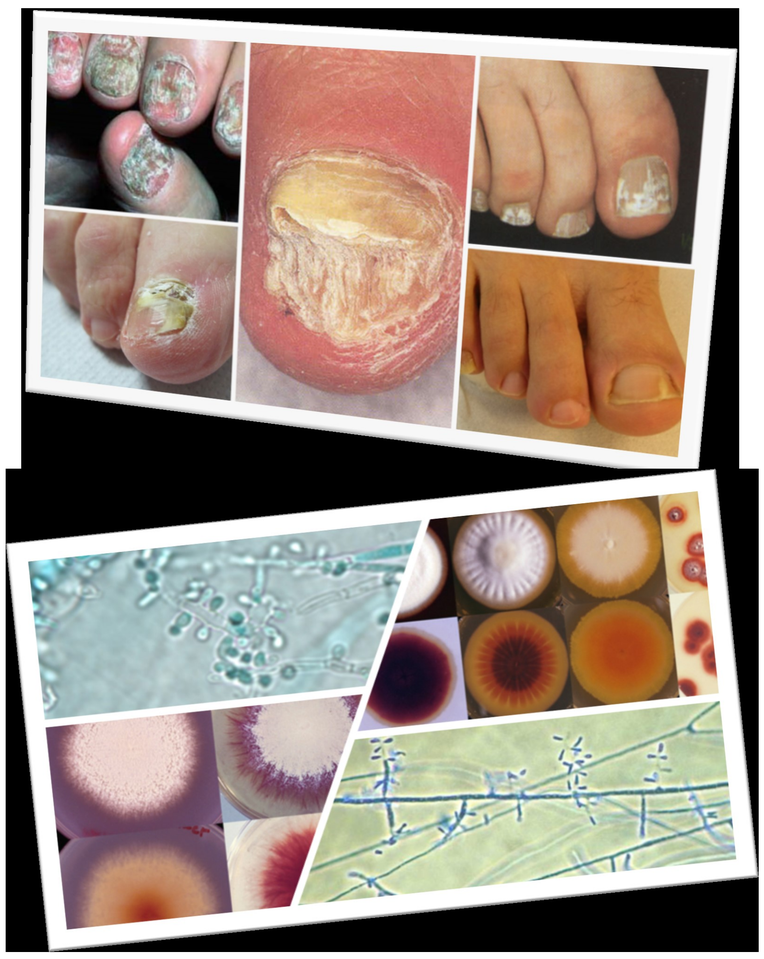
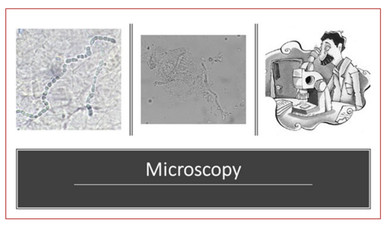
INTRODUCTION

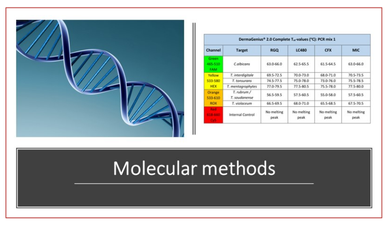
Onychomycosis represents one of the most frequent mycoses in the world with a incidence progressively increases with age up to a prevalence of 20% between 40 and 60 years and exceeds 40% in the elderly. The main etiological agents are dermatophytes, including *Trichophyton rubrum* and *Trichophyton mentagrophytes*, but yeasts and non-dermatophyte moulds can also be involved. The laboratory diagnosis of dermatophytosis is currently based on microscopy and culture assays although these methods have low to moderate sensitivity. Molecular methods have been developed to overcome these problems and recently several commercial kits have been validated for the detection of dermatophytes in nails.

The aim of the present study was to evaluate the real-time multiplex PCR for detecting and identifying dermatophytes in the nail plate compared with microscopy and culture methods.



Laboratory diagnosis





MATERIALS AND METHODS

Over period between January and May 2019, 96 patients were observed. Nail material was taken through scraping from clinically abnormal nails. The nail scrapings was divided into three parts: one for microscopy in 15% potassium hydroxide, one for culture in Sabouraud's dextrose agar medium supplemented with 0.5% cycloheximide and 1% chloramphenicol and the remaining part for DNA extraction and PCR assay. The DermaGenius® Nail real-time multiplex PCR (PathoNostics, The Netherlands) was use for the detection the most clinical prevalent dermatophytes species. The DNA was extracted by using the PathoNostics Extraction Kit following the manufacturer's instructions.

RESULTS

Techniques	Positive PCR: Dermatophyte	Positive PCR: *C. albicans*	Positive PCR: Dermatophyte + *C. albicans*	Negative PCR	Total
Microscopy					
Positive	38 (39.6%)	0	0	14 (14.6%)	52 (54.2%)
Negative	13 (13.5%)	0	0	31 (32.3%)	44 (45.8%)
Culture					
Dermatophytes	29 (30.2%)	0	0	0	29 (30.2%)
Candida albicans	0	0	0	1	1 (1%)
NDM° + Yeasts°°	0	0	0	16 (16.7%)	16 (16.7%)
Negative	22 (22.95)	0	0	28 (29.2%)	50 (52.1%)
Microscopy + Culture					
Positive	42 (43.7%)	0	0	17 (17.7%)	59 (61.4%)
Negative	9 (9.3%)	0	0	28 (29.2%)	37 (38.5%)
Total	51 (53.1%)	0	0	45 (46.7%)	96 (100%)

°non-dermatophyte moulds

**yeasts other than *Candida albicans*

A total of 61 (63.5%) cases of onychomycosis were confirmed. The microscopy was positive in 52/96 samples (54.2%) among which 37 specimens had a fungal culture positive for dermatophytes (25), yeasts (2), or nondermatophyte moulds (10). The fungal cultures were positive in 46/96 samples (48%) and in particular 29 were dermatophytes, 3 were yeasts and 14 were positive for a non-dermatophyte moulds. The PCR was positive in 51/96 nail samples (53.1%), among which 38 where microscopy positive (38/51, 74.5%) and 29 where culture positive for dermatophytes (29/51, 56.9%). The PCR assay was positive in 9 samples that showed microscopy and culture negative and in 13 samples with negative culture for a dermatophytes but with a positive microscopy showing its ability to identify non growing fungal agents.

CONCLUSIONS

This study makes a further contribution to the clinical evaluation of DermaGenius® Nail real-time multiplex PCR, a commercial multiplex PCR assay for the diagnosis of onychomycosis. The data obtained show that the PCR assay is a reliable test that shortens the time to diagnosis and can unmask the presence of non growing fungal pathogens in nails.