



Improving the diagnosis of dermatophytes in gaza strip by using Nested PCR

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Abstract

Dermatophytes are a very related to keratinophilic fungi that can invade keratinized humans and animal tissues such as skin, hair and nails causing dermatophytosis. They are the important cause of superficial fungal infection.

Conventional identification methods like potassium hydroxide (KOH) microscopy and fungal culture lacks the ability to make an early and specific diagnosis. In this study it is taken into consideration to evaluate nested polymerase chain reaction (NPCR) using primers targeting dermatophyte specific sequence of chitin synthase 1 (CHS1) gene and compared with conventional method by potassium hydroxide (KOH) microscopy test that carried out in Rimal clinic in Gaza city.

A total of ninety nine patients were clinically suspected with dermatophytosis including 16 skin specimens 16 nail specimens and 67 hair specimens. For each specimen KOH, PCR and NPCR tests were carried out.

The output results of NPCR sequencing was compared with the wild-type gene which was obtained from the National Center for Biotechnology Information (NCBI). The comparison indicated that the product of NPCR is CHS1 gene. Additionally, it was considered to compare the results of NPCR with KOH for dermatophytes which showed that 41.4% are positive indication based on KOH while 18.18% only was positive indication according to NPCR.

After carrying out the statistical analysis using SPSS for both tests, it was found that 30% of the total samples should be included for treatment based on KOH test, although this percent of the sample doesn't need to undergo treatment according to NPCR test. It is also shown that 6% of the samples are excluded for treatment in KOH method, in spite of the NPCR indicated that this percent must be included in the treatment.

The prominent controversy between the test results (KOH and NPCR) was found particularly in the nails diagnosis.

The study results approved that the NPCR test should be considered in dermatophytes test in Gaza Strip medical laboratories along with KOH test particularly in nails.

Moreover, to improve the quality of test results, it was recommended to conduct training session for lab technicians to develop their capacity in the diagnosis of dermatophytes by KOH test.

Keywords: Dermatophytes, KOH method, PCR, Nested PCR

1. Introduction

The dermatophytes are a group of closely related fungi that have the capacity to invade keratinized tissue (skin, hair, and nails) of humans and other animals to produce an infection, dermatophytosis, commonly referred to as ringworm. Infection is generally cutaneous and restricted to the nonliving cornified layers because of the inability of the fungi to penetrate the deeper tissues or organs of immunocompetent hosts. Reactions to a dermatophyte infection may range from mild to severe as a consequence of the host's reactions to the metabolic products of the fungus, the virulence of the infecting strain or species, the anatomic location of the infection, and local environmental factors [1-3].

The etiologic agents of the dermatophytoses are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum* and *Trichophyton*, of anamorphic class Hyphomycetes of the Deuteromycota (Fungi Imperfecti) [1]. Geophilic dermatophytes are found mainly in soil, where they are associated with decomposing hair, feathers, hooves and other keratin sources. They infect both humans and animals (*Epidermophyton*). Zoophilic dermatophytes are mainly found in animals but can be transmitted to humans (*Microsporum*). Anthropophilic dermatophytes are mainly found in humans and are very seldom transmitted to animals (*Trichophyton*) [4].

Because dermatophytes require keratin for growth, they are restricted to hair, nails, and superficial skin. Thus, these fungi do not infect mucosal surfaces. Dermatophytoses are referred to as "tinea" infections. They are also named for the body site involved. Some dermatophytes are spread directly from one person to another (anthropophilic organisms). Others live in and are transmitted to humans from soil (geophilic organisms), and still others spread to humans from animal hosts (zoophilic organisms). Transmission of dermatophytes also can occur indirectly from fomites (e.g., upholstery, hairbrushes, hats). Anthropophilic organisms are responsible for most fungal skin infections. Transmission can occur by direct contact or from exposure to desquamated cells. Direct inoculation through breaks in the skin occurs more often in persons with depressed cell-mediated immunity. Once fungi enter the skin, they germinate and invade the superficial skin layers. In patients with dermatophytoses, physical examination may reveal a characteristic [2].

The dermatophytosis caused by various dermatophyte species cannot be easily differentiated on the basis of clinical manifestations methods. For many years, conventional laboratory methods based on the detection of phenotypic characteristics, such as microscopy and *in-vitro* culture, have played an essential role in dermatophyte identification.

However, these procedures generally suffer from the drawbacks of being either slow or non-specific. Recent developments and applications of nucleic acid amplification technology have provided the opportunity to enhance the quality and speed of dermatophyte diagnosis. This method by use polymerase chain reaction (PCR) for diagnosis after that using nested PCR [5]. Nested PCR is a variation of the polymerase chain reaction (PCR), in that two pairs (instead of one pair) of PCR primers are used to amplify a fragment. The first pair of PCR primers amplifies a fragment similar to a standard PCR. However, a second pair of primers called nested primers (as they lie / are nested within the first fragment) bind inside the first PCR product fragment to allow

amplification of a second PCR product which is shorter than the first one.

The advantage of nested PCR is that if the wrong PCR fragment was amplified, the probability is quite low that the region would be amplified a second time by the second set of primers. Thus, Nested PCR is a very specific PCR amplification. The aim of this study was to Improving the diagnosis of dermatophytes in Gaza Strip by using Nested PCR.

2. Materials and Methods

2.1 PCR primers

Table 1: list of PCR primers used in this study.

Nucleotides [nt]	Sequence	Primer name
70 to 89	5'-CAT CGA GTA CAT GTG CTC GC-3'	CHS1 1S
485 to 504	5'-CTC GAG GTC AAA AGC ACG CC-3'	CHS1 1R
111 to 130	5'-GCA AAG AAG CCT GGA AGA AG-3'	CHS1JF2
378 to 398	5'-GGA GAC CAT CTG TGA GAG TTG-3'	CHS1JR2

2.2 Study Area

The study was performed at Al-Rimal Clinics at Ministry of Health (MOH) and Gene Medical Labs in Gaza Strip.

2.3 Samples

A total of 100 samples from patients clinically suspected with dermatophytosis were included in the study irrespective of their age or gender.

2.4 Specimens Collection

For skin dermatophytoses the clinical specimens collected were epidermal scales. The scales were scrapped from near the advancing edges of the lesions after disinfecting the lesions with 70% alcohol. When the advancing edges were not evident, scrapings were collected from areas representing the whole infected area.

In hair sample dermatophytoses, the basal root portion of hair was collected by plucking the hair with sterile forceps. In cases with black dot, scalpel was used to scrape the scales and excavate small portions of the hair roots.

2.5 Specimens Division.

According to the modified procedures of Garg *et al.*, (2009), the collected specimens were divided into two portions. The first portion of the specimens was examined microscopically using 20% potassium hydroxide (KOH) and with 40% dimethyl sulfoxide. The second portion was used for DNA extraction to be used in PCR and nested PCR [6].

2.6 Specimens Identification

2.6.1 Direct microscopy

This method aids visualizing hyphae and confirmation of the diagnosis of dermatophyte infection. The scale from the active border of a lesion was obtained, and several loose hairs from the affected area were pulled out. In the case of nails, sub-ungual debris was obtained. A moist cotton swab was rubbed vigorously over the active border of a lesion works as well as a scalpel blade is safer. The scale, hair, or debris was transferred to a glass slide, and a few drops of 20% KOH were added. For nail material or hair, the slide was gently warmed.

The wet-mount preparation was then examined under a microscope (400X) with back-and-forth rotation of the focus knobs. This technique aided the visualization of hyphae (branching, rod-shaped filaments of uniform width with lines of separation [septa]). In tinea capitis, the hairshaft may be uniformly coated with minute dermatophyte spores [2].

2.7 Molecular Techniques

2.7.1 DNA Extraction

The crushed specimen were cut and put in Eppendorf tube and 200 µl buffer (0.02g Ca HCO₃, 30 µl HCl, add water to 10 ml) were Added. Then the following steps were followed.

1. Add 5 of (proteinase K)
2. Incubation for 2-3 hours at 65°C
3. Then using MasterPure™ Genomic DNA Purification Kit for Blood (Epicentre Technologies Co., USA) according to the following procedure:
 - a. Add 250 precipitation solution (5M Sodium perchlorate (dissolve 70 g of sodium perchlorate in 80 ml distilled water make upto 100 ml)
 - b. Mixed by vortex for at least 30 sec, then centrifugation at 14,000 Xg for 7 min.
 - c. The supernatant was poured into a new Eppendorf tube, and 700 µl of isopropanol were added. The tube was inverted gently 30-40 times to visualize the DNA strings.
 - d. The DNA was precipitated by centrifugation at 14,000 Xg for 10 min.
4. DNA was washed twice with 75% ethanol, by adding 200 µl of 70% ethanol followed by centrifugation at 14,000 Xg for 3 min.
5. DNA pellet was air dried, resuspended in 100 µl of TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA) buffer, and then incubated overnight at room temperature (or incubation for 10 min at 37°C).
6. Finally, the DNA was mixed, quantified using agarose gel electrophoresis for semi quality and DNA quality evaluation.

2.7.2 Polymerase Chain Reaction (PCR)

The polymerase chain reaction is an *in vitro* technique which allows the amplification of a specific DNA fragment that lies between two regions of known DNA sequence [7]. The amplification of DNA is achieved by using a short single stranded DNA molecules which are complementary to the ends of a defined sequence of the DNA template (known as primers), that hybridize to opposite strands and flank the target DNA sequence that is to be amplified. Under suitable reaction conditions and in the presence of deoxynucleoside triphosphate (dNTPs), a DNA polymerase extends the primers annealed to a single stranded DNA template. As a result, a new DNA strands complementary to the template strands are synthesized [7-8]. Repetitive cycles involving template denaturation, primer annealing, and extension of the annealed primers by Taq DNA polymerase results in exponential accumulation of a specific DNA fragments. In other words, the number of target DNA copies approximately doubles every cycle, since the primer extension products synthesized in a given cycle can serve as a template in the next cycle [9].

2.7.3 First PCR

The sequence of primers used for specific amplification was performed using primer pairs CHS1 1S (5'-CAT CGA GTA CAT GTG CTC GC-3'; nucleotides [nt] 70 to 89) and CHS1 1R (5'-CTC GAG GTC AAA AGC ACG CC-3'; nt 485 to 504). These primers amplify a 435-bp DNA fragment of the dermatophyte-specific CHS1 gene sequence of *Arthroderma benhaemiae*, a teleomorph of *Trichophyton mentagrophytes* (DDBJ accession no. AB003558) [6].

2.7.4 Nested PCR

Nested PCR was performed by designing a novel set of primers, JF2 (5'-GCA AAG AAG CCT GGA AGA AG-3'; nt 111 to 130) and JR2 (5'-GGA GAC CAT CTG TGA GAG TTG-3'; nt 378 to 398), amplifying a DNA fragment of 288 bp from the internal sequence of the amplicon obtained from first-round PCR [6].

2.7.5 PCR Mixture

The PCR mixture (25 μ l) for first-round PCR contained 12.5 μ l of green mix (need information) 1 μ l each of primers 0.1MG/ML CHS1 1S and CHS1 1R (Operon, Cologne, Germany), and 3 μ l of DNA template. Deionised water was added subsequently to achieve the final volume (Table 2). The reaction mixture was initially denatured at 94°C for 30 s, followed by 31 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. This was followed by a final extension step for 5 min at 72°C in a thermal cycler (HYBAID, Omnigene) (Table 3). The PCR mixture for nested PCR consisted of of 1 μ l primers JF2 and JR2 and 2 μ l diluted product of the primary cycle as the DNA template; the rest of the constituents were the same as those described above (Table 4). The running conditions of nested PCR were similar to the first-round PCR except that 35 cycles were used (Table 5). Double-distilled water and DNA of positive controls were used as the negative and positive controls, respectively.

Table 2: First PCR reaction mixture for 25 μ l, the amounts given are per reaction

Volume (μ l)	Reagents
12.5 μ l	Go tag polymerase (ready mix)
1 μ l	Primer forward
1 μ l	Primer Reverse
3 μ l	DNA
7.5 μ l	Water
25 μ l	Total mix
2 μ l	We take DNA Template

Table 3: Temperature cycling program for first PCR

PCR Machine Cycling Parameters For first PCR Rounds	
94°C/30 sec.	initially denatured
94°C/30sec. 60°C/30 sec. 72°C/ 60 sec.	31 cycles:
72°C/ 5 min	extension step
4°C	Hold:

Table 4: Nested PCR Master Mix for 25 μ l reactions, the amounts given are per reaction.

Volume (μ l)	Reagents
12.5 μ l	Go tag polymerase (ready mix)
1 μ l	Primer forward
1 μ l	Primer Reverse
2 μ l	DNA Template
6.5 μ l	Water
25 μ l	Total mix

Table 5: Temperature cycling program for NPCR

PCR Machine Cycling Parameters For nested PCR Rounds	
94°C/30 sec.	initially denatured
94°C/30sec. 60°C/30 sec. 72°C/ 60 sec.	35 cycles:
72°C/ 5 min	extension step
4°C	Hold:

2.7.6 Agarose Gel Electrophoresis

The amplified PCR product were resolved by electrophoresis on a 2% agarose gels and stained with ethidium bromide for analysis. The agarose gel (Life Technologies, Scotland) was prepared in 1X Tris-Acetate EDTA (TAE) buffer (40 mM Tris base, 40 mM acetic acid, 1mM EDTA), then stained with ethidium bromide (final concentration is 0.5 μ g/ μ l). The gel casting tray containing the gel is placed into the electrophoresis chamber (Owl Scientific Plastics, Inc.). Stained PCR products and DNA molecular weight marker were loaded into the agarose gel. Then the gel was run at 80 volt (constant voltage) for 45-75 min, according to the gel size used. After that, the ethidium bromide-stained DNA was detected by ultraviolet radiation using UV Transilluminator (Dinco & Rheunium Industries Ltd.). Amplicon of 288 bp was taken as positive for dermatophytes and photographed by digital Camera for documentation.

2.7.7 DNA Sequencing

Direct DNA sequencing for PCR products that contain the known mutations and polymorphisms in -(AB003558) JF2 (5'-GCA AAG AAG CCT GGA AGA AG-3'; nt 111 to 130)

and JR2 (5'-GGA GAC CAT CTG TGA GAG TTG-3'; nt 378 to 398), amplifying a DNA fragment of 288 bp from the internal sequence of the amplicon obtained from first-round PCR.

The amplified products were separated on 2% agarose gel and purified using GFX™ PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech).

3. Results

3.1 Potassium hydroxide (KOH) method

This method aided visualizing hyphae or spores and confirmed the diagnosis of dermatophyte infection. In *Tinea capitis*, the hair shaft may be uniformly coated with minute dermatophyte spores (Figure 1).

As shown in Figure (1) and Table (6) the positive result in KOH method were 41 out of 99 samples which considered as 41.4%. These results distributed as 3 from skin out of 16, 9 from nails out of 16 and 29 from scalp out of 67. The results also showed that the highest percentage of positive results was from the nails (Table 6).

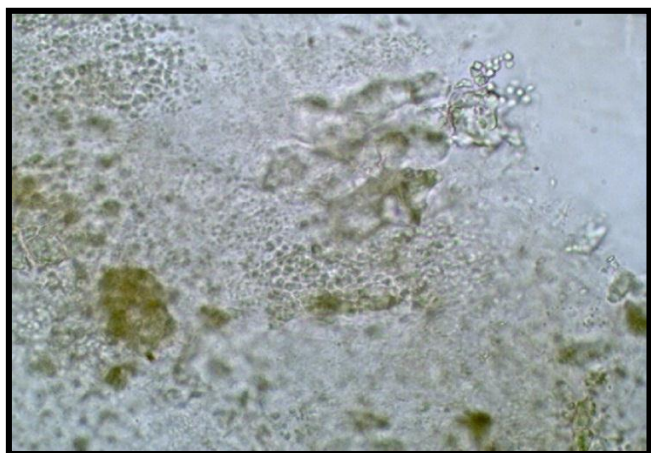


Fig 1: Microscopic appearance of positive sample (spores) from hair in KOH method.

3.2 PCR Result

The amplified PCR product were resolved by electrophoresis on a 2% agarose gels and stained by ethidium bromide for analysis (Figure 2).

3.2.1 First PCR

As shown in Figure (8) the positive result in FPCR were 2 out of 99 samples which considered as 2.02% only.

3.2.2 Nested PCR

As shown in Figure (8) and Table (6) the positive result in NPCR were 18 out of 99 samples which considered as 18.18%. These results distributed as 1 from skin out of 16, 3 from nails out of 16 and 14 from scalp out of 67 (Table 6).

The results also showed that the highest percentage positive results were from the scalp which constitutes 20% of all suspected scalp samples.

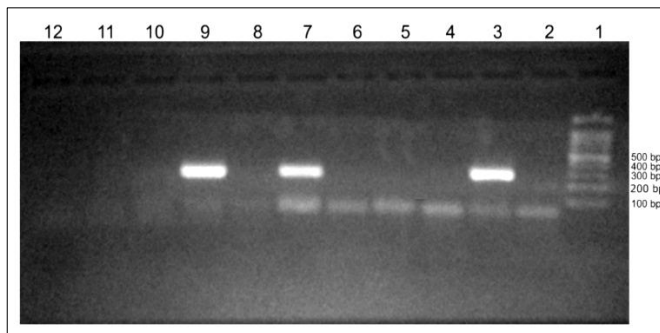


Fig 2: Results of first and nested PCR of clinical specimens of dermatomycosis. Lane 1, 100 bp DNA ladder (Molecular Marker); Lane 2, 4, 6 first PCR negative cases; Lane 3, 7 nested PCR positive cases; Lane 5, nested PCR negative case; Lane 8, 9 first and nested PCR positive control, respectively (288 bp); Lane 10, 11 first and nested PCR negative control; Lane 12 blank.

3.3 Gene Sequencing

Figure (4) showed the gene sequencing of PCR gene product. By comparing our result with the wild-type gene obtained from the NCBI gene bank accession number GI: AB 003558), we found 95% homology with the reference sequencer GI: AB 003558. This comparison confirmed PCR product to be indeed the product of CHS1 gene and confirmed the PCR product specificity of the CHS1 gene (Figure 3).

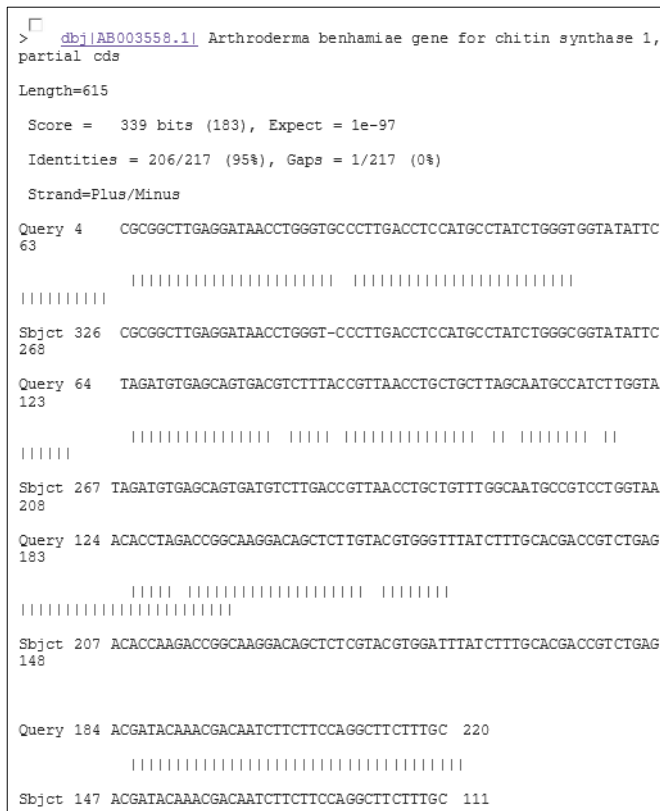


Fig 3: The wild-type gene obtained from the NCBI gene bank accession number GI: AB 003558)

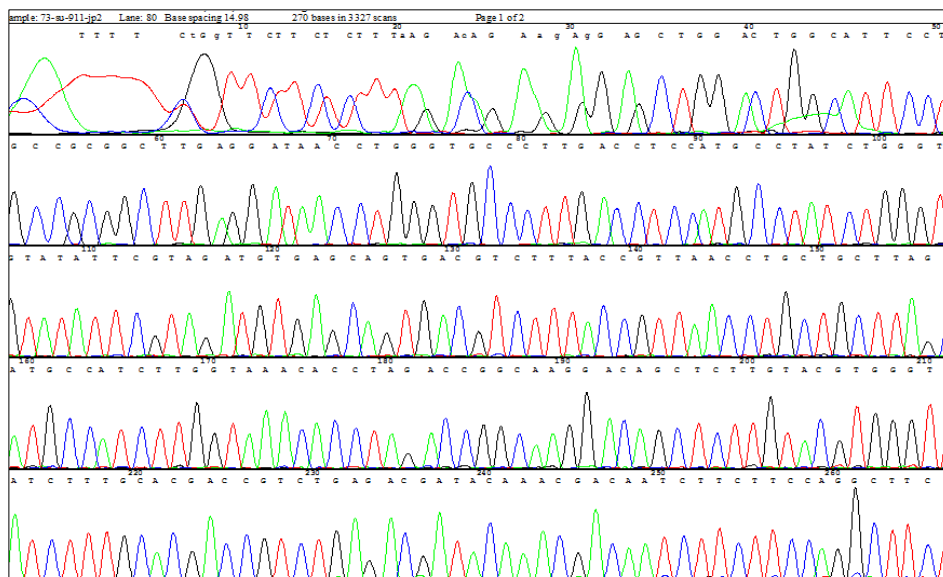


Fig 2: The DNA sequencing result of CHS1 out put

3.4 Statistical analysis

Of the 99 clinically suspected cases of dermatophytosis, 41.4% were diagnosed as positive for fungal elements by KOH microscopy. Dermatophytes were detected in 18.18% of the specimens by nested PCR.

3.4.1 Sample classification

As shown in Figure (5) 46.32% of our clinical samples were from male. The sources of the clinical samples were distributed as 16.16% from both skin and nails and 67.68% from scalp (Table 6).

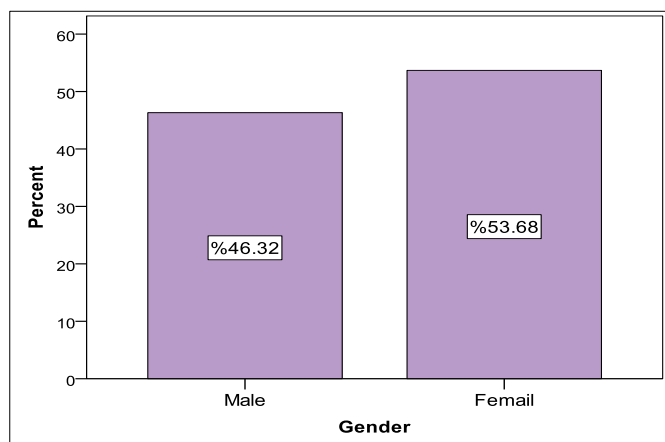


Fig 3: Sample classification of gender

As inferred from the Questionnaire (Appendix) about the presence of domestic animals in the houses of the suspected patients, cats were the highest percentage which was 70.4% (Figure 6).

The percentage of positive results among the suspected patients holding domestic animals at their houses was 33.3% while it was 22% for the suspected patient not holding domestic animals (Table 7).

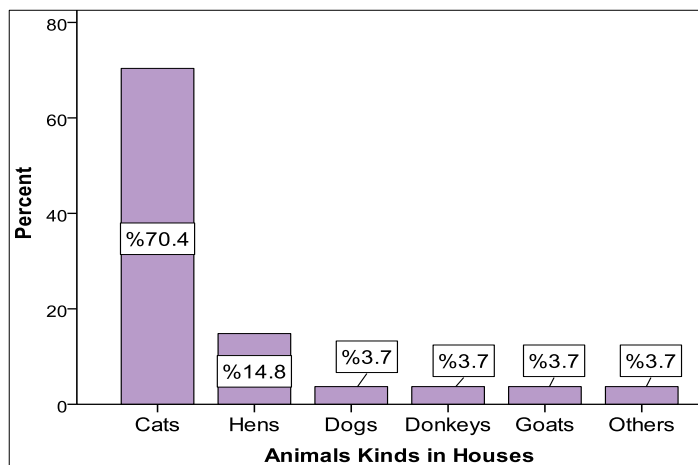


Fig 6: The distribution of the domestic animals in patients house.

The age distribution of the cases is ranged from 1 year to higher than 35 years. The highest age distribution was ranged between 1-10 years which constituted 64.52% (Figure 7).

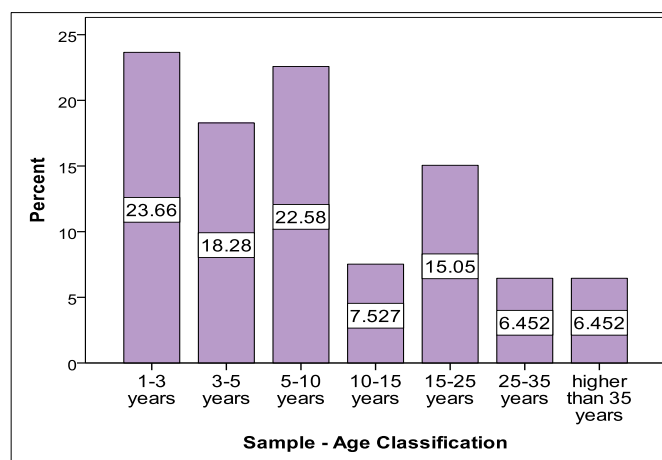


Fig 7: Age group distribution.

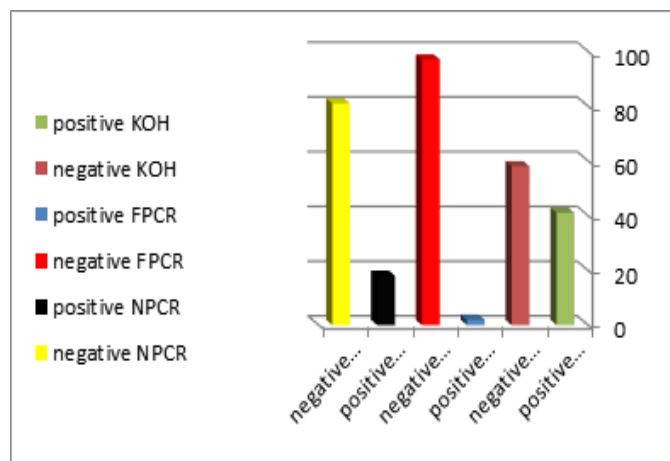


Fig 8: The results of KOH, FPCR, NPCR classification.

3.4.2 Relative absolute error

Table (7) shows a tabulating of the samples according to the sample type and the Lab diagnosis methods with the relative absolute error.

Table 7: Sample type and lab diagnosis methods (KOH or NPCR).

Sample Type	KOH Result		Nested PCR Result		Total	% Error
	Positive	Negative	Positive	Negative		
Skin	3	13	1	15	16	12.50
Nail	9	7	3	13	16	37.50
Scalp	29	38	14	53	67	22.39
Total	41	58	18	81	99	23.23

As shown in Table (8) a tabulating of the samples according to holding animals in the house and the Lab diagnosis methods with the relative absolute error.

Table 8: Holding animals in the house relative to KOH & NPCR.

holding animals	KOH Result		Nested PCR Result		Total	% Error
	Positive	Negative	Positive	Negative		
Yes	14	13	5	22	27	33.33
No	24	39	10	53	63	22.22
Total	38	52	15	75	90	25.56

3.4.3 Lab diagnosis exclusion and inclusion errors

Inclusion and Exclusion errors refer to discrepancies between the diagnosis by KOH method and NPCR method. Exclusion errors represent the percentage of negative samples by KOH method that was positive according to NPCR method. Inclusion errors represent the percentage of negative samples by NPCR method which was positive in KOH method (Table 9).

Table 9: Lab diagnosis exclusion and inclusion errors.

Nested PCR Result vs. KOH Result				
Total	KOH Result		Nested pcr Result	
	negative	positive		
18	6	12	positive	Total
81	52	29	negative	
99	58	41		

- Exclusion error = 6/99 = 6.06%
- Inclusion error = 29/99 = 29.29%

4. Discussion

Dermatophytes are among the few fungi causing communicable diseases; previously most dermatophyte strains had relatively restricted geographical distribution. However recently, dermatophytosis has become one of the most common human infectious diseases in the world and is cosmopolitan in distribution. Dermatophytosis cannot be easily diagnosed on the basis of clinical manifestations as a number of other conditions mimic the clinical presentation. The differential diagnosis of dermatophytoses includes seborrhoeic dermatitis, atopic dermatitis, contact dermatitis, psoriasis, candidal intertrigo, erythrasma, eczema etc [2]. Further, it is more difficult to diagnose dermatophytosis in immunocompromised patients, as clinical presentation is often atypical [10].

It is essential that good laboratory methods are available for rapid and precise identification of the dermatophytes involved, in order to apply appropriate treatment and prevention measures. The conventional methods of fungal detection have their own drawbacks; for e.g. KOH microscopy has low specificity and fungal culture is associated with low sensitivity and takes long time. Further dermatophyte isolates from patients on antifungal treatment generally do not show characteristic morphology on culture, thus further compromising the results of culture isolation [5]. The changing profiles of human dermatophytoses among countries have further necessitated the development of improved diagnostic methods for identification of dermatophytes [5]. Thus newer fungal diagnostic methods are needed as identification of the etiological agent is required not only for accurate diagnosis, but also for post-therapeutic strategies [11].

Very few studies have compared KOH microscopy with direct PCR of clinical specimens. In a case study, Nagao *et al.* detected *Trichophyton rubrum* by nested PCR targeting internal transcribed spacer gene 1 (ITS1) in a patient with trichophytia profunda acuta, which was negative by both KOH microscopy and culture [12]. Yan *et al.*, (2007) evaluated arbitrary primed PCR with conventional methods in 50 tinea corporis and 58 tinea cruris patients and showed that arbitrary primed PCR is a rapid sensitive and specific detection method for dermatophytes from skin scrapings [13]. Recently bergman *et al.*, performed a PCR-reverse line blot assay on 819 clinical samples (596 nail, 203 skin and 20 hair) and demonstrated a positive PCR-RLB result in 93.6% of 172 culture-positive and microscopy-positive samples [14]. In Garg *et al.*, (2007) study involving 152 clinically suspected patients with onychomycosis it was established that nested PCR might be considered as gold standard for the diagnosis of onychomycosis, where the etiological agents are dermatophytes [15]. In Garg *et al.*, (2009) study results indicated that nested PCR may be considered as gold standard for the diagnosis of dermatophytosis and can aid the clinician in initiating prompt and appropriate antifungal therapy [6].

In this study, a total of ninety nine patients were clinically suspected with dermatophytosis including 16 skin specimens, 16 nail specimens and 67 hair specimens. For each specimen both of KOH and NPCR test were carried out.

Having compared the output results of NPCR sequencing with the wild-type gene which is obtained from the NCBI gene bank. The comparison indicates that the product of NPCR is CHS1 gene according to (NCBI) gene bank. Additionally, it is considered to compare the results of NPCR with KOH for

dermatophytes which gave that 41.4% of positive indication based on KOH and 18.18% of positive indication according to NPCR. Our results reflected the accuracy of NPCR method and eliminated the false-positive and reconsider few of the false-negative as positive samples (Table 7).

After carrying out the statistical analysis using SPSS for both results obtained from NPCR and KOH diagnosis methods, it was found that 30% of the total samples have to be included for treatment based on KOH test, although this percent of the samples did not need to undergo treatment according to NPCR test. It was also shown that 6% of the samples are excluded for treatment in KOH diagnosis method, and the NPCR indicated that this percent should be included in the treatment (Table 9). Correct diagnosis of dermatophytic onychomycosis and identification of the causal agent is a major importance as it allows appropriate antifungal treatment to be promptly instituted. Diagnosis of onychomycosis is currently performed by direct mycological examination and culture on Sabouraud dextrose agar medium. The precise identification of the dermatophyte in cause is based on the macroscopic and microscopic characters of the grown colonies. However, false negative results of direct examination occur in 5 to 15% of cases, depending on the skill of the observer and the quality of sampling [16, 17]. Furthermore, dermatophyte hyphae are very difficult to distinguish from those of non dermatophytic fungi like molds which often only occur as transient contaminants and not as the actual etiological agent of the disease [17-19].

The present study aimed at evaluating a PCR technique based on the amplification of the CHS1 gene which is one of the most widely used target in the molecular diagnosis of dermatophytic onychia in humans [16, 20-23].

The prominent controversy between the results of KOH method and NPCR was found in the nails diagnosis.. This complies with previous studies but differs in the nature of results as the positive results higher in KOH test method and its may related to:

- Labs equipment shortage.
- No advanced training for workers in the labs.
- Technicians unqualified to carry out perfect tests to discriminate between pathogenic fungus and normal flora, contaminant, bubbles or oil.
- Nails thickness without being treated enough in KOH.

On the other hand, our results showed that people who got contact with animals (pets) were most likely to have dermatophytosis more than other people. This finding was in good agreement with most previously reported studies [24, 25].

5. Conclusions & Recommendations

5.1 Conclusions

By comparing the output results of NPCR sequencing with the wild-type gene which was obtained from the NCBI gene bank, it was indicated that the product of NPCR was CHS1 gene.

The results of KOH diagnosis method for dermatophytes gave 41.4% positive indication and 18.18% was positive indication according to NPCR diagnosis method.

The statistical analysis using SPSS for both test results obtained from NPCR and KOH diagnosis methods, it was found that 30% of the total sample has to be included for treatment based on KOH test, although this percent of the sample didn't need to undergo treatment according to NPCR test. It was also shown that 6% of the samples are excluded for

treatment in KOH test, in spite the NPCR indicated that this percent should be included in the treatment.

The prominent controversy between the results of KOH and NPCR diagnosis methods were found in the nail samples.

5.2 Recommendations

1. Sending directives to the Ministry of Health in the Gaza Strip by introducing screening NPCR part of routine testing for dermatophytes.
2. Conduct training session for lab technicians to develop their skills in the diagnosis of dermatophytes by KOH test.
3. Community awareness in taking necessary measures when dealing with domestic animals.
4. Inviting researchers to take into account the studies on dermatophytes in the Gaza Strip

6. Acknowledgment

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