Identification of 5S rRNA gene of *Enterobius vermicularis*

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الخلاصة

أجريت هذه الدراسة من بداية تشرين الأول 2012 إلى نهاية ايلول 2013، من اجل دراسة بعض الجوانب الوبائية والمناعية والجزيئية لطفيلي الدودة الدبوسية.
شملت هذه الدراسة جمع 224عينة براز من الاشخاص المصابين الذين تراوحت أعمارهم بين سنة واحدةو60سنة،وتم الكشف عن وجود الطفيلي بالبراز باستخدام طريقة المسحة الرطبة المباشرة وطريقة التركيز ثم التصبيغ بالايودين iodine stain وقد شخص الطفيلي بطور البيضة واليرقة والبالغة تحت المجهر الضوئي باستخدام العدسةx40,x10و العدسة الزيتية (100x) وأظهرت النتائج ان146(65%)عينة من أصل 224 كانت موجبة للفحص المجهري ،وقدحفظت عينات البراز بدرجة حرارة -20° م.
أما فيما يخص الجانب الجزيئية استخدمت في هذه الدراسة تقنية تفاعل انزيم البلمرة (PCR) المتسلسل للكشف عن جين 5sRNAفي الدودة الدبوسية في المصابين بالدودة الدبوسية وباستخدام البادئات الخاصة لهذه العوامل (primers) حيث أظهرت النتائج ان126(56.3%)عينة كانت موجبة من أصل 224عينة مفحوصة. وفي المقارنة بين الفحص المجهري وتقنية الPCR اوضحت النتائج ان 146 (65%)عينة كانت موجبة بالفحص المجهري بينما126(56%)عينة كانت موجبة بالPCR من اصل 224 عينةمع اهمية تقنية ال PCR في تشخيص المرض الغامض في 30(23%) .

ABSTRACT

Background: The present study was carried out from October 2012 to the end of September 2013.
The study included a collection of two hundred -twenty four stool sample from deferent ages, Ages were vary from one to sixty years old for detection *Enterobius vermicularis* **.**Stool samples were used direct wet mount ,concentration method and stained with iodine ((the eggs ,larvae and adult)) had been noticed by microscop , 10x ,40x and oil immersion lens (100x). The results showed that 146 (65%) out of 224 were microscopically positive. The stool samples were store in -20°C until use.
In molecular study of this parasite PCR technique was conducted, to detect one gene 5sRNA by using specific primers for *Enterobius vermicularis*, the results showed that 126(56.3%) samples out of 224 were positive.
In comparison between microscopic and PCR methods the results showed that 146 (65%), 126(56.3%) out of 224 were positive respectively,with important of PCR technique in diagnosis occult enterobiasis 30(23%).
Materials and methods: stool samples were used in this study to detect the presence one specific 5SrRNA gene . 224 randomly stool sample were determined by the polymerase chain reaction method. **Results:** The 5SrRNA gene may be facilities in diagnosis occult and non- occult Enterobiasis .**Conclusion:** Polymerase Chain Reaction technique was useful for identification of *Enetrobius Vermicularis* and occult cases.This study aimed to role of Polymerase chain reaction(PCR) technique used to prove the DNA of *Enerobius vermicularis*.

Keywords: *Enterobius vermicularis*; Enterobiasis,5S rRNA gene; Polymerase chain rection.

Introduction

The organism was first identified in 1758 by karlLinnaeus, who named it *Oxyuyris vermicularis*, it is an obligate parasite and human represent the only natural host of this worm ,it is measured approximately 10 mm in length and it lives with their heads embedded in the right hemicolon and adjacent bowel, infection that it is known as Enterobiasis ,it is more commonly associated with children between the ages 5 to 15 years old.the prevalence of Enterobiasis greatly depends upon socio-economic status, education levels and personal hygiene **(1)**. These parasites are still causing many health and economic problems in different parts of the world for both children and adults .It is endemic parasite a worldwide pandemic, it is estimated that pinworms infect more than 400,000,000 people throughout the world (10% of humans), Enterobiasis may remain asymptomatic or cause perianal pruritus stimulated by movement of female, and the albuminous substance that surrounds the eggs ,other symptoms associated with entrobiasislike insomnia, restlessness, irritability, and rarely, impetigo of scratched skin, or enuresis **(2)**. It may cause progressive eosinophilia and elevated in IgE ,although rare occurrence ,female pinworm may also eventually invade the human genital tract and cause vulvovaginitis **(3).** Ribosomal RNA (rRNA) sequences have been used extensively as model genes for phylogenetic inference and microbial speciation , although the small gene encoding 5s rRNA in nematodes is extremely conserved, the intergenic region between repeating 5S rRNA coding regions appears to vary greatly in size and nucleotide composition .The organization of the 5s rRNA gene has been studied in detail in *Cuenorhubditis elegms, Brugia malayi, Ascaris Iumbricoides and Entrobius vermicularis***(4).** The 5S rRNA spacer region has been used for systematic, diagnostic and phylogenetic inferences in nematodes due to its variability in size and sequence **(5).**Combined analyses of the nuclear 5S rRNA facilitated elucidation of the phylogenic relationship between pin from captive champanzes and those from human **(6).**

Materials and methods

A 224 patients were included in this study. All those patients undergo full history (a questioner and full information were obtained from the patient like age, address, and asking about clinical symptoms like abdominal pain, anal itching and found adult worm in stool) Those patients undergo stool examination by microscopic and PCR. Also laboratory investigation was taken (general stool examination). The patients excluded from the study are those with features of CBP, and patients with allergy.

DNA extraction and genotyping. Genomic DNA was extracted according to the manufacturer's protocol from 100-200 mg of the frozen stool sample using a DNA Extraction Kit (Bioneer / Korea). The polymorphic region was amplified by PCR. Amplification reaction preformed in 0.2 ml tube of Accu Power PCR Premix tube according to the bioneer's corporation then the thermocycling condition for this reaction carried out and products analyzed by 1% agarose gel electrophoresis. PCR products used according to the manufacturer's protocol, and analyzed by 2% agarose gel electrophoresis **(7).**

Table (1): The specific primers and their sequences

Sequence	Orientation	Size of PCR product (bp)
5'- **CACTTGCTATACCAACAACA C** -3'	Forward	420
5'- **GCGCTACTAAACCATAGACG** -3'	Reverse	

Table (2): Thermocycling condition for 5sRNA gene detection

Step	Temperature/^{0}C	Time (second)	Number of cycle
Initial denaturation	98	300	1
Denaturation	98	30	35
Annealing	50-55	30	1
Extension	72	30	1

Results

The samples used in PCR were DNA isolated from 224 randomly stool sample , this samples used to detect the presence of of specific 5SrRNA gene, primers that used in this assay provided by Bioneer Co. Korea which ,this gene successfully used as a PCR target for molecular study of *E.vermicularis***.** The optimum conditions in this experiment were corresponding to standardization of other previous studies(**8**) , the result were detected by electrophoresis and nanodram system that used to estimate DNA weight. The results of this technique revealed that the amplified DNA has (420 bp) for 5S rRNA gene identified in 126(56.3%) from 224 stool samples, with important of PCR technique in diagnosis occult Enterobiasis in 30(23%).

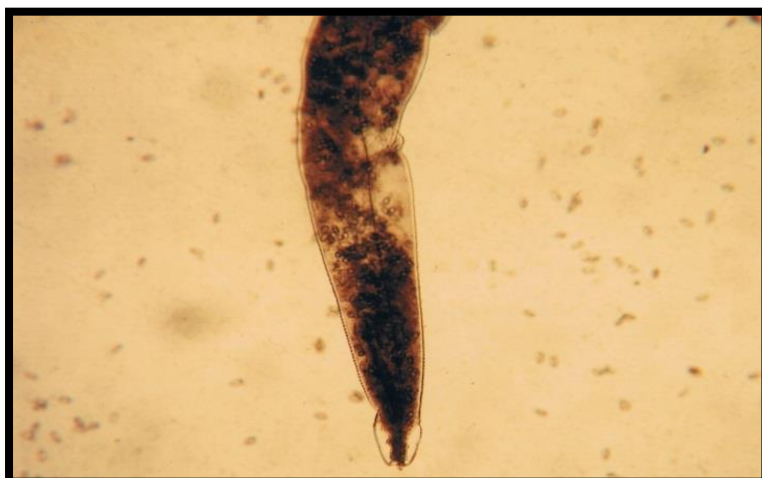

Figure (1): Anterior end of Enterobius.

Figure (2): Posterior end of Enterobius with eggs.

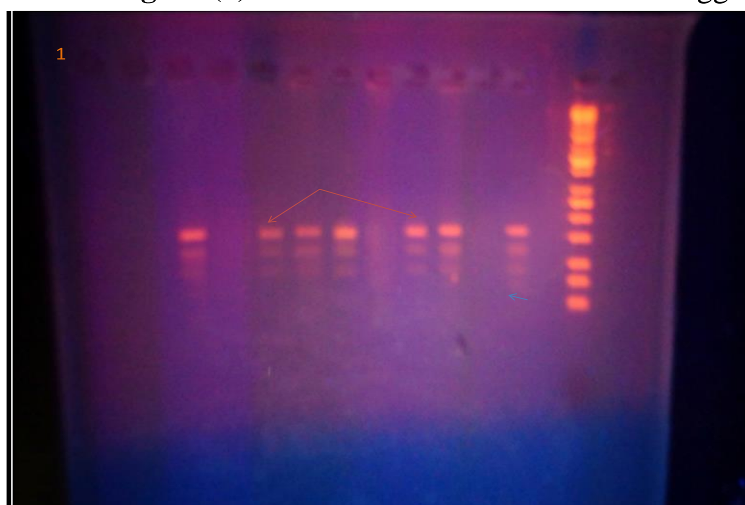

Figure (3): Ethidium bromide stained agarose gel shows PCR amplification product (420 bp) for *E.vermicularis* **Lines: 1, 3, 4, 6, 7, 10 show positive result with *E.vermicularis gene*. Lines: 2,5,9 shows negative result with *E.vermicularis gene*.**

Discussion

In present study The amplification result confirmed the specific nature of the diagnostic bands with 420 bp length this result agree with **(9),**who *Enterobius vermicularis* amplification was performed by nested PCR in 420bp.PCR results of this estimation revealed that the amplified DNA has (420bp) for 5S RNA gene in 32 samples from origin 88 sample examined , although from percentage infection in PCR result less from percentage infection in microscopic diagnosis ,but PCR remained good tool to diagnosis parasite specially that founded in human body without symptoms and that failed diagnosis microscopically this agree with **(10)**,who demonstrated that a molecular biology approach provides a specific and sensitive diagnostic tool and the opportunity to access a parasite's genetic information.

This result demonstrated the importance of the identification of the gene that rests in the central role in diagnosis occult Entrobiasis this result agree with **(11)**,whose resulting 5S rRNA intergenic spacer(gene) was successfully used as a PCR target for *E.vermicularis* diagnosis in Amerindian coprolites and **(12)** and **(13)**, whose noted the 5S rRNA spacer region has been used for systematic, diagnostic and phylogenetic inferences in nematodes due to its variability in size and sequence.

Conclusions:

Polymerase Chain Reaction technique was useful for identification of *Enetrobius Vermicularis* and occult cases.

Recommendations

Polymerase Chain Reaction technique test very useful for occult cases.

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