IL-17 family gene polymorphisms association with *Echinococcosis* risk in Babylon population, Iraq.

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Abstract

Echinococcosis **is the name given to a prominent silent chronic helminthes zoonotic infection caused by infection with the adult or larval stage of the dog tapeworm** ***Echicoccous granulosus*****, Based on molecular characteristics, the species/strains of E. granulosus have been taxonomically revised as:** ***E. granulosus*** **(G1–G3),** ***E. equinus*** **(G4),** ***E. ortleppi*** **(G5),** ***E. canadensis*** **(G6–G10), and E. felidis** ***Echinococcus granulosus*** **has been divided into several strains according to the host.**

Keywords: Interleukin 17 gene, *E. granulosus*, Polymorphism of Interleukin 17 family.

Accepted on September 28, 2021

Introduction

Echinococcosis is the name given to a prominent silent chronic helminthes zoonotic infection caused by infection with the adult or larval stage of the dog tapeworm *Echicoccous granulosus*, which belongs to the Taeniidae family and genus *Echinococcus Hydatids* have been known to humans for thousands of years with documentation of epidemic fever [1-6].

PCR and DNA sequencing have subsequently been used to construct a phylogenetic tree of the genus *Echinococcus*, with intraspecific variation in *E. granulosus* being identified.

Based on molecular characteristics, the species/strains of *E. granulosus* have been taxonomically revised as:

E. granulosus sensu stricto (G1–G3), *E. equinus* (G4), *E. ortleppi* (G5), *E. canadensis* (G6–G10) and *E. felidis*. *Echinococcus granulosus* has been divided into several strains according to the host.

Echinococcus granulosus has been researched extensively and discussed in detail as it is the species that is most widely distributed throughout the world, it has six strains which have been identified as G1 genotype (common sheep strain), G2 genotype (Tasmanian sheep strain), G4 genotype (horse strain),

G3, G5 genotypes (cattle strain), G6 genotype (camel strain), G7 genotype pig strain [7,8].

Materials and Methods

Samples collection

The present study included collection of 60 samples of human (40 patient and 30 control) from of Babylon hospitals. From October 2019 till October 2020. The blood samples were drawn from all individual by using disposable syringe (5 ml).

Five mL of blood was obtained from each subject by vein puncture and slowly pushed into two tube (2.5 mL blood in EDTA- tube for genetic study and used in Polymerase Chain Reaction (PCR) and genetic sequencing to detect dominant parasite strains.

PCR technique

PCR technique was performed for in detection and genotyping of *Echinococcusgranulosus* hydatid cyst based on IL17 family gene in isolates. This technique was carried out according to method described by as following steps [9].

Genomic DNA extraction: Genomic DNA from hydatid cyst samples was extracted by using GSYAN DNA Extraction Kit Geneaid.

Genomic DNA examination: The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260/280 nm).

PCR product analysis: The PCR products of IL17 family genes were analyzed by agarose gel electrophoresis, PCR and gene sequencing techniques were applied for investigating of some isolate of *E. granulosus* collected from human. This procedure was carried out according to [10].

IL-17 A, B, RA, RB genes amplification for PCR analyses: PCR amplification of DNA was carried out in final reaction mixture volume of 25 µl and within 35 cycles.

Products of PCR amplification were electrophoresed by 1.5% agarose gel and then visualized under UV-trans illuminator after staining with ethidium bromide at 100v for 60 minutes.

PCR mixtures and PCR conditions of this assay was summarized in (Table 1).

PCR mixtures		PCR conditions		
Contents	Volume	Type of cycle	Condition	No. of cycles
Master mix	12.5 µl	Initialization	95°C for 5 min	1
Forward primer	1.5 µl	Denaturation	94°C for 1 min	35
Reverse primer	1.5 µl	Annealing	55°C for 1 min (rs2275913) 56°C for 1 min (rs375208) 58°C for 1 min (rs879576) 54°C for 1 min (rs1025689)	
Template DNA	3 µl	Extension	72°C for 1 min	
Nuclase-free water	6.5 µl	Final Extension	72°C for 10 min	1

Table 1. *Uniplex PCR mixtures and conditions for identification of IL17 SNPs.*

Sequencing of PCR products: All PCR products obtained above were cleaned and submitted for sequencing as follows. The PCR product was cleaned of amplification primer using the Gel/PCR DNA Fragments extraction kit (Geneaid, USA) as per manufacturer's instructions.

Purified DNA was sequenced at Macrogen Company (Korea) with the sequencing primers for each gene as outlined in Table 2-4. Sanger sequencing method was carried out on an applied biosystems 3730xl DNA Analyzer (Applied Biosystems, Foster city, CA, USA). Because of the DNA sequencing method consider the gold standard for SNPs detection in IL-17gene [11].

Bio informatics and statistical analysis: The raw sequence data was trimmed and aligned to the control sequences. The standard sequences for alignment were taken from GenBank sequences for IL17 genes at NCBI.

Multiple alignments were done by using Clustal W v2.0, of Geneious Prime Software V2021.1 (Biomatters, Inc., North America) to identify SNPs, Allele frequency and genotypes. All other bioinformatic and statistical analysis were done according to Xavier [12,13].

Results

Detection of gene polymorphism (SNPs) of IL-17 family

Genotyping characterization of (rs 2275913) by Sequencing: The results of genotyping for (40) patients, and (30) controls subjects for the amplicon of the PCR product as achieved by sequencing for four primers IL-17A and IL-17RA, IL17B and IL 17RB are presented in (table 1,2) respectively,

showing the three genotypes (GG, GA, AA) for IL-17A of the patients.

Results showed that for IL-17A (rs2275913) gene polymorphism frequency of the G allele carriers was significantly decreased in hydatid cyst patients than that in healthy controls (38 *vs*. 29%).as shown in (Table 2). These results suggested that G allele might play a protective role against hydatid cyst for IL-17A (rs 2275913).

SNP	Allele	Frequency	Controls	Patients	P Value	OR (95% CI)
rs2275913 IL17 A	G	68 (0.96)	29 (0.97)	38 (0.95)	0.733	1.526
	A	2 (0.04)	1 (0.03)	2 (0.05)		(0.132-17.663)
	P value	<0.0001*	<0.0001*	<0.0001*		
	Genotype					
	G/G	32 (0.91)	14 (0.93)	18 (0.9)	0.95	1
	G/A	3 (0.09)	1 (0.07)	2 (0.1)	---	1.09 (0.08-14.660
	A/A	0 (0)	0 (0)	0 (0)		----
	P value	<0.0001*	<0.0001*	<0.0001*		

Table 2. *rs2275913 SNP distribution frequencies in the screened population (Control and Patients)*: Represent a significant difference at p<0.05.*

According to our results showed that the IL-17A (allele A) was insignificantly difference associated with susceptibility to E.granulosus infection. Individuals with two A alleles (homozygous for the AA) were not significantly represented among the patients with E.granulosus 0(0%) p-value=0.0001,

as compared with healthy control subjects, 3(8%) and Individuals with two G alleles (homozygous for the GG) had

an increased risk of developing HC infection than other two genotypes.

In contrast, the individuals with two GA alleles (Heterozygous for two different allele) were obviously more presented among patient individuals, 0.1% when compared with control.

Genotyping characterization of (rs879576) by sequencing

The distribution of each allele and genotype is shown in Table 3. In the genotype and allele frequencies in rs879576polymorphism, there was not difference between hydatid disease patients and healthy controls,

which was shown in Table 3. In addition, all subjects carrying GG genotype have significantly higher risks of hydatid disease compared with GA genotype (P<0.05).

The results revealed that subjects with the G allele were more likely to get hydatid disease compared with those bearing the A allele (OR, 1.1161; 95% CI, p=0.801). Further analysis demonstrated the higher risk of GG genotype and G allele mainly existed in female P (0.05).

SNP	Allele	Frequency	Controls	Patients	P value	OR (95% CI)
rs879576 IL17RA	G	55 (0.79)	24 (0.8)	31 (0.78)	0.801	1.161 (0.363-3.713)
	A	15 (0.21)	6 (0.2)	9 (0.22)		
	P value	<0.0001*	0.001*	0.001*		
	Genotypes					
	G/G	20 (0.57)	9 (0.6)	11 (0.55)	0.81	1
	G/A	15 (0.43)	6 (0.4)	9 (0.45)		1.19
	A/A	0 (0)	0 (0)	0 (0)		0.30-4.73
	P value	0.398	0.439	0.655		

Table 3. *rs879576 SNP distribution frequencies in the screened population*: Represent a significant difference at p<0.05.*

Dominant and recessive model also demonstrated the similar results that G allele of rs2275913 and rs 879576 increase the risk of HC.

Genotyping characterization of (rs375208) by sequencing

Molecular genetic study of DNA sequences, genotype and gene sequences was used to discover the genetic patterns of granulocytes. These are E. granulosus strains, G1-G3 genotypes are included in the granulosus sensu stricto,

sequencing analysis of fragment of IL17B gene to determine genotyping of E.granulosus in hyper-endemic areas of Babylon province.

sThis work done by randomly choosing of samples. All three variants were significant difference at p<0.05.

SNP	Allele	Frequency	Controls	Patients	P	OR (95% CI)
rs375208	A	65 (0.93)	27(0.9)	38 (0.95)	0.421	0.474 (0.074-3.031)
	G	5 (0.07)	3 (0.1)	2 (0.05)		
	P value	<0.0001*	<0.0001*	<0.0001*		
	Genotypes					
	A/A	30 (0.86)	12 (0.8)	18 (0.9)	0.38	1 0.42 (0.06-3.02)
	A/G	5 (0.14)	3 (0.2)	2 (0.1)		
	GG	0 (0.000)	0 (0)	0 (0)		
	P value	<0.0001*	0.02*	<0.0001*		

Table 4. *rs375208 SNP distribution frequencies in the screened population (control and patients)*: Represent a significant difference at p<0.05.*

Genotyping characterization of (rs1025689) by Sequencing

In normal host immunological responses, the IL-17 cytokine family and its receptors play critical functions. Their abnormal

expression has been linked to a variety of human diseases, including inflammation and cancer.

The SNP (rs1025689) in the IL17RB gene showed significant difference between the hydatid disease patients group and the control group as show in (Table 5).

SNP	Allele	Frequency	Controls	Patients	P value	OR (95% CI)
rs1025689	C	44 (0.63)	21 (0.7)	23 (0.57)	0.284	1.725 (0.634-4.694)
	G	26 (0.37)	9 (0.3)	17 (0.42)		
	P value	0.031*	0.028*	0.343		
	Genotypes					
	C/C	9 (0.26)	6 (0.4)	3 (0.15)	0.12	1
	C/G	26 (0.74)	9 (0.6)	17 (0.85)		3.49
	G/G	0 (0)	0 (0)	0 (0)		(0.6917.76)
	P value	0.004*	0.439	0.002*		

Table 5. *rs1025689 SNP distribution frequencies in the screened population (Control and Patients)*: Represent a significant difference at p<0.05.*

The polymorphisms within the IL17RB C allele confer an increased risk of susceptibility to extensive forms of hydatid disease, especially with an early onset of disease. In this SNP, the C/C, GG and GG genotype frequencies are reported respectively to be 0.26, 0.74, and 0. The IL17 RB rs1025689 C allele effect was consistent in the three cohorts and the association improved after the meta-analysis,

showing statistically significant results (P=0.12, OR =1.00, 95% CI, under a fixed-effects meta-analysis) after Bonferroni correction.

Discussion

Human can be infected by all E. granulosus genotypes except G4 Thus, various animals can play a role in the epidemiology of human echinococ-cosis/hydatidosis and design an appropriate strategy to control the infection requires a good understanding of transmission cycle in each region. However, Polymorphisms in IL-17 cytokines alter the activity of interleukins and may alter cytokine function, thus, dysregulating IL-17 expression.

The distribution of each allele and genotype is shown in (Table 3). In the genotype and allele frequencies in rs879576 polymorphism, there was not difference between hydatid disease patients and healthy controls. Uncontrolled inflammation has been widely accepted as a hallmarker of hydatid disease, however, the regulation of genes within the inflammatory pathways is not well understood yet, any changes in structure and expression, may be due to genetic variations, might affect cytokine production and hydatid disease development. A common type of genetic variation in the genome is single nucleotide polymorphism (SNP). IL-17 have many SNPs, among which rs879576(A/G), rs 1025689(C/G) rs2275913 (G>A) and rs375208 (G/A) loci are located in 6p12.1 chromosome and have been shown to correlate with aggressive disease and poorer survival in recent series with inflammatory disease [14,15].

Recently, molecular identification of *E. granulosus* strains has emerged as the most important method for obtaining accurate information about taxonomic units in order to study the epidemiology of common animal diseases and, as a result, develop appropriate control and control programs to prevent transmission and completion of their life cycle. In Iraq, molecular investigations are being conducted to determine the types and strains of *E. granulosus* are few [16].

The identified *Echinococcus granulosus* genotypes isolates were submitted into of NCBI-GenBank, The genotype distribution and relative allele frequencies of rs375208 (A/A), (G/G), and (G/G) polymorphisms at the IL17 B gene in the study subjects are showed in (4). The action and expression of IL-17RB on the sex has not been evaluated yet but in CG was evaluated between them (0.71, 0.29 *vs.* 0.44,0.56) respectively as show (Table 5). Therefore, expression of IL-17RB in the sex and its role in pathophysiology of hydatid disease should be investigated in the future [17].

IL-17RB is found in endocrine tissues and epithelial cells throughout the body, including the kidney, liver, and mucosal tissues. Elevated IL-17RB expression is also found lung tissues from asthmatic patients and in skin lesions from patients with atopic dermatitis. IL-17RB expression in human innate type 2 lymphocytes, Natural Killer T (NKT) cells, and Th2 cells suggests a potential role in immune cells. In these human cells IL-17B promotes IL-33-driven type 2 immune responses, a function shared with IL-17E, but not withIL-17A [18-20].

Conclusion

This research as the genetic characterization of *E. granulosus* in the Babylon province showed that il17 family gene is the common species/genotypes of *E. granulosus* in human. Thus further genetic studies are needed to exactly determine the genotypes of *E. granulosus* in the region. Eventually, this information may be considered when implementing hydatidosis control programs, be-cause antigenic variation and

differences in other biological characteristics of *Echinococcosis* species were reported.

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