



The Effect of *Echinococcus granulosus* on TP53 and CTNNB1 genes Sequence Alteration among hepatocellular Carcinoma Patients

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Abstract

Echinococcus granulosus is one of the small tap worms that causes Hydatid cyst. This study was planned for detection of the incidence among cancer patients at Tumor unit in Baghdad medical city. Eleven samples of liver biopsy out of 40 specimens taken from patients with liver cancer in this study. All patients were suffering from Hydatid cyst infection Patients of migratory encampment in and around Baghdad city Kirkuk, Diyala, and Tikrit Hospitals, suffering from hydatidosis. The present study showed that the predominance of hydatidosis was in the patients whose ages ranged between ($\leq 20-60$) years. The sensitivity and specificity of ELISA with hydatid cyst IgG were 96%. While Casoin test (immediate hyper sensitivity skin test) was 100%. The majority incidence was recorded between (50-60) years. The study showed that the predominance of hydatidosis was higher in females 28(70%), than males 12 (30%). Analysis of the gene sequence showed that there were genetic mutations occurred in the hepatic cells, and several locations underwent change in genetic sequence including TCC to TCT in exon 147(5), GGT to AGT in exon 152(5), ACT to CCT in exon 160(5), TAT to TGT in exon 203(6), ATG to CTG in exon 235(7).

Keywords: TP53 gene, Mutation, Tumor, Hydatid cyst, Gene sequencing.

Introduction

Hydatid cyst caused by *Echinococcus granulosus*, is an important zoonotic infection causing high morbidity and mortality in human [1]. A great many intraspecific variants or strains of *Echinococcus granulosus* have been described from different intermediate host species or geographical areas [2]. *Echinococcus granulosus* is known to exist as a biologically distinct sub specific variants or strains which may vary in their infectivity to man [3].

There is clear evidence that within *Echinococcus* species, genetic heterogeneity is commonly found, and that these intraspecific variations perhaps have important roles in regard epidemiology. A strain of *Echinococcus* is a group of individuals which differs statistically from other groups of the same species in gene frequencies [4]. Although morphological and biological studies have provided extremely useful information for strain identification, it has to be considered that despite biological and

morphological studies presented very important information on strain detection we have to observe the variability of these features [5]. They might be affected by environmental and host factors and might not show distinct genetic feature [6]. The parasite that has effect on the tumor suppressor protein which is encoded by the TP53 gene contribute to the multiple cellular pathways such as DNA repair, angiogenesis cell cycle control, cellular senescence, cell differentiation, apoptosis induction, and cell proliferation control [7].

In spite of the dependence of frequency on the Tumor type, site and stage TP53 gene mutation is mostly sporadic and happens in about 50% of all human malignancies [8]. Mutations of TP53 germ line lead to Li-Fraumeni syndrome (LFS), which rare predisposition syndrome of cancer related dramatically high prevalence and early onset and different primary neoplastic disease e.g. breast cancer, brain cancer (including MB),

soft tissue and bone carcinoma as well as adrenocortical carcinoma [9]. By using PCR amplification HCCs were analyzed for point mutations in exons 5-8, using PCR amplification, direct DNA sequencing and single-strand conformation polymorphism, all these HCCs have been tested for exon 5-8 point mutation [7]. Mutations were found in 11 HCC patients. The study aimed to detection of the Hydatid cysts in infected patients, and to determine of the mutations occurred in *TP53* and *CTNNB1* genes in liver cancer patients.

Materials and Methods

This study was carried out in Central Health Laboratory/Baghdad from 2st January 2018 to 30th 1st September 2018. Every sample was examined by light microscope to confirm the fertility of the cysts that mean protoscoleces. To detect specific IgG antibodies in serum of echinococcosis patients with EcAg by using ELISA technique. The immediate hyper sensitivity skin test (Casoni test) was done. Fatherly they were measured the size of cysts were measured as a diameters. DNA extraction from hepatic cells was a primary and crucial step which has a principle effect on PCR results.

In the current study 11 simple methods for DNA extraction from hepatocytes cells were used DNA extraction of liver biopsy samples were localized by histological examination of tissue sections stained with H&E. Paraffin sections were dewaxed and rehydrated before further processing. Tumor areas from several (frozen or paraffin) sections were micro dissected with a scalpel blade, pooled, and soaked overnight at 4°C in 200 µl of buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, and 1% (w/v) SDS. DNA extraction was performed essentially as described by Shafritz *et al.*; [11] PCR.

The analyses of p53 gene mutations in liver tumor induced in human cancers the majority of p53 point mutations are localized within these evolutionarily conserved regions that code for the DNA-binding domain of the protein. The shifted SSCP fragments were collected and submitted to a standard PCR followed by an asymmetrical PCR, to amplify either the sense or the antisense strand. For both the asymmetrical PCR and DNA sequencing, the same primers were used as for amplification of genomic DNA.

The primer used was 5'-ATGGAACCAGACAGAAAAGC-3'; 439 F: 5'-GCTACTTGTTCTTGAGTGAAG-3'. DNA isolation. Tumor areas in the liver samples were localized by histological examination of tissue sections stained with H&E. Several consecutive microtome unstained sections were then prepared. Paraffin sections were dewaxed and rehydrated before further processing. Tumor areas from several (frozen or paraffin) sections were micro dissected with a scalpel blade, pooled, and soaked overnight at 4°C in 200 µl of buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, and 1% (w/v) SDS. DNA extraction

Statistical Analysis

Statistical analysis was computer assisted using Chi square [10].

Results

In Table 1, the results showed that in the distribution of Hydatid cyst among age groups, that mean (32.03 ± 17.44) showed no significant difference P=0.272, and a highly significant difference was between genders P=0.000, While a significant differences was seen between rural and urban P=0.000.

Table 1: Distribution of Socio-Demographical Characteristics in Patients with Hydatid Cyst

Variables	Groups	No.	%	C.S.P-value
Age Groups Per yrs.	20 -	4	10	χ ² = 6.364 P=0.272 NS
	30 -	8	30	
	40 -	12	20	
	50 - 60	16	30	
Mean ± SD		32.03 ± 17.44		
Gender	Female	28	70%	P=0.000 HS
	Male	12	30%	
Residence	Rural	32		Urban P=0.000 HS
			8	

Immunity		Positive	Negative	
IgG		38	2	P<0.01
		95%	4%	
Casoni test		40	0.0	P<0.01
		100%	0.0	

⊙ HS: Highly Sig. at P<0.01; NS: Non Sig. at P>0.05
 The distribution of hydatid cyst according to their size

The majority of cysts 20 (50%) had (>10cm) (15%) had (3-5cm) size. While 8cysts (20%) size, 6cyst (15%) had (6-8cm) size, 6cysts were (9-10cm) size.

Table 2: Distribution of hydatid cyst according to their size

Variable	Responding	No.	%	C.S. (⊙) P-value
Single HC	3 - 5 cm.	6	15	χ ² = 19.303 P=0.000 HS
	6 - 8 cm.	6	15	
	9 - 10 cm.	8	20	
	> 10 cm.	20	50	
	Total	40	100	

⊙ HS: Highly Sig. at P<0.01

Detection the Mutation of Liver Tumor with Hydatid Infection

Tumor induction ASL and HCCs were obtained from human complaining from

Hydatid cyst (. The 11 samples were from the following carcinogenicity bioassays. BT1, BT2, to B11 tissue samples had been either frozen in liquid nitrogen and stored at 80Â°C or fixed in alcohol and embedded in paraffin.

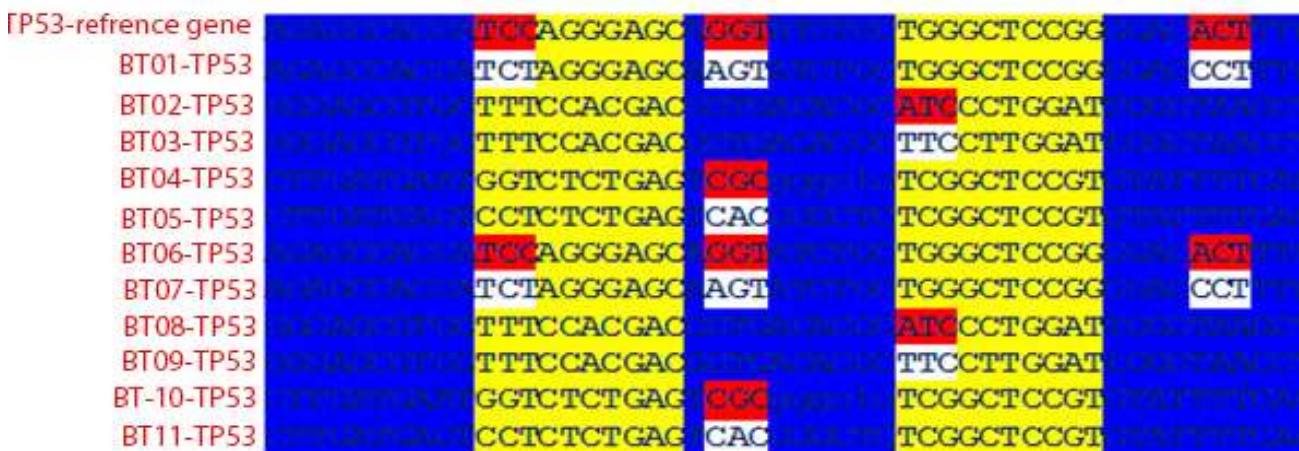


Figure 1: The sequence of TP53 gene, Nucleotides changes shown in TP53 gene Exon 147,152,160.203, and 235 amplified from tumor lever cancer samples. The mutation occurred on nucleotides TCC, GGT, ACT, CGC and ATC of TP53 gene

Table 2: Tumor obtained from patients with Hydatid cysts infection, 6 out of 11 samples was found mutation in numbers of exons in different position of TP53 gene

Sample	DNA sequence change	Exon
1	TCC to TCT	147
	GGT to AGT	152
	AGT to CCT	235
3	ATC to TCC	203
5	CGC to CAC	160
7	TCC to TCT	147
	GGT to AGT	152
	AGT to CCT	235
9	ATC to TCC	203
11	CGC to CAC	160

Discussion

Echinococcus granulosus is one of risky parasites which may lead to carcinoma and this finding agreed with Brawly O. (2016) who found that the most interesting liver cysts have personally seen were a series of hydatid cysts caused by *Echinococcus* [12].

The liver is the first portal to infiltrate its infection with Hydatid cyst which may expose it to cancer, and this report applies to that deduced by Zhang, R. *et al*, (2017) who found that the ideal experimental model should orthotopically induce hydatid disease in the most affected organ, *i.e.*, the liver [13], the model should resemble the natural infection route and course with a stable and predictable growth pattern [13].

The diagnoses of 11 cancer cases of patients with this parasite, demonstrates co-infection with cancer disease. The study found that most of the 11 patients are female 7(70%) than male 3(30%). This finding agreed with Brunttei, E. who reported that the estrogen hormone plays an important role in dissolving egg shells and facilitating hatched encosphere of the parasite to penetrate host females tissues [14].

The size of the cyst was >10 cm. in 50% of Hydatid cyst located inside hepatic with a highly significant difference $P=0.000$. The reached study agreed with Al- shamary 2010 who found that the size of the cyst reached to >10 cm in liver tissue because the liver cells have the ability to inflation and

accommodate the large size of the cyst [15]. The genetic mutations obtained at several sites on Tp53 gene are indicative of liver cancer, and the change in occurred the nucleotides sequence in the top of the TP53 exons: TCC to TCT in exon 147(5), GGT to AGT in exon 152(5), ACT to CCT in exon 160(5), ATG to ATG in exon 235(7), TAT to TGT in exon 203(6), ATC to TTC in exon 253(7), CGC to CAC in exon 246(7) and GAG to GTG in exon 283 (8).

This is a clear evidence that there is a cancerous tumor spread and because of this change cell and became an abnormal mass Rivlin, N. 2011 [16]. This study is the first of its kind in Iraq and possibly in the world which explained that it is possible for Hydatid cyst to cause the cancer.

The study has shown that there is a genetic change in TP53 gene in exons5-7 and the occurrence of carcinogenesis in hepatocytes due to B virus and C virus, Lina, M.2013 [17]. This means that hepatic cells caused by many pathogens, viruses and bacteria, as well as parasites that infect those organs [16].

Here, the parasite played an important role in co-occurring with the observed mutations in the gene sequence in those oxons. One other hand, there was no change in the CTNBB1 gene pathway and this finding disagreed with Friemel, J. *et al*; 2016 who found that genetic mutations have occurred in TP53 gene but not occurred in CTNBB1 gene [18].

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