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Prevalence of Hydatid Diseases between Sheep and Human in Diyala Province

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Abstract

In this study, the results of demographical picture showed no significant differences between ages among hydatid cyst patients (P = 0.06), while the distribution of hydatid cyst according to residency showed that the distribution rate was 24 (80.0%) in the rural area when compared with its distribution rate in the urban area 6 (20.0%), with a significant difference (P = 0.007). Also, the distribution rate of fertile human hydatid cyst was 18 (60.0%) in comparison with the sterile human hydatid cyst 12 (40.0%), with no significant difference, and this distribution rate was close to the fertile sheep hydatid cyst 16 (53.3%) compared to the distribution rate of the sterile sheep hydatid cyst 14 (46.7%), with no significant difference too. The mean anti Human-IgM antibodies level was (2.31 ± 1.38) in hydatid cyst patients compared to the control group (0.09 ± 0.12), with a highly significant difference (P ≤ 0.0001). The mean anti Human-IgG antibodies level was (14.47 ± 7.15) in hydatid cyst patients compared to the control group (0.05 ± 0.09), with a highly significant difference (P ≤ 0.0001). Also, the mean Human-IL-32 level was (26.42 ± 10.07) in hydatid cyst patients compared to the control group (1.26 ± 0.12), with a highly significant difference (P ≤ 0.0001), while the mean Human-TGF β level was (18.16 ± 8.52) in hydatid cyst patients compared to the control group (5.1273 ± 1.49), with a highly significant difference (P ≤ 0.0001). The mean anti-Sheep-IgG antibodies level was 18(2.10 ± 1.20) in fertile hydatid cysts compared to its mean in the sterile hydatid cyst 12(1.88 ± 0.86), with no significant difference (P = 0.57). The mean anti Human-IgM antibodies level was 18(14.37 ± 6.24) in the fertile hydatid cyst compared to its mean in the sterile hydatid cyst 12(2.62 ± 8.64), with no significant difference (P = 0.32). The mean anti- Human-IgG antibodies level was 18(2.11 ± 1.41) in the fertile hydatid cysts compared to its mean in the sterile hydatid cyst 12(14.64 ± 8.64), with no significant difference (P = 0.92). A mutation occurred with IL-32 gene ID 9235 in SNPs, rs12922880. The variation of wild CC To T > C was changed to TT, TT, TT, TC, TC, TT, TT, TC, TT, TC in comparison with the control group. Also in SNPs, AA to A > C was changed to AC, CC. AC, CC, CC, CC, AC, AC, CC, CC in comparison with the control group.

Keywords: Hydatid diseases, Sheep, Human, Diyala province

1. Introduction

Hydatid cyst is a parasitic and zoonotic disease which is widely distributed in the world. In animal model studies and cell culture investigations, anticancer impacts of hydatid cyst have been demonstrated (Daneshpour *et al.*, 2019). The anti-cancer impact mechanism of the hydatid cyst fluids has not been clearly explained, and there may be a role of apoptosis induction in this respect (Motavallihaghi *et al.*, 2023). Thus, it can be transmitted by hand to mouth following handling of dogs or things contaminated with the eggs, or due to consumption of contaminated water or food (University of Gondar, 2020). The larval stage of the tapeworm can be lodged in different parts of the body where they produce a sac filled with fluid called the hydatid cyst. Within a period of time, these cysts hold immature tapeworm forms and their sizes begin to increase from 5 to 10 cm or more (University of Gondar, 2020). Some of these cysts may die, but others may continue to survive for several years. The cysts also contain (daughter cysts) which, if liberated, can be spread to other body parts (Khuroo's, 2021). Hydatid diseases occur globally and common found in grazing areas. Since 2008, observation of human hydatid disease has not been required in Queensland. However, there were between 4–13 observations yearly from 2000 to 2008, (Al Malki &

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Ahmed, 2022). It can take months to several years from egg ingestion to symptom development of the hydatid disease. For detection of the hydatid cyst existence in the body, ultrasound or CAT scans can be used. Blood examinations for investigating the human's immune response to the disease can be beneficial, but are not usually positive even in case of the presence of the infection (Carnero *et al.*, 2017). Samples are occasionally taken from cysts to insure the existence of the tape worms. Hydatid cysts are most frequently detected in the livers and lungs, however, they may also be found in other organs, muscles and bones (Gessesecor, 2020). The cysts can live for tens of years, and nonspecific signs involve anorexia, weakness and loss of weight (University of Gondar, 2020). Other symptoms and signs are governed by the hydatid cyst location and the pressures exerted on the adjacent tissue; which may involve abdominal pains, vomiting and dyspnea. Serious allergic reactions and even deaths can occur when the cysts rupture or leak (Hanalioglu *et al.*, 2022). Treatments are usually complicated and may vary depending cyst's location and. There is usually a need to remove the cyst surgically. In certain conditions, the drug which kills the tapeworm is also used and injection of such drug into the cyst may be required. In some cases, treatment is not required, but the patients will need monitoring for long periods of time (Sharma & Gupta, 2021; Sutrave & Richter, 2023). Dingoes, domestic dogs, and foxes are able to carry several tiny adult tape worms in their bowels without any infection signs. The eggs of tapeworms can pass in the dog's faces and can live in soils, fields and gardens for many months (Sutrave & Richter, 2023). Animals like goats, sheep, cattle, pigs, kangaroos, camels, horses, wallabies and wombats are infected via ingestion of grasses contaminated with eggs in dog faces. Hydatid cysts are formed in the organs and tissues of those animals, and their cycle is ended when dogs become infected through ingestion of cysts in the undercooked meat and offals of an infected animal (Abdulhameed *et al.*, 2018). About 5–7 weeks after being infected, the dogs will begin passing eggs. Even if the dogs are not treated, the infection will recover within (6–12) months; however, dogs may be re-infected when they consume contaminated meats. When humans swallow tape worm eggs, they will become infected (Riahi *et al.*, 2020). This can happen by touching the dog then touching the mouth, by dog kissing, via contacting contaminated soils or by ingestion water or foods contaminated with dog faces that contain the worm eggs (Riahi *et al.*, 2020). In such interactive responses, there will be a significant role of the transfo rming growth factor β (TGF-β). Nevertheless, nowadays, studies on hydatid cyst disease mainly focus on adults, with few studies on its incidence among children (Qin *et al.*, 2022). It is uncertain whether hydatidosis in children involves the same molecular regulation mechanisms. The pro-inflammatory cytokine Interleukin-32 (IL-32), stimulates other cytokines intricated in inflammations. Recently, it has been proposed that IL-32 plays an essential role in host defense against pathogenic organisms, in addition to the chronic inflammation pathogenesis [13]. Our study aimed to determie the distribution of Hydatid diseases between Sheep and Human and its effect on both TGF-β and IL-32.

2. Materials and method

In the present study, (30) venous blood samples were taken from sheep expected to it be infected with Hydatidosis. Samples were taken from the auricular vein of these sheep before slaughter. In the second step, the sheep were slaughtered and parts of their intestines affected by Hydatid cysts, the livers and lungs, were taken. The blood samples were centrifuged, and the serum was taken until it was used for examination and stored at −20°C. The organs (Liver and Lungs) were excised and the fluid located inside the cysts was withdrawn, then stored with preservatives until use. The samples were taken to the typical veterinary clinic in Baqubah. Five ml from hepatic hydatid cyst fluid of humans as well as other cysts (ranged from 8–14 cm), from affected sheep organs, the liver and lung, were drawn by the surgeon in the operative room, separated by centrifugation at 3000 rpm for 3 min, and were measured by colorimetric assay and by direct microscope exam X40 for detection of fertility. Five ml of venous blood were collected from 20 patients suffering from echinococcosis, and other 20 samples from healthy humans as a control group, and 20 serum samples after one year from cyst removal, separated by centrifugation at 5000 rpm for 5 min. then 5 ml of blood were taken from the ear vein of the sheep for the purpose of serological tests. All the samples were taken in Alkalus general Hospital during the period from 1st July 2023 to 1st February 2024. The *Echinococcus granulosus* IgM and IgG were measured in biological samples using the ELISA Kit. The levels of tumor growth factor was measured using the double Antibody Sandwich ELISA technique. ELISA kit was used to measure the IL-32 using the quantitative sandwich enzyme immunoassay technique. Molecular diagnosis was performed by PCR technique. The primer of IL-32 used was rs7044343-F: TGTAAAAC GACGGCCAGTTGTCTCACCAGAGGGATTT, rs7044343-R: CAGGAAACAGCTATGACCATCAA

Table 1. Demographical picture of studied groups (n = 45).

Parameters		Case	Control	Total	P-value
Age range (Years)	(17–31)	10 (33.3%)	2 (13.3%)	12 (26.7%)	0.06
	(32–46)	14 (46.7%)	5 (33.3%)	19 (42.2%)	
	(47–61)	6 (20.0%)	8 (53.3%)	14 (31.1%)	
	Total	30 (100.0%)	15 (100.0%)	45 (100.0%)	
Residency	Rural	24 (80.0%)	6 (40.0%)	30 (66.7%)	0.007
	Urban	6 (20.0%)	9 (60.0%)	15 (33.3%)	
	Total	30 (100.0%)	15 (100.0%)	45 (100.0%)	
Human haydatide cyst types	Frertile	18 (60.0%)	–	18 (60.0%)	–
	Sterile	12 (40.0%)	–	12 (40.0%)	
	Total	30 (100.0%)	–	30 (100.0%)	
Sheep haydatide cyst types	Frertile	16 (53.3%)	–	16 (53.3%)	–
	Sterile	14 (46.7%)	–	14 (46.7%)	
	Total	30 (100.0%)	–	30 (100.0%)	

CACCGTCACCTTAC. Gene sequence was done by signer sequencer.

Statistical analysis: For data analysis, the SPSS-20 software program (Mean ± S.D) was used including the t-test. The ($P < 0.05$) value was considered as significant.

3. Results

The results of demographical picture in Table 1 showed no significant differences between ages among hydatid cyst patients ($P = 0.06$), while the distribution of hydatid cyst according to residency showed that the distribution rate was 24 (80.0%) in the rural area when compared with its distribution rate in the urban area 6 (20.0%), with a significant difference ($P = 0.007$). Also, the distribution rate of fertile human hydatid cyst was 18 (60.0%) in comparison with the sterile human hydatid cyst 12 (40.0%), with no significant difference, and this distribution rate was close to the fertile sheep hydatid cyst 16 (53.3%) compared to the distribution rate of the sterile sheep hydatid cyst 14 (46.7%), with no significant difference too.

The mean anti Human-IgM antibodies level was (2.31±1.38) in hydatid cyst patients compared to the control group (0.09 ± 0.12), with a highly significant difference ($P \leq 0.0001$). The mean anti Human-IgG antibodies level was (14.47 ± 7.15) in hydatid cyst patients compared to the control group (0.05 ± 0.09), with a highly significant difference ($P \leq 0.0001$). Also, the mean Human-IL-32 level was (26.42 ± 10.07) in hydatid cyst patients compared to the control group (1.26 ± 0.12), with a highly significant difference ($P \leq 0.0001$), while the mean Human-TGF β level was (18.16 ± 8.52) in hydatid cyst patients compared to the control group (5.1273 ± 1.49), with a highly significant difference ($P \leq 0.0001$), as shown in Table 2.

The mean anti- Sheep-IgG antibodies level was 18(2.10 ± 1.20) in fertile hydatid cysts compared to its mean in the sterile hydatid cyst 12(1.88 ± 0.86), with no significant difference ($P = 0.57$). The mean anti Human-IgM antibodies level was 18(14.37 ± 6.24) in the fertile hydatid cyst compared to its mean in the sterile hydatid cyst 12(2.62 ± 8.64), with no significant difference ($P = 0.32$). The mean anti- Human-IgG antibodies level was 18(2.11 ± 1.41) in the fertile hydatid cysts compared to its mean in the sterile hydatid cyst 12(14.64 ± 8.64), with no significant difference ($P = 0.92$), as shown in Table 3.

Table 2. The mean levels of Human-IgM, IgG and Human-IL32, Human-TGF in patients in comparison with control group.

Parameter	Group	Mean	SD	T-test	P-values
Human-IgM	Case	2.31	1.38	8.77	≤0.0001
	Control	0.09	0.12		
Human-IgG	Case	14.47	7.15	11.02	≤0.0001
	Control	0.05	0.09		
Human-IL32	Case	26.42	10.07	13	≤0.0001
	Control	1.26	2.33		
Human-TGF-β	Case	18.16	8.52	8.13	≤0.0001
	Control	5.1273	1.49		

The mean of anti Sheep-IgG antibodies levels in firtile hydatid cysts was 16(2.24 ± 1.27) compared to the sterile hydatid cyst 14(1.76 ± 0.75), with no significant differnce ($P = 0.21$). The mean of Human-IL32 levels in firtile hydatid cysts was 16(26.04 ± 12.17) compared to the sterile hydatid cysts 14(26.87 ± 7.40), with no significant differnce ($P = 0.82$). The mean of Human-TGF β levels in firtile hydatid cysts was 16(16.04 ± 7.44) compared to the sterile hydatid cysts 14(20.59 ± 8.64), with no significant differnce ($P = 0.14$), as shown in Table 4.

The results of this study demonstrated that there was weak negative correlations ($r = -0.101, -.227$) between the human haydatid cyst and sheep haydatid cyst types with the levels of sheep-IgG respectively (−.101,−.227), and these correlations were non-significant ($P = 0.595,0.228$) respectively. The results of this study found that there were a weak positive correlation ($r = .183$) between the levels of

Table 3. The mean levels of Sheep-IgG and Human-IgM, IgG with types of haydatide cysts.

Parameters	Haydat cyst type	N	Mean	SD	T-test	P-value
Sheep-IgG	Fertile	18	2.10	1.20	0.57	0.57
	Sterile	12	1.88	0.86		
Human-IgM	Fertile	18	2.11	1.41	0.99	0.32
	Sterile	12	2.62	1.33		
Human-IgG	Fertile	18	14.37	6.24	0.09	0.92
	Sterile	12	14.64	8.64		

Table 4. The mean levels of Sheep-IgG and Human-IL32, TGF-β cyytokens with types of haydatid cysts.

Parameteres	Sheep haydatid cyst type	N	Mean	SD	T-test	P-value
Sheep-IgG	Fertile	16	2.24	1.27	1.27	0.21
	Sterile	14	1.76	0.75		
Human-IL32	Fertile	16	26.04	12.17	0.22	0.82
	Sterile	14	26.87	7.40		
Human-TGF-β	Fertile	16	16.04	9.05	1.51	0.14
	Sterile	14	20.59	7.44		

Table 5. Correlation analysis of studied parameters with types of hydatid cysts.

	Human haydaidet cyst		Sheep haydatide cyst	
Parameters	r	p	r	p
Sheep-IgG	−.101	.595	−.227	.228
Human-IgM	.183	.333	−.245	.192
Human-IgG	.019	.922	.154	.417
Human-IL32	.098	.605	.042	.826
Human-TGF-β	.179	.343	.271	.147

Human-IgM with Human haydaid cyst with (P = 0.333), while there was a weak negative correlation (r = −.245) with sheep haydatid cyst with (P = 0.192). The results of the current study showed that there was a weak positive correlation between the levels of Human-IgG, Human-IL32 and Human-TGF-β with both type of haydatid cysts (human and sheep) with (r = .019, .098, 179), (.154, .042, .271) respectively, and these correlation were statistically non-significant for three mentioned above tests (P-value ≥ 0.05).

Table 6 and Fig. 1 showed that a mutation occurred with IL-32 gene ID 9235 in SNPs, rs12922880. The variation of wild CC To T > C was changed to TT, TT, TT TC, TC, TT, TT, TC, TT, TC in comparison with the control group. Also in SNPs, rs2283468. AA to A > C was changed to AC, CC. AC, CC, CC, CC, AC, AC, CC, CC in comparison with the control group.

Table 6. Variation of wild SNPs of IL-32 gene ID 9235.

IL32 GENE ID 9235		
SNPs	rs12922880	rs2283468
Wild	CC	AA
Variation	T > C	A > C
Samples		
1	TT	AC
2	TT	CC
3	TT	AC
4	TC	CC
5	TC	CC
6	TT	CC
7	TT	AC
8	TC	AC
9	TT	CC
10	TT	CC
C1	TC	AA
C2	CC	AA
C3	CC	AC
C4	TC	AA
C5	CC	AA

4. Discussion

The results of hydatid disease distribution based on residential location, our results were in agreement with (Baruah *et al.*, 2020) who reported that 80% of those infected with Hydatid are residents of rural areas due to the abundance of animals and dogs. The mean of anti Human-IgM and IgG antibodies levels of hydatid diseases was raised compared to control group with highly significant differences P ≤ 0.0001. AL-Masoudi *et al.* (2021) reported that there is a very high increase in the levels of IgM and IgG antibodies in the sera of hydatid patients with acute and chronic phases in Babylon Province. Also the mean of Human-IL-32 levels was raised with Hydatid diseases compared to the control group with a highly significant difference (P ≤ 0.0001), and these results matched with (Rahdar *et al.*, 2018) who reported that there is an effect on the levels of interleukin-32 in the sera of people with hydatid disease, and there is a very noticeable increase because this interleukin is a pro-inflammatory cytokine and it is affected by parasitic worms. The mean of anti Sheep-IgG antibodies levels with fertile hydatid cyst was 16(2.24 ± 1.27) compared to the sterile hydatid cyst 14(1.76 ± 0.75), with no significant difference (P = 0.21).

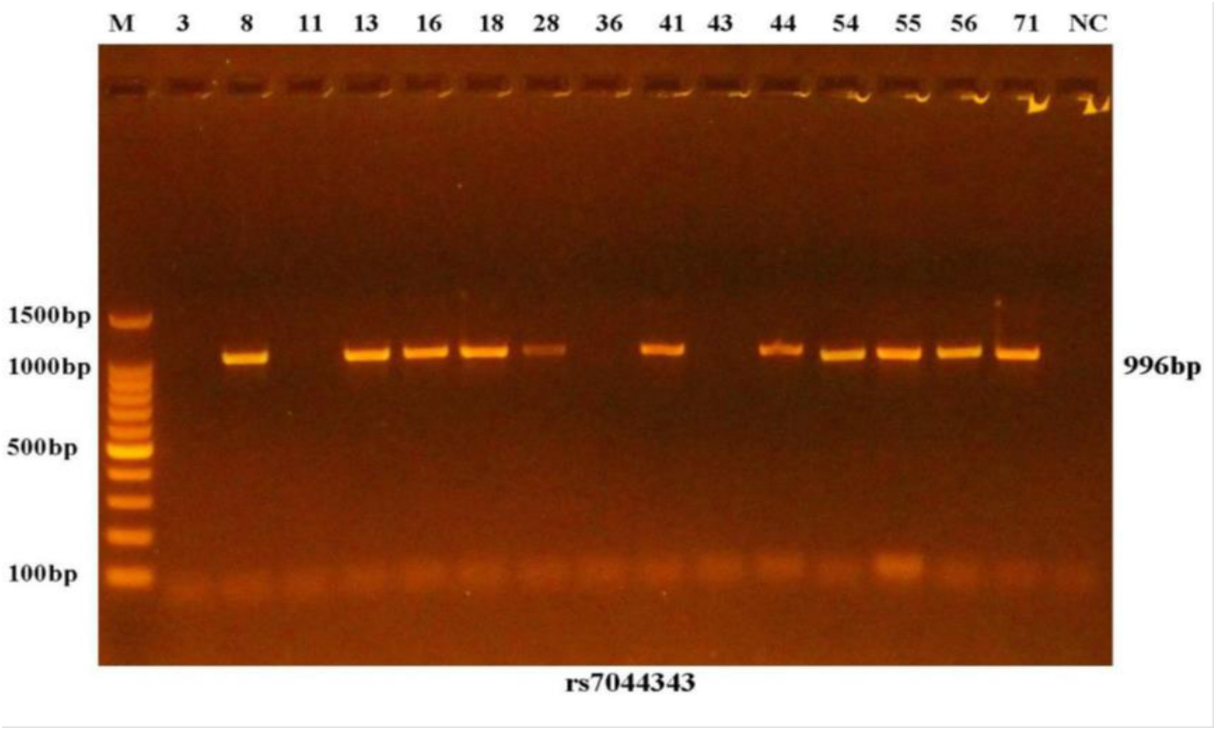

Fig. 1. Results of amplifications of rs7044343 specific regions of Human IL32 were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder markers. Lanes (8, 13, 16, 18, 28, 41, 44, 54, 55, 56 and 71) resembling 996bp PCR product.

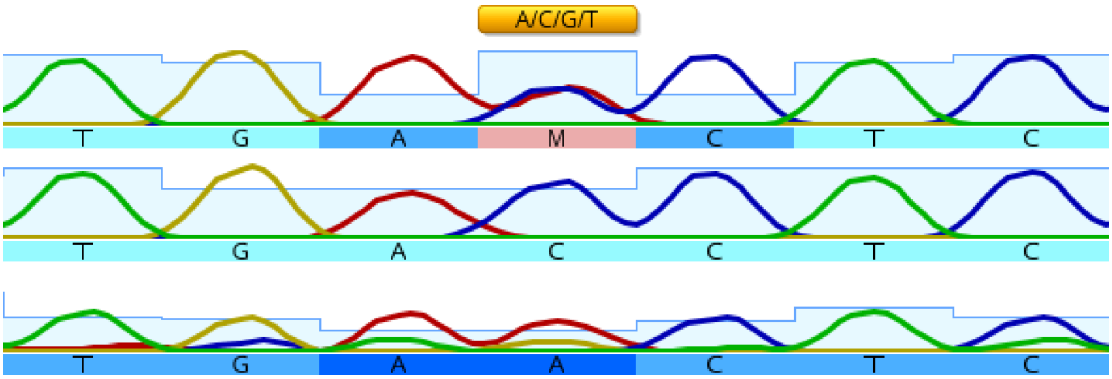

Fig. 2. Analysis of rs2283468 SNP of IL32 gene using Sanger sequencings. Single "C" peak indicates a C homozygous alleles. Single "A" peak indicates a A homozygous alleles. Presence of "C" and "A" peak indicates C/A heterozygous alleles.

Al-Nuaimi & Al-Hassani (2023) stated that there were no significant differences in distribution of fertile and sterile Hydatid cyst fluids, and the distribution is almost equal and there is no noticeable difference found between infections in Babylon Province (Al-Nuaimi & Al-Hassani, 2023). With Hydatid diseases, a mutation occurred with Human IL-32 gene ID 9235 in SNPs, rs12922880. The variation of wild CC To T > C was changed to TT, TT, TT TC, TC, TT, TT, TC, TT, TC in comparison with the control group. Also, in SNPs, AA to A > C was changed to AC, CC. AC, CC, CC, CC, AC, AC, CC, CC in comparison with the control group. For the first time in Iraq, we targeted a gene for interleukin-32 to see the occurrence of repeated genetic mutations on many sites of the spike. This proves a direct effect on this interleukin. It has been found that there is research on other parasites that targeted interleukin-32, with cutaneous leishmaniasis (Santos *et al.*, 2020). These results disagreed with (Davide De Biase *et al.*, 2023) who proved the immuno-histochemical analysis was done by the use of primary antibodies anti-TGF-β, anti-Iba1, anti-CD3, anti-CD20 and anti-MMP9. Lastly, the real time PCR was carried out to estimate the concentrations of IL-10, IL-12, TNF-α, INF-γ and TGF-β. The immuno-histochemical examination revealed a

diffuse immune-labeling of mononuclear cells of Iba-1 & TGF-β and a higher amount of CD20+ B cells in comparison with CD3+ T cells in both B and C groups. No significant statistical differences were shown in the levels of expression for Th-1-like immune cytokines TNF-α, INF-γ and IL-12 (Davide De Biase *et al.*, 2023; Siracusano, 2024).

5. Conclusions

With Hydatid diseases, a mutation occurred with Human IL-32 gene ID 9235 in SNPs, rs12922880. The variation of wild CC To T > C was changed to TT, TT, TT TC, TC, TT, TT, TC, TT, TC in comparison with the control group. Also, in SNPs, rs2283468. AA to A > C was changed to AC, CC. AC, CC, CC, CC, AC, AC, CC, CC.

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