

Association of Serum TIMP-1 Levels and Gene Polymorphisms in Patients with Hydatid Disease

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ABSTRACT

Cystic echinococcosis (CE), caused by the larval forms of *Echinococcus granulosus*, is a significant global health issue and a primary parasitic disease affecting agricultural animals. This study investigates the serum levels of tissue inhibitor of metalloproteinases-1 (TIMP-1) and the association of specific single nucleotide polymorphisms (SNPs), rs1043428 and rs41454248, with hydatid disease. TIMP-1, an extracellular matrix protein and zinc-dependent endopeptidase inhibitor, plays a role in regulating matrix metalloproteinases (MMPs), connective tissue turnover, and vascular wall remodeling. The study included 62 patients diagnosed with hydatid disease (18 males and 44 females) and 75 healthy controls (29 males and 46 females). TIMP-1 levels and the two SNPs were analyzed and compared between the groups. Results showed no significant difference in age between patients and controls ($p > 0.05$). TIMP-1 concentrations were significantly elevated in patients with hydatid disease (717.08 ± 31.61 pg/ml) compared to the control group (518.05 ± 32.56 pg/ml, $p < 0.05$). Regarding the rs41454248 SNP, the GG genotype and G allele exhibited slightly higher frequencies in patients with hydatid disease (9.7% and 55.0%, respectively) compared to controls. However, these differences were not statistically significant ($p = 0.5$). The odds ratio (OR) associated with the GG genotype and G allele was 0.29, suggesting a potential, though non-significant, risk factor for hydatid disease. The rs1043428 SNP showed genotypes deviating from Hardy-Weinberg equilibrium in patients. The CC genotype was significantly more frequent in patients (1.6%) compared to controls (0%), with an OR of 0.05 and a highly significant p-value ($p = 0.001$). The elevated frequency of the CC genotype suggests a reduced protective role in hydatid disease. In conclusion, this study highlights the elevated TIMP-1 levels in patients with hydatid disease and suggests potential associations between specific TIMP-1 gene polymorphisms and disease susceptibility. These findings provide insights into the molecular mechanisms of hydatid disease and may guide future research into diagnostic and therapeutic strategies.

Keywords: Serum Level, TIMP-1, Hydatid Disease

INTRODUCTION

Hydatidosis, is a disease occur by the immature forms of tapeworms related to the *Echinococcus* genus. The two primary species of *Taenia echinococcus* that infect humans are *Echinococcus granulosus* and *Echinococcus multilocularis*. Additional species of *Echinococcus* that can infect humans include *Echinococcus vogeli* and *Echinococcus oligarthrus* [1]. In 1690, Hartmann discovered the presence of the adult worm in the dog's intestine, as well as human infection with its larvae. The infected and transmission to humans happened by ingested contaminated food and water with its eggs. In human body, the worm affects organs like liver and lungs. The development of cysts due to parasitic infection poses a significant threat, since they have the potential to attain considerable dimensions and inflict severe harm upon the organs they affect [2].

Hydatid illness, caused by the parasite *Echinococcus granulosus*, is found worldwide, especially in regions with a high prevalence of livestock raising and frequent interaction between humans and diseased animals. Hydatid disease is prevalent in various regions, including as South America, Africa, The Mediterranean region, the Middle East, Central Asia, as well as Australia and New Zealand. The prevalence is higher in rural and pastoral areas characterized by insufficient access to proper hygiene and sanitation. Nevertheless, as a result of worldwide travel and trade, instances can also arise in locations that are not naturally prone to the disease, as a result of the introduction of diseased animals or tainted food products. Hence,

although the disease may exhibit higher occurrence rates in specific areas, it possesses the capacity to impact populations globally [3]. *Echinococcus granulosus* infection primarily affects the liver and lungs. Nevertheless, the parasite has the capacity to infect virtually any organ in the body, such as the spleen, kidneys, heart, brain, and bones, albeit these occurrences are less frequent [4]. The immunity to *Echinococcus granulosus*, include both innate and adaptive immunity. Innate immune response is responsible for the first defense line to the parasite also adaptive immunity also had role in defense [5]. cellular immunity and humoral responses ad role in clearance pathogen by of stimulateating T cells, B cells, and production antibodies, specifically IgG. cytokine response to *E. granulosus* is significantly influenced like interferon-gamma (IFN- γ) [6]. Tissue inhibitor metalloproteinases (TIMPs) are glycoproteins that breakdown of extracellular components [7]. TIMP-1 is a member of the zinc-dependent endopeptidase group complex. as noted by Forte *et al.*, [8]. TIMP-1 activated of mitogen-activated protein kinase signaling pathways, specifically MAPK-5 [9]. this study was to evaluation concentration and gene variations of TIMP-1, specifically rs41454248 and rs1043428, in studied groups patients and healthy individuals. Cystic echinococcosis (CE) is a global public health problem. It is disease from farm animals [10]. TIMP-1 is an extracellular matrix protein that belongs to the zinc-dependent endopeptidase family, works to prevent MMPs activity by degrading the

protein of connective tissues, and plays roles in degeneration and aneurysms of blood vessel walls. The study aims to assess the levels of TIMP-1 and the single nucleotide polymorphisms (SNPs) rs41454248 and rs1043428 in hydatid disease patients compared to a control group of healthy individuals. The study included 62 patients with hydatid disease (18 males and 44 females) and a control group of 75 individuals (29 males and 46 females). TIMP-1 levels, along with the rs41454248 and rs1043428 SNPs, were analyzed in both groups. The results showed no significant differences in the average age between the studied groups at a significance level of 0.05. TIMP-1 concentrations were increased in the hydatid disease patients compared to the control groups (19.43 ± 3.90 pg/ml vs. 527.54 ± 100.75 pg/ml) for the rs41454248 (SNPs), genotype GG and G allele showed a low frequency in hydatid disease group compared to the control group (9.7% vs. 55.0%, OR: 0.93, $p=0.5$).

Despite the high OR value, it was not statistically significant, suggesting it may not be a risk factor. The rs1043428 (SNPs) showed that genotypes were not in Hardy-Weinberg equilibrium in the patient group, while they were in equilibrium in the control group. The CC genotype had a significant increase in frequency in the control groups in compared the hydatid disease groups (1.6% vs. 25.3%, OR: 0.5, $p=0.001$). The C allele also showed a non-significant increase (50.0% vs. 55.0%, OR: 0.81, $p=0.05$), indicating it might be protective factor for hydatid disease.

Subjects

Sample Collection

Blood collected from patients infected with hydatid disease as well as from the healthy control group who visited Baqubah Teaching Hospital/Baqubah City - Diyala Governorate, Al-Muqadadiya Hospital, Khanaqin Hospital. after the personal consent of groups, which was taken before collecting the samples according to the science and ethics of scientific research, with approval from the Ministry of Health, samples were collected from December 2022 to June 2023. The study

included 62 patients with hydatid disease (18 males and 44 females) with an average age of (36.76 ± 1.27) years, compared to healthy of 75 individuals (29 males and 46 females) with an average age of (34.20 ± 1.20) years. Each participant provided 5 ml of venous blood: 2 ml put in an EDTA tube for genomic analysis of TIMP-1 gene polymorphisms (rs41454248 and rs1043428), and 3 ml put in a gel tube to separate the serum for TIMP-1 level measurement. Individuals with chronic or transmissible diseases were excluded following a questionnaire to identify any existing conditions.

Extraction of Genomic DNA

The extraction was performed according to the company INTRON, Korea. The concentration of the extracted DNA was 50-100 ng/μl, with A260/A280 purity ratios of 1.8–2.0, using a namedrop spectrophotometer for quantification.

Preparation of Primers

Primer Design Primers were designed using the online NCBI Primer-Blast website. Primer synthesis was done according to Alpha-DNA Company, Baghdad, Iraq specifications as shown in Table 1&2.

Polymerase Chain Reaction (PCR) Protocol

The allele-specific primer assay was for the detection of single nucleotide polymorphisms (SNPs) within the TIMP-1 gene, specifically rs1043428 and rs41454248. The PCR was carried out using two Eppendorf tubes per sample. Each tube received 12.5 μl of master mix, 2 μl of DNA, and a 2 μl aliquot of primers (1 μl forward plus 1 μl reverse for the first tube) (1 2 μF reverse plus 1 μL common forward for the second tube). This volume was made up to 25μL with nuclease-free water. The thermocycler protocol for both TIMP-1 SNPs was as follows: Denaturation at 95°C for 35 seconds, initial denaturation at 160°C35 seconds annealing at 160°C35 seconds, and extension at 172°C35 seconds repeated for 135 cycles. Final extension at 172°C10 minute. The PCR amplicons were observed by electrophoresis on a 1.5% agarose gel, stained with Red Safe stain and bands observed under UV transillumination.

Table 1: TIMP-1 Primers Information and Conditions Rs:41454248.

Rs41454248	Sequence (5'>3')	Product length
Forward Primer 1	GCTGCTTGTCGGTCCCA	236 bp
Forward Primer 2	GCTGCTTGTCGGTCCCG	
Reverse Primer	CAAGAAACAGCCACGATT	

Table 2: TIMP-1 Primers Information and Conditions Rs1043428

Rs1043428	Sequence (5'>3')	Product length
Forward Primer 1	CGGTTTCGTCTACACCCG	191 bp
Forward Primer 2	CGGTTTCGTCTACACCCG	
Reverse Primer	GCTGCTTGTCGGTCCCAATTC	

Statistical Analysis

Boundary homogeneity, normality, and boundary normal distribution, mean and standard deviation Student's t-test using IBM SPSS version 26.0. This was considered unlikely when <0.05 Pearson's chi-square used to determine Probability for non-parametric data. Also in individual percentage is 95% confidence interval, and Fisher Exact Probability was calculated using WinPepi version 11.65 [11]. For calculate of genotyping frequencies and alleles The online Hardy-Weinberg calculator was used [12].

Results

Study included 62 patients with hydatid disease (18 males and 44 females) compared to a control group of 75 individuals (29 males and 46 females). No significant difference in age between studied groups (Table 3). 0.05. present study also found the concentration rates of TIMP-1 in the sera of the study groups, as they indicated increased concentrations, Table (3) among the infected compared to the control group (36.76 ± 1.27 , 34.20 ± 1.20 pg/μl).

There is no significant difference regarding the place of residence, whether in urban or rural areas, as the percentage was equal for both residential areas according to table (3). In this study, it was found that

there is significant increase with a probability level of (0.05), in concentration of TIMP-1 in patients in hydatid cyst disease. Refer to table (4).

Table 3: The Demographic Data of the Studied Groups

		Patients Group	Control Group	Probability
Sexes No. (%)	Males	18 (29.0)	29 (38.7)	P > 0.05
	Females	44 (71.0)	46 (61.3)	
	Total	62 (100.0)	75 (100.0)	
Living Location	Urban	31 (50.0)	50 (66.7)	P < 0.05
	Rural	31 (50.0)	25 (33.3)	
	Total	62 (100.0)	75 (100.0)	
Age Mean $\pm$ SE (Years)		36.76 $\pm$ 1.27	34.20 $\pm$ 1.20	P > 0.05

Table 4: Serum Levels in TIMP-1

TIMP-1 Level Mean $\pm$ SE (Pg/ml)		Probability
Patients Group	Control Group	
320.74 $\pm$ 43.81	518.05 $\pm$ 32.56	P < 0.001

The Single Nucleotide Polymorphism rs41454248 of TIMP-1

The genetic variation results for TIMP-1 single nucleotide polymorphisms (SNPs) rs41454248 revealed the presence of two alleles, A and G, lead to three genotypes: GG, GA, and AA. Using the polymerase chain reaction technique, the genotypes were analyzed as follows: the homozygous GG genotype produced a single band on the

agarose gel when electrophoresed, in the well loaded with forward primer 1.

The heterozygous GA genotype displayed two bands on the agarose gel; one in the well with forward primer 1 and another in the well with forward primer 2. The homozygous AA genotype appeared as a single band in the well loaded with forward primer 2 (Figure 1).

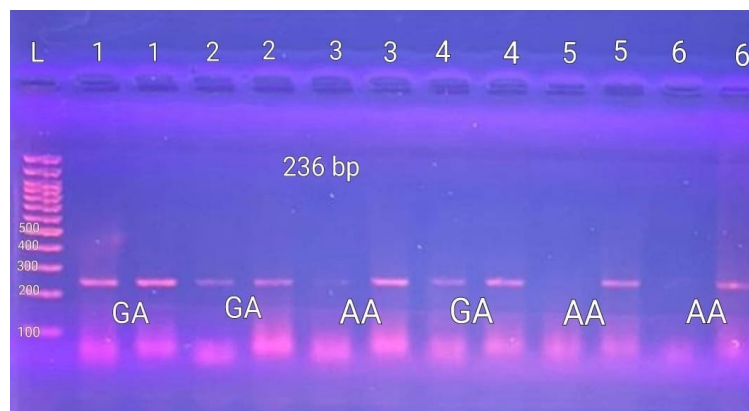


Figure 1: Gel Electrophoresis of TIMP-1 Single Nucleotide Polymorphism (SNP) rs41452448 Polymerase Chain Reaction (PCR) Product on 1.5% Agarose Gel Stained with Red Safe Stain (Intron Company, South Korea)

Current study showed frequency ratios of genotypes and alleles for TIMP-1 rs41454248 were consistent with Hardy-Weinberg equilibrium, as shown in Table (5). Frequencies of genotypes GA and AA, as well as the A allele, were increased with non-significantly in the hydatid cyst patient group to the control group (90.3% vs. 49.53%, 45.0% vs. 43.0%,

respectively) see table (5). The frequency of GG genotype and G allele were, however, higher in the patient group than in the healthy control group (9.7% vs. 26.7%, OR 0.29, $p < 0.05$; 55.0% vs. 57.0%, OR 0.93, $p > 0.05$). An elevated odds ratio (OR) like that for the GG genotype and G allele could mean a potential risk factor for hydatid cysts (Table 6).

Table 5: Genotyping and Allele Frequencies of TIMP-1 rs41454248 SNPs

Genotypes	Patients Group (N=62) - CE		Control Group (N=75)	
	Observed	Expected	Observed	Expected
GG	6 (9.7)	18.65 (30.07)	20 (26.7)	24.08 (32.11)
GA	56 (90.3)	30.71 (49.53)	45 (60.0)	36.83 (49.11)
AA	0 (0.0)	12.65 (20.40)	10 (13.3)	14.08 (18.78)
Total	62 (100.0)	62 (100.0)	75 (100.0)	75 (100.0)
P-HWE	P < 0.001		P > 0.05	

P-HWE: the Probability of Hardy-Weinberg Equilibrium, OR: Odds Ratio, 95% CI: 95% Confidence Intervals

Table 6: TIMP-1 Genotyping and Allele Frequencies of rs41454248 between the Groups.

Genotypes	Patients Group (N=62)	Control Group (N=75)	χ^2	OR (95% CI)	Fisher's Exact Probability
G	68 (55.0)	85 (57.0)	0.092	0.93 (0.58-1.50)	P > 0.05
A	56 (45.0)	65 (43.0)	0.092	1.08 (0.67-1.74)	P > 0.05
GG	6 (9.7)	20 (26.7)	6.371	0.29 (0.11-0.78)	P < 0.05
GA	56 (90.3)	45 (60.0)	16.109	6.22 (2.40-16.14)	P < 0.001
AA	0 (0.0)	10 (13.3)	8.918	0.05 (0.0-0.85)	P < 0.001
Total	62 (100.0)	75 (100.0)			

Table 7: TIMP-1 Levels Among rs41454248 Single Nucleotide Polymorphism (SNP) between the Groups.

Genotypes	TIMP-1 Level Mean $\pm$ SE (Pg/ML)		Probability
	Patients Group (N=62)	Control Group (N=75)	
GG	19.43 $\pm$ 3.90	527.54 $\pm$ 100.75 ^A	P < 0.001
GA	353.03 $\pm$ 46.49	530.22 $\pm$ 31.09 ^A	P < 0.05
AA	-	444.33 $\pm$ 23.85 ^A	
Probability	P < 0.05		

The Single Nucleotide Polymorphism rs1043428 of TIMP-1

Two alleles at the TIMP-1 rs1043428 (SNPs) were evaluated, related to three genotypes CC, CG, GG which were identified using PCR-ASP technique if person has homozygote CC Genotype, it revealed a single band in agarose gel (Figure 2). While if it has CG heterozygote genotype, it appeared two bands in the gel: one in the well loaded with forward 1 primer and the second in the well loaded with forward 2 primers. Also, when the subject has homozygote GG genotype, it appearing as a band of the well loaded with forward 2 primers in agarose gel. The high OR value of GG genotype and G allele, as a result,

may be a risk indicator for hydatid cysts see table (8) also the high OR value of CC genotype and C allele as a result may be a risk indicator for hydatid disease. Results of this study reflected that frequency ratio regarding genotypes and alleles of TIMP-1 rs1043428 for both groups are in equilibrium with Hardy-Weinberg table (8). Also genotypes result found non-significant decrease in patients with hydatid cysts in compare other group for genotypes (CC, CG, GG) and alleles (C, G) (1.6 vs. 25.3 and 96.8 vs. 60.0 and 49.43), and the odds ratio and probability for genotypes CC, CG (OR0.05, P=0.001 and OR: 20, P=0.001 respectively). as in table (9).

**Figure 2:** Gel Electrophoresis of TIMP-1 Single Nucleotide Polymorphism (SNP)

Rs1043428 Polymerase Chain Reaction Product on 1.5% Agarose Gel Stained with Red Safe Stain (Intron company, South Korea). 1-6: Numbers for samples electrophoresed; the single band for the CC and GG genotypes (Wild and mutant homozygote, respectively) the double bands for the CG genotype (mutant homozygote).

Table 8: The Genotyping and Alleles Frequencies of TIMP-1 rs1043428 SNPs

Genotypes	Patients Group (N=62)		Control Group (N=75)	
	CE		CE	
	Observed	Expected	Observed	Expected
CC	1 (1.6)	15.50 (25.0)	19 (25.3)	22.96 (30.62)
CG	60 (96.8)	31.0 (50.0)	45 (60.0)	37.07 (49.43)
GG	1 (1.6)	15.50 (25.0)	11 (14.67)	14.96 (19.95)
Total	62 (100.0)	62 (100.0)	75 (100.0)	75 (100.0)
P _{HWE}	P < 0.001		P > 0.05	

Table 9: TIMP-1 Genotyping and Allele Frequencies of rs1043428 between the Groups.

Genotypes	Patients Group (N=62)	Control Group (N=75)	χ^2	OR (95% CI)	Fisher's Exact Probability
C	62 (50.0)	83 (55.0)	0.775	0.81 (0.50-1.30)	P > 0.05
G	62 (50.0)	67 (45.0)	0.775	1.24 (0.77-1.99)	P > 0.05
CC	1 (1.6)	19 (25.3)	15.318	0.05 (0.01-0.37)	P < 0.001
CG	60 (96.8)	45 (60.0)	25.640	20 (4.59-87.06)	P < 0.001
GG	1 (1.6)	11 (14.67)	7.237	0.10 (0.01-0.75)	P < 0.05
Total	62 (100.0)	75 (100.0)			

Table 10: TIMP-1 Levels among rs1043428 Single Nucleotide Polymorphism (SNP) between the Groups.

Genotypes	TIMP-1 Level Mean $\pm$ SE (Pg/ML)		Probability
	Patients Group (N=62)	Control Group (N=75)	
CC	11.09	426.71 $\pm$ 24.43 ^A	P < 0.001
CG	316.14 $\pm$ 43.89	515.86 $\pm$ 30.78 ^A	P < 0.001
GG	906.58	684.78 $\pm$ 174.26 ^A	P > 0.05

Conclusion

According to the present findings, TIMP-1 serum levels were insignificantly different between the studied groups but were higher in

1 patient group. As for genotyping and allele frequencies, GG genotype and G allele of rs41454248 showed a nonsignificant increase in frequency of patient group; high odds ratio (OR) for GG genotype and G allele indicates that they might be potential risk factors for hydatid

disease. For rs1043428, CC genotype was increased in patient group, while C allele showed a significant decrease in patient group. The low OR value for CC genotype and C allele indicates that they might have a protective role against hydatid disease. Furthermore, CG genotype had a significant increase in patient group, indicating it to be a risk factor for the disease. The GG genotype increased only in the control

group. There was a non-significant increase in the patient group for the G allele compared to the control group.

The OR value for hydatid disease is 2.62 (95% CI = 1.39-4.94) for GG when other genotypes are compared with it and 1.75 (95% CI = 1.01-3.02) for the G allele, which shows a possible risk factor for this disease.

References

- [1] W. Zhang, H. Wen, J. Li, R. Lin, and D. P. McManus, "Immunology and immunodiagnosis of cystic echinococcosis: an update," *Journal of Immunology Research*, vol. 2012, no. 1, p. 101895, 2012.
- [2] K. A. H. Al-Khalidi, H. R. Al-Abodi, H. K. Jabbar, and B. A. Hmood, "Echinococcus granulosus," in *Overview on Echinococcosis*. IntechOpen, 2020.
- [3] S. N. Khan *et al.*, "Cystic echinococcosis: an emerging zoonosis in southern regions of Khyber Pakhtunkhwa, Pakistan," *BMC Veterinary Research*, vol. 17, pp. 1-11, 2021.
- [4] A. Casulli, "Recognising the substantial burden of neglected pandemics cystic and alveolar echinococcosis," *The Lancet Global Health*, vol. 8, no. 4, pp. e470-e471, 2020.
- [5] D. McManus, "Immunodiagnosis of sheep infections with Echinococcus granulosus: in 35 years where have we come?," *Parasite Immunology*, vol. 36, no. 3, pp. 125-130, 2014.
- [6] K. Rasheed, S. A. Zargar, and A. A. Telwani, "Hydatid cyst of spleen: a diagnostic challenge," *North American journal of medical sciences*, vol. 5, no. 1, p. 10, 2013.
- [7] R. Asgari, K. Mansouri, A. Abdolmaleki, and M. Bakhtiari, "Association of matrix metalloproteinases with male reproductive functions; with focus on MMP2, 7, and 9," *Meta Gene*, vol. 29, p. 100906, 2021.
- [8] D. Forte *et al.*, "The tissue inhibitor of metalloproteinases-1 (TIMP-1) promotes survival and migration of acute myeloid leukemia cells through CD63/PI3K/Akt/p21 signaling," *Oncotarget*, vol. 8, no. 2, p. 2261, 2017.
- [9] E. S. Khamaes, N. Y. Al-Bayati, and A. H. Abbas, "Tissue inhibitor of metalloproteinase-1 (TIMP-1) serum level and genetic polymorphisms associated with cutaneous leishmania infections," *Human Gene*, vol. 33, p. 201049, 2022.
- [10] M. Ren *et al.*, "Polymerization of MIP-1 chemokine (CCL3 and CCL4) and clearance of MIP-1 by insulin-degrading enzyme," *The EMBO journal*, vol. 29, no. 23, pp. 3952-3966, 2010.
- [11] J. H. Abramson, "WINPEPI updated: computer programs for epidemiologists, and their teaching potential," *Epidemiologic Perspectives & Innovations*, vol. 8, no. 1, pp. 1-9, 2011.
- [12] D. G. Andrews, *An introduction to atmospheric physics*. Cambridge University Press, 2010.



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