

Comparative assay of protease enzyme activity in protoscolices (*Hydatid cyst*) and liver tissues

Narges Anasori¹, Ali Farahnak¹*, Taghi Golmohamadi², Mohamadreza Eshraghian³,
Mohamadbagher Molaei Rad¹

¹Department of Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Iran.

²Department of Biochemistry, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran.

³Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

* Corresponding author: E-mail address: Farahnak@tums.ac.ir (A. Farahnak)

ABSTRACT

Hydatidosis is a zoonosis disease caused by the larva of *Echinococcus granulosus* parasite in man and animals. The Proteases enzymes are necessary for nutrition, migration and evasion of immunity of parasite into host. The aim of this study was to determine the protease activity of hydatid cyst protoscolices (PSC), healthy and cystic liver tissues in order to compare of this biomarker for host and parasite in hydatid disease. In this study, PSC were collected from sheep liver tissue infected with hydatid cysts at an abattoir and washed 3 times with PBS buffer. PSC samples were freeze-thawed and sonicated while collected liver tissues were homogenized. Extract solution samples were centrifuged and stored at - 20°C. Protease enzymes activity was measured in the extract solutions of PSC and sheep liver tissue samples (healthy and cystic livers). Samples protein concentrations and protein bands were detected using Bradford and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) methods respectively. To determine significant difference between two groups, statistical t-test was performed. The values of protease enzyme activities in healthy, cystic and PSC were determined 0.0028, 0.0087 and 0.50U/ml/mg respectively. Elevation of protease enzyme activity in cystic liver as compared with healthy was not significant. Statistical T-test showed higher protease enzyme activity for PSC as compared to healthy ($p < 0.05$). SDS-PAGE confirmed 24 kDa and 54 kDa bands for protease enzyme in PSC samples and 24 kDa band in liver samples. Protease enzyme activity and molecular weight as compared to healthy liver tissues could be concerned as a comparative metabolic biomarker for host and parasite in hydatidosis.

Keywords: Protease; Hydatid Cyst; Protoscolices; Liver

INTRODUCTION

Infection with *Echinococcus granulosus* parasite may be naturally transmitted between humans and animals. Hydatid cyst disease (Hydatidosis) is term used to refer to infection with the parasite larva in humans and echinococcosis should be limited to infectivity with the adult stage in carnivores [1]. Proteases are a group of enzymes whose breakdown proteins. They are also called proteolytic enzymes or proteinases [2]. The role of secreted proteases on successful migration, nutrition and evasion of the immune system by *Angiostrongylus cantonensis*, *Opisthorchis viverrini* and *Fasciola* spp parasites have been reported [3, 4, 5]. Increased supernatural count of protease enzymes (cathepsin B) in hydatid cyst, supports the

importance of enzyme as an indicator for infertility in hydatid cyst [6].

In the present study, protease enzyme activities of PSC, healthy and cystic liver tissues were determined in order to compare activity level of this biomarker for host and parasite in hydatidosis.

MATERIALS AND METHODS

Protoscolices (PSC) extract preparation

PSC samples were obtained from 10 liver infected with hydatid cysts of sheep slaughtered at Karaj abattoir (Karaj, Iran). PSC parasites were washed 3 times with PBS buffer, pH 7.2. PSC were freeze-thawed 3-6 times in liquid nitrogen and were sonicated in a 150W ultrasonic disintegrator, 10 sec vibrations and 5 sec rest on ice until no intact PSC were visible

microscopically (approximately 15 min). Then suspension was centrifuged (10000g for 30 min at 4°C) and supernatant was stored at -20°C [7].

Liver extract preparation

Sheep livers (10 healthy livers and 10 infected samples) were obtained at abattoir and washed 3 times with PBS buffer pH 7.2. Then they were homogenized with 3 volumes of homogenizing buffer in a glass homogenizer, so the suspensions were centrifuged (10000g for 30 min at 4°C) and supernatant stored at -20°C [8].

Protein assay in the PSC and liver solutions

The concentration of protein in the extract solutions of PSC and sheep liver tissues were measured by the method of Bradford using bovine serum albumin as the standard [8].

Protease activity assay in the PSC and liver solutions

Protease enzymes digest casein as substrate and release tyrosine. Folin & Ciocalteu's phenol reagent primarily react with free tyrosine and produce a blue color. In this work, to enzyme assay, casein solution prepared and incubated in 37 °C for 5 min, and then samples were added to casein solution tube and incubated for 10 min. The reaction stopped with trichloroacetic acid (TCA). Samples were added to blank tubes and incubated for 30min.

Then all tubes centrifuged for 5 min at 13000×g at 25°C. Finally, the supernatant was poured into the tube and added sodium carbonate solution and Folin & Ciocalteu's phenol reagent, and was incubated for 30 min at 37°C. Then solution tubes were centrifuged (as above) and their Absorbances were measured spectrophotometrically at 660 nm. The protease activities were compared to a standard curve and reported as Units /ml [9].

SDS-PAGE analysis of PSC and liver samples

To separate and stain the protein components of samples, Sodium dodecyl sulfate polyacrylamide

gel electrophoresis (SDS-PAGE) and coomassie blue staining were used. Samples were mixed with sample buffer and were run on 10% acrylamide gels. Finally, the gel was stained with coomassie blue R-250. Molecular weights of sample proteins were compared with respect to the protein marker [8]. To detect the molecular weight, Ratio factor of ladder bands was calculated and molecular weights of proteins were determined against standard curve in Excel software. The proteins of gels were identified by using protein database (<http://web.expasy.org>).

Statistical analysis of samples

Statistical T-test was performed to compare the mean values of protein concentrations and enzyme activities between healthy and infected liver tissues or parasite and liver tissues groups. Statistical comparisons were carried out using statistical software [10].

RESULTS

Protein concentration, enzyme activities and statistical analysis results

The mean values of protein concentrations and enzyme activities for PSC, healthy and infected liver samples are presented in table 1. Protein concentration of healthy liver was higher than infected liver ($p < 0.05$).

Elevation of protease enzyme activity in infected liver as compared to healthy liver was not significant ($p > 0.05$). T-test analysis showed higher Protease enzyme activity for PSC as compared to healthy/ infected livers ($p < 0.05$).

SDS-PAGE analysis results

Extract samples of PSC, healthy and infected liver tissues were analyzed by SDS-PAGE electrophoresis and the results are shown in figure 1. SDS-Page gel shows protein band with 24 kDa and 54 kDa for protease enzyme in PSC samples and 24 kDa in liver samples.

Table 1. The mean values of protein concentrations and Protease enzyme activity for PSC, healthy and cystic liver samples

Samples	Protein concentration(mg/ml)	Protease total activity (U/ml)	Protease specific activity (U/ml/mg)
Healthy liver	5.0±0.7	0.014±0.003	0.0028±0.0
Cystic liver	2.3±0.5	0.020±0.004	0.0087±0.003
PSC extract	0.1±0.09	0.050±0.004	0.50±0.085

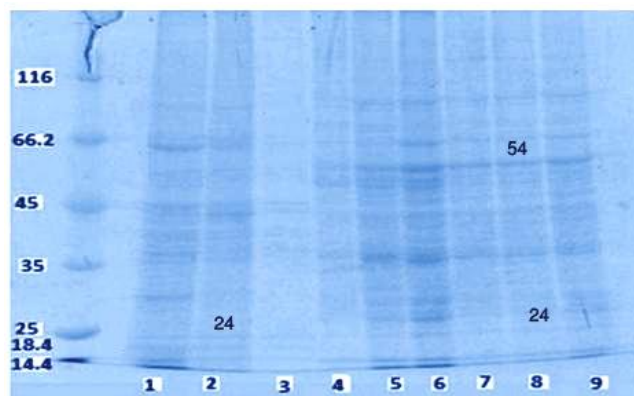


Figure 1. SDS- PAGE analysis of proteins from the extracts of protoscolices (PSC) of hydatid cyst and liver tissues. Lanes 1-3 are healthy liver samples. Lanes 4-6 are infected liver samples. Lanes 7-9 are PSC samples.

DISCUSSION

Liver tissue is the most important source for protein synthesis and detoxification. Two major types of liver cells are paranchymal cells and sinusoid cells. Function of hepatocytes should be disturbed in the presence of infections [11]. In our study the protein concentration of infected tissue was decreased. Hydatid cyst cause to reduce protein production by liver tissues and finally results in decreasing amount of protein concentration. Protease enzyme could be changed during liver autolysis. The partial increasing of protease enzyme in infected liver should be resulted from tissue autolysis in the presence of hydatid cyst infection. Autolysis phenomena cause to protease elevation to broke lysis proteins [12].

In this work, we found that specific activity of protease enzymes of protoscolices was more than healthy tissue. This higher activity level can be referred to requirements of parasite for nutrition, migration and evasion of immune system into a presence and or next host (3). Recently, the role of protease enzymes for survival of schistosome parasite under oxidative stress have been shown[13].

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The range of proteinases, including cathepsin B, cathepsin L1, cathepsin L2, cathepsin D, cathepsin C and legumain are assumed to be concerned in the catabolism of host haemoglobin in helminth pathogens[14]. The different cathepsins enzymes (cystein protease) identified based on their migration distance due to their molecular weights [15]. In this research two protein bands 24 kDa and 54 kDa was found in PSC samples and one protein band 24 kDa was found in both liver samples. These proteins are very important from comparative proteins in host and parasite_point of view.

CONCLUSION

Briefly, level of PSC enzyme activity as compared to liver tissues could be concerned as a comparative metabolic biomarker for host and parasite in hydatidosis from comparative biochemistry point of view.

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