Original Article

Assessment of Alkaline Phosphatase Activity in Hydatid Cyst Protoscolices and Liver Tissue as a Pathological Biomarker

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Introduction: Hydatid cyst disease is caused by the protoscolices of *Echinococcus granulosus*. Alkaline phosphatase (ALP) enzyme is required for metabolism, physiology, immunology, and nutrients absorption in parasite. The aim of this study was to compare the level of ALP activity (as a pathological biomarker) in hydatid cyst protoscolices (HCP) with that of sheep liver tissue and to determine the effect of cystic infection on the enzyme activity. **Methods:** HCPs were collected from sheep livers with hydatid cysts at a local abattoir and washed 3 times with PBS buffer. HCP samples were freeze-thawed and sonicated, while the collected liver tissues were homogenized. Then, extract solutions were centrifuged and stored at -20°C. ALP activity was measured in the extract solutions of HCP and healthy and infected sheep liver tissue samples. The amounts and bands of protein samples were detected using Bradford method and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), respectively. To determine the significant difference between the two groups, independent two samples T-test was used. **Results:** The mean values of ALP-specific activity of healthy and infected livers and HCP were estimated 0.019, 0.175, and 1.28 U/ml/mg, respectively. Higher ALP activity level was observed in cystic liver compared to healthy liver ($p<0.05$). T-test analysis showed higher ALP enzyme activity for HCP compared to healthy liver ($p<0.05$). SDS-PAGE demonstrated a protein band with molecular weight of 59 kDa in HCP samples, which was identified as ALP. **Conclusion:** ALP activity in HCP and healthy liver indicates the importance of this enzyme in comparative biochemistry of liver and parasite. Higher level of ALP enzyme activity in cystic liver in comparison with healthy liver could be considered as a pathological biomarker for diagnosis of hydatid cyst disease with other hydatid disease parameters. *J Med Microbiol Infec Dis, 2014, 2 (2): 68-70.*

Keywords: Alkaline phosphatase, Hydatid cyst, Liver, Parasite.

INTRODUCTION

Echinococcus granulosus infection may be transmitted between humans and animals. Hydatid disease refers to an infection with the parasite larva in humans, and echinococcosis is restricted to infection with the adult stage in carnivorous animals [1]. Alkaline phosphatase (ALP) plays an important role in metabolism, physiology, immunology, and nutrients absorption of the cystic echinococcosis [2, 3]. ALP catalyzes the hydrolysis of phosphate monoesters in an alkaline environment [4]. Alkaline and acid phosphatase activities have shown to reduce during the progression of the disease [5]. Diagnosis of hydatid disease is made by a combination of imaging techniques, clinical signs, serological tests, cyst fluid examination, and molecular techniques [6].

Therefore, the aim of this study was to determine ALP enzyme activity in hydatid cyst protoscolices (HCP) and in both healthy and infected sheep liver tissue. The impacts of hydatid infection influence on ALP enzyme, as a pathological biomarker in hydatid cyst, in liver tissue was also evaluated.

MATERIAL AND METHODS

Protoscolices extract solution preparation. Protoscolices were collected from livers of 10 sheep infected with hydatid cysts from a local abattoir in Karaj, Iran. The protoscolices were washed 3 times with PBS (pH 7.2) and then recollected. Protoscolices were resuspended in PBS and freeze-thawed 3-6 times in liquid nitrogen and sonicated in a 150 W ultrasonic disintegrator,10 sec ON and 5 sec OFF on ice until no intact Protoscolices were visible microscopically (approximately 15 min). Then, the suspensions were centrifuged (10000×g for 30 min at 4°C) and supernatants were stored at -20°C [7] until used.

Preparation of Liver extract solution. Ten healthy and 10 infected sheep liver samples were washed 3 times with PBS buffer (pH 7.2). Each sample was homogenized in 3 volumes of homogenizing buffer in a glass homogenizer, and then the suspensions were centrifuged at 10000×g for 30 min at 4°C. The supernatants were recovered and stored at -20°C [8].

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Protein assay in the solutions. The amount of protein in the extract solutions of both protoscolices and sheep liver tissues were estimated by the Bradford method using bovine serum albumin as standard [8].

ALP activity assay in the solutions. ALP activity was determined using alkaline phosphatase assay kit (Ref. number 10-503R20). The kit uses *p*-nitrophenyl phosphate (*p*NPP) as a phosphatase substrate, which turns yellow once dephosphorylated by ALP. To assay enzyme, 20 µl of each sample was added to a cuvette containing 1 ml of 37°C working solution .The samples were mixed well in a water bath at 37°C and absorbance was measured after one, two, and three min at 405 nm by a spectrophotometer. After subtracting subsequent Abs values, mean value was calculated and then ALP activity was estimated [9].

SDS-PAGE analysis of samples. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and coomassie blue staining were used to separate and stain the protein components of samples. Samples were mixed with sample buffer and run on acrylamide gels (10%). Then, the gel was stained with coomassie blue R-250. Molecular weights (MWs) of sample proteins were measured in regard to the protein marker [8]. To detect the MW, ratio factor (Rf) of ladder bands was calculated and MWs of proteins were determined against standard curve in Excel software. Moreover the proteins of gels were identified using protein database as mentioned in the following (http://-web.expasy.org).

Statistical analysis. Independent two sample T-test was performed to compare the mean values of protein concentrations and enzyme levels between healthy and infected liver tissues. Statistical comparisons were performed using statistical software named Evans research associates, 2008 [10].

RESULTS

Protein concentration, enzyme activity and statistical analysis results. The mean values of protein concentrations and enzyme activities in HCP and healthy and infected liver samples are shown in Table 1. The Protein concentration of healthy liver was higher than infected liver ($p<0.05$). A significantly higher level of ALP specific activity was observed in cystic liver samples compared to healthy livers ($p<0.05$). Also two-sample T-test analysis showed higher ALP activity in HCP compared to healthy livers ($p<0.05$).

SDS-PAGE analysis results. The extract samples of HCP and healthy and infected liver tissue were analyzed by SDS-PAGE (Figure 1). SDS-Page gel showed a protein band of 59 kDa for ALP enzyme in the parasite samples. The same protein band is seen in the liver tissues; however, this protein has not been reported in EXPASY protein database.

Table 1. The mean values and standard error of protein amount and ALP enzyme activity for HCP, and healthy and infected liver samples

Samples	Protein concentration (mg/ml)	ALP total activity (U/ml)	ALP specific activity (U/ml/mg)
Healthy liver	**5.0±0.7**	**0.097±0.0**	**0.019**±0.0
Infected liver	**2.3±0.5**	0.404±0.09	**0.175**±0.0
HCP extract	**0.1**±0.09	**0.128**±0.0	**1.280**±0.3

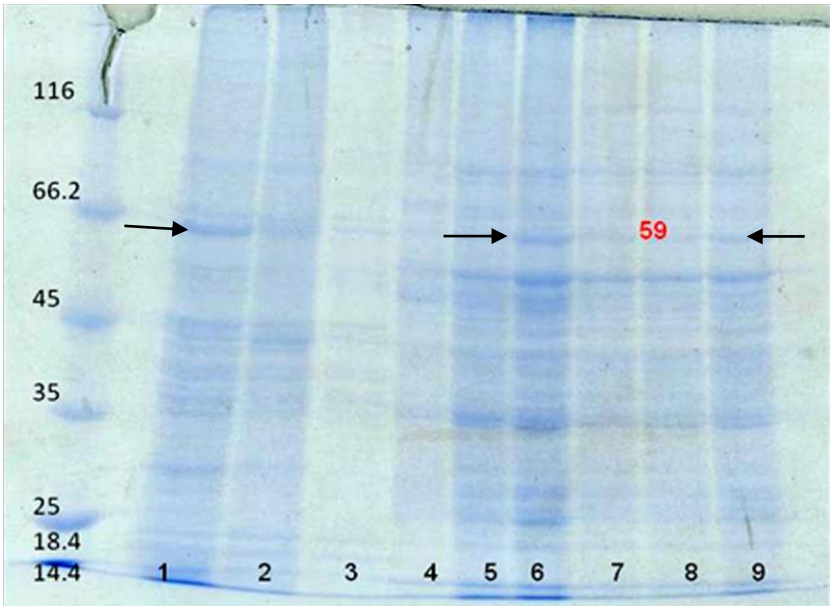

Fig. 1. SDS-PAGE analysis and molecular weight (kDa) detection of proteins of the extracts of hydatid cyst protoscolices (HCP) and liver tissues. Lanes 1-3 are healthy liver samples; lanes 4-6 are infected liver samples; lanes 7-9 are HCP samples.

DISCUSSION

Liver tissue is a vital source of protein synthesis and detoxification. Two major types of liver cells are parenchymal or hepatocytes and sinusoidal. Function of hepatocytes may be disturbed in the presence of infections [11]. In the present study, the reduction of protein synthesis as a result of hydatid cyst leads to a decrease in protein concentration of infected tissue. Zvesdina *et al.* indicated disturbed protein synthesis in hepatocytes by chelating agent [12].

Alkaline phosphatases are a group of enzymes in the cells lining the biliary ducts of the liver [4]. The presence of hydatid cyst causes irritation of biliary ducts. Hydatid infection stimulates biliary cells to excrete ALP. Our results showed that hydatid cyst increase enzyme activity in liver tissue, which could be considered as a pathological reaction to parasite.

In this study, we found that ALP-specific activity in HCP was more than healthy liver tissue. This higher activity level can be attributed to requirements of parasite for biochemical adaptation to present and next hosts. Also, Sarciron *et al.* and Wijdan *et al.* have confirmed the above finding [13, 14].

The results of the protein bands of SDS-PAGE analysis and their search in protein database showed ALP enzyme (59 kDa), acetylcholine transferase (84 kDa.), and glutathione S-transferase (GST) (24 kDa) in the parasite sample. Of these enzymes, GST has been previously reported in Iranian isolates of hydatid cyst protoscoloices [15]. The 59-kDa protein band in parasite is very important from a comparative biochemistry point of view. Higher level of enzyme activity in cystic liver could be considered as a pathological biomarker in hydatid cyst disease.

ALP enzyme activity in parasite HCP and healthy liver shows the importance of this enzyme in comparative biochemistry of liver and parasite. Higher level of enzyme activity in cystic liver compared to healthy liver could be considered as a pathological biomarker in hydatid cyst disease in comparison with other hydatid disease parameters.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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