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Short Communication

Activity Assay of Glutathione S-Transferase (GSTs) Enzyme as a Diagnostic Biomarker for Liver Hydatid Cyst in Vitro

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Abstract

Background: The aim of this study was to detect the Glutathione S-Transferase(GST) enzyme activity of healthy / cystic liver as a diagnostic biomarker for hydatidosis. In order to compare with liver tissue, the level of the GSTs enzyme activityof parasite was also determined.

Methods: Parasites were collected from sheep liver tissue with hydatid cysts at a local abattoir and washed with PBS buffer. Collected parasites and liver tissues were sonicated or homogenized respectively. Extract solution samples were centrifuged and stored at - 20°C. GSTenzyme activities were measured in the extract of parasite and liver tissue samples (healthy and infected livers). Protein amounts 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,two-sample*t*-test was performed.

Results: GST specific activities of healthy / infected livers and parasites were estimated 304, 1297 and 146 U/ml/mgrespectively. Significant higherGST specific activities in cystic liver than healthy liver was observed ($P<0.05$). T-test analysis showed GST activity of parasite was lower than healthy liver tissue. SDS-PAGE showed GST protein bands with 24 kDa in parasite samples and25 kDa in liver tissues.

Conclusion: GST activity incystic liver tissue could be concerned as a biomarker for hydatid cyst diagnosis with other hydatid disease parameters.

Keywords: Glutathione S-Transferase, Hydatid cyst, Protoscolices, Liver, Parasite

Introduction

Infection with *Echinococcus* parasite may be naturally transmitted between humans and animals. Hydatidosisis term used to refer to infection with the parasite larva in humans and echinococcosis restricted to infection with the adult stage in carnivorous animals (1).

Recently, the genetics, structure and function of the human cytosolic GST enzyme with emphasis on their roles in the cellular metabolism has been defined (2).This enzyme protect cells against toxicants by conjugating the glutathione as substrate to xenobiotics. GST activity was detected in most mammalian tissues, especially in the liverwhich plays a key role in detoxification. There are different classes of GST isozymes that diverge in their-specificity to xenobiotic or endogenous substrates (3). Enzymes are essential for survival, migration and metabolism of parasites. GST enzymes involved in the cellular detoxification of a broad range of chemical substrates (4). Apart from reac-

tion from their endogenous metabolism, GSTs of helminth parasite may protect against exogenous xenobiotics as a result of immune effectors mechanisms from the host (5).Glutathione transferase activity has been determined in cestodes, digeneas and nematodes. Significantly higher activity has been found in intestinal cestodes and digeneas, compared with parasitic nematodes (6). GSTs activity assay has been demonstrated in the cytosol of protoscolices from sheep hydatid cysts (7).

The liver is the most common organ involved by hydatidosis (8). Diagnosis of hydatid disease is done by a combination of clinical signs, imaging techniques, cyst fluid examination, serological tests and molecular techniques (9).The prevalence of liver hydatid cyst was reported 4.7% in people of Peru country using recombinant antigen, EpC1 glutathione S-transferase [rEpC1-GST], in western blot technique (10). However, the hydatid cyst diagnosis technique is under developing due to specificity and sensitivity problems.

In the present study, GST enzyme activity of hydatid cyst protoscolices (parasite), healthy and cystic liver tissues were compared and mentioned GST enzyme importance for diagnostic biomarker in hydatid cyst disease.

Materials and Methods

Preparation of protoscolices (parasite) extracts solution

Ten samples of parasites were obtained from 10 liver infected with hydatid cysts of sheep slaughtered at a localabattoir (Karaj, Iran). Parasites samples were washed 3 times with PBS buffer,pH 7.2, freeze-thawed 3-6 times in liquid nitrogenand and water bath 37°C respectively and sonicated in a 150W ultrasonic disintegrator,10 sec ON and 5 sec OFF on ice until no intact PSC were visible microscopically (approximately15 min). Then resulted suspension was centrifuged (10000g for 30 min at 4°C) and supernatant was stored at -20°C (11).

Preparation of Liver extracts solution

Sheep livers (10 health liver samples and 10 infected samples) were obtained at a local abattoir andwashed 3 times with PBS buffer pH 7.2. Then they were homogenized with 3 volumes of homogenizing buffer, PBS pH 6.5, in a glass homogenizer, so the suspension were centrifuged (10000g for 30 minat 4°C) and supernatant stored at -20°C (12).

Protein assay in the solutions

The protein concentration in the extract solutions of protoscolices and sheep liver tissues were estimated by the method of Bradford using bovine serum albumin as the standard (12).

GSTs activity assay in the solutions

GSTs activity was assayed spectrophotometrically at 25°C with reduced glutathione (GSH) and 1-chloro-2, 4-dinitrobenzene (CDNB) as substrates. This was done by watching an increase in absorbance at 340nm. Protosolices and liver extract samples were removed from -20°C freezer and allowed to thaw on ice. CDNB 100 mM from 4°C and GSH 100 mM from -20°C freezer were removed and allowed to thaw at room temperature, when thawed, incubated at 30°C in water bath. For each assay was prepared one ml of assay cocktail (980µlPBS pH 6.5, 10µl of 100 mM CDNB and 10 µl of 100 mM GSH), then removed 100 µl of cocktail and its remaining placed 900 µl of it into 1.5 ml cuvette. To zero spectrophotometer, was used 1 ml of distilled water and to the blank cuveet added 100µl PBS to 900 µl of cocktail and measured absorbance at 340 nm, every1 minute, for 3 min. To the test cuvette was added 100 µl of sample to 900 µl cocktail, mixed and measured absorbance at 340 nm as above (13).

SDS-PAGE analysis of samples

SDS-PAGE and coomassie blue staining were used to separate and stain the protein components of samples respectively. 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 (12).To detect the molecular weight, Rf(Ratio factor) of ladder bands was calculated, standard curve

was prepared in Excel software and finally proteins MW were determined. The proteins of gels were identified primitively by using protein database. (http://web.expasy.org).

Statistical analysis

Independent *t*-test was performed to compare the mean values of protein concentration and enzyme activity between healthy and cystic liver tissues or parasite and liver tissues. Statistical comparisons were carried out using statisticalsoftware (14).

Results

Protein concentration, enzyme activities and statistical analysis results

The mean values of protein concentrations and enzyme activities for parasite, healthy and infected liver samples are presented in Table 1.

Protein concentration of healthy liver was higher than infected liver ($P<0.05$).Significant higher GST specific activities in cystic liver samples was observed as compared with healthy liver ($P<0.05$). Statistical *t*-test showed GST enzyme activity of parasite was lower than healthy liver tissues ($P<0.05$).

SDS-PAGE analysis results

Extract samples of protoscolices, healthy and cystic liver tissues were analyzed by SDS-PAGE electrophoresis and the results are shown in Fig. 1. SDS-PAGE gel shows GST protein bands with 24kDa in parasite and 25kDa in healthy liver samples. Similar cross protein bands is observing in parasite and liver sample but has been not recorded by databse. Identified proteins are presented in table 2, 3.

Table 1: The mean values of protein amounts and GSTs activity for parasite, healthy and cystic liver samples

Samples	Protein amounts (mg/ml)	GST Total activity (U/ml)*	GST Specific activity(U/ml/mg/protein)
Healthy liver	5.0 ±0.7	1522±0.27	304.4±0.09
Cystic liver	2.3±0.5	2984±0.61	1297±0.16
Parasite extract	0.1±0.09	14.60±0.0	146±0.0

**One unit of GST activity is the amount of enzyme which produces 1 µmol of GS-DNB conjugate/min under the conditions of the assay*

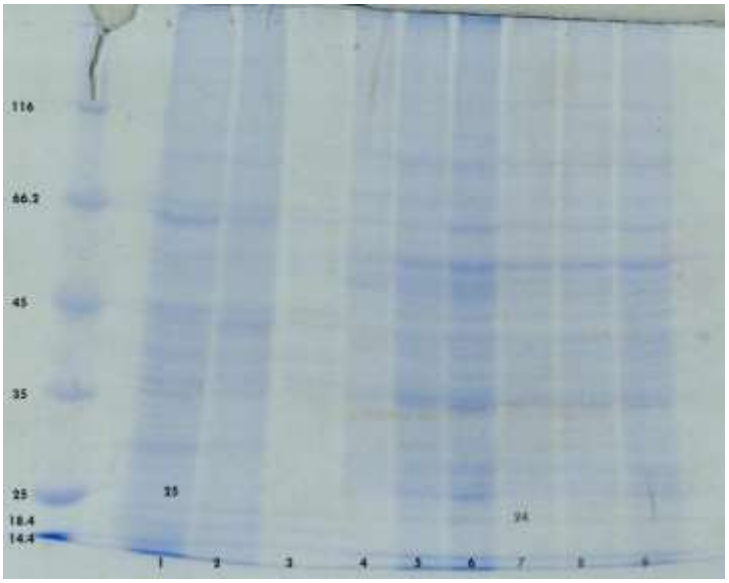

Fig. 1: Molecular weight of proteins (kDa) from the extracts of parasite and liver tissues in SDS-PAGE. Lanes 1-3 are healthy liver samples. Lanes 4-6 are cystic liver samples. Lanes 7-9 are parasite samples (Protein marker, peqGOLD I, Lot-No. 64072)

Table 2: Identified proteins of parasite according to molecular weight by using protein database(http://web.expasy.org)

MW according to Ratio factor (Rf)	MW according to database	Protein
80.537	84.086	Acetyl choline transferase
	77.460	Smde D
75.857	72.271	Phosphoenolpyruvate carbaxylase
	75.392	UDP-N-acetyl-D-galactosamin: popypeptide-N0acetyl Galactosaminyltransferase
65.012	65.213	AntigenII/3
	65.261	CYtosckeleton/extrinsic tomembrane (eg10)
	65.465	TGR Thiroidoxinreductaseserin/Threonine-protein Phosphatase
	66.310	
59.429	59.187	NADHdehydrogenase subunit5
	59.522	ALP
	60.100,60.200,60.205,60.242,60.300	COX1
	60.301,60.304,60.318	∂-Glutamyl – transferase
	61.040	
52.751	51.389	RNA poly II (rpb2)
	54.705	AMP activatedproteinkinasea
	54.714	PhosphoenolpyruvateCarboxylase (pepk)
	54.849	α- amylase
	54.875	Ag5 (Serin-type endopeptidase activity)
47.863	45.978	NASH-ubaqiunoneoxidoreductase chain 4 (ND4) Enolase
	46.561	Antigen EG13
	46.689	NADH-ubiqiunone
	47.782	Ixidoreduvtase chain 4
	48.857	Elnogation factor 1-α(ef1a)
23.067	22.571	NADH-ubiqiunone Oxidoreductase chain 1(ND1)
	22.573	NADH-Ubiqiunone Oxidoreductase chain 1 (ND1)
	23.050	Ra I-like protein
	23.881	ZW5
	24.147	COX3
	24.226	Potative cysteine peptidase
	24.301	GST2
19.230	18.382	Arginine N-methyl
	18.502	Transterasel
	18.991	Actin-1 (ACT1)
	19.046	Calcineurine B(calB)
	19.356	Calcium B like protein
	19.677	22KDa antigen 5
	19.705	ATPase subunite 6
	19.733	ATP synthease F subunite 6
	19.747	ND1(atp6)
	19.881	CO1

Table 3: Identified proteins of sheep liver tissue according to molecular weight by using protein database(http://web.expasy.org)

MW according to Ratio factor (Rf)	MW according to database	Protein
65.012	65.675	Betacaroten axygenase2
	65.235	Polactin receptor (PRL-R)
59.429	60.623	Coatomer Protein Complex∂2
	59.230	Cytochrom P4501A1(CYP1A1)
52.751	52.026	NADH dehydrogenasesubunite4
	52.970	6phosphogluconate dehydrogenase
	53.025	Serine hydroxyl methyl transferase
47.863	45.985	α – 1 anti proteinase, α- 1 anti trypine
	46.716	Thyroid hormone receptor beta
	48.056	Corticostroid-binding globuline Serpin A6, Transcortin
23.067	23.272	Secreted phosohoprotein24
	23.601	Copperchaperone of SODI
	23.608	Cathestin L1 (CTSL)
	25.244	GST
19.230	19.121	NADH – ubiquinone oxidoreductase chain 6
	19.842	Cathelicidine2, (Bactenecine-5) (Bac5),(CaTHL2)

Discussion

Liver tissue is the most important source for protein synthesis and detoxification. Two major types of liver cells are hepatocytes and sinusoid cells.Function of hepatocytesmay be disturbed in the presence of infections (15). In our study the protein concentration of infected tissue was reduced. The reduction of protein synthesis as a result of hydatid cyst causes to decrease protein concentration.

GSTs can make up to 10% of cytosolic protein in some mammalian organs (16). Hepatic cells contain high levels of GSTenzyme which has been found to be an indicator of hepatocyte injury in transplantation, toxicity and infections (17).The hydatid cyst infection stimulates oxidative stress and toxin production in hepatic cells. From the point of biochemical defense view GST enzyme be able to neutralization of these toxins, therefore we expect to increase activity level of this enzyme. Increase of liver protein and GST in infected mice indicates the occurrence of oxidative stress in hepatocytes due to infection (18). α-glutathione S-transferase(GSTA) is distributed homogeneously in the liver tissue.Serum GSTA is a more sensitive marker than transaminases (Alanine aminotransferase, Asparatate aminotransferase and Alkaline transferase) for monitoring and as an early analystof hepatic damage(19). Therefore GST activity difference between healthy and infected host liver tissue could be concerned for hydatid cyst diagnosis. However, other infections cause to hepatocyte damage, thus GST elevation must be evaluated with other clinical and paraclinical parameters of hydatid cyst disease.

Mammalian cytosolic GSTs are dimeric, with both subunits being from the same class of GSTs, although not necessarily identical. The monomers are approximately 25 kDa in size(20). GST enzyme molecular weight of parasite is reported 24-27 kDa (13).In this research protein bands with 24kDa and 25kDa were found in parasite and liver tissues respectively. This protein is very important from parasite survival point of view.

Conclusion

GST activity in cystic liver tissue could be concerned as a biomarker for hydatid cyst diagnosis with other parameters of hydatidosis.

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