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Research Article

EVALUATION OF MATRIX METALLOPROTEINASE (MMP) ACTIVITY IN CYSTIC FLUID OF *CYSTICERCUS TENUICOLLIS* FROM GOATS THROUGH GELATIN ZYMOGRAPHY

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ABSTRACT
Cysticercus tenuicollis cysts (a larval stage of *Taenia hydatigena*) were collected from goats slaughtered at local abattoir and were washed thoroughly with PBS (pH 7.4). The cyst fluid was aspirated, centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatants were used for further study. On gelatin zymographic analysis, it was observed that prominent bands at 220 kDa MMP-9, 92 kDa MMP-9 and 72 kDa MMP-2 in the cystic fluid of *Cysticercus tenuicollis*. The 135 kDa MMP-9, was observed as a fainter band. Among the four bands, 92 kDa MMP-9 band was showing the greatest gelatinolytic activity. The 72 kDa MMP-2 band was very prominent in cystic fluid of *Cysticercus tenuicollis* and found along with its active forms (62 kDa) as doublets. The relative amount of 92 kDa MMP-9 band was found to four times greater than that of 72 kDa MMP-2.

Keywords*: Cysticercus Tenuicollis, Cystic Fluid, Gelatin Zymography, MMP, Goats*

INTRODUCTION
Matrix metalloproteinases (MMPs) are a family of enzymes, comprising at least 18 members of enzymes, capable of degrading Extracellular matrix (ECM) during several physiological and pathological conditions. (Hu *et al.,* 2007). MMPs were considered only for the ability to degrade extracellular matrix (ECM) molecules (e.g., collagen, laminin, fibronectin) and to release hidden epitopes from the ECM. MMP-2 and MMP-9 are endopeptidases of the MMP family produced by neutrophils, macrophages and monocytes, having a significant effect on immunity. Involvement of MMPs activity, particularly, gelatinases in both protozoan and helminth infections is evident.

Host invasion and tissue migration of several nematodes have been linked to the expression and release of parasite-derived proteases. In nematodes, MMPs are the proteases which are thought to play an important and essential role in these migratory and invasive phenomena (McKerrow *et al.,* 1990). Multiple enzyme activities of MMPs with various molecular weights in different helminthiasis was noticed. MMP mediated histolysis of skin and intestinal walls through substrate impregnated zymographic analysis of extracts and ES products of different nematode parasites (Tort *et al.,* 1999) and degradation of ECM proteins was observed. However, the works on cystic fluid of *Cysticercus tenuicollis* are very scanty. Hence, the present work was carried out to study the gelatinase activity in cystic fluid of *Cysticercus tenuicollis* through gelatin zymography.

MATERIALS AND METHODS
The *Cysticercus tenuicollis* cysts were collected goats slaughtered at local abattoirs in Orathanadu, Pattukkottai and Thanjavur areas. The cysts were washed thoroughly with PBS (pH 7.4). and after careful preservation in PBS, the positive samples were used for further study. For preparation of cystic fluid antigens, the fluid was aspirated from the cyst with the help of a sterilized syringe and needle and collected directly in centrifuge tube. It was centrifuged at 10,000 rpm for 15 minutes at 4°C (Skeurman and Hillard, 1966). The supernatants thus collected were stored at -20 °C till further use. The protein content was estimated by Lowry's method (1951). Gelatin zymography of cystic fluid was carried out as

per the method of Heussen and Dowdle (1980) with some modifications and the procedure was as follows. SDS-PAGE was carried out, as described by the method of Laemmli, (1970). The resolving gel (8%) was co-polymerized with 0.3% gelatin solution (final concentration of gelatin in gel was 0.15%) and the electrophoretic run was carried out at 100 V until tracking dye reaches the bottom. Then, renaturation was carried out with renaturation solution (2.5% Triton X -100) for 3 hours on a mechanical shaker with mild agitation. Then, developing was carried out by incubating the gel in developing buffer (10 mM $CaCl_2$, 0.15 M NaCl and 50 mM Tris (pH 7.5))for 18 hours at 37ºC and then stained with 0.2 % Coomassie blue for 2 hours, followed by destaining for 1 hour with destaining solution and then, further destaining was carried out with distilled water. Then, calibration of the gelatin zymogram was carried out with human capillary blood gelatinases, as per the procedure suggested by Makowski and Ramsby (1996). A drop of human capillary blood (15-20 µL) was obtained by fingerstick puncture and placed in a tarred polypropylene tube. The weight of the blood was determined in an analytical balance and 20 volumes of non-reducing Laemmli buffer was immediately added. The sample was then vortex mixed (30S) and aliquots stored (-20°C) and the preparation were found to be stable for at least 3 months.

RESULTS AND DISCUSSION

The presence of MMP activity in the cystic fluid was assessed by gelatin zymography as shown in the following figure.

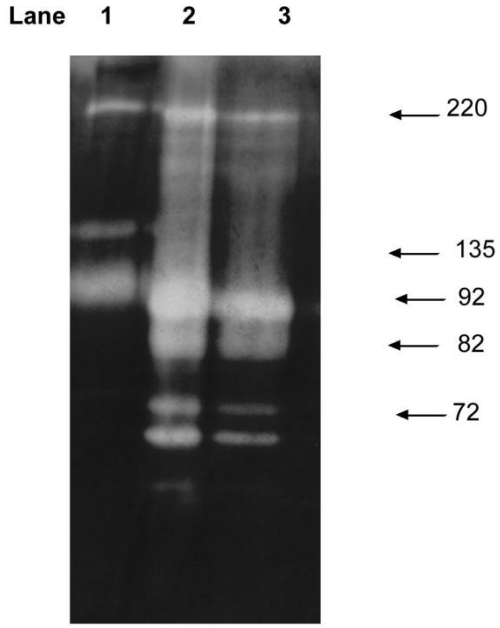

Figure 1: Gelatin zymography of the cystic fluid of *Cysticercus tenuicollis*
Lane 1- Human capillary blood gelatinases (MW in kDa)
Lane 2- Cystic fluid (50 micorliters)
Lane 3- Cystic fluid (25 microliters)

Gelatin zymography revealed the presence of prominent bands at 220 kDa MMP-9, 92 kDa MMP-9 and 72 kDa MMP-2 in the cystic fluid of *Cysticercus tenuicollis*. The 135 kDa MMP-9, was observed as a fainter band. Among the four bands, 92 kDa MMP-9 band was showing the greatest gelatinolytic activity. The 72 kDa MMP-2 band was very prominent in cystic fluid of *Cysticercus tenuicollis* and found along with its active forms (62 kDa) as doublets.

The relative amount of 92 kDa MMP-9 band and to that of 72 kDa MMP-2 was 4 and the 72 kDa band in both the forms of pro and active forms were observed as distinct bands with a clear active form of MMP-

2 (62 kDa). The gelatinolytic activity of 220, 92 and 72kDa proteinases were at least five times higher. A band at 220 kDa was appearing as single band having more gelatinolytic activity than that of human standard 220 kDa band. Below the 220 kDa band, the fainter bands were observed at 180, 160 and 40 kDa sizes.

A doublet containing pro and active form of MMP-9 differing in size of 10 kDa (92 kDa and 82 kDa bands) was observed. All the three forms of MMP-9 and MMP-2 were catalytically active. The gelatinase activity of MMP-9 and MMP-2 was inhibited by 10 mM EDTA al 1, 10-phenanthroline.

The presence of strong gelatinolytic activity in the cystic fluid antigens of *Cysticercus tenuicollis* was authentically confirmed for the first time.

The presence of MMP-9 and MMP-2 in human serum was already documented in parasitic infections in the Central Nervous System (Bruschi and Pinto, 2013). and the authors reported the presence of both 220 kDa and 95, 82 of MMP-9 and 72 kDa of MMP-2 in patients with neurocysticercosis as well as in healthy control.

The gelatinase activities in ES product of *Angiostrongylus cantonensis* were well documented and it was suggested that these MMPs secreted by larvae could be associated with parasites spreading and pathogenesis in host. They further proposed that the MMPs were secreted by the parasite not by the host (Lai *et al.*, 2005). In our study, the gelatinase activity of MMP- 9 was purely from cystic fluid of *Cysticercus tenuicollis* as the cystic fluid was subjected into high speed centrifugation, the possibility of liberation of MMP- 9 by the leucocyte was eliminated.

In our study, the presence of both pro and active forms of MMP- 2 and an increased activity of MMP- 9 could be related to the activator role of MMP- 2 on pro MMP. The ratio of MMP-9 /-2 was observed as 1.5 in cystic fluid of *Cysticercus tenuicollis* suggesting the active role of MMP- 2 in regulating the activity of MMP -9. The presence of very low level of MMP 2 to that higher level of MM -9 is a suggestive of the regulatory role of MMP- 2 on MMP- 9. The ratio of MMP- 9 to MMP- 2 was decreased from its normal state to a diseased condition (Freidman *et al.*, 1995). Further studies involving advanced confirmatory techniques like Gas Chromatography-Mass Spectrometry (GC-MS) are highly warranted to reassure the nature of these MMPs in cystic fluid. Much more work is also needed to find out the activity of gelatinases in other cestode infections.

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