

A COMPARATIVE GELATIN ZYMOGRAPHY OF MATRIX METALLOPROTEINASES IN SERUM OF NATIVE SHEEP BREEDS OF TAMIL NADU

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Abstract: A comparative study was undertaken to study the gelatinase activity through gelatin zymography in serum of various native sheep breeds of Tamil Nadu. Six adult male and female native breed sheep viz. Ramnad white, Sevvadu, Kilakaraisal, Vembur, Mecheri and Pattinam were selected. Blood samples were collected, homogenated, filtered and subjected to gelatin zymography. In gelatin zymography, the presence of three prominent bands at 220, 92 kDa of MMP-9 and 72 kDa of MMP-2 were obviously observed for all sheep breeds. All the three forms are proteolytically active, degraded the gelatin. Both latent forms of MMP-2 and MMP-9 were exhibited and the latent bands were observed as thicker bands than the active form. The intensity of 72 kDa of MMP-2 was 3-5 times higher than 92 kDa of MMP-9. The level of expression of 72 kDa of MMP-2 was constant as compared to 92 kDa of MMP-9. In all the groups, 135 homodimer or MMP-9 was also observed. Further, in Mecheri and Pattinam groups showed maximum gelatinolytic activity as compared to marker by showing more intensity in 72 kDa of MMP-2. In addition, the 62 kDa of MMP-2 was also observed. The level of expression of 72 kDa band was constant compared to that 92 kDa. It was concluded that there was no-significant difference between the expression of MMP-9 and MMP-2 in both the sexes of each breed. Further, there is more up regulation of MMP-2 mediated through MMP-9 activity observed in sheep serum. It was inferred that the expression of MMP-2 and MMP-9 were to be correlated with reproductive status of individual animal as MMP played extensive role in tissue remodelling and extra cellular degradation.

Key words: Metalloproteinases, Sheep breeds, Gelatinase

INTRODUCTION

Sheep is a multi facet animal known for its utility like wool, meat, milk, skin and manure, forms an important component of rural economy particularly in the arid and semi-arid and mountainous areas of country. The total sheep contributes around 12.71% of the total

livestock population. The total number of sheep in the country as per 2012 Census is 65 millions [1]. Matrix Metallo Proteinases (MMP) are a group of structurally related proteins that degrade extra cellular membrane (ECM) and basement membrane (BM) components and their specific evolutionary DNA sequence in a zinc dependent manner at

physiological pH [2]. Currently, more than 25 different types of MMPs have been identified among vertebrates and the majority is expressed and has similar functions in humans. Nevertheless, MMPs have many similarities in their structure, sharing considerable homology within their major domains including signal peptide, propeptide, catalytic and hemopexin like domains [3]. MMP activity is regulated at the level of gene transcription and the synthesis of pro – MMPs. Most proteases are classified as serine, cysteine, aspartic or matrix metalloproteinases (MMPs) according to their catalytic mechanism and inhibitor sensitivities [4]. The MMPs or matrixins were first described by Gross and Lapiere [5] and are mostly known by their ability to degrade components of the ECM. This family of endoproteases has been considered essential in a number of normal physiologic processes as well as pathological events. Hence, the present study was conducted to find out the presence of gelatinases in various sheep breeds of Tamil Nadu.

MATERIALS AND METHODS

The proposed study was carried out at the Department of Veterinary Physiology and Biochemistry, TANUVAS - Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India. The institute is located at an altitude of 30m feet above the mean sea level, at a latitude of 10.6° North and a longitude of 79.3° east.

On each breed, four healthy animals of Ramnad white, Sevvadu, Kilakaraisal, Vembur, Mecheri and Pattinam were selected. Blood samples from each animal were collected in a heparinised vacutainer during early morning before feeding the animals. The samples were transported to the laboratory immediately and evaluated for protein content using standard procedure of Lowry's method [6]. The blood samples were centrifuged at 3000 rpm for 15 min and the separated serum was analyzed for protein content by photometric estimation of blue colour by using spectrophotometer. The standard curve was built by using various concentrations of bovine serum albumin (BSA) as standard. The serum samples were stored at -20°C for further analysis.

The serum samples were subjected to modified SDS-PAGE (modification of Laemmli's method [7] carried out by Heussen and Dowdle [8] by the addition of

co-polymerizing substrate of gelatin (0.3%) (final concentration was 0.15% to the resolving gel (8%). The samples were electrophoresed at 100V for 20 min. Renaturation was carried out with 2.5% triton X-100 for 3 hrs on a mechanical shaker with a mild agitation. Then developing was done by incubating the gel in 10 mM CaCl₂, 0.15 M NaCl and 50 mM Tris pH 7.5 for 18 hrs at 37°C. The gel was stained with 0.25% coomassie brilliant blue for 2 hrs, followed by destaining with destaining solution for 1 hr and finally the gel was washed with distilled water.

Human capillary blood gelatinase was used as the standard marker for comparing the zymogram bands as per the procedure carried out by Makowski and Ramsby [9]. Using a fingerstick puncture, the blood was collected from a capillary and weighed in a tarred polypropylene tube using analytical balance. Samples were added with 20X volume of Laemmli buffer and thoroughly mixed. Then the aliquots were stable for 3 months at -20°C.

RESULT AND DISCUSSION

On gelatin zymography, it was confirmed that MMP-2, MMP-9 were present in the serum samples of all sheep breeds, and the results were depicted in Fig. 1.

In gelatin zymography, the presence of three prominent bands at 220, 92 kDa of MMP-9 and 72 kDa of MMP-2 were obviously observed in both the sexes of all sheep breeds. All the three forms are proteolytically active and fully degraded the gelatin. Both the latent forms of MMP-2 and MMP-9 were exhibited and the latent bands were observed as thicker bands than the active form. Similar results were observed by various authors [10-13].

In our earlier study, Pandiyan et al. [11], Balamurugan et al. [14] and Prakash Krupakaran et al. [13] observed 220, 92, and 135 kDa of MMP-9 and 72 kDa of MMP-2 was observed in serum and compared with the other species of domestic animals. In the present study, the existence of gelatinase was confirmed in serum of sheep breeds. Similarly, in lamb model, elevated level of MMP-9 (220 kDa; dimer), pro-MMP-9 (92 kDa; monomer) and pro MMP-2 (72 kDa) were detected after the implantation of tissue engineered vascular graft by Cummings et al. [10]. The elevated level of MMP-2 and MMP-9 was observed due to remodelling of tissues during

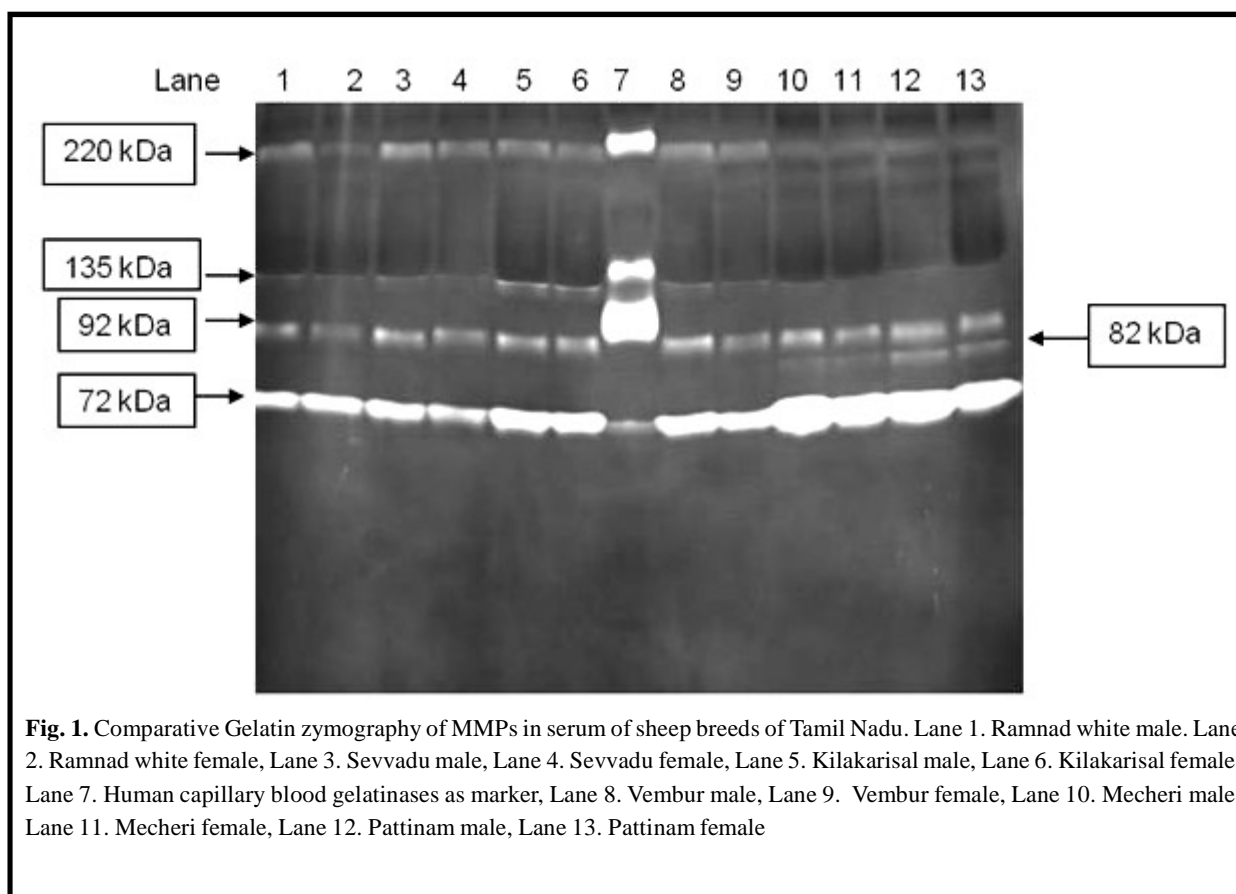


Fig. 1. Comparative Gelatin zymography of MMPs in serum of sheep breeds of Tamil Nadu. Lane 1. Ramnad white male, Lane 2. Ramnad white female, Lane 3. Sevvadu male, Lane 4. Sevvadu female, Lane 5. Kilakaraisal male, Lane 6. Kilakaraisal female, Lane 7. Human capillary blood gelatinases as marker, Lane 8. Vembur male, Lane 9. Vembur female, Lane 10. Mecheri male, Lane 11. Mecheri female, Lane 12. Pattinam male, Lane 13. Pattinam female

implantation. In another study by Ilhan et al. [15] demonstrated that MMP-9 expression was increased in sheep during Listerialmeningoencephalitis infection as compared to normal sheep.

In the present study, MMP-2 (72 kDa) was very prominent in all the species as compared to human markers (lane 7 and 8). The intensity of 72 kDa of MMP-2 was 3-5 times higher than 92 kDa of MMP-9. The level of expression of 72 kDa of MMP-2 was constant as compared to 92 kDa of MMP-9. In all the groups, 135 homodimer or MMP-9 was also observed.

Wilson et al. [16] examined levels of MMP's in post-MI (myocardial infarction) in sheep model and found that there was a significant rise in MMP expression above the normal level with respect to pathological remodelling. Hence, the MMP's which were present in normal level increased during external pressure or during internal physiological changes.

In another study, Bannikov et al. [17] reported the serum gelatinase (MMP-9) activity was increased in acute septicemic and chronically ill animals as

compared normal animals. They further concluded that the level of MMP-9 in acute and chronic metabolic disease was identical and normal in healthy animals.

In the present study, the latent form of 72KDa MMP-2 and active form 62KDa MMP-2 band were observed was in concordant to Daniele et al. [18]. In this study the expression of MMPs in serum of patients with metastatic and non metastatic breast cancer and compared with controlled group and reported that MMP-2, MMP-9 were significantly higher in metastatic breast cancer than non metastatic breast cancer, and in normal control.

Further, in Mecheri and Pattinam groups showed maximum gelatinolytic activity as compared to marker by showing more intensity in 72 kDa of MMP-2. In addition, the 62 kDa of MMP-2 band was observed. The level of expression of 72 kDa band was constant compared to that 92 KDa. Similarly, Chegeni et al. [19] observed that total MMP-9 was higher in dogs affected with dilated cardiomyopathy (DCM) than the control group and further concluded that the active form of MMP-9

was detected only in patients with DCM. Foda and Zucker [20] carried out studies in abdominal aortic aneurysm (AAA) patients and healthy patients, predominate form was active MMP-9, active MMP-2 found higher in AAA patients than the normal population.

It was inferred that the expression of MMP-2 and MMP-9 was to be correlated with the reproductive status of individual animal as MMP played extensive role in tissue remodelling and extra cellular degradation. It was concluded that there was no-significant difference between the expression of MMP-9 and MMP-2 in both the sexes of each breed. Further, there is more up regulation of MMP-2 mediated through MMP-9 activity observed in sheep serum.

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