Original article

Comparative study of the kidneys from dystrophic mice

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Abstract

The Duchenne Muscular Dystrophy (DMD) is a recessive genetic disease linked to chromosome X. This disease is characterized by an absence or dysfunction in the expression of dystrophin. Experimental models *mdx* are widely used for the development of research addressing the DMD. The objective of this research is to contribute to a detailed study of possible renal morphological changes resulting from DMD. We used five pairs of kidneys from *mdx* mice and five from normal mice, which were subjected to measurement, light microscopy, and scanning electron microscopy. The morphological findings of kidneys from *mdx* mice are within the patterns described in animal studies with severe dehydration, which exhibit signs of diffuse hemorrhage in the cortical and medullary area, while the glomeruli in the cortical region showed a decrease in urinary space, located between the Bowman's capsule and the inner cell mass of the glomeruli. However, future experiments with animals in different ages can assist in the proving of the morphological changes found here.

Keywords: dehydration, Duchenne Muscular Dystrophy, glomeruli, kidneys.

1 Introduction

The Muscular dystrophy is a term often used to refer to the primary disease from skeletal muscles, resulting in progressive degeneration, limited regeneration and fibrosis of the muscle fibers (BERGMAN, INZANA, MONROE et al., 2002). These dystrophies form an heterogeneous group of genetic diseases that generate an increasing loss of muscle fibers caused by mutation or deletion of structural proteins from skeletal muscles which can trigger systemic effects (WALLACE and MCNALLY, 2008).

From the systemic effects in patients with Duchenne Muscular Dystrophy (DMD), Gatti (2007) cites renal failure as a secondary cause. In these individuals, renal failure is established when three quarters of the nephrons from one or both kidneys are affected, with no more symptom of compensatory hypertrophy.

The kidneys are organs formed by the renal tubules, surrounded by fat and partially covered by peritoneum on its ventral surface. Anatomically, the lateral margin has a convex curvature, and the medial, nearly straight, where is an opening called hilum, through which, blood vessels, nerves from kidneys and ureters are communicating (DYCE, SACK and WENSING, 2010).

These organs have an important role in blood filtration, ensuring the homeostasis of body fluids, through mechanisms of glomerular filtration, reabsorption and tubular secretion performed in nephrons (GATTI, 2007).

Proteinuria, glucosuria, and cylindruria are some changes that affect renal function, being that evaluation of renal epithelial cells in the urinary sediment may be indicative of this commitment (GATTI, 2007).

Several preclinical researches related to DMD, used *mdx* mice to evaluate the impairment morphophysiological from this disease, although the disease is less aggressive in this species, the frequency of the use of this model is justified by the ease reproduction and manipulation of its genome (COLLINS and MORGAN, 2003; SANTOS, OLIVEIRA, BERTASSOLI et al., 2013).

To better understand the impact of possible changes in systemic DMD, become necessary additional studies of all organs that can be affected by muscular dystrophy. Therefore, the aim of this research is to contribute with a detailed study of renal morphological changes from mdx mice by associating to possible dysfunctions resulting from dystrophy.

2 Material and Methods

2.1 Animals

We used five normal mice (*Mus musculus*) and five *mdx* mice, males, aged five months, donated from Science Biomedical Institute (ICB) by São Paulo University, São Paulo.

2.2 Euthanasia

The animals were euthanized by anesthetic overdose, as standardized by the bioethics committee of the Faculty of Veterinary Medicine from USP.

2.3 Microscopic analysis

The kidneys were collected with the aid of tweezers and bistoury and analyzed macroscopically. The measurements were performed with a caliper and following the kidneys were weighed on a precision balance and stored in bottles containing paraformaldehyd solution 4%

The photo documentation was performed using a Sony Mavica 3.2.

2.4 Light microscopy

The samples were processed by routine histological techniques, starting with an increasing concentrations of ethanols (70 to 100%) and diaphanized in xylene, followed by paraffin embedding. The blocks were cut on a Leica microtome RM 2165, and then stained with hematoxylin/eosin.

The documentation was performed on a Leica DM 2000 photomicroscope.

2.5 Scanning Electron Microscopy (SEM)

Tissue fragments were fixed in glutaraldehyde (Propylene oxide EM Grade - Polysciences, Inc., USA), washed in 0.1 M phosphate buffer at a pH of 7.4, and post-fixed in 1% osmium tetroxide (Spurr's Kit – Electron Microscopy Sciences Co., USA). Then, tissue fragments were dehydrated in a graded ethanol series (50%, 70%, 90%, and 100%) and dried in a critical point dryer (Balzers PCD 020). Tissues fragments were fixed in metal supports (stubs) and then sputter coated with gold (Emitech K550). The analysis was performed on an electron microscope, model Leo 435 VP.

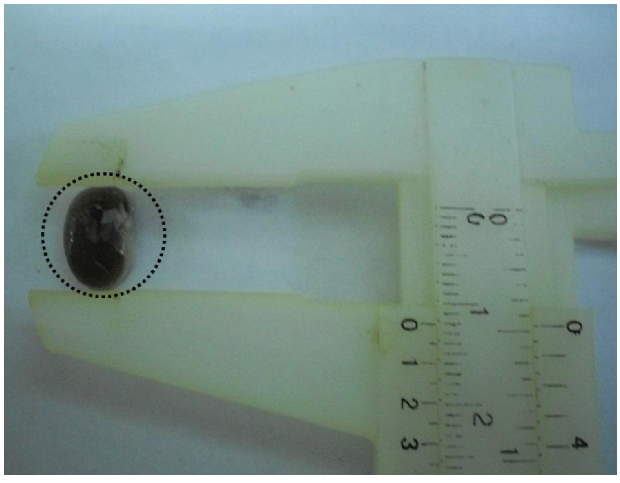

Figure 1. Morphometry, performed with the aid of a caliper.

2.6 Nomenclature

The nomenclature used was referred to according to the International Committee on Veterinary Gross Anatomical Nomenclature (INTERNATIONAL..., 2005).

3 Results

3.1 Macroscopic analysis

In both groups, the length of the left kidney (Figure 1) was relatively larger than the right kidney. The measures of the kidneys of animals of this experiment is displayed in Table 1.

The weight of the kidneys and the relationship between the affected and control group showed minor changes where the right and left kidneys of the control group had heavier than the kidneys from affected group. In both groups, the right kidneys were heavier than the left kidney (Table 1).

3.2 Microscopic analysis

The renal histology of the kidneys from affected group showed a decrease in the space located between Bowman's capsule and the inner cell mass of the glomeruli, compared to the normal group (Figure 2 and 3).

In SEM analysis (Figure 4), no abnormal structure was evident. Normal and affected kidney, showed glomeruli and proximal and distal convoluted tubules, without change.

4 Discussion

In the animals of this study, the right kidneys were located in the right renal fossa of the caudate lobe of the liver and were positioned most cranial than the left and morphologically within of the normality, according with that described in the domestic animals (DYCE, SACK and WENSING, 2010).

The morphometry of the kidneys from affected animals are similar when compared to the control group (OLIVEIRA, KERSUL, PRADO et al., 2011)

Macroscopic morphology of the kidneys from normal and dystrophic mice showed no significant variations among themselves nor with that described for other mice (OLIVEIRA, KERSUL, PRADO et al., 2011).

The renal capsule appears as a smooth and thin layer, while the cortex is thicker than medullary area. The medullary diverticulum is divided into segments as described in literature (KONDE, WRIGLEY, PARK et al., 1984).

In both groups the length and thickness of the left kidney is relatively larger than the right, but the kidneys from affected and control group does not show significant changes.

The right and left kidneys of control animals are heavier than the kidneys from mdx. In both groups the right kidney

Table 1. Mean and standard deviation from weight, length and thickness of the kidneys from normal mice and *mdx* mice.

kidney	Weight kg	Standard deviation	lenght cm	Standard deviation	Thickness cm	Standard deviation
Normal right	0.275	±0.007	1.00	±0.070	0.58	±0.044
Affected right	0.274	±0.009	0.96	±0.114	0.54	±0.054
Normal left	0.250	±0.020	1.08	±0.083	0.54	±0.054
Affected right	0.249	±0.021	1.14	±0.089	0.56	±0.054

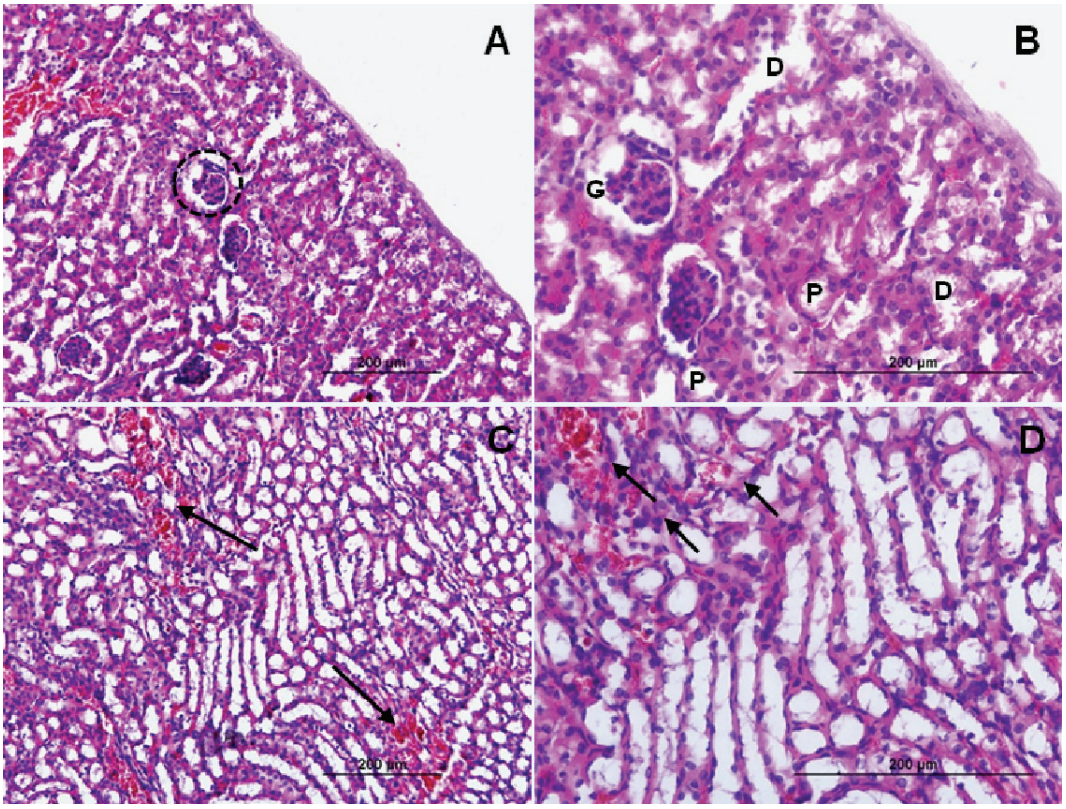

Figure 2. Photomicrograph of kidneys from normal mice. A) glomeruli (circle) without changes. B) proximal (P) and distal (D) tubules and glomeruli (G) with intact nuclei. C) hemorrhagic areas (arrow). D) Hemorrhagic areas (arrow) bar: 200µm. HE.

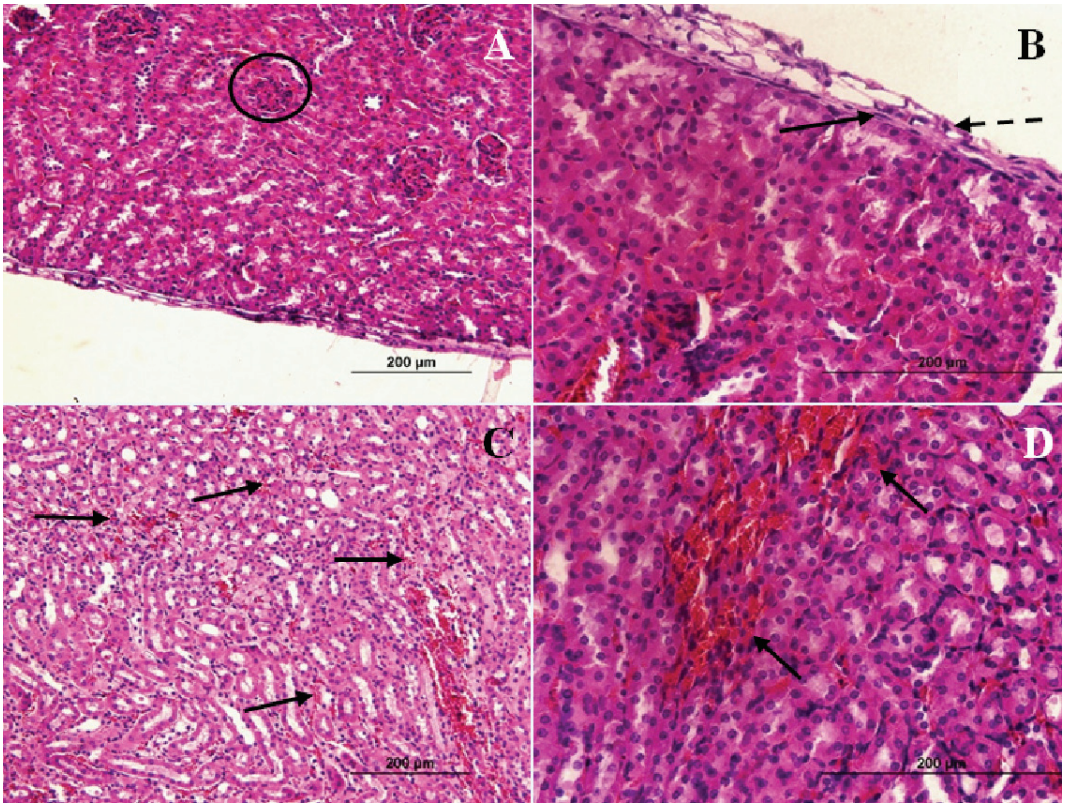

Figure 3. Photomicrograph of kidneys from dystrophic mice. A) The glomeruli presenting reduced urinary space (circle) in the cortical zone. B) The outer layer of the capsule (dotted arrow) and inner layer of the capsule (arrow) from cortical zone. C) Medullary zone showing hemorrhagic areas (arrows). D) Medullary zone presenting hemorrhagic areas (arrows). Bar: 200µm. HE.

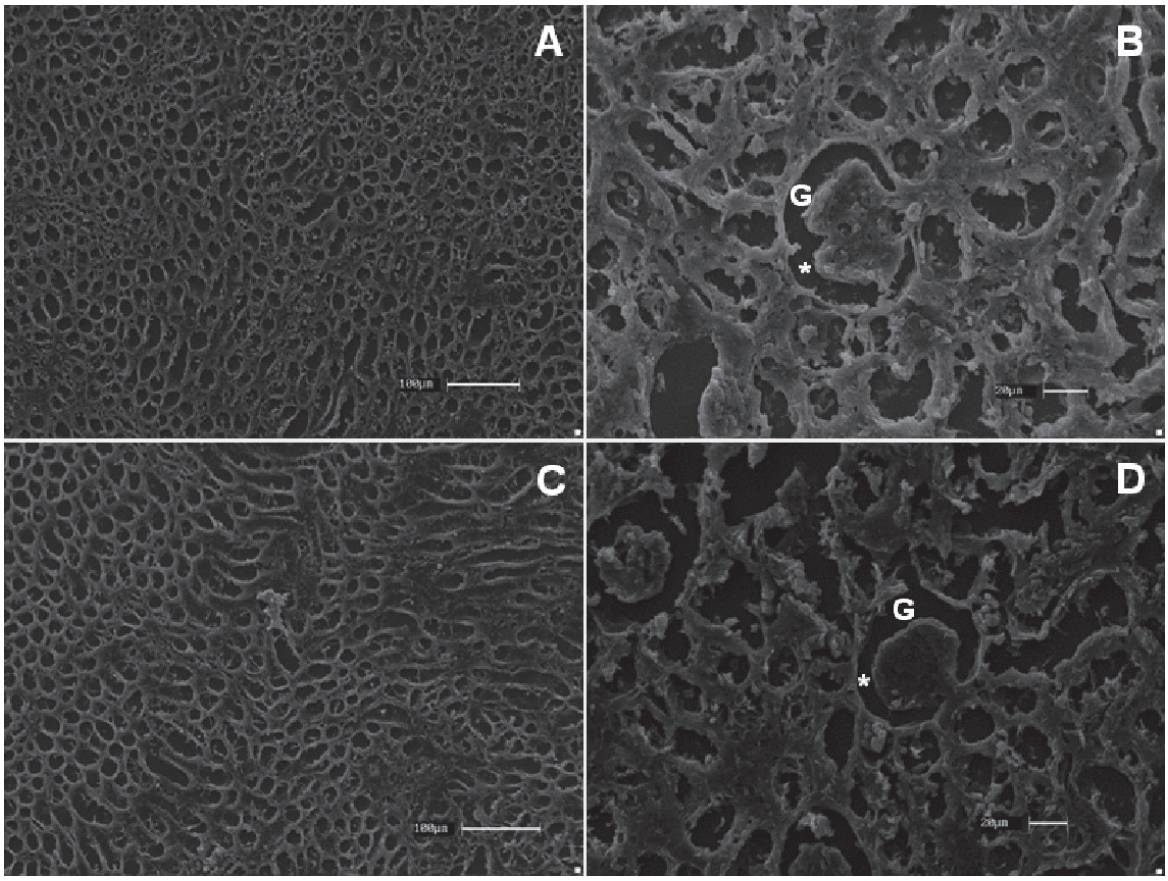

Figure 4. SEM from normal mice kidney (A and B) and mdx (C and D). A) No structural changes in distal and proximal convoluted tubules. B) Glomerulus (G) and urinary space (*). C) No structural changes. D) Glomerulus (G) and urinary space (*), both of which have no changes.

are heavier than the left kidney. Some studies have shown that reducing in weight of the kidneys may be characteristic of a possible renal atrophy, but in this study we cannot say that (GATTI, 2007; OLIVEIRA, KERSUL, PRADO et al., 2011).

Microscopically, the kidney of dystrophic animals (Figure 3) showed signals of diffuse hemorrhage in the cortical and medullary zone, while the glomeruli in the cortical zone showed decrease in the urinary space, located between the Bowman's capsule and the inner cell mass of the glomeruli.

Oliveira, Kersul, Prado et al. (2011) in an experiment with mice alcoholism-induced, cited similar findings to the affected group studied here. This decrease in the urinary space in the glomeruli may be linked with the feeds or the state of dehydration of the animals (GATTI, 2007; OLIVEIRA, KERSUL, PRADO et al., 2011). The state of dehydration found here corroborates with the findings in weighing from the kidneys of affected animals.

The SEM of the kidneys from dystrophic mice does not show changes, but by light microscopy, it is observed that the lumen of the convoluted tubules is reduced in dystrophic animals. This finding is related to an intense vacuolization in the tubular cells. The accumulation of vacuoles within or adjacent to the cells is caused by a hydric degeneration, caused by hydroelectric disorder, caused by accumulates of cell electrolytes (GATTI, 2007; OLIVEIRA, KERSUL, PRADO et al., 2011).

According Haenggi, Schaub and Fritschy (2005), the renal structure depends primarily on protein complexes such as dystrophin, utrophin, and its isoforms, especially Dp 71 which is decreased in the kidneys from *mdx* mice, and that these proteins are essential for maintaining the normal structure of epithelial cells from membranes of kidneys.

Thus, future immunohistochemical studies may elucidate possible morphological and functional changes caused by this decrease from the lumen of distal and proximal convoluted tubules.

5 Conclusion

The renal morphology of the animals here studied shows that changes may be related to dehydration. However possible associations with a renal failure pre-established require further study.

Further studies about animals with different ages will allow better morphological evaluations of the kidney.

Future studies on renal failure in DMD, need to take into consideration the age of the animals since the DMD is a progressive disease. In addition, other factors would be also important, such as evaluation of urea and creatinine serum levels associated with other examinations, such as, urinalysis tests, immunohistochemistry and microscopy electron transmission.

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