Computer-Aided Image Analysis in Neuro-Pathology

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Abstract: - ***Introduction*:** We propose a simple new method for quick quantitative assessment of severity of Duchenne Muscular Dystrophy (DMD). The method is based on computer-aided analysis of histopathological images obtained from biopsies of dystrophic muscles. Information contained in such slides that results from staining tissues from biopsies with different dyes helps to reveal details that might otherwise not be apparent. ***Materials and Methods*:** The method is based on **c**olor **f**iltration **p**ixel-by-**p**ixel of the whole virtual slides (*CFPP method*) and semi-quantitative evaluation of morphological structure of the muscular tissue. All calculations are performed in Matlab. ***Results*:** We demonstrated that with appropriate image preprocessing CFPP method helps in neuropathological diagnosis in assessing severity of DMD or differentiating DMD from normal muscle. We have used similar method for assessment and grading of DLBCLs in oncopathology. ***Conclusions*:** The presented method allows to distinguish DMD from normal muscle tissue, as well as gives a possibility to evaluate the severity of DMD.

Key-Words: - digital pathology, biopsy, dystrophy, DMD, CFPP method

1 Introduction

Duchenne Muscular Dystrophy (DMD) is a severe genetic disease characterized by a progressive muscle weakness and loss in structural integrity of muscle fiber membranes, caused by deficiency of the dystrophin protein [1]. Respiratory complications and cardiomyopathies are common causes of DMD patients premature death .

Despite the introduction of a full range of genetic diagnostic tests and sophisticated techniques in modern pathology, interpretation of histopathological images obtained from muscle biopsies remains important in the daily practice of neuropathology since it can give indications of the severity and the rate of progression of neuromuscular disease.

Healthy muscle has a characteristic appearance, and is made up of closely-packed fibers, which are more or less evenly sized. Dystrophic changes on muscle biopsy include variation in fiber size, increased internal nuclei and increased connective tissue [2] and the presence of regenerating and degenerating fibers [3]. As the disease progresses the microscopic images will show a greater proportion of connective tissue. In later pathology the image shows an increase in internal nuclei and increased variability of myofiber size [4].

Digital pathology helps in neuropathological diagnostics [5]. Here we propose a simple and quick method of quantitative assessment of DMD. The method is based on computer-aided analysis of histopathological images obtained from biopsies of dystrophic muscles.

2 Materials and methods

In the present study, we analyzed 13 digital microscopic images of DMD muscle tissue. The images were obtained from 2 subjects in moderate (8 slides) and 2 subjects in advanced DMD stage (5 slides) at the Department of Neurology, Medical University of Warsaw. The images were acquired with x400 magnification, from original microscopy slides coming from biopsies. The biopsies were performed as a part of diagnostic procedure, so no IRB approval was required for current retrospective analysis. Additionally, we have analyzed 3 microscopic slides of normal muscle tissue.

The images were analyzed on a standard PC with Intel i7 dual core processor and 8GB RAM. All analyses were performed in MATLAB 2016b.

2.1 Standard method of specimen assessment

Among many features, severity of dystrophic muscles is assessed based on the amount and distribution of irregular myocytes, and the amount

of fatty and connective tissue in the microscopic images obtained from biopsy (*whole specimen*). When grading the severity of dystrophy, pathologist concentrates his/her attention on some fragments of the image. The evaluation based on the whole specimen is frequently compared to the evaluation in the manually chosen and magnified *regions of interests (ROIs)* containing *objects* (cells' nuclei) that are hematoxilin-stained (H) and so turned blue, and *objects* (myocytes) that are eosin-stained (E) and so turned pink (cf. Fig. 1.).

Manual grading, based on visual assessment, is frequently time consuming. It requires patience and accurate analysis of the chosen *ROIs*. Hence, our objective is to develop a new simple method for quick assessment of microscopic slides of dystrophic muscles.

2.2 CFPP method

Herein, we propose a new dedicated method for quick semi-automatic assessment of severity/stage of Duchenne Muscular Dystrophy (DMD) from dyes-stained microscopic images. The method is based on **c**olor **f**iltration **p**ixel-by-**p**ixel of the whole virtual slides (*CFPP method*) and semi-quantitative evaluation of morphological structure of the muscular tissue.

The method is simple and quick – it does not require either time consuming manual marking of artifact-free representative *ROIs* or complicated detection of myocytes to grade the dystrophic tissue. Instead, we propose to count the numbers of pixels belonging to the H-stained nuclei (L_H), to the E-stained myocytes (L_E), and to the E-stained connective tissue including sarcolemma and not stained regions belonging to the adipocytes (L_C) in the given whole specimen.

We define two quantities characterizing dystrophic changes that will be called the *connective tissue indices*:

$$CI_H = (L_C + L_H) / (L_C + L_E) \qquad (1)$$

$$CI_C = L_C / (L_C + L_E) \qquad (2)$$

The calculations are easily done with MATLAB. Each whole specimen is appropriately color filtered pixel by pixel. Color-coding of computer images most often uses RGB system - the color of a pixel is expressed as a triplet, *(r,g,b)* i.e. *(read, green, blue)*, each component of which can vary from *0* for the darkest one to *255* for the brightest one.

For a single .tiff image opened in MATLAB, we count pixels belonging to the nuclei that are H-stained: we choose pixels with *(r,g,b)* components

greater than minimum values further denoted with index *Hn,* but smaller than maximum values further denoted with index *HN, i.e.* pixels fulfilling the condition

$$(r_{Hn}<r<r_{HN}) \; AND \; (g_{Hn}<g<g_{HN}) \; AND \; (b_{Hn}<b< b_{HN}) \qquad (3)$$

To count pixels belonging to the E-stained connective tissue (adipocytes or fat cells) we choose pixels with *(r,g,b)* components greater than minimum values further denoted with index C*m* but smaller than maximum values further denoted with index *CM, i.e.* pixels fulfilling the condition

$$(r_{Cm}<r<r_{CM}) \; AND \; (g_{Cm}<g<g_{CM}) \; AND \; (b_{Cmc}<b< b_{CM}) \qquad (4)$$

In general, to count pixels belonging to the E-stained myocytes we choose pixels with *(r,g,b)* components greater than minimum further denoted with index *Em* but smaller than maximum values further denoted with index *EM, i.e.* pixels fulfilling the condition

$$(r_{Em}<r<r_{EM}) \; AND \; (g_{Em}<g<g_{EM}) \; AND \; (b_{Em}<b<b_{EM}) \qquad (5)$$

But quite often, like in the presented case, for the number of pixels belonging to E-stained myocytes, L_E, one can take all the remaining pixels in the image

$$L_E = L - L_H - L_C \qquad (6)$$

where *L* denotes the total number of pixels in the given specimen.

It is important that for at least one component, *r* or *g* or *b,* the intervals of values for pixels belonging to H-stained (2) and to E-stained (3, 4) structures should be completely disjoint. Such color filtration also filters out the remainings of the empty background and majority of artefacts.

When a fragment of a slide is viewed in MATLAB then clicking on a pixel shows *(r,g,b)* components of this pixel. To choose *Hn* values one clicks on several dark-blue pixels belonging to the nuclei, one writes down the shown *(r,g,b)* values, and for calculation of L_H one takes average value of the corresponding component as the limits in (2); similarly, to choose *HN* values one clicks on several light-blue pixels. To choose *Em* or *Cm* values one clicks on dark pixels belonging to myocytes or connective tissue, respectively; to choose *EM* or *CM* values one clicks on some light pixels belonging to myocytes or

connective tissue, respectively. One writes down the shown (r,g,b) values and for calculation of L_E or L_C one takes average values of the corresponding component as the limits in (4) and (5) (cf. Fig. 1.).
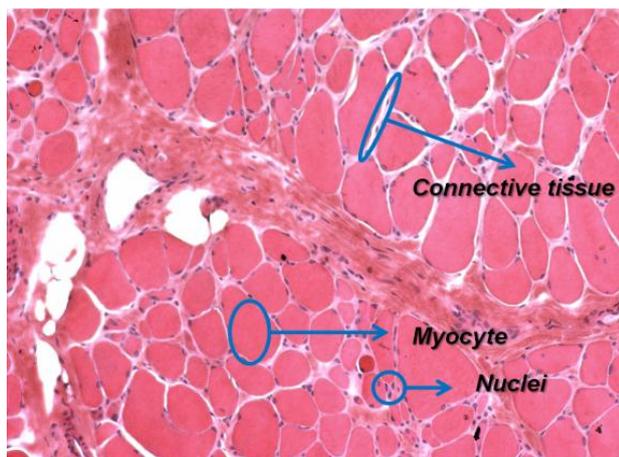

Fig. 1. Example of regions: Nuclei - H-stained, counted into L_H ; Myocyte – E-stained, counted into L_M ; Connective tissue – partially E-stained sarcolemma with not stained adipocytes, counted into L_C . These regions were used to establish the limits of (r,g,b) in (7)-(8).

In the presented case, to count the number of pixels belonging to H-stained nuclei, L_H , we choose the following (r,g,b) intervals:

$$(<100 , 200> , <50 , 80> , <115 , 160>) \qquad (7)$$

To count the number of pixels belonging to E-stained connective tissue, L_C, we choose the following (r,g,b) intervals:

$$(<205 , 255> , <100 , 255> , <165 , 255>) \qquad (8)$$

To calculate L_H , L_E and L_C the whole specimen may be subdivided into any number of disjoint parts or even read into RAM pixel by pixel. It is important since it makes possible to analyze specimens even on a PC with 16 GB RAM. L_H , L_E and L_C are used to calculate the connective tissue indices, CI (1),(2).

3 Results

We have calculated connective tissue indices CI_H and CI_C for 13 slides of 4 patients in two grades of DMD and for 3 images of normal muscles. Examples are shown on Figs. 2.-4. and mean values and standard deviation of the connective tissue indices moderate and advanced DMD compared to normal muscle are shown on Figs. 5.-6.

Index CI_H (1) better differentiates stages of DMD than CI_C index (Figs, 5.-6.) but further research are needed.
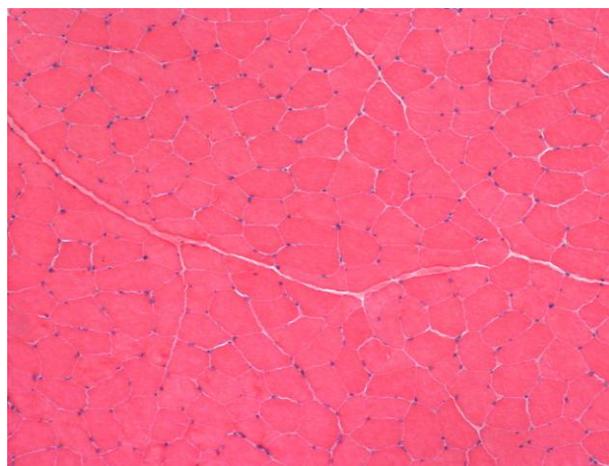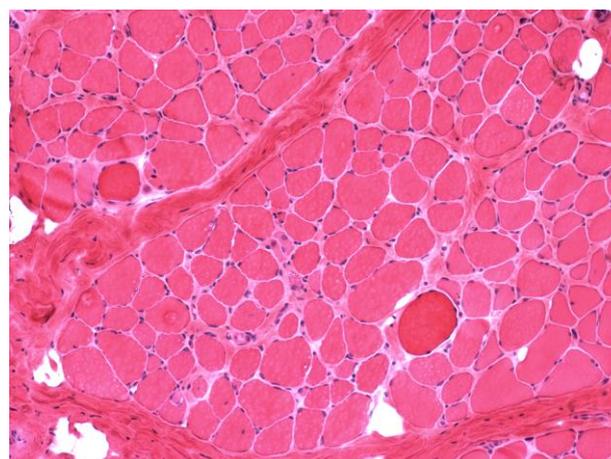

Fig. 2. Normal muscle. $CI_H = 0.032 \pm 0.001$.

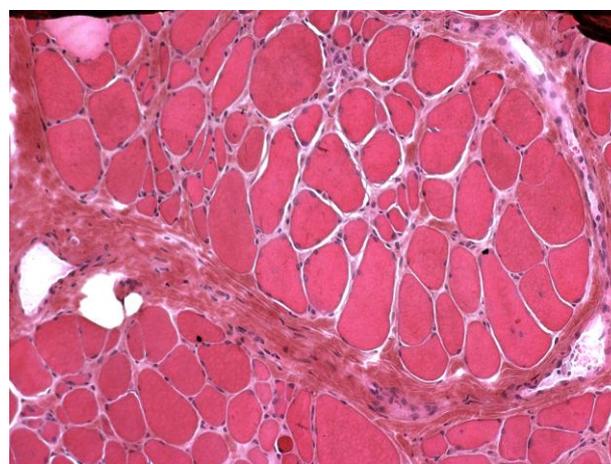Fig. 3. Moderate stage of DMD, $CI_H = 0.17 \pm 0.03$.

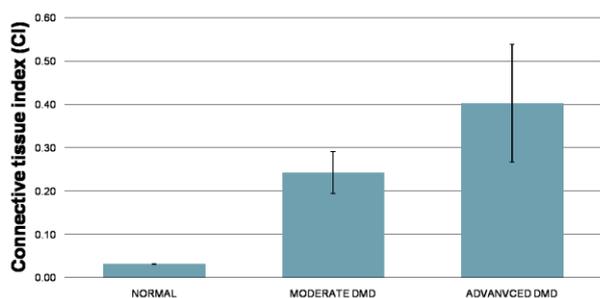Fig. 4. Advanced stage of DMD, $CI_H = 0.32 \pm 0.09$.

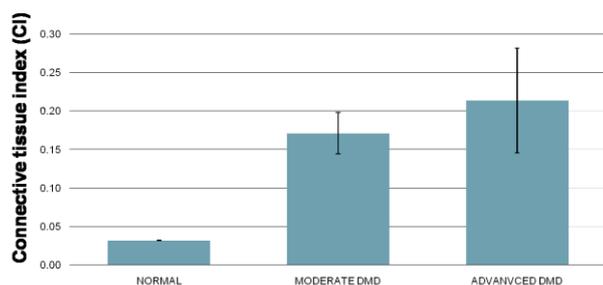Fig. 5. Connective tissue indices CI_H for the analyzed DMD and normal muscle tissue images.

Fig. 6. Connective tissue indices CI_C for the analyzed DMD and normal muscle tissue images.

4 Conclusions

CFPP method is simple and not time consuming. The method allows to distinguish DMD from normal muscle tissue, as well as potentially gives a possibility to evaluate the severity of DMD. The method is dedicated to DMD microscopic images, but its general principle is applicable to other histological images.

Presented results are promising and further research are planned on a large data set, including microscopy images from different types of dystrophy. Moreover, initial step assessing the quality of analyzed microscopy images is foreseen to be implemented for CFPP method.

CFPP method is simple and not time consuming.

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