Relation between blood CD49d and muscle ultrasonography in children with Duchenne Muscular Dystrophy

Thesis

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Fatma Hasan Hasan Besar

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List of Abbreviations

Abbr.	Full-term
DMD	: Duchenne Muscle Dystrophy
DGC	: Dystrophin-associated glycoprotein complex
MUS	: Muscle ultrasound
QMUS	: Quantitative muscle ultrasound
KD	: Kilodaltons
n NOS	: Neuronal nitric oxide synthase
I NOS	: Inducible nitric oxide synthase
SDB	: Sleep-disordered breathing
REM	: Rrapid eye movement sleep
WAIS	: Wechsler Adult Intelligence Scale
IQ	: Intelligence Quotient
CK	: Creatine kinase
EMG	: Electromyography
ATPase	: Adenosine triphosphatase
MLPA	: Multiplex ligation-dependent probe amplification
PCR	: Polymerase chain reaction

FISH : Fluorescence in situ hybridization
DHPLC : Denaturing high performance liquid chromatography
SCAIP : Single-condition amplification internal primer sequencing
DGGE : Denaturing gradient gel electrophoresis
DOVAMK-S : Detection of virtually all mutations - single-strand
DCM : Dilated cardiomyopathy
NIPPV : Intermittent positive pressure ventilation
DXA : Dual energy x-ray absorptiometry
25OHD : 25-hydroxyvitamin D
AAN : American Academy of Neurology
rAAV : Recombinant adeno-associated viral
FDA : Food and Drug Administration
EMG : Electromyography
CT : Computed tomography
MRI : Magnetic resonance imaging
ECM : Extracellular matrix
VCAM-1 : Vascular cell adhesion molecule-1
FN : Fibronectin
CBC : Complete blood Count
ELISA : Enzyme-linked immunosorbent assay
ROM : Range of motion
10MWT : Ten Meters Walk Test
QBA : Quantitative backscatter analysis
GSL : Grayscale levels

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Abstract

Background: Duchenne Muscular Dystrophy (DMD) an X-linked recessive disorder affecting 1 in 3500- 6000 male births. It is caused by the absence of functional dystrophin, due to mutations in the dystrophin gene. Although the cause of DMD is genetic, there is accumulating data suggesting that an immune response may play a role in pathophysiology of this disease and also contributes to disease progression in DMD patients. Alpha integrins (CD49d) can drive T cells to the site of inflammation favoring migration and adhesion to the muscle tissue and muscle damage by interacting more strongly with the fiber and extracellular matrix proteins. DMD patients are clinically heterogeneous and the functional defect cannot be correlated with genotype. Therefore, it is important to be able to define reliable noninvasive inflammatory biomarkers. **Methods:** A case control study was carried out at neurology outpatient clinic, Children's Hospital, Ain Shams University . The study included 20 male patient (mean age of 10.35 years), compared to 20 healthy children age, sex and socioeconomic matched served as control group. Enrolled subjects underwent peripheral blood sampling (for measuring CD49d, CBC and CPK), motor grading and muscle ultrasound**. Results:** Patients had a significant higher CD 49 levels than controls. Cutoff values was 0.250 with 90% sensitivity and 70% specificity. **Conclusions**: Our data indicates that CD49d expression in high levels involved in the inflammatory process seen in DMD patients.

Key words: blood CD49d, muscle ultrasonography, children, Duchenne Muscular Dystrophy

Introduction

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disease of muscle characterized by a progressive loss of functional muscle mass and replacement with fibrofatty tissue ***(Sussman and Michael, 2002).***

The incidence of DMD is approximately 1 in 3500 live male births ***(Gulati et al., 2005)***. It is caused by mutations in the *DMD* gene. This gene encodes a protein called dystrophin ***(Blake et al., 2002)***, which localizes to the cytoplasmic face of the sarcolemma (plasma membrane) of the skeletal muscle ***(Ervasti , 2007)***, forming one component of a large glycoprotein complex (dystrophin-associated glycoprotein complex). The majority of mutations are intragenic deletions, which account for 65-72% of all DMD patients ***(Aartsma-Rus et al., 2006).*** Point mutations, small deletions or insertions account for 20% of patients without deletions or duplications ***(Prior and Bridgeman , 2005).***

The precise mechanism of how dystrophin deficiency leads to degeneration of muscle fibers remains unclear. Absence of dystrophin at the plasma membrane leads to delocalisation of dystrophin-associated proteins from the membrane, disruption of the cytoskeleton with resultant membrane instability and increased susceptibility to mechanical stress ***(Deconinck et al., 2007).***

Duchenne muscular dystrophy is the most common fatal genetic disorder diagnosed in childhood, and it does shorten life. Because the muscle weakness increases gradually over the years, complications eventually develop. The breathing or heart problems usually become more serious for older teenagers or people in their 20s. In the past, most people with DMD did not live beyond their early 20s. Improvements in treatment have meant that life expectancy has increased. At present, average life expectancy for people with DMD is 27 years. However, there is a lot of individual variation in the severity of DMD and the individual life expectancy ***(Kliegman et al., 2011)***.

There is no cure for DMD at this time, but there continues to be a tremendous amount of research taking place across the world ***(Jefferies et al., 2005)***. Treatment aims to control symptoms to improve quality of life. Steroid drugs can slow the loss of muscle strength. They may be started when the child is diagnosed or when muscle strength begins to decline. Physical therapy is used to promote mobility and prevent contractures (***Finder et al., 2004).***

Ultrasound imaging has proved to be a useful, non invasive screening tool in the investigation of children with neuromuscular disease. Muscular dystrophies were associated with an increase in the intensity of echo reflected from the muscle substance, with corresponding loss of bone echo. Severity of changes on the scan did not relate to

functional disability, and some children had good function yet strikingly abnormal scan. the degree on the scan correlated with the degree of disruption of muscle architecture on biopsy ***(Wattjes et al., 2010).*** Muscle ultrasound (MUS) is potentially an attractive follow up tool for DMD because it reflects the severity of the dystrophic process without the need for invasive procedures, by quantifying echo intensity (i.e. mean grey level of muscle images) and muscle thickness ***(Jansen et al., 2012).***

DMD patients are clinically heterogeneous and the functional defect cannot be correlated with genotype. Therefore, it is important to be able to define reliable noninvasive biomarkers to better define the disease progression ***(Pinto-Mariz et al., 2015).*** The non-invasive biomarkers obtained at early stages of the disease are found to be highly predictive for the loss of ambulation before 6 months of age. An elevation in the number of circulating $CD49d^{hi}$ T cells is found to be strongly associated with the severe clinical form of the disease. This factor can be used as predictive tests for screaning to separate them into groups with slow or fast disease progression before their inclusion into a therapy ***(Barthélémy et al., 2014)***.

Moreover, anti-CD49d peptides or antibodies can be used as a therapeutic approach to decrease inflammation-mediated tissue damage in DMD.