Cytokines and growth factors in Duchene muscular dystrophy patients

Iman Abdel-Meguid[1], Ekram Abdel-Salam[1] and Soheir Korraa[2]

[1]Department of Pediatrics, Genetic-Unit, Faculty of Medicine - Cairo University, [2]Department of Radiation Health, National Centre for Radiation Research and Technology, Atomic Energy Authority

ABSTRACT

Introduction: Dystrophin deficiency associated with Duchene muscular dystrophy (DMD) results in chronic inflammation and severe skeletal muscle degeneration, where the extent of muscle fibrosis contributes to disease severity. The microenvironment of dystrophic muscles is associated with variation in levels of cytokine and growth factors. Most of the current researches test for such cytokines and growth factors in tissue biopsies, which is an invasive technique.

The Aim: Of the present study is to investigate whether cytokines and growth factors, as indicators of inflammatory response, can be detected in blood of DMD patients as non-invasive technique.

Patient and Methods: Accordingly the cytokine tumor necrosis factor alfa (TNF TNF-α), as well as the growth factors basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) were measured in blood of 24 boys with DMD diagnosed clinically and at the molecular level versus 20 age matching healthy boys.

Results: Showed a significant increases in TNF-ά (30.2 ± 9.5 vs. 3.6 ± 0.9 pg/ml) and bFGF (21.7 ± 10.3 vs. 4.75 ± 2.2 pg/ml.), while VEGF was significantly decreased (190 ± 115 vs. 210 ± 142 pg/ml) in blood of DMD patients compared to controls.

Conclusion: Results provide further proof that inflammatory response is associated with DMD pathogenesis and favours the use of biomarkers in blood of such patients as a non invasive technique.

Key Words:

(basic fibroblast growth factor (bFGF), duchene muscular dystrophy, necrosis factor alfa (TNF TNF-α), vascular endothelial growth factor (VEGF)).

Corresponding Author:

Dr: Iman E Meguid
E-mail: moni_ehsan@hotmail.com

INTRODUCTION

DMD is an X-linked recessive disorder, primarily characterizebd by progressive muscle weakness and waisting. Mutations in dystrophin gene are the prime

cause for muscle degeneration associated with DMD[1]. Normally dystrophin interacts with several members of the dystrophin glycoprotein complex, which forms a mechanical as well as signaling link from the extracellular matrix to the cytoskeleton[2]. Mutations in dystrophin result in membrane damage, allowing massive infiltration of immune cells, chronic inflammation, necrosis, and severe muscle degeneration[3]. Normally, muscle cells possess the capacity to regenerate in response to injury signals[4], however, this ability is lost in DMD, presumably due to an exhaustion of satellite cells during ongoing degeneration and regeneration cycles[5]. Although dystrophin mutations represent the primary cause of DMD, it is the secondary processes involving persistent inflammation and impaired regeneration that likely exacerbate disease progression. This results in chronic inflammation and severe skeletal muscle degeneration, where the extent of muscle fibrosis contributes to disease severity. Elevated numbers of inflammatory cells are known to be present at the sites of muscle injuries and interact together for cytokine and growth factors signaling.[6-9]

TNF-α is an early and potent pro-inflammatory cytokine that stimulates the inflammatory response. Even minor trauma to muscle will increase levels of TNF-α by release from mast cells; TNF-α is also produced by neutrophils, macrophages and lymphocytes that accumulate rapidly at the site of injury. TNF-α increases rapidly within damaged myofibers and is expressed by myoblasts and myotubes[10-12]. TNF-α is greatly elevated in injured normal damaged myofibers[10,11] and myopathic skeletal muscle[13], it is chemotactic for myoblasts[14] and is mitogenic for satellite cells in vivo, suggesting a direct role in myogenesis of regenerating muscle.[10]

Muscle tissue repair is a complex biological process that crucially involves activation of stem cells. Skeletal muscle contains two different stem cell types: 1 myogenic stem cells, so-called satellite cells (SCs), that reside beneath the basal lamina of muscle fibers[15,2] interstitial multipotent stem cells, which are extralaminal, exhibit fibroblastic morphology and do not express myogenic markers[16]. SCs represent the pre-eminent muscle stem cell type used for muscle growth, repair and regeneration[17]. They are primarily quiescent in skeletal muscle, can self-renew and upon activation, proliferate and further differentiate to become fusion-competent myoblasts and ensure muscle regeneration[18]. Interstitial "muscle-derived" stem cells give rise to several lineages after transplantation and in this setting, contribute to synchronized reconstitution of blood vessels (pericytes, smooth muscle cells [SMCs], and endothelial cells), peripheral nerve (Schwann cells), and muscle cells (myofibers and SCs;[16]. However, participation of multipotent interstitial stem cells in physiological muscle repair appears to be limited.

Satellite cells could excrete growth factors including VEGF that would induce angiogenesis and improve cell survival.[19]. The VEGF is the prototypic member of a family of secreted, homodimeric glycoproteins with endothelial cell-specific mitogenic activity and the ability to stimulate angiogenesis in vivo[20]. On the other hand, a number of growth factors such as fibroblast growth factor (FGF) can promote the activation and proliferation of skeletal satellite cells.[21]

Basic FGF contributes to promote proliferation and to inhibit differentiation of skeletal muscles.[22]

The aim of the present study is to investigate levels of cytokines (TNF-α) and growth factors (VEGF and bFGF) involved in muscle regeneration in blood of DMD patients compared to controls.

PATIENT AND METHODS

Subjects were[23] DMD boys diagnosed clinically and at the molecular level (mean age (8.1 ± 1.9) years versus 20 age and socioeconomic matching healthy boys (mean of age 8.2 ± 2.2). Patients and controls were chosen to be free from any infection and receiving no therapeutic treatment known to increase the oxidative stress. Blood samples were drawn after their parents' consent.

Plasma analysis was performed for cytokine (TNF-ά) using elisa/PCR (24), growth factor VEGF using the ACCUCYTE Human VEGF immunoassay kit [24] and bFGF using human bFGF immunosorbant assay (ELIZA) Quantitin kit.[25]

Statistical Analysis:
Each experimental condition was performed and expressed as mean ± SD. Comparisons were made by Student's t-test (two-tailed for independent samples).

RESULTS

TNF and bFGF were significantly higher in Blood of DMD patients compared to controls (Table 1, Figures 1 & 2)TNF was (30.2 ± 9.5 vs. 3.6 ± 0.9 pg/ml) and bFGF was (21.7 ± 10.3 vs. 4.75 ± 2.2). In contrast, VEGF significantly lower in blood of DMD patients compared to controls. (190 ± 115 vs. 210 ± 142).

Table 1: TNF, bFGF and VEGF in blood of DMD patients compared to controls.

	TNF pg/ml	bFGF pg/ml.	VEGF pg/ml
DMD patients	30.2 ± 9.5	21.7± 10.3	190 ± 115
Controls	3.6 ± 0.9	4.75 ± 2.2	210 ± 142
t	14	17	4.2
p	p <0.00001	< 0.00005	< 0.005

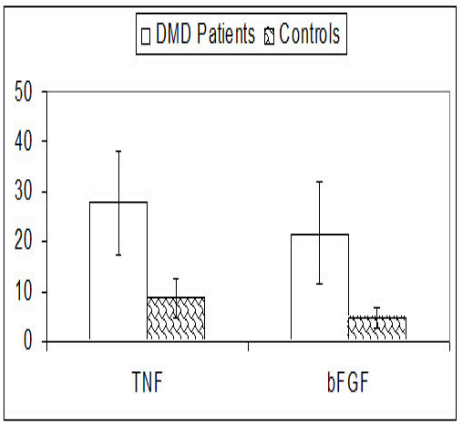

Fig. 1: TNF, and bFGF in Blood of DMD patients compared to controls.

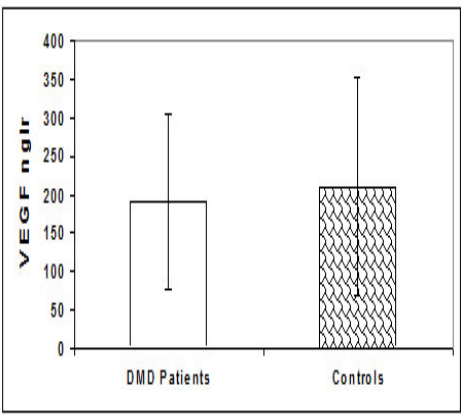

Fig. 2: VEGF in Blood of DMD Patients Compared to Controls.

DISCUSSION

In normal skeletal muscles, damage due to contractile force is followed by an inflammatory response involving multiple cell types that subsides after several days. This transient inflammatory response is a normal homeostatic reaction to muscle damage that induces muscle repair. However in DMD patients a persistent inflammatory response in their skeletal muscles leads to an altered extracellular environment, including an increased presence of inflammatory cells (e.g., macrophages) and elevated levels of various inflammatory cytokines and growth factors. Unfortunately, the signals that lead to successful muscle repair in healthy muscle may promote muscle waisting and fibrosis in dystrophic muscles[26]. Our results point to increase in inflammatory cytokines TNF-ά and growth factor bFGF in blood of DMD patients, while VEGF exhibited a significant decrease compared to controls.

TNF-α is an important mediator of inflammatory and autoimmune diseases. It was reported that the mean serum TNF-αconcentration in Duchenne muscular dystrophy patients was approximately 1,000 times higher than that in healthy subjects[10] and that TNF-α levels are upregulated in dystrophic muscles from animal models and DMD patients[4,6]. Among its pleiotropic effects, TNF-α acts as a potent inducer of the inflammatory response transcription factor NF-κB[27,28]. Although dystrophin mutations represent the primary cause of DMD, it is the secondary processes involving persistent inflammation and impaired regeneration that likely exacerbate disease progression. The microenvironment of dystrophic muscles consists of elevated numbers of inflammatory cells that act as a complex interface for cytokine signaling[4, 6]. It is emphasized that TNF contribute to myofibre necrosis, where antibody depletion of host neutrophils resulted in a delayed and significantly reduced amount of skeletal muscle breakdown in young dystrophic mdx mice.[29]

Results of the present study showed increased levels of bFGF compared to controls. Growth factors, represent essential elements in the modulation of muscle cell regeneration and differentiation[4].Interestingly, many growth factors, including basic fibroblast growth factor (bFGF), has been shown to be upregulated in mdx mice[30] and serum levels has been shown to increase in DMD patients compared to controls[31]. However, bFGF role in DMD pathogenesis is still unclear. Moreover, disruption of muscle membrane repair machinery results in progressive muscular dystrophy in the presence of a functional dystrophn glycogen[32]. It is suggested that DMD lack dystrophin and as a result their skeletal muscles show extensive muscle fiber damage and fibrosis, and regeneration[33]. This is also supported by data obtained from experiments carried out on mdx mice, which are larger than normal mice. Mdx have been noted to show larger limb muscles than normal mice; however, the larger muscles of mdx mice are actually weaker than those of normal mice due to extensive fibrosis and the lower potential for force generation by newly regenerated fibers[34,35]. Mdx mice have a large number of degenerating and regenerative muscle fibers in the first 4 months of life, after which the number of degenerative and necrotic fibers declines.[35]

In the present study VEGF was significantly lower in DMD patients compared

to controls. Exercise is known to increase muscle VEGF mRNA[36-38] and DMD patients usually have limited physical activity, which can explain the lower level of VEGF in our DMD patients. Data obtained from comparative studies on young and ageing muscle and on exercised and sedentary muscles, indicated that in aged compared with young men, muscle capillary contacts and capillary-to-fiber perimeter exchange index were lower and that VEGF muscle protein decreases with ageing[39,40]. Replicative aging of myogenic cells (satellite cells), owing to enhanced myofiber turnover, is an accepted common explanation of the progression of DMD pathogenesis[41]. Studies supporting our finding can be obtained from a previous study that showed that intramuscular delivery of VEGF using recombinant adeno-associated virus (rAAV) vectors in mdx mice induced an increased forelimb strength and strength normalized to weight[42]. Another example supporting replicative ageing theory is that mdx mice transplant injected with muscle precursor cells into tibialis exhibited Graft success. This was evaluated as the percentage of improvement of hybrid fibers by 1.9-fold after swimming 3 times per week during 4 weeks. It was suggested that exercise induced fiber breaks, which improved muscle precursor cells recruitment and fusion and increased long-term graft success and also transverse and longitudinal distribution of hybrid fibers.[43]

CONCLUSION

Growth factors and cytokines are associated with DMD pathogenesis, where TNF, bFGF and VEGF can give a reflection of the severity of DMD pathology. Detecting such growth factors and cytokines biomarkers in blood of DMD patients represents for the first time a non invasive technique compared to the invasive technique of muscle biopsy previously used.

REFERENCES

1. Emery AEH. Duchenne muscular dystrophy. 2nd ed. USA: Oxford University Press; 1993.

2. Durbeej M, Campbell KP. Muscular dystrophies involving the dystrophin-glycoprotein complex: An overview of current mouse models. Curr.Opin. Genet.Dev. 2002; 12 (3): 349-61.

3. Emery AE. The muscular dystrophies. Lancet 2002 23; 359 (9307): 687-95.

4. Tidball JG. Inflammatory processes in muscle injury and repair. Am.J.Physiol. Regul.Integr.Comp.Physiol. 2005; 288 (2): R345-53.

5. Blake DJ, Weir A, Newey SE, Davies KE. Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol. Rev. 2002; 82 (2): 291-329.

6. Gorospe JR, Tharp MD, Hinckley J, Kornegay JN, Hoffman EP. A role for mast cells in the progression of Duchenne muscular dystrophy? Correlations in dystrophin-deficient humans, dogs and mice. J.Neurol. Sci. 1994; 122 (1):44-56.

7. Spencer MJ, Tidball JG. Do immune cells promote the pathology of dystrophin-deficient myopathies? Neuromuscul.Disord. 2001; 11 (6-7): 556-64.

8. Spencer MJ, Montecino Rodriguez E, Dorshkind K, Tidball JG. Helper

(CD4(+)) and cytotoxic (CD8(+)) T cells promote the pathology of dystrophin-deficient muscle. Clin. Immunol. 2001; 98 (2): 235-43.

9. Cai B, Spencer MJ, Nakamura G, Tseng Ong L, Tidball JG. Eosinophilia of dystrophin-deficient muscle is promoted by perforin-mediated cytotoxicity by T cell effectors. Am.J. Pathol. 2000; 156 (5): 1789-96.

10. Collins RA, Grounds MD. The role of tumor necrosis factor-alpha (TNF-alpha) in skeletal muscle regeneration. Studies in TNF-alpha(-/-) and TNF-alpha(-/-)/LT-alpha(-/-) mice. J.Histochem.Cytochem. 2001; 49 (8): 989-1001.

11. Warren GL, Hulderman T, Jensen N, McKinstry M, Mishra M, Luster MI, et al. Physiological role of tumor necrosis factor alpha in traumatic muscle injury. FASEB J. 2002; 16(12): 1630-2.

12. Li YP. TNF-alpha is a mitogen in skeletal muscle. Am.J.Physiol.Cell.Physiol. 2003; 285 (2): C370-6.

13. Kuru S, Inukai A, Kato T, Liang Y, Kimura S, Sobue G. Expression of tumor necrosis factor-alpha in regenerating muscle fibers in inflammatory and non-inflammatory myopathies. Acta Neuropathol. 2003; 105 (3): 217-24.

14. Torrente Y, El Fahime E, Caron NJ, Del Bo R, Belicchi M, Pisati F, et al. Tumor necrosis factor-alpha (TNF-alpha) stimulates chemotactic response in mouse myogenic cells. Cell Transplant. 2003; 12 (1): 91-100.

15. Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, et al. Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J.Cell Biol. 2000 11; 151 (6): 1221-34.

16. Tamaki T, Akatsuka A, Ando K, Nakamura Y, Matsuzawa H, Hotta T, et al. Identification of myogenic-endothelial progenitor cells in the interstitial spaces of skeletal muscle. J.Cell Biol. 2002 13; 157 (4): 571-7.

17. Dhawan J, Rando TA. Stem cells in postnatal myogenesis: Molecular mechanisms of satellite cell quiescence, activation and replenishment. Trends Cell Biol. 2005; 15(12): 666-73.

18. Hawke TJ, Garry DJ. Myogenic satellite cells: Physiology to molecular biology. J.Appl. Physiol. 2001; 91 (2): 534-51.

19. Xia JH, Xie AN, Zhang KL, Xu L, Zheng XY. The vascular endothelial growth factor expression and vascular regeneration in infarcted myocardium by skeletal muscle satellite cells. Chin.Med.J.(Engl) 2006 20; 119 (2): 117-21.

20. Germani A, Di Carlo A, Mangoni A, Straino S, Giacinti C, Turrini P, et al. Vascular endothelial growth factor modulates skeletal myoblast function. Am.J.Pathol. 2003; 163 (4): 1417-28.

21. Vandervelde S, van Luyn MJ, Tio RA, Harmsen MC. Signaling factors in stem cell-mediated repair of infarcted myocardium. J.Mol.Cell. Cardiol. 2005; 39 (2): 363-76.

22. Liu HZ, Li Q, Yang XY, Liu L, Liu L, An XR, et al. Expression of basic fibroblast growth factor results in the decrease of myostatin mRNA in murine C2C12 myoblasts. Acta Biochim.

Biophys.Sin.(Shanghai) 2006; 38 (10): 697-703.

23. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999; 13 (1): 9-22.

24. Saito K, Kobayashi D, Sasaki M, Araake H, Kida T, Yagihashi A, et al. Detection of human serum tumor necrosis factor-alpha in healthy donors, using a highly sensitive immuno-PCR assay. Clin.Chem. 1999; 45 (5): 665-9.

25. Gosselin LE, McCormick KM. Targeting the immune system to improve ventilatory function in muscular dystrophy. Med.Sci.Sports Exerc. 2004; 36 (1): 44-51.

26. Baired A, Bohlen P. Fibroblast growth factors. In: Sporn MB, Roberts AB, editors. Peptide growth factors and their receptors: Springer; 1991. p. 369.

27. Porreca E, Guglielmi MD, Uncini A, Di Gregorio P, Angelini A, Di Febbo C, et al. Haemostatic abnormalities, cardiac involvement and serum tumor necrosis factor levels in X-linked dystrophic patients. Thromb. Haemost. 1999; 81 (4): 543-6.

28. Porter JD, Khanna S, Kaminski HJ, Rao JS, Merriam AP, Richmonds CR, et al. A chronic inflammatory response dominates the skeletal muscle molecular signature in dystrophin-deficient mdx mice. Hum.Mol. Genet. 2002 1; 11 (3): 263-72.

29. Hodgetts S, Radley H, Davies M, Grounds MD. Reduced necrosis of dystrophic muscle by depletion of host neutrophils, or blocking TNFalpha function with Etanercept in mdx mice. Neuromuscul. Disord. 2006; 16 (9-10): 591-602.

30. D'Amore PA, Brown RH,Jr, Ku PT, Hoffman EP, Watanabe H, Arahata K, et al. Elevated basic fibroblast growth factor in the serum of patients with Duchenne muscular dystrophy. Ann. Neurol. 1994; 35 (3): 362-5.

31. Blake DJ, Weir A, Newey SE, Davies KE. Function and genetics of dystrophin and dystrophin-related proteins in muscle. Physiol. Rev. 2002; 82 (2): 291-329.

32. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, et al. Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 2003 8; 423 (6936): 168-72.

33. Carlson CG, Makiejus RV. A non-invasive procedure to detect muscle weakness in the mdx mouse. Muscle Nerve 1990; 13 (6): 480-4.

34. Connolly AM, Keeling RM, Mehta S, Pestronk A, Sanes JR. Three mouse models of muscular dystrophy: The natural history of strength and fatigue in dystrophin-, dystrophin/utrophin- and laminin alpha2-deficient mice. Neuromuscul. Disord. 2001; 11 (8): 703-12.

35. Roig M, Roma J, Fargas A, Munell F. Longitudinal pathologic study of the gastrocnemius muscle group in mdx mice. Acta Neuropathol. 2004; 107 (1): 27-34.

36. Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ. Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. Am.J. Physiol. 1999; 276 (2 Pt 2): H679-85.

37. Gavin TP, Robinson CB, Yeager RC, England JA, Nifong LW, Hickner RC. Angiogenic growth factor response to acute systemic exercise in human skeletal muscle. J.Appl. Physiol. 2004; 96 (1): 19-24.

38. Nico B, Corsi P, Vacca A, Roncali L, Ribatti D. Vascular endothelial growth factor and vascular endothelial growth factor receptor-2 expression in mdx mouse brain. Brain Res. 2002 25; 953 (1-2): 12-6.

39. Nico B, Mangieri D, Crivellato E, Longo V, De Giorgis M, Capobianco C, et al. HIF activation and VEGF overexpression are coupled with ZO-1 up-phosphorylation in the brain of dystrophic mdx mouse. Brain Pathol. 2007; 17 (4): 399-406.

40. Croley AN, Zwetsloot KA, Westerkamp LM, Ryan NA, Pendergast AM, Hickner RC, et al. Lower capillarization, VEGF protein and VEGF mRNA response to acute exercise in the vastus lateralis muscle of aged vs. young women. J.Appl.Physiol. 2005; 99 (5): 1872-9.

41. Ryan NA, Zwetsloot KA, Westerkamp LM, Hickner RC, Pofahl WE, Gavin TP. Lower skeletal muscle capillarization and VEGF expression in aged vs. young men. J.Appl. Physiol. 2006; 100(1): 178-85.

42. Oexle K, Kohlschutter A. Cause of progression in Duchenne muscular dystrophy: Impaired differentiation more probable than replicative aging. Neuropediatrics 2001; 32(3): 123-9.

43. Messina S, Mazzeo A, Bitto A, Aguennouz M, Migliorato A, De Pasquale, M G, et al. VEGF overexpression via adeno-associated virus gene transfer promotes skeletal muscle regeneration and enhances muscle function in mdx mice. FASEB J. 2007; 21 (13): 3737-46.