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Serum Calcium in Duchenne/Becker Muscular Dystrophy Patients of Gujarat Population

KEYWORDS: Duchenne/Becker muscular dystrophy, dystrophin gene, calcium, exon

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ABSTRACT *The dystrophinopathies, DMD and the allelic BMD are the most common forms of muscular dystrophy in humans. Duchenne Muscular Dystrophy (DMD) is one of the most common inherited neuromuscular disease affecting only males. It is an X-linked recessive disorder caused by mutation in the dystrophin gene. The present study aimed to rule out the pathological role of serum calcium (Ca) level in D/BMD patients and their correlation with gene deletion and age. Out of total 80 suspected D/BMD patients, 58 (72.5%) patients were confirmed using M-PCR for evaluation of 26 exons. The serum Ca level showed a significant ($p<0.05$) decrease in DMD, as well as inverse correlation with advancing age in DMD only. In addition, study also revealed no correlation between Ca and deletion pattern of exon/s. The present observations thus conclude that Ca level with clinical diagnosis could be meaningful from the etiologic and therapeutic points of view.*

INTRODUCTION

Muscular dystrophy (MD) is a group of genetically determined muscular disorders characterized by muscle weakness, necrosis and exemplify by muscle degeneration. Duchenne muscular dystrophy (DMD) [OMIM #310200] is an X-linked recessive, rapidly progressive form of muscular dystrophy which affects 1 in 3500 males[1]. Becker muscular dystrophy (BMD) [OMIM #300376] is a milder form of the disease with a later age of onset and a slower clinical progression[2]. DMD and the allelic BMD together are termed as dystrophinopathies. D/BMD caused by mutations of the dystrophin gene [OMIM #300377] is located at locus Xp21.2. The dystrophin gene remains the only known human metagene, measuring 2.4 Mb[3,4]. The dystrophin gene, translates into the dystrophin protein, a large protein (427 kD) that works as anchoring between the extracellular matrix and the cytoplasmic cytoskeleton[5]. It is assumed that the this protein in association with the dystrophin-glycoprotein complex (DGC), has a crucial role in maintaining sarcolemmal stability during contraction of muscle [6,7].

In muscular dystrophy, almost all dystrophin associated proteins are also greatly reduced in their relative density[8,9]. However especially in dystrophinopathies, gene deletion leads to the absence or truncation of dystrophin protein, and the consequent destabilization of the DGC[6,9], which in turn, results in a loss of linkage property between the extra cellular matrix and the actin membrane cytoskeleton, lead to an alteration of ion channel physiology, Ca homeostasis[10] and structural organization of myocyte[11,12]. The genetic defects of dystrophin gene is not directly related to proteins of the Ca cycle, but disturbed intracellular Ca pathway are most likely involved in the pathophysiology of Duchenne myofibers [13-15].

Ca is the most ubiquitous and universal intracellular signaling molecule, which regulate a wide array of physiological processes. But excess Ca is also a cellular toxin and ultimately causes a variety of pathological changes including necrosis[13-16]. Moreover, various studies suggested an elevated cytosolic Ca level in dystrophin deficient myofibers[17-20]. An increased Ca level in these fibers is due to a raised Ca influx or decreased efflux that results from a non-specific permeability increase to all cations[21]. Increased levels of proteolysis[22,23] and reactive oxygen species (ROS)[24] cause destructive cycle of proteolysis and membrane damage that ultimately lead to muscle degeneration[18-20].

Until today, various studies suggest that multiple factors contribute to muscle damage in dystrophic muscle. An elevated intracellular Ca is widely thought to be a crucial initiating event in the pathophysiology of Duchenne/Becker muscular dystrophy (D/BMD)[10,13-16]. However there is still a need to know the effect of these mechanisms at serum level. Hence, we attempted to investigate whether muscle degenerations in D/BMD are associated with changes in serum Ca levels, as well as correlated these indices with age and deletion pattern of exon/s in proband.

In present study, total 80 clinical suspected D/BMD and 40 healthy age matched control individuals were reviewed who were consulted at Gujarat Genetic Diagnostic Center (GenDiCe) and Indian Muscular Dystrophy Society (IMDS) Ahmedabad, Gujarat, India (2011 - 13). The cases were diagnosed based on clinical examinations and laboratory findings. Patients found to have any infection, recent trauma or surgery at the time were not considered in this study. Information pertaining to use of any drug and therapies taken at the time of blood collection were also considered. The study was approved by the Institutional ethics board. Informed consent was obtained from each patient or the parents of individuals <18years.

Serum Ca level in D/BMD patients was measured using the fully automated biochemical analyzer (COBAS INTEGRA 400, Roche) [25]. The reference range of Ca level was settled as 8.10 to 10.40 mg/dl[26]. We have found that mean serum Ca was significantly ($p<0.05$) decreased in DMD than in control subjects with no significant decrease in BMD. Our investigation also revealed that the serum Ca level was declined in D/BMD patients compared to controls but within normal limit which is in agreement with the data of others[27]. Moreover study data revealed a decreased Ca level in serum with advancing age, which could be due to result of reduced efflux and/or progressive elimination of dystrophic muscle fibers in DMD probands (Graph 1). Various

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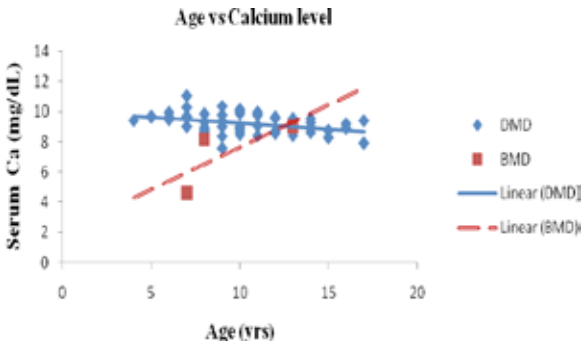
studies have also reported an increased level of creatine phosphokinase (CPK), myoglobin, Lactate dehydrogenase (LDH) and transaminases in serum which could be due to damaged muscle membranes[28-31].



Graph 1: Graph representing calcium relationship to age in Duchenne/Becker muscular dystrophy.

TABLE – 1
Serum Calcium level

	Control (n=40)	DMD (n=55)	BMD (n=3)
Ca (mg/dl)	9.59±0.08	9.23±0.08*	7.28±1.36^{ns}
Values are given Mean ± SEM; P<0.05= *, ns = non significant;			

Ca = Serum calcium;

DMD = Duchenne muscular dystrophy;

BMD = Becker muscular dystrophy;

Additionally, the genomic DNA was extracted from peripheral blood lymphocytes by phenol chloroform extraction method[32]. DNA was subsequently applied for multiplex polymerase chain reactions (M-PCR) for deletion detection, it was performed by three separate PCR reactions sets, according to Chamberlain et al.[33], modified by Beggs et al.[34] allowing amplification of exon 46 plus the original set, Beggs et al.[35] and Kunkel et al.[36] allowed screening for deletions of 26 exons, using the condition recommended at the Leiden Muscular Dystrophy pages.

Out of 80 clinically suspected cases of D/BMD boys, the diagnoses of D/BMD confirmed by M-PCR (26 exons) in 58 (72.5%) in which 55 (94.8%) DMD and 3 (5.2%) BMD patients. The majority proband 79.3% (46/58) had deletion in the central rod domain between exon 45-52 and out of these 25.9% (15/58) deletion started with exon 45. We also found most frequent deletion was of exon 50, 56.9% (33/58) at the central deletion hot spot, which was also coincided with previous Western Indian study[37,38]. Our observation indicated that this part of the gene is more prone to deletion in Gujarat population. Moreover one of the longest deletion include exon 3 to 44 was found in one BMD proband. The comparison between deletion pattern and Ca level is given in Table 2.

Our observation emphasizes that there was no apparent correlation between the size and/or location of the deletion pattern and serum Ca level as well as severity or succession of the disease condition in D/BMD patients. It is clear that the result indicated highest Ca level (11.05 mg/dl) was noticed in one of the DMD proband with single exon 60 deletion, while in one BMD proband with longest deletion (exon 3-44) showed Ca level (8.18 mg/dl) within the reference limit (Table 1).

TABLE – 2
Correlation between serum calcium level and deletion pattern among D/BMD patients.

Deletion	Patients	Phenotype	Mean Calcium (mg/dl)
del 3	1	BMD	4.61
del 3-6	1	DMD	8.54
del 3-13	1	DMD	8.26
del 3-19	1	DMD	9.00
del 3-43	1	DMD	9.55
del 3-44	1	BMD	8.18
del 41-43	1	BMD	9.05
del 41-45	2	DMD	9.77
del 44	1	DMD	9.41
del 44-52	1	DMD	10.28
del 45	6	DMD	9.28
del 45-50	3	DMD	9.39
del 45-52	6	DMD	9.35
del 46-47	3	DMD	8.71
del 46-48	1	DMD	8.90
del 46-49	1	DMD	8.61
del 46-51	2	DMD	8.67
del 46-52	5	DMD	9.39
del 48-50	4	DMD	9.43
del 48-52	4	DMD	8.84
del 49-50	3	DMD	9.06
del 49-52	2	DMD	8.79
del 50	2	DMD	9.16
del 50-52	1	DMD	9.64
del 51	2	DMD	9.50
del 51-52	1	DMD	8.86
del 60	1	DMD	11.05

CONCLUSION:
The study was carried out on 80 patients using M-PCR for deletion screening in addition to serum calcium level. Location and size of deletion mutations found in the dystrophin gene has not clearly indicated any correlation with serum calcium level. It is reduced with age, but falls in the references range. Further, though calcium level may not have diagnostic value, it is useful for better management of proband and it requires in-depth study to investigate pathogenesis and drug therapy of the patients.

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