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Phenotypic and Biochemical variability in Calpainopathy families of Pakistan

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ABSTRACT
Calpainopathy(LGMD2A) is the most common form of recessive LGMD. Creatine phosphokinase (CPK) a specific enzyme for muscles, clinically utilizes for acute and chronic muscles diseases detection; one of the diagnostic tools for calpainopathy. Aim of the present study is to determine the interfamily biochemical variability in calpainopathy of Pakistani families before which is not reported. All the subjects were divided into two groups namely A & B. CPK analysis of normal and affected individuals of both families (A&B) was performed on chemistry analyzer Hitachi 912 (Roche) and digital X-rays films of one affected individual from each family were taken.Affected individuals of family A showed 2 to 3 folds increased in CPK while affected individuals of family B showed from normal to 32 folds increased in CPK regardless of the sex involved.Interfamily clinical course of calpainopathy vary considerably suggesting complete profile of genetic analysis, clinical signs and symptoms. Biochemical and radiological examination are needed for excluding calpainopathy.
Key words: *Biochemical analysis, radiological analysis, calpainopathy, LGMD.*

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INTRODUCTION

The term limb girdle muscular dystrophy (LGMD)generally defines autosomal recessive defect of the muscles of proximal limb in which muscles are progressively weakened. The disease symptoms may appear from early childhood to late adult age.It is not a congenital disease [2]. The causative gene for Calpainopathy (LGMD2A) is calpain 3 (CAPN3), a calcium dependent protease and mutations occur at 15q15.1-15.3 and about 20-40% of all LGMD population were affected from calpainopathy. In most countries, Calpainopathy is the most common LGMD [7, 9, 10]. The main common clinical features of calpainopathy are scapular winging, atrophy, involvement of the posterior muscles of thigh, hip adductors weakness and joint contractures [6]. Variable phenotypes and asymptomatic increased CK value may often lead to misdiagnosis [1, 8]. Sometimes muscles inflammation was observed with eosinophilic infiltrates[4] or phenotypes like of Becker muscular dystrophy (BMD).Eosinophils can be found in LGMD2C[8]and LGMD2A [5].

Creatine kinase (CK) or creatine phosphokinase (CPK) enzyme converts creatine to phosphocreatine, a specific enzyme for muscles, clinically utilized for acute and chronic muscles' diseases detection.The enzyme has B and M subunits, may exist as heterodimer MB or homodimers BB and MM, derived from different genes. MM mainly occur in skeletal muscles, BB occurs in thyroid and brain, while MB occurs in cardiac muscles [10]. Analysis of CKlevel in blood seems to be a satisfactory screening method, if clinically specified.

Aim of the present study is to determine the inter-families biochemical variability in calpainopathy of Pakistani families before which were not reported.

MATERIAL AND METHODS

Two calpainopathy families (A, B) were identified and enrolled into this study at the neurology department of *Lady Reading Hospital Peshawar.*Calpainopathywas diagnosed by Electromyogram (EMG) showing small polyphasic potentials.This study was performed in accordance with the *declaration of*

Helsinki and was approved by the ethics committees of the participating institutes. Peripheral blood was obtained from all available affected and unaffected members of the families. Genomic DNA was extracted with standard phenol chloroform method and stored at -80.

Genetic mapping byhighly polymorphic microsatellite markers (average heterozygosity> 75%) spanning the linkage interval of CAPN3 gene on chromosome 15q15.1-q21.1 were carried out. Microsatellite markers used for linkage mapping are given in Table 1. After obtaining the positive and convincing linkage to a known gene in both families, corresponding gene was sequenced to identify functional variant causing the disease phenotype in the families remain inconclusive. The whole exome sequencing is required to elucidate the variant.

Blood samples were collected for CPK, analysis from three normal (III-1, IV-1, V-5), and three affected individuals (V-4, V-6, V-7) of the family A, eight affected individuals (IV-1, IV-2, IV-4, IV-5, IV-6, IV-7, IV-10, IV-11) and four normal individuals (III-4, III-5, IV-8, IV-12) of the family B in gel tubes (BD Vacutainer®, Franklin Lakes NJ, USA).

Serum enzyme CPK, were measured using by Hitachi 912 (Roche) chemistry. The levels of serum enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. The upper limits of normal for CPK was 170 U/L for Female and 190 U/L for Male.Creatine phosphate and ADP of the reagent kit react in the presence of serum CK convert the DP to ATP and creatine, the ATP in turns react with the D-glucose in the reagent and the hexokinase catalyse the reaction converting the glucose to phosphorylated glucose which reduce the NADP of the reagent to NADPH. The rate of the NADPH formation is directly proportional to the catalytic CK activity. It is determined by measuring the increase in absorbance.

Digital X-ray radiography was performed for all the affected participant of the study. The images were reviewed by expert radiologist; the results were obtained and interpreted.

RESULTS AND DISCUSSION

We studied twocalpainopathy families with 11 affected individuals and7 normal individuals for phenotypic and biochemical variability. Among the 11 affected individuals, age range from 15 to 40 years. The mean age at onset of calpainopathyin these patients was 27.5years.First symptoms were toe walking, weakness in the lower limbs, proximal weakness, and scapular winging. There was no clear correlation between age of onset and clinical course.In family A, all affected individuals are no longer able to walk. The severity of diseasewas more in family A as compare to family B. The same ages of affected individuals in family B, one affected individual is still toe walking while some affected individualscan walk with the help of mechanical suppor.

Affected individuals of family A showed 2 to 3 folds increased in CPK levels regardless of the sex involved (Table 1). According to X-rays report, the bones of patient(V-4) appeared over tabulated with thinning of cortices. Joint deformity was due to permanent contractures. However, since long bones and joints were well developed to normal configuration, it appeared that the problem or disease started late in life. It appeared that the bones were secondarily involved to some muscular disorders.

Affected individuals of family B showed from normal to 32 folds increased in CPK levels regardless of the sex involved (Table 1).Radiographic analysis was performed for one affected member (IV-7). According to X-rays report the patient's bones appeared ostepenic. All bones were developed to the adult size. Acetabuli appeared to be ill defined. Ischial bones appeared hypoplastic. All other joints appeared to be of normal configuration. Apparent deformity was due to the presence of muscle contractures. Since bones were well developed to the normal size, the disability was probably related to some neuromuscular disorders.

For the study, two amilies (A &B) showing calpainopathy already reported by Khan *et al*. [3] were recruited from Khyber Pakhtunkhwa, province of Pakistan. In each family, at least one affected individual was radiographically examined by expert radiologist at Pakistan Institute of Medical Sciences (PIMS) Islamabad.

Biochemical investigations revealed inter and intra-familial variability of calpainopathy. The CPK was analyzed for all affected, parents and normal individuals of both families. Families of group A and B showed a variable values for CPK. Affected members in family A showed 2.2 to 2.6 fold increased in CPK values while affected members in family B showed from normal to 32 fold increased in CPK values regardless of the sex involved.De paula*et al*. reported that the mean CPK values in males were about 24 fold above the normal value while in female 14 fold above the normal values [1] but Zatz *et al*. reported that all affected individuals showed normal or borderline increase of CPK [12].

Table 1: Level of Creatine Phosphokinase in normal and affected individuals of family A and B

Family A			
S. No.	Studied individuals	CPK values (U/L)	Ratio of CPK (analyzed and actual)
1	IV-1 (N)*	145	1X
2	IV-2 (N)	275	1.6X
3	V-4 (A)**	430	2.2X
4	V-5 (N)	255	1.5X
5	V-6 (A)	425	2.5X
6	V-7 (A)	505	2.6X
Family B			
1	III-4 (N)	86	1X
2	III-5 (N)	50	1X
3	IV-1 (A)	94	1X
4	IV-2 (A)	1629	9.5X
5	IV-4 (A)	6049	32X
6	IV-5 (A)	1140	6X
7	IV-6 (A)	638	3.3X
8	IV-7 (A)	490	2.5X
9	IV-8 (N)	164	1X
10	IV-10 (A)	186	1X
11	IV-11 (A)	571	3.3X
12	IV-12 (N)	81	1X

CONCLUSION

Interfamily clinical course of calpainopathy vary considerably suggesting environmental and other genetic factors which influence the disease course. Although CK level in serum is an adequate screening methods but only CK and radiological examination is not enough for excluding calpainopathy. A complete profile of genetic analysis, clinical signs and symptoms, biochemical and radiological examination are needed for excluding calpainopathy.

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