Carrier Detection of Duchenne Muscular Dystrophy by CPK Activity Testing and Conventional Needle EMG.

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Abstract

Background: Duchenne Muscular Dystrophy (DMD) is a dismal disease, which exhibits an X-linked mood of inheritance, characterized by progressive proximal muscular weakness, beginning in early childhood, wheel chair dependency by early teens and death from cardiopulmonary complications by the end of the second or third decade. Although the majority of carriers of DMD and Becker muscular dystrophy (BMD) are asymptomatic but they can be identified through minor clinical changes like limited weakness or occasionally bulky calves, and some may have elevated CPK levels and mild EMG changes, and as DMD is incurable disease, and carrier detection and genetic counseling are an important aspect of the global approach to limiting the number of patients with DMD and BMD and of carriers.

The Objective of the study is to test the value of CPK and conventional needle EMG muscle testing in detecting carriers in a group of Iraqi females with their sons having DMD and BMD.

Patients and Methods: The study was conducted in AL-Kadhimiyah Teaching Hospital Section of Neurology from October 2002 to December 2003, where a group of 20 female carriers of DMD and BMD, from 15 families were studied and compared to other Control group of 20 females randomly picked up. To each female studied full medical history, neurological examination, including manual muscle power testing, and

Pedigree analysis taken, and to each female in the study CPK testing, ECG, with cardiac Echo, and Conventional needle EMG were done.

Results: Only one female carrier (5%) had mild proximal muscle weakness, cardiac involvement only one (5%), had mildly dilated left ventricle but with normal systolic function.

11 (55%) female carriers had mildly elevated CPK above the upper reference range (170 U/L); 10 (58.8%) DMD, and 1 (33.33%) of BMD. And there is significant ($p<0.05$) difference in CPK activity between the two groups.

There is negative correlation between the age of female carriers and the CPK activity.

9 female carriers (45%) total, (52.94%) of DMD had proximal myopathic EMG changes, which were more prominent in the upper limbs. And there is significant ($P< 0.05$) difference in mean amplitudes of motor unit action potentials of Biceps Brachii, and Vastus Medialis muscles.

Conclusion: As the CPK and EMG testings are simple, non costly and readily available tests, and as they can be positive to some extent in a proportions of carriers so they can be performed on all possible carriers in the families of DMD & BMD as a simple screening test, especially the CPK, better to perform at an earlier age, and the EMG at an older age because it requires cooperation.

This has a significant impact on genetic counseling, aiming at preventing the spread of this bleak disease.

Keywords: Duchenne, Becker, Musclar dystrophy, Female carriers, CPK and Conventional EMG.

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Introduction:

Duchene muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked recessive traits. Thus males carrying the gene are affected, whilst heterozygous females are carriers but often unaffected. Carrier females will pass on the condition to 50% of their sons and 50% of their daughters that will be carriers, i.e. a 25% chance that a carrier will have an affected son [(1)].

Gowers was the first to deduce the genetic basis for the disease. In 1986 100 years after Gowers' Kunkel identified the DMD gene and provided molecular

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genetic confirmation of the inheritance pattern [2].

The primary purpose of identifying carriers is to provide accurate information to women and their husbands about the risk that any of their children will inherit the disease [3]. So carrier detection is an important aspect of the care and evaluation of patients with DMD and BMD and their family members [2]. Before the discovery of Dystrophin gene in 1986, carriers can be identified from pedigree information, from physical examination, and from indirect laboratory tests, the simplest and most reliable being the Creatinin Phosphokinase (CPK) test[3].

Mild muscle weakness or enlarged calves are occasionally encountered in heterozygous females [3]. Roses and colleagues have studied the female carriers and report a slight weakness and enlargement of the calves as well as elevated CPK values and abnormalities of the electromyogram (EMG) and muscle biopsy –all-slight in degree- in over 80% of the patients [4]. It has long been known that carriers of DMD may also have symptoms of the disease. Summaries of case reports until 1970 were reported by Penn and colleagues [5]. A separate group known as manifesting carriers is the result of incomplete Lionization of the maternal X-chromosome [1]. The clinical picture of carriers with symptoms can vary from muscle pain and cramp on exertion at one end of the spectrum, to severe muscle weakness leading to wheel chair dependency on the other end [6, 7].

If weakness is present, it is commonly mild, predominantly asymmetric, and proximally distributed [6, 8, 9]. The pelvic girdle is more frequently and earlier affected than the shoulder girdle. Age of onset is also variable, ranging from the first to the fourth decade. Onset before the age of 15 years usually leads to severe involvement [6, 10]. A small minority of female carriers is symptomatic [2]. Clinically apparent muscle weakness occurs in 2.5 to 20 percent of female carriers of a mutated Dystrophin gene [11]. Carriers of BMD rarely have symptoms-since the first report by Moser in 1974 [3], only few instances have been described [8].

A major advance in clinical diagnosis was the discovery by Sibley and Lehninger (1949), amplified by Schapira et al. (1953), that serum activity of the enzyme, aldolase, was greatly increased in boys with progressive muscular dystrophy. After a number of other serum enzymes were found to be elevated, the CPK test introduced by Ebashi et al (1959) proved to be the most sensitive, and it was soon established that young boys with DMD invariably had extremely large elevations of serum CPK that were rarely found in autosomal recessive muscular dystrophy [3]. Serum CK is also increased in approximately 70 and 50 percent of Duchenne and Becker carriers, respectively [12]. The elevations are usually mild, up to three times the upper limit of normal, ranging from 2-10 times the upper limit of normal [11,13].

For many years CPK testing was the best method for carrier detection [2] as about two-third of definite carriers have abnormally increased serum CPK, but a normal CPK test is not conclusive evidence against the carrier state[3]. There is some evidence that the detection rate for carriers is higher in childhood than in adults [3].

EMG In Muscular Dystrophy: Even though not diagnostic, narrows the differential diagnosis by effectively excluding primarily neurogenic processes such as spinal muscular atrophy. In general, the proximal muscles of the lower extremities may exhibit the more prominent EMG findings. The MUAPs in patients with DMD or BMD are typically of short

duration, particularly the simple (i.e, non polyphasic) MUAPs.

MUAPs amplitudes are variable (normal to reduced) and they are typically polyphasic from the variability in muscle fiber diameters, resulting in longer MUAPs durations [4]. Quantitative EMG (using measurement of duration, amplitude, and number of phases of photographed muscle action potentials) has revealed abnormalities in 38 to 42% of definite and probable carriers. Occasionally, the EMG is abnormal in a woman with normal CPK activity [3].

Electrocardiography (ECG) In Muscular Dystrophy: Among patients with DMD extensive fibrosis of the posterobasal left ventricular wall may result in the characteristic ECG changes of tall right precordial R waves with an increased R/S ratio and deep Q waves in leads I , aVL, and V5-6. The disorder is also associated with conduction disturbances, especially intra atrial leading to a variety of atrial arythmias. Intra atrial conduction defects are more common than AV or infra nodal defects in DMD. Since 1967, abnormalities in ECG, like those seen in DMD patients, have been recognized in carriers of DMD [14, 15]. It has become clear that severe cardiac involvement- dilated cardiomyopathy- can also occur in carriers, and may or may not be accompanied by muscle weakness [15, 16, 17].

Patients and methods:

The study was conducted in Al-Kadhimiya teaching hospital section of neurology from October 2002 to December 2003. Where we received families with cases of DMD and BMD, after assessment of their sons and re-evaluation by senior neurologist, CPK testing, EMG and muscle biopsy, we managed to enroll 15 families with clear history and diagnosis of DMD or BMD, 13 families with DMD and 2 families with BMD. From these 15 families 20 mothers of affected sons with DMD or BMD were studied. We called them carrier's group. And for the purpose of comparison we randomly picked other group of 20 females with comparable ages who were mothers of normal sons and had no history of muscle diseases or systemic illness and no family history of muscular dystrophy. And all of them where instructed and informed about the aim of the study we called them control group. A signed consent was taken from all subjects in both groups for inclusion in the study.

Methods:

To each female in the 2 groups' full medical history, neurological examination including muscles strength assessment by manual muscle testing using the medical research council scale, and calf circumference had been measured using special anthropometrics tape adapted from (BSN-JOBST,inc.) also the height and weight had been measured and family pedigree details taken from each mother.

And from each female in the 2 groups, 3ml of venous blood was taken for CPK testing (upper reference limit 170 U/L) and measured according to the RANDOX Enzymatic Colorimetric Method, also to each female ECG taken and in case of any abnormality transthorasic cardiac Echo was done, to each of the female studied a conventional needle electrode EMG, for the Deltoid, Bicepse Brachii, Triceps, and Abducter pollisis brevis (for the upper limbs, and Vastus Medialis, Rectus Femoris, Gastrocnemeus, and Tibialis Anterior muscles, (for the lower limbs) using Medtronic keypoint EMG machine.

Statistical Analysis:

We used the student t test to compare means of calf circumference, weight, height, ages, CPK and mean value of amplitude and duration between carrier and control groups and we used the correlation regression for the relation

between CPK activity and the age of the carriers.

Results:

The Group of Carriers: From the 15 families (13 DMD and 2 BMD), 20 female carriers (17 DMD and 3 of BMD), by family pedigree analysis we found that 12 females (60%) are obligate (definite) carriers and 8 (40%) are of sporadic cases. Their ages ranged (28-60 years), average 36.65, SD 7.52, No one had significant symptoms of muscle weakness, apart from one (5%) who had G 4+ proximal weakness in the pelvic girdle muscle. 4 carriers (20%) had calf hypertrophy (calf circumference >15inches) and only 2 (10%) had rubbery or resilient calf muscle texture. Only one carrier (5%) had history of palpitation on moderate-severe exertion. And 3 carriers (15%) had ECG changes, 2 of them had R-wave in V1 and deep S-waves in V5-6. And only one had LVH and Echocardiographic changes of mildly dilated left ventricle, but with normal ejection fraction (normal systolic function**).** 11 female carrier (55%) had mildly elevated CPK activity above the upper reference limits of 170 U/L, 10 (58.82%) of DMD, and one (33.33%) of BMD.9 carriers (45%), all of them carriers of DMD (52.94%), had proximal myopathic EMG changes of low mean duration and amplitudes of MUAPs and >15% polyphasia as shown in table (1). **The Control Group:** 20 healthy mothers, their age's ranges (20-46 years), average 31.5 SD 8.15 they were age, height and weight matched. No one had calf enlargement (calf circumference. >15 inches). Their mean calf circumference was 14.35 inches range (13.5-15), SD 0.46, compared to 15.05 inches ranges (13.5-17.5), SD 1.05 in the carrier group, but there is no significant difference in calf circumference between the two groups. Only two mothers from the control group (10%) had borderline CPK of 172, 175 U/L. And their mean CPK was 97.9 ranges (36-175 U/L), SD 42.7 Compared to 200.9 U/L as a mean, average (88-303 U/L), SD 74.76 in the carriers' group. And there is significant change in CPK values between the two groups. Also we found a negative correlation between age of carriers and CPK activity. Only 10 females in the control group had done conventional EMG successfully with good cooperation while the others were reluctant to do the test and three of them were uncooperative during the procedure. While all mothers in the carrier group were cooperative during the procedure. The EMG findings were normal and devoid of abnormalities in the control group. While the carrier group 9 females (45%) had low mean duration, and amplitudes of the MUAPs with polyphasic potentials >15%. With no significant difference ($p>0.05$) was found between the carrier group and the control group. But we found a significant difference ($p <0.05$) in the amplitudes of Biceps Brachii, and Vastus Medialis muscles, between the two groups. As shown in table (2) 9 of the carriers (45%) of the total (52.94%) of DMD showed > 15% polyphysia, 5 carriers (25%) only in the proximal upper limb muscles, 3 (15%) had polyphysia in both upper and lower proximal muscles and only 1 (5%) had polyphysia only in the proximal lower limb muscles.

Table (1): the characteristics of carriers

Characteristics of carriers	Carriers of DMD	Carriers of BMD	TOTAL NO.
Sign/symptoms of muscle weakness	1 (5.88%)	0	1 (5%)
ECG changes	3 (17.64%)	0	3 (15%)
Dilated LV	1 (5.88%)	0	1 (5%)
Calf hypertrophy	3 (17.64%)	1 (33.33%)	4 (20%)
Resilient calf	2(11.76%)	0	2 (10%)
CPK> 170 u/l	10 (58.82%)	1 (33.33%)	11 (55%)
Myopathic EMG	9 (52.94%)	0	9 (45%)

Table (2): the difference in the mean duration and amplitude of the MUAPs between carriers and control groups.

Parameter	Muscle	Carriers Mean±SD	Control Mean±SD	P Value
Duration (msec)	Deltoid	8.8±2.3	10.8±0.42	NS
	Biceps	9.12±1.92	10.63±0.38	NS
	Triceps	10.76±1.65	11.94±0.34	NS
	Vastus Med.	9.43±1.8	10.8±0.4	NS
	Gastro.	10.22±0.33	9.98±0.35	NS
Amplitude (mv)	Deltoid	0.529±0.285	0.98±0.113	NS
	Biceps	0.598±0.31	0.975±0.135	S
	Triceps	0.69±0.3	1±0.12	NS
	Vastus Med.	0.68±0.26	1.075±0.17	S
	Gastro.	1.12±0.17	0.945±0.36	NS

NS (not significant) $p>0.05$
S (significant) $p<0.05$

Discussion: In this study we tried to include as many carriers within each family as possible to increase the total number of definite carriers to keep ascertainment bias to a minimum. As four of the carriers came with their sisters who also had affected sons with DMD, and one carrier of BMD came with her daughter who also had an affected son. In our 20 female carriers 12 had definite X-linked pattern by pedigree analysis, and 8 mothers had only one or more affected sons (sporadic cases), in the past they were considered as probable carriers but new techniques to identify carrier status suggest that a

significant proportion of apparently sporadic cases are in fact the offspring of previously unrecognized carriers [1]. So from the 15 pedigrees 8 pedigrees with sporadic cases and 7 pedigrees with familial cases, as it has long been apparent that isolated cases of DMD are very common; Duchenne, himself, failed to recognize the familial nature of the disease in his original ten patients [3]. The question here is whether the female carriers of sporadic case is a true carrier of X-linked recessive or autosomal recessive dystrophy, and in our study all the families taken lacking female affections which excludes this possibility. In this study the cases of DMD were assessed by clinical examination, CPK testing, EMG and Muscle biopsy and we found that all the cases had early involvement around the age of 2-3 years with evident course of progressive muscle weakness and calf hypertrophy, elevated CPK and other query cases were excluded from the study. only one carrier had mild muscle weakness (5%).

the explanation of this low figure, might be due to the relatively small sample , and also we depend on the manual muscle power testing which has the drawback of being highly subjective. While in the Hoogerwaard, et al study they disclose more carriers with muscle weakness by the application of hand held dynamometry [11].

As there is no significant difference in calf circumference between the group of carriers and the control group but still there is relatively high measurements >15 inches in 4 (20%) which still can be a relatively high figure while only two carriers (10%) had rubbery calf muscle texture and this can be explained due to the subcutaneous fat in females which can give a false impression of soft texture. No carrier had cardiac symptoms apart of one who had palpitation on severe exertion, and only three (15%) had ECG changes 2 had R-waves in V1 and relatively deep S in V5-V6 and one had evidence of LVH, but cardiac Echo were completely normal apart from mildly dilated left ventricle but with normal systolic function, this mild cardiac involvement might explained by skewed X-inactivation which is tissue specific.

CPK was in the past the best method for carrier detection, but the results can be difficult to interpret in ethnic and racial groups with normally elevated CPK levels e.g. blacks have a higher reference range than whites; CPK levels of blacks may exceed the laboratory-stated normal limits without the presence of any pathology [3,2], so in our study we take a randomly selected sample of mothers who had normal children and have no history of muscular dystrophy nor of muscle weakness or any systemic illness , for purpose of comparison . As our female sample all are Iraqi, Arabic Muslims, and we only found that the CPK activity in the control group was borderline only in two females (172, 175 U/L) which might be regarded as trivial, but also refer to that it is a non specific test. And many factors affect the results and may give high readings, so we take into consideration these factors like strenuous exercise or muscle trauma, Even I.M injection or EMG needle (so the blood sample should be taken before the EMG examination) also any history of taking drugs that raise the CPK, should be sought in the history (like statins e.g.) Also haemolysed serum will give falsely elevated results.

In our study we found that the CPK, activity was mildly elevated in 11 (55%) of the total carriers, and 10 (58.8%) of carriers of DMD,While it is elevated in only one (33.33%) carrier of BMD. And these findings correlate with the Hoogerwaard et al study [11], also we found that the results of CPK, were statistically significant.

We found that there is a negative correlation between the age and CPK

activity of carriers, which support the opinion that the detection rate of CPK for carriers is higher in childhood than in adults [3] due to the possibility of selective loss of dystrophin-negative fibers with advancing age [13].

The quantitative MUAP analysis was carried out as described by Buchthal [18], in all subjects in the two groups, where we found there is a relatively low mean duration and amplitude, and >15% polyphasia, in the proximal muscles in 9 carriers (45%) in total, while it is (52.94%) in carriers of DMD, while it is normal in the control group and also in the carriers of BMD, which might be explained by the mild involvement in BMD. There is no significant difference in the mean durations and amplitudes between the two groups, but the mean amplitudes of Biceps Brachii, and Vastus Medialis muscles were found to be of significance.

Also we found that polyphasia >15% was found more frequently in the proximal upper limbs muscles (Deltoids, Biceps Brachii) it is found in 8 carriers (40%) , but present in 5 carriers (25%) in the Triceps ,but it was only found in 3 carriers in the Vastus muscle, which is against of what is known of the early and more involvement of the pelvic-girdle muscles in carriers of DMD, but it agrees with the Hoogerwaard et al[11] which showed more involvement of weakness of the shoulder girdle muscles. The EMG had the drawback of being relatively an invasive procedure, and requires patients cooperation, and was difficult in children, so our patients all are adults, and only 10 females from the control group had done the procedure with good cooperation and 7 refused the test and 3 were uncooperative so we consider only 10, while the carrier group all the mothers had good cooperation with the test, which might be that they felt commitment to their affected sons.

Conclusion: As the CPK and EMG testings are simple, non costly and readily available tests, and as they can be positive to some extent in a proportions of carriers so they can be performed on all possible carriers in the families of DMD & BMD as a simple screening test, especially the CPK, better to perform at an earlier age, and the EMG at an older age because it requires cooperation.

This has a significant impact on genetic counseling, aiming at preventing the spread of this bleak disease.

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