Genetical and Biochemical Study in Duchenne muscular dystropy carriers

H. I. Ibrahim and A. H. Hasson
Department of Biology, College of Education, Ibn Al-Haithiam University of Baghdad.

Abstract

Six females definite Duchenne carriers and 8 females of possible carriers were examine in this study by serum C.P.K after exercise and muscle Biopsy for histochemical study. This study was compared with that of normal person.

The serum C.P.K was found to be highly elevated in all the 14 carriers, while slight - moderate elevation was found in normal control.

All the definite carriers and 6 expected gave abnormal histochemistry in their muscles, the other 2 gave no definite abnormality.

So these tests can be used in the future for assessment of Duchenne carriers.

Introduction

Duchenne MD is x- linked recessive disease affects the muscle primarly , so this disease usually affects males and the females usually carriers for the disease.

Appear as a detection of these carriers is very important in order to prevent this miserable disease by making advice to their relatives.

For women known to be carrier of the gene for one of the x- linked dystrophy, there is a 1 in 4 chance that any pregnancy will result in an affected son.

Examination of the family history can show three types of carrier (1):

1- Definite carriers; those mothers of an affected son who have also an affected brother, maternal uncle, sister's son or other male relative in the female line of inheritance.

2- Probable carriers; those with 2 or more affected sons without other affected relative.
3- Possible carriers; are the mothers of isolated cases and the sisters and other female relatives of affected male.
It is a fact that many, and perhaps all, true carriers have slight degree of myopathy which usually can be detected by investigation (2).
The estimation of C.P.K in the sera of these carriers was also tried. (3). High level was noticed only in (70- 75%) of definite carriers (4), others give normal level (20-25%) .
Those with high C.P.K. were more seen during childhood, decline is noticed in the second and 3rd decades (4). There are tow anaerobic ways by which muscles can acquire ATP , one of these ways is depends on creatine phosphate ,a high energy compound built up when a muscle is resting (5). so the measurement of serum C.P.K. is of value in the diagnosis of disorders affecting skeletal and cardiac muscle as well as in studies of families affected with muscle dystrophy. Nonmuscular tissue other than brain do not contain high levels of C.P.K.(6).
Eelectromyographic study (EMG) may be also helpful. (Sampling of multiple muscles this was recorded by smith, Amick and Johnson 1966 (7,8) and others. However 50-70% of definite carriers only, gave abnormal EMG study and this needs careful comparism with normal control subjects.
Lastly muscle histology and electron microscopy (9) was tried and the result was variable and non specific and not conclusive.
In this study biochemical and histochemical evaluation was tried for both C.P.K. in the serum and muscle histochemistry for muscle biopsy samples from females who proved to be carriers for Duchenne M.D gene.
Comparism study was done in both rest and exercise state.

Materials and methods

six females at age (20- 30 years) definite carriers and 8 expected carriers of the same age were examined in this study. Normal females of the same age were used as control from those undergoing orthopaedic surgery.
Serum was taken from all and sends for C.P.K. study at rest.

Then all were subjected to exercise in the form of going up and down stairs for 5 time. (about 3 meter hight), and another serum was taken for new C.P.K. value.

Muscle Biopsy was taken from the left Vastus medialis to all 14 females, and a histochemical study was done by:

- Eosin / Haematoxyline stain.
- Stain for SDH (10)
- Stain for NADH .

Result

- Serum C.P.K.

It was increased by 2 folds after exercise., when compared with the control.

- Muscle histochemistry

a. Eosin / haematoxyline study

All the muscle examined showed various degree of myopathic charges in the form of variation in fibers size and shape, fibers atrophy, neuclear changes etc, when compared with normal control muscle.

b. The results of SDH & NADH

Good differentiation into fibers types was noticed in the examined muscle and selective a trophy was noticed in type I which was stained light violet by SDH and light blue by NADH

Type II fibers that stained Deeply for these two enzymes were of normal size.

The activities of these two enzymes were increased in the muscle of the carriers when compared with normal.

Discussion

Positive SDH and NADH activity was noticed in both type I & II muscle fibers, i.e. they are cytoplasmic enzymes. The high concentration of their activity in type II was explained according to the metabolic pathway of these fibers (kreb cycle) and the oxidative activity of these enzymes.(11). So that good differentiation was noticed into types of fibers.

When compared with the control muscle, atrophy seen in the carrier muscle fibers and selectivcly affecting type I, which is a classical criteria of myopathy.

The increased activity of these enzymes in the carrier muscle add another criteria in addition to the classical criteria for detection of carriers

M. C.P.K is an intracellular enzyme its level in the serum reflect the turn over of the muscle activity (12). The 2 folds increase in its level usually explained according to the fact of leakage phenomenon. Usually accepted in myopathic muscle fibers.

Exercise usually aggravate this phenomenon.

Conclusion

The above results (both C.P.K after exercise and muscle histochemistry in the muscle of carriers) showed changes regarding the carrier when compared with normal, and so these can be used to evaluated Duchenne carriers female specially for those possible or suspicious definite carriers for the purpose of family planning.

References

1. Murphy, S.F.(1967). Oculo - pharangeal syndrom. J. of American Medical Association . 203, 1003.
2. Emery, A. E. H.(1963). Clinical manifestation in two Duchene M.D Carriers. Lancet .
3. Schapira, F. and Dreyfus, J.(1960). Revue Francaise d' Etudes Biologiques. 5.
4. Gradner – Medwin, D. (1970). Mutation rate in Duchenne MD.J. of Medical genetics.7, 334.
5. Mader, S.(2001). Human biology . 6th edition. McGraw- hill, U.S.A.
6. Harper, H.; Rodwell, V. and Mayes. P. (2000). Reviewof physiological chemistry .18th edition. McGraw- hill, U.S.A
7. Smith, H. L.; Amick, L. D. and Johnson, W. W. (1966). Detection of carriers and subclinical state of Duchenne MD. J. of Paediatries. 69, 67.
8. Gradner – Medwin, D. (1979). Controversies about Duchenne MD. Developmental medicine and child neurology 21, 390.

9. Afifi, A. K.; Bergman, R. A. and Zellweger, H.(1973). A possible role of electron microscopy in detection of carriers of duchenne MD. J of neurology. 36, 643.
10. Nachlas, M. M.; Tsou, K. C. and Seligman, A. M.(1957). Cytochemical demonstration of SDH .J. Histochemistry & Cytochemistry. 5: 420- 436.
11. Dubowitz , V.(1973). Muscle Biopsy : Amodern approach.
12. Emery, A. E. H. and Marton, R.(1968). Genetic counseling in lethal x- linked disorder. Genitica et Statistica Medica . 18, 534.

دراسة وراثية وكيميائية حيوية لبعض الحاملين لصفات مرض دوشن (اعتلال العضلات الابتدائي)

حقي اسماعيل ابراهيم و عذراء حميد حسون
قسم علوم الحياة ، كلية التربية ابن – الهيثم، جامعة بغداد

الخلاصة

ست اناث من الحاملين للصفات الوراثية الاكيدة لمرض دوشن وثمان من المحتملات اجرين الفحص ،عن طريق اجراء فحص سيرولوجي لانظيم C.P.K في الدم بعد الاجهاد وكذلك الفحص النسيجي الكيميائي للعضلات مع المقارنة بالاناث الطبيعين.

لوحظ من الدراسة ارتفاع عال في نسبة الانظيم C.P.K في دم الحاملين للصفات الوراثية للمرض مقارنة بالطبيعيين ، وقد أعطت نتائج فحوصات العضلات ان جميع الحاملين الأكيدين للصفات الوراثية للمرض وحوالي ست من الحاملين المتوقعين أعطوا نتائجا غير طبيعية . أما الاثنتين الباقيتين فلم يعطوا نتائجا غير طبيعية أكيدة .

لذا يمكن استخدام هذه الفحوصات لغرض استبيان حاملي الصفات الوراثية لهذا المرض ومن ثم إعطاء الصورة عن وضع الأسرة المستقبلي.