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CYTOGENETIC APPROACH FOR DIAGNOSIS OF PATIENTS WITH MENTAL RETARDATION

MENTAL RETARDASYONLU HASTALARIN TEŞHISINDE SITOGENETIK YAKLAŞIM

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ABSTRACT

The completion of the human genome project and advances in molecular genetics have facilitated the diagnosis of genetic diseases. The information gained over the last 20 years has enabled rapid and reliable identification of genetic diseases. The discovery of DNA, obtaining metaphase plates from lymphocyte cell culture, and preparation staining methods have led to the visualization of numerical and structural defects in chromosomes under a microscope. There have been great developments in this field which is defined as cytogenetics in the last 50 years. In this study, an optimized laboratory procedure has been given with emphasis on the identification of fragile X mental retardation cases related to X chromosome by cytogenetic methods. In addition, sister chromatid exchange method used in the determination of chromosome fractures is mentioned. The original photographs of the stained metaphase plates obtained by these methods are shown in the study.

We believe that each laboratory can achieve better results by modifying the blood culture and staining procedure steps according to their own conditions.

Keywords: Fragile X staining, Blood culture, Lymphocyte isolation, SCE staining

ÖZ

İnsan genom projesinin tamamlanması ve moleküler genetikteki gelişmeler genetik hastlalıkların teşhisini kolaylaştırmıştır. Son 20 yılda bu konuda elde edilen bilgiler hızlı güvenilir bir biçimde genetik hastalıkların belirlenmesine olanak tanımıştır. DNA'nın keşfi, lenfosit hücre kültüründen metafaz plaklarının elde edilmesi ve preparat boyama yöntemleri, kromozomlarda oluşan sayısal ve yapısal düzensizleri mikroskop altında görülmesini sağlamıştır. Sitogenetik olarak tanımlanan bu alanda son 50 yılda büyük gelişmeler yaşanmıştır. Bu çalışmada daha çok X kromozomu ile ilgili Frajil X mental retardasyon olgularının sitogenetik yöntemler ile belirlenmesi üzerinde durularak, optimize edilmiş laboratuvar prosedürü verilmiştir. Ayrıca kromozom kırıklarının belirlenmesinde kullanılan sister kromatid exchange metoduna değinilmiştir. Bu yöntemler ile elde edilen boyanmış metafaz plakların orjinal fotoğrafları çalışmada gösterilmiştir. Kan kültürü ve boyama prosedürü aşamalarını her laboratuvar kendi koşullarına göre modifiye ederek daha iyi sonuçlar elde edebileceğini düşünmekteyiz.

Anahtar Kelimeler: Frajil X boyama, Kan kültürü, Lenfosit izolasyonu, SCE boyama

1. INTRODUCTION

Fragile X Syndrome is the most common hereditary cause of mental retardation and learning difficulties. The gene that causes fragile X syndrome is called FMR1. It occurs as a result of mutation in FMR1 gene on X chromosome. Epilepsy is also associated with one fourth of the patients. Hyperactivity and autistic symptoms (social shyness, avoidance of eye contact) are also common. What is the Frequency of Appearance? Clinical features of fragile X syndrome can be summarized in three main areas: cognitive, physical and behavioral. Men are generally more severely affected than women. Fragile X-linked mental retardation is seen in approximately 1 in 3600 men and 1 in 4000 to 6000 women. Approximately 1 in 2,000 people have milder problems [1]. Common Behavioral Characteristics; - Lack of attention, - Excessive mobility, - Inability to make eye contact, - Hand flapping, - Social anxiety, - Abnormal shyness. Common Physical Properties; Adult males usually have long faces, large and / or prominent ears and large testicles (macroorchidism). Problems with connective tissue are common, such as flat-footed and mitral valve prolapse that can cause heart murmur. Women and young children may have some of these characteristics or may not be different from the general population. Wide forehead, Strabismus, Long face, Large and prominent ears, High palate, Hyperextensibility of joints, Muscle laxity, Large testes, Flat insoles, Delayed speech and developmental retardation are a finding that needs attention and attention for family and follow-up physicians. Chromosome analysis and Fragile X- DNA analysis should be performed after exclusion of common causes such as hypothyroidism.

At the beginning of the normal fragile X gene, a small portion repeats several times. In people with fragile X syndrome, this recurrent part of the gene is larger than normal [2].

Fragile X syndrome does not occur in individuals with up to 60 repeats in the initial portion of the FMR1 gene and the FMR1 gene of these children is normal. Fragile X syndrome does not occur in people with 60 to 200 repetitions, but it can be said that they are carriers of pre-mutation. Men with more than 200 repetitions have a full mutation and fragile X syndrome. These men may have some of the problems described above [3].

Premutation carriers are unlikely to encounter any learning and behavioral disorders seen in fragile X syndrome, but early menopause (premature ovarian failure) in female carriers and Parkinson's disease-like hands tremors and gait and balance disorders (ataxia) may develop after 50 years of age in male carriers. . Fragile X DNA testing is also useful for correct diagnosis and treatment, accurate genetic counseling, and the risk of mental retardation-learning disability-autism in subsequent children [4].

Fragile X syndrome (FRXS) is a common hereditary disorder that causes development and learning disability. In humans, 30-50% of hereditary MRI is thought to result from mutations in genes on the X chromosome [5]. There is a fracture on the X chromosome structurally in the region defined as fragile X (FRAX) (Xq 27.3). In studies with MRI, FRXS was identified in 5.9% of male patients and 0.3% of female patients. FRXS has been described especially in male patients and is a common form of MRI in men. There are characteristic facial findings. Long face, broad forehead, prominent chin, long broad soft ears. It is difficult to detect in childhood and becomes more pronounced in adult patients [6]. Fifty percent of girls with fragile X syndrome have normal intelligence, but may have learning difficulties and emotional problems. 50% may show borderline or severe MRI. The disease is dominated by X-linked. Cytogenetic analysis should be the first step in the identification of index cases since other chromosomal abnormalities in addition to Rajil X may cause MR and developmental disturbances [7].

2. MATERIAL and METHOD

The fragile region index is always present in the case or in the family at the same point of the chromosome. But 100% is not seen in all cells. Other than fragile regions such as 16q22, others may be observed under special culture conditions or when inducers are added [8].

For example: Folate sensitive ones: 2q11, 2q13, 6p23, 7p11. 8q22, 9p22, 9q23, 10q23, 11q13, 12q13. 16p12, 22q13, Xq27. Distamycin A induced: 16q22, 17p12.

BrdU induced: 10q25, 6q13, 9p21. Afidicoline induced: 2q31, 3p14, 6q26, 7q32, 16q23, Xp22. 5-Azacitidine induced: 1q42, 19q13.

FraX (q27) is the most important fragile region of clinical significance in the given fragile regions and is related to mental retardation [9]. Therefore, only methods related to fragile Xq27 will be given.

Method 1. Solutions used: Preparation of medium: Med 199 (Gibco) 46 ml, FCS (Gibco) 2 ml, Phytohemaglutinin (Gibco) 1.7 ml, Penicillin 0.05 ml, Streptomycin 0.05 ml

Procedures: Chromosome production, Giemsa banding and light microscopy consists of examination stages. 100 cells must be analyzed during the examination under light microscope. If it supports clinical findings and is not seen in 100 cells, another 100 cells should be examined. Because this fragile region is susceptible to folic acid, the incidence is often very low. The minimum rate for diagnosis is 4% [10].

Method 2: The superiority of this method to method 1 is to induce the incidence of fragile Xq27 by adding a chemical agent. The solutions used are metatrexate (MTX) solution. Procedures: After the blood is drawn from the patient with a heparinized syringe, the needle is removed and 5 drops are added to the culture tube with the medium. 37 ° C is left in the oven. After 48 hours, 0.2 ml of stock MTX solution is added. 0.05 ml colchicine is added at 70 hours. Unlike chromosomes, it is kept in hypotonic solution for 15 minutes. Giemsa banding is applied. Metaphase frequency is low in the examined preparations. But fragile Xq27 is observed with a frequency of 10-12%.

Brdu Staining

Required Solutions: Brdu Stock: 15.37 mg BRDU + 10 ml distilled water (filtered)

32258 Hoechst stock solution: 0.5 mg / ml (50 microg / ml) prepared with bisbenzimide distilled water (lasts 2 weeks in the dark at +4 ºC) 32258 Hoechst working solution: 1 ml hoechst stock solution + 99 ml PBS solution 10 ml culture 0.1 ml of BRDU + 1 ml of heparinized blood is added. Incubate for 70 hours. 5 min in PBS. (room temperature) in PBS Hoerst solution in dark environment 20 min. (room temperature) in dark environment, UV light at a height of 13 cm in PBS solution for 25 minutes. Distilled water is passed. At 65 º C in 2x SSC solution for 15 min. It is suspended. distilled water. 5% giemsa is stained for 20 minutes. distilled water after examined under light microscobe (x200) (Fig.2-3).

3. FINDINGS

According to Krawczun, (1985), The majority of metaphase chromosomes had a net Gbanding motif. Figure 1 shows representative X chromosomes in which each has a portion of the Giemsa Xq27 positive band below the fragile site, while most of this dark band remains near the fragile site. This trend was clearly observed in more than 200 X chromosomes of the three subjects analyzed in this study. These observations established that the fragile site was in the Xq27.3 sub-band. When the length of the chromosome was compared to the expression of fra (X), the data clearly indicated that, for all subjects, the tendency was to show the greatest expression of the fragile Xq27 site in less extensive chromosome preparations. The percentage of frailty progressed steadily as the study of longer chromosomes progressed. Above 850 bands per haploid level, identification of the X chromosome with clear Xqter visualization was rarely possible [11].

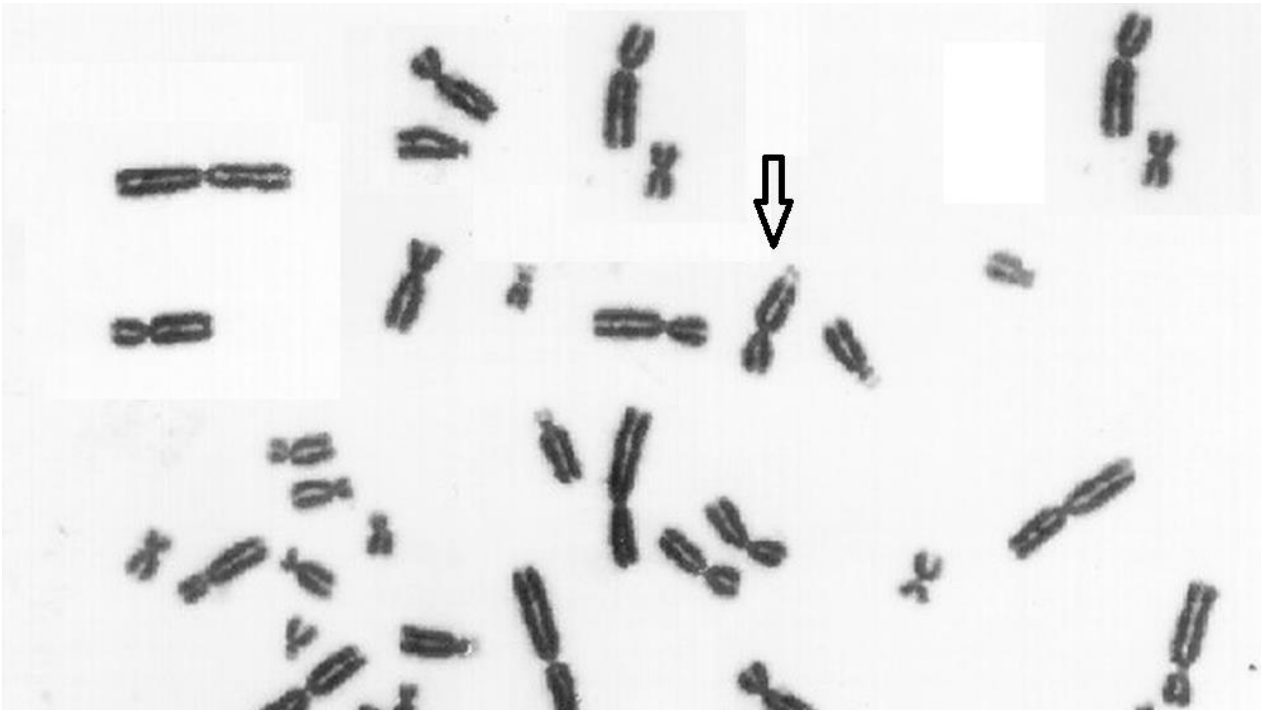

Figure 1. Metaphase area stained with fragile X method. X chromosome is indicated by an arrow.

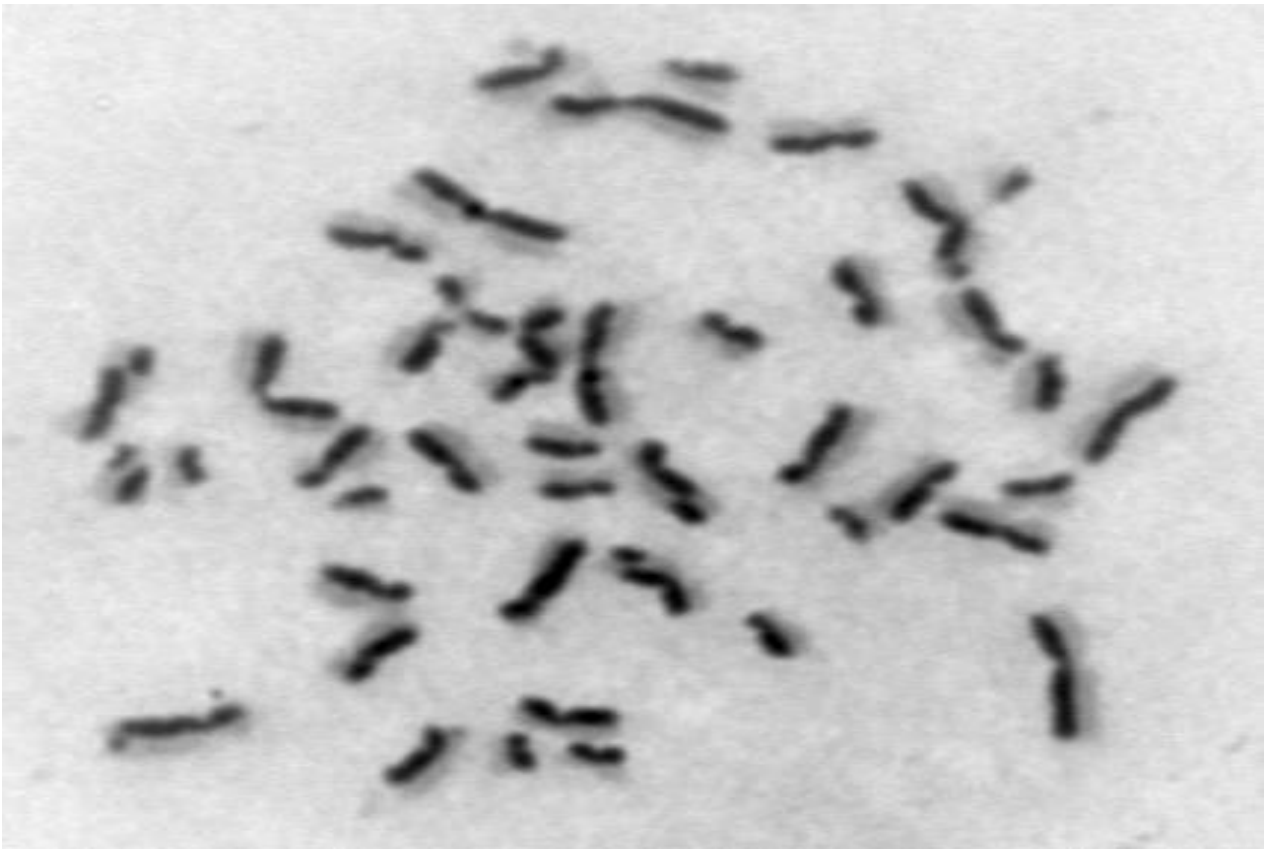

Figure 2. A metaphase field with chromatite fracture number 5

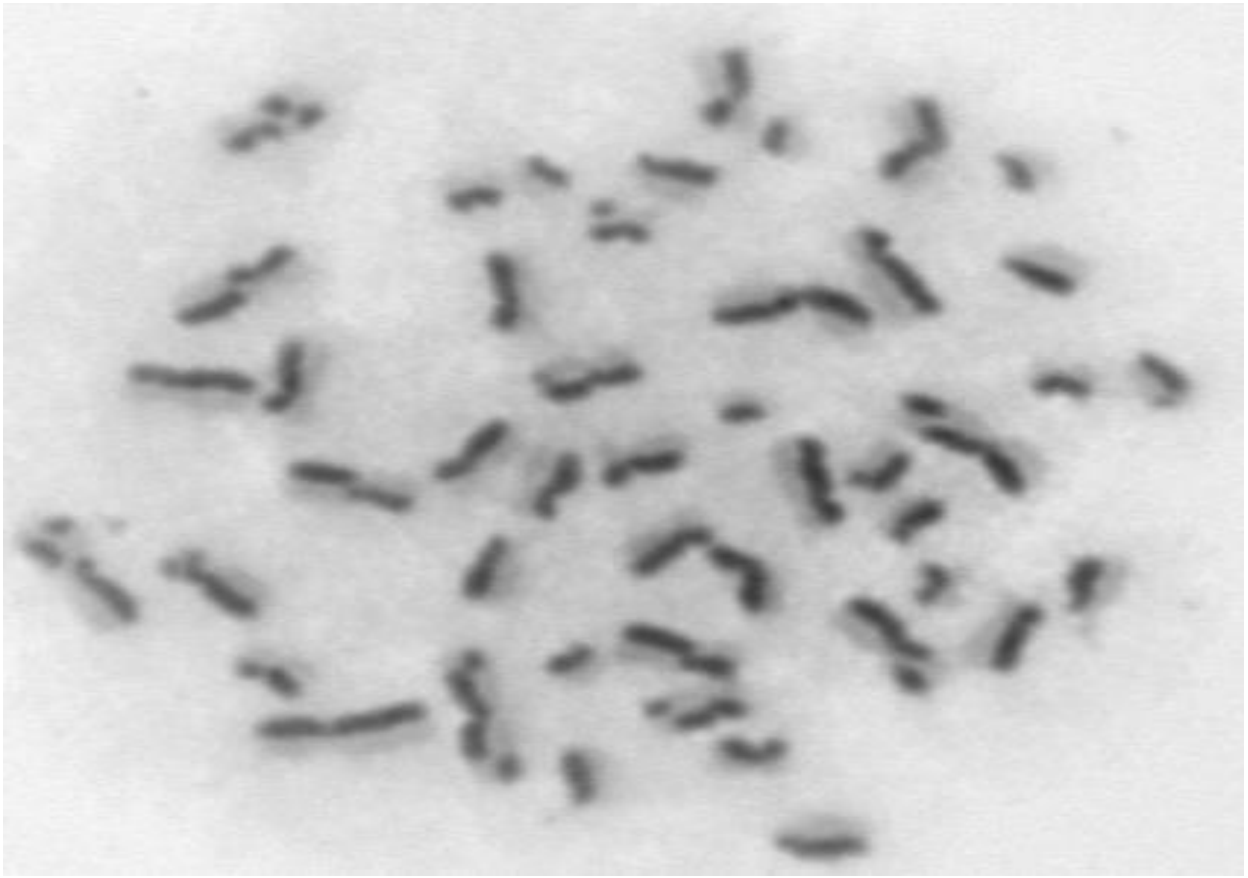

Figure 3. A metaphase field with chromatite fracture number 21

Today, although there is no definitive treatment to eliminate Fragile X disease, there are many applications for treatment [12]. These applications include special education, speech and language therapy, skills training therapy, and physical therapy. With applications such as sensory integration therapy, motor coordination, joint stability, visual, auditory and tactile information are aimed to be converted into appropriate motor responses.

Drugs are often used to treat hyperactivity and short attention span. There are also drugs used in the treatment of aggression, anxiety and depression. Providing the best possible education and therapy can only be achieved through close monitoring of the developmental stages and well-informed parents [13,14,15].

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