Assessment of Genetic Mutations DMD, DYSF, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1 Genes Induction Duchenne Muscular Dystrophy

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Abstract

Background Importance and Aim: Our aim is to investigate the genetic and genetic patterns of Duchenne muscular dystrophy.

Methodology: In this study we have analyzed 20 people. 10 patients Duchenne muscular dystrophy disease and 10 persons control group. The genes DMD, DYSF, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1 analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with nervous disorders, Duchenne muscular dystrophy disease.

Results and Conclusion: In fact, of all people with Duchenne muscular dystrophy disease. 10 patients Duchenne muscular dystrophy disease had a genetic mutation in the genes DMD, DYSF, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1 Duchenne muscular dystrophy disease. Any genetic mutations in the target genes control group did not show.

Keywords: Genetic study; Duchenne muscular dystrophy disease; Mutations The genes DMD, DYSF, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1

Introduction

Duchenne muscular dystrophy (DMD) is a severe type of muscular dystrophy.[1] The symptom of muscle weakness usually begin around the age of four in boys and worsens quickly. [2] Typically muscle loss occurs first in the upper legs and pelvis followed by those of the upper arms. This can result in trouble standing up.[1] Most are unable to walk by the age of 12.[2] Affected muscles may look larger due to increased fat content. Scoliosis is also common. Some may have intellectual disability. Females with a single copy of the defective gene may show mild symptoms.[1]

The disorder is X-linked recessive. About two thirds of cases are inherited from a person's parents, while one third of cases are due to a new mutation. It is caused by a mutation in the gene for the protein dystrophin. Dystrophin is important to maintain the muscle fiber cell membrane. Genetic testing can often make the diagnosis at birth. Those affected also have a high level of creatine kinase in their blood.[1]

Figure 1: Schematic View of Child with Duchenne Muscular Dystrophy.

No cure for muscular dystrophy is known. Physical therapy, braces, and corrective surgery may help with some symptoms.[2] Assisted ventilation may be required in those with weakness of breathing muscles.[1] Medications used include steroids to slow muscle degeneration, anticonvulsants to control seizures and some muscle activity, and immunosuppressants to delay damage to dying muscle cells.[3,4]

DMD affects about one in 5,000 males at birth. It is the most common type of muscular dystrophy.[1] The average life expectancy is 26; however, with excellent care, some may live into their 30s or 40s. [5-8] Gene therapy, as a treatment, is in the early stages of study in humans.[9,10][Figure 1]

Materials and Methods

In this study, 10 patients with Duchenne muscular dystrophy disease, and 10 persons control group were studied. Peripheral blood samples from patients and parents with written permission control were prepared. After separation of serum, using Real Time-PCR technique of tRNA molecules was collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solution in ratio of 1to5with the peripheral blood of patients and controls were mixed. After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 min at 400 Gera. The cell sediment with PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1to2 onficole (Ficol) was poured in the 480G was centrifuged for 34 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes have a molecular weight greater than Ficol and deposited in test tubes. [11-18]

The supernatant, which contained the mono nuclear cells, was removed, and the 400 Gera was centrifuged for 12 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 34 minutes incubation at 5 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription genes DMD, DYSE, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1, and were kept. [19]

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 4-5 × 10³ Neuroglial cells were transfer red to1.5ml Eppendorf tube and then were centrifuged at 2000 rpm for 7minutes at time. Remove the supernatant culture medium and there maining sediment, 100µl of PBS buffer was added. After adding 5-10µl PE monoclonal anti body to the cell suspension for 60 min at 4°C incubated and read immediately by flow cytometry. For example, rather than control anti body Neuroglial cells PE, igg1 negative control solution was used. [20]

Total mRNA Extraction Procedure Includes

1. 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200µl chloroform solution to target mix, and then transfer the micro tubes was added, and the shaker well was mixed for 15

seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4°C and was centrifuged at 13200 rpm era. Remove the upper phase product was transfer reductase new micro tube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C was incubated. [21-27]

2. Then for 45min at 4°C and was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C and by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20µl sterile water and at a later stage, the concentration of extracted mRNA was determined. [28]

To assessment the quality of mi-rna, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentase K1622) and 1µl oligoprimers 18 (DT) was performed. Following the PCR reaction 2µm dNTP, 1µg cDNA, ferment as PCR buffer 1X, 0 / 75µm mgcl2, 1.25 U / µL Tag DNA at 95°C for 4min, 95°C for 30s, annealing temperature 58°C for 30s, and 72 °C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophoresis with ethidium bromide staining and color was evaluated. [29-30]

Results

The results of the PCR and RT-PCR reaction for Duchenne disease target genes are as follows:[Figure 2-8]

Figure 2: Diagnosis of DYSE gene expression in patients with Duchenne Muscular Dystrophy.

Figure 3: Schematic view of the nucleotide sequence of target mutated genes in patients with Duchenne scrotum dystrophy compared to normal group.

Figure 4: Schematic representation of the expression mutated genes expression diagram in patients with Duchenne scrotum dystrophy with MHC expression changes compared to normal group.

Figure 5: Schematic view of the formation of a target mutated genes band in patients with Duchenne scrotum dystrophy with a pattern of bond formation in parent genes carrying Duchenne muscle dystrophy target mutations.

Figure 6: Schematic View of the Formation of the Dystrophin-mutated Gene Genes in Patients with Duchenne Sciaticular Dystrophy with the Bond Formation in the Genes of the Mothers and Daughters of the Duchenne Muscular Dystrophy Mutations.

Figure 7: Schematic view of the pattern of altered mirna expression of dystrophin in patients with Duchenne Muscular Dystrophy.

Figure 8: Images of Children with Duchenne Muscular Dystrophy.

Discussion

According to studies by Goemans NM and colleagues in 2011, similar results were obtained with the results of this study in the

study of genetic mutations effective in Duchenne disease. Since this study was the first in Iran, so the need for further research into the Duchenne disease should be created by other scholars of the world for different human races in the world.[Figure 9]

Figure 9: schematic view of an X Linked-recessive hereditary pattern that Duchenne muscular dystrophy also follows this pattern.

Conclusion

According to the results of sequencing the genome of patients with Duchenne muscular dystrophy disease, and the genetic mutations DMD, DYSF, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1 genes found that about 100% of patients with Duchenne muscular dystrophy disease, they have this genetic mutations. Patients with Duchenne muscular dystrophy disease, unusual

and frightening images in the process of Duchenne muscular dystrophy disease, experience. Lot epigenetic factors involved in Duchenne muscular dystrophy disease. But the most prominent factor to induce Duchenne muscular dystrophy disease, mutations is DMD, DYSF, EMD, LMNA, DUX4, DMPK, ZNF9, PABPN1 genes. This gene can induce the birth and can also be induced in the adulthood.

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