Assessment of Genetic Mutation Gene HPRT1 in induce Lesch-Nyhan Syndrome in 20 patient Tabriz, Iran

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Abstract

In this study we have analyzed 20 Lesch-Nyhan Syndrome (LNS) and 20 control group. The gene HPRT1 analyzed in terms of genetic mutation made. In this study, people who have genetic mutation were targeted, with nervous disorders, Lesch-Nyhan Syndrome (LNS). In fact, of all people with Lesch-Nyhan Syndrome (LNS) 20 Lesch-Nyhan Syndrome (LNS) had a genetic mutation in the gene HPRT1 Lesch-Nyhan Syndrome (LNS). Any genetic mutations in the target genes control group did not show.

Introduction

Lesch-Nyhan Syndrome (LNS), also known as Nyhan’s syndrome and juvenile gout, [1] is a rare inherited disorder caused by a deficiency of the enzyme Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT), produced by mutations in the HPRT gene located on the X chromosome.

LNS affect about one in 380,000 live births [2]. The disorder was first recognized and clinically characterized by medical student Michael Lesch and his mentor, pediatrician William Nyhan, who published their findings in 1964 [3] (Figure 1).

The HGPRT deficiency causes a build-up of uric acid in all body fluids. This results in both hyperuricemia and hyperuricosuria, associated with severe gout and kidney problems. Neurological signs include poor muscle control and moderate intellectual disability. These complications usually appear in the first year of life. Beginning in the second year of life, a particularly striking feature of LNS is self-mutilating behaviors, characterized by lip and finger biting. Neurological symptoms include facial grimacing, involuntary writhing, and repetitive movements of the arms and legs similar to those seen in Huntington’s disease. The etiology of the neurological abnormalities remains unknown. Because a lack of HGPRT causes the body to poorly utilize vitamin B12, some boys may develop megaloblastic anemia [4].

LNS is an X-linked recessive disease; the gene mutation is usually carried by the mother and passed on to her son, although one-third of all cases arise de novo (from new mutations) and do not have a family history. LNS are present at birth in baby boys. Most, but not all, persons with this
deficiency have severe mental and physical problems throughout life. There are a few rare cases in the world of affected females [5] (Figure 2).

The symptoms caused by the buildup of uric acid (gout and renal symptoms) respond well to treatment with drugs such as allopurinol that reduce the levels of uric acid in the blood. The mental deficits and self-mutilating behavior do not respond well to treatment. There is no cure, but many patients live to adulthood. Several new experimental treatments may alleviate symptoms [6,10] (Figure 3).

**Signs and Symptoms**

LNS is characterized by three major hallmarks: neurologic dysfunction, cognitive and behavioral disturbances including self-mutilation, and uric acid overproduction (hyperuricemia). Damage to the basal ganglia causes sufferers to adopt a characteristic fencing stance due to the nature of the lesion. Some may also be afflicted with macrocytic anemia. Virtually all patients are male; males suffer delayed growth and puberty, and most develop shrunken testicles or testicular atrophy. Female carriers are at an increased risk for gouty arthritis but are usually otherwise unaffected [11,14].

**Over Production of Uric Acid**

One of the first symptoms of the disease is the presence of sand-like crystals of uric acid in the diapers of the affected infant. Overproduction of uric acid may lead to the development of uric acid crystals or stones in the kidneys, ureters, or bladder. Such crystals deposited in joints later in the disease may produce gout-like arthritis, with swelling and tenderness [15-18].

The overproduction of uric acid is present at birth, but may not be recognized by routine clinical laboratory testing methods. The serum uric acid concentration is often normal, as the excess purines are promptly eliminated in the urine. The crystals usually appear as an orange grainy material, or they may coalesce to form either multiple tiny stones or distinct large stones that are difficult to pass. The stones, or calculi, usually cause hematuria (blood in the urine) and increase the risk of urinary tract infection. Some victims suffer kidney damage due to such kidney stones. Stones may be the presenting feature of the disease, but can go undetected for months or even years [19-22].

**Nervous System Impairment**

The periods before and surrounding birth are typically normal in individuals with LNS. The most common presenting features are abnormally decreased muscle tone (hypotonia) and developmental delay, which are evident by three to six months of age. Affected individuals are late in sitting up, while most never crawl or walk. Lack of speech is also a very common trait associated with LNS [23,26] (Figure 4).

Irritability is most often noticed along with the first signs of nervous system impairment. Within the first few years of life, extra pyramidal involvement causes abnormal involuntary muscle contractions such as loss of motor control (dystonia), writhing motions (choreoathetosis), and arching of the spine (opisthotonus). Signs of pyramidal system involvement, including spasticity, overactive reflexes (hyperreflexia) and extensor plantar reflexes, also occur. The resemblance to athetoid cerebral palsy is apparent in the neurologic aspects of LNS. As a result, most individuals are initially diagnosed as having cerebral palsy. The motor disability is so extensive that most individuals never walk, and become lifelong wheelchair users [27-30].

**Materials and Methods**

In this study, 20 patients with Lesch-Nyhan Syndrome (LNS) and 20 control groups 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 Hydroxyl Ethyl Starch (HES) was used. At this stage, HES solution in ratio of 1to5 with 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 1 to 2 on ficole (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 ficole and deposited in test tubes.

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 LSMAC. Then the cells were washed with PBS and attached to the column LSMACSS Pam Stem cell culture medium containing the transcription gene HPRT1, and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 4-5 × 10^6 Neuroglial cells were transfer red to 1.5 ml Eppendorf tube and then were centrifuged at 2000 rpm for 7 minutes at a time. Remove the supernatant culture medium and there manning sediment, 100 µl of PBS buffer was added. After adding 5-10 µl 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.

**Total RNA extraction procedure includes**

1) 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200 µl 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 was centrifuged at 13200 rpm era. Remove the upper phase product was transferred to a new micro tube and to the one times the volume of cold ethanol was added. The resulting mixtures for 24 hours at -20°C were incubated.

2) Then for 45min at 4°C and was centrifuged at 12000 rpm era. Remove the supernatant and the white precipitate, 1 ml 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 20 µl sterile water and at a later stage, the concentration of extracted mRNA was determined.

To assessment the quality of mi-RNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and 1 µl oligoprimers18 (dT) was performed. Following the PCR reaction 2 µM dNTP, 1µg cDNA, Fermentas PCR buffer1X, 0 / 75 µM MgCl2, 1.25 U / µL Tag DNA at 95°C for 4 min, 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 colors were evaluated (Figures 5,6,7).

**Discussion and Conclusion**

According to the results of sequencing the genome of patients with Lesch-Nyhan Syndrome (LNS), and the genetic mutation HPRT1 gene found that about 100% of patients with Lesch-Nyhan Syndrome (LNS), they have these genetic mutations. Patients with Lesch-Nyhan Syndrome...
Syndrome (LNS), unusual and frightening images in the process of Lesch-Nyhan Syndrome (LNS), experience. Lot epigenetic factors involved in Lesch-Nyhan Syndrome (LNS). But the most prominent factor to induce Lesch-Nyhan Syndrome (LNS), mutation is HPRT1 gene. This gene can induce the birth and can also be induced in the adulthood.

References