Ankara Bahçelievler Rotary Club

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RI D2430 ANKARA BAHÇELİEVLER ROTARY CLUB

Global Grant Project:
Laboratory Diagnosis Of Lysosomal Storage Diseases

OUR CLUB

Bahcelievler Rotary Club has been founded by Ulus Rotary Club and the founding president Rtn.Dr.Berdan Akalın on April 5th, 1985 by gathering people living or working at Bahcelievler. May 22nd, 1987 was the date our club has taken its charter from Rotary International and the first meeting was made at Etap Mola Hotel at Kizilay. In this meeting it was decided to make the weekly meetings on Thursday noon. Later on in 1990-1991 term, the meetings were shifted to evenings. Bahcelievler Rotaract Club was founded in 1994, Tandogan Rotary Club in 1996 and Kastamonu Rotary Club in 2003, Cayyolu Rotary Club in 2013 all under the supervision of Bahcelievler Rotary Club.

ANKARA BAHCELIEVLER ROTARY CLUB

GLOBAL GRANT PROJECT

LABORATORY DIAGNOSIS OF LYSOSOMAL STORAGE DISEASES

ANKARA UNIVERSITY, FACULTY OF MEDICINE,

DEPARTMENT OF PEDIATRIC METABOLISM

1.GENERAL INFORMATION ABOUT THE PROJECT

1.1.PURPOSE AND IMPORTANCE OF THE PROJECT

Lysosomal storage diseases (LSDs) are one of the group of inborn metabolic diseases. LSDs were first described by Hers H.G. in 1965. Hers first diagnosed Pompe diseases characterized by glycogen accumulation in lysosomes as a result of alpha glucosidase deficiency. Today, more than 60 LSDs have been identified according to the type of material that accumulates in the cell, its location and the enzyme that is deficient. Accumulation increases with age. LSDs are in the group of rare diseases with a ratio of 1/7000-1/8000. Hundreds of inborn metabolic disease have been identified with advancing technology and increasing recognition technology. New diseases continue to be recognized. Because LSDs are a multisystemic disease, the diagnosis can take up to 7 years if there is no suspicion. Even if the symptoms are mild, it will extend to 30 years. Most of LSDs are caused by severe clinical conditions affecting infants or children, causing permanent mental retardation, phsysical developmental delay and death. Early identification of LSDs is very important in terms of approach to treatment and prevention of permanent sequelae.

Most of the LSDs are inherited autosomal recessive. Due to the high number of consanguineus marriages in our country (21%), the incidence of inborn metabolic diseases is quite high. The population of Turkey is 80 million. The number of people suffering from metabolic disease is approximately 7-8 million in our country. 50% of these patients are children and 50% of patients die before the age of five if they can not be diagnosed.

Inadequate and late diagnosis in these rare inborn metabolic diseases is still the major obstacle to treatment today. Annual infant birth rates are also high in our country. The population of Turkey is 80 million and 1.177.000 babies are born every year in Turkey. For example from an European country, the population of Italy is 61 million and 576.000 babies are born every year in Italy. There are 40 national newborn screening laboratories for inborn metabolic diseases in Italy but we have only 2 laboratories in Turkey. Although metabolic diseases appear to be much less in many countries in Europe than in our country, approximately 15-20 diseases can be screened. However, only 2 inborn metabolic diseases (Phenylketonuria and Deficiency of Biotinidase Deficiency) can be screened in our country by national health system. LSDs are being added in many newborn screening programs in USA and many European countries. Countries which neonatal screening is not possible, the clinical findings of the patient are determined by the physician. If LSDs are suspected, the disease-specific enzyme deficiency is diagnosed by showing lack of enzyme in leukocyte, fibroblast culture or the affected tissues. Genetic pathogen mutations can also be used to confirm the diagnosis. However; in our country, measuring of spesific lysosomal enzyme levels are performed in a few number of centers. Because of difficulty of achieving to these tests, patients are being diagnosed late. Furthermore, they are not being diagnosed. Therefore, treatment of these disorders are delayed. Lysosomal diseases can not be screened in our country, thus we aimed to evaluate chitotriosidase activity (method is written in title 1.2.3.1.) for early diagnosis of lysosomal diseases. For this purpose, we will evaluate at least 3000 patients who have symptoms and signs of LSDs (symptoms and signs are given below table 1) within 2 years by chitothyrosidase blood test. If the chitothyrosidase level is high, we will study 4 different LSDs-specific enzymes which are more common and have treatment options than other LSDs (especially β-glucosidase for Gaucher, sphingomyelinase for Nieman Pick, α-glucosidase for Pompe, acid lipase for Wolman-Cholesterol ester depot diseases, α-galactosidase for Fabry) using intra-leukocyte enzyme measurements. In order to measure these enzymes and chitotriosidase activity, spectrofluorometric measuring device is needed.

Especially in the last 20 years, the researches on the pathophysiology and treatment of LSDs have been very successful. As a result of these studies, some therapeutic treatments have been used for some LSDs. Enzyme replacement therapy, which was first used in non-

neuronopathic Gaucher's disease, was later used in Mucopolysaccharidosis Type I, II, IV and VI, Pompe, Fabry and Wolman-Cholesterol Ester Depot diseases. Hematopoietic stem cell transplantation can be performed in some LSDs, for example Krabbe. Studies on gene therapies continue today. Our main goal in this project is to diagnose our patients to give them the chance for treatment. At the same time, it is very important to diagnose our patients for giving genetic counseling to the parents who have high risk of recurrence in the next pregnancy. Healty babies can born through the genetic counseling.

Early suspicion and diagnosis in infants and early treatment provide a chance for healthy life or less sequelae. Intervention of neurocognitive functions without deterioration is very important. Healthy babies is important both in terms of the national economy and reducing infant mortality as indicators of country developmental level. The ability to see daily needs alone is the most important indicator of quality of life. Treatment in childhood is directly effective on the health and ability of adulthood. However, some LSDs do not have a good prognosis despite sufficient treatments. Even if the patient dies, it is important that the mother provides a prenatal diagnosis in subsequent pregnancies.

1.2.LYSOSOMAL STORAGE DİSEASES

- Sphingolipidoses
- Ceramidase
    - Farber disease
    - Krabbe disease
        - Infantile onset
        - Late onset
- Galactosialidosis
- Gangliosides: gangliosidoses
    - Alpha-galactosidase
        - Fabry disease (alpha-galactosidase A)
        - Schindler disease (alpha-galactosidase B)
    - Beta-galactosidase / GM1 gangliosidosis

        - Infantile
        - Juvenile
        - Adult / chronic
    - GM2 gangliosidosis
        - AB variant
        - Activator deficiency
        - Sandhoff disease
            - Infantile
            - Juvenile
            - Adult onset
        - Tay–Sachs
            - Juvenile hexosaminidase A deficiency
            - Chronic hexosaminidase A deficiency
- Glucocerebroside
    - Gaucher disease
        - Type I
        - Type II
        - Type III
- Sphingomyelinase
    - Lysosomal acid lipase deficiency
        - Early onset
        - Late onset
    - Niemann–Pick disease
        - Type A
        - Type B
- Sulfatidosis
    - Metachromatic leukodystrophy
        - Saposin B deficiency
    - Multiple sulfatase deficiency

Mucopolysaccharidoses

- Type I
  - MPS I Hurler syndrome
  - MPS I S Scheie syndrome
  - MPS I H-S Hurler–Scheie syndrome
- Type II (Hunter syndrome)
- Type III (Sanfilippo syndrome)
  - MPS III A (Type A)
  - MPS III B (Type B)
  - MPS III C (Type C)
  - MPS III D (Type D)
- Type IV (Morquio)
  - MPS IVA (Type A)
  - MPS IVB (Type B)
- Type VI (Maroteaux–Lamy syndrome)
- Type VII (Sly syndrome)
- Type IX (hyaluronidase deficiency)

Mucolipidosis

- Type I (sialidosis)
- Type II (I-cell disease)
- Type III (pseudo-Hurler polydystrophy / phosphotransferase deficiency)
- Type IV (mucolipidin 1 deficiency)

Lipidoses

- Niemann–Pick disease
  - type C
  - Type D
- Neuronal ceroid lipofuscinoses
  - Type 1 Santavuori–Haltia disease / infantile NCL (CLN1 PPT1)
  - Type 2 Jansky–Bielschowsky disease / late infantile NCL (CLN2/LINCL TPP1)
  - Type 3 Batten–Spielmeyer–Vogt disease / juvenile NCL (CLN3)

    - Type 4 Kufs disease / adult NCL (CLN4)
    - Type 5 Finnish Variant / late infantile (CLN5)
    - Type 6 Late infantile variant (CLN6)
    - Type 7 CLN7
    - Type 8 Northern epilepsy (CLN8)
    - Type 8 Turkish late infantile (CLN8)
    - Type 9 German/Serbian late infantile (unknown)
    - Type 10 Congenital cathepsin D deficiency (CTSD)
- Wolman disease

- Alpha-mannosidosis
- Beta-mannosidosis
- Aspartylglucosaminuria
- Fucosidosis

Lysosomal transport diseases

- Cystinosis
- Pycnodysostosis
- Salla disease / sialic acid storage disease
- Infantile free sialic acid storage disease

Glycogen storage diseases

- Type II Pompe disease
- Type IIb Danon disease

Other

- Cholesteryl ester storage disease

1.2.SYMPTOMS AND SIGNS SUGGESTING LYSOSOMAL STORAGE DISEASES

The symptoms of LSD vary depending on the particular disorder and other variables such as the age of onset, and can be mild to severe.

In infants and children presenting with the findings in the table below, it is necessary to carry out metabolic tests for diagnosis of LSDs (Table 1).

Table 1. Symptoms and Signs of LSDs.

General

- Developmental delay
- Abnormal facial appearance (sometimes combined with macroglossia)

Central Nervous System

- Movement disorders
- Seizures
- Loss of acquired abilities
- Dementia

Pulmonary problems

- Dyspnoea
- Apnea

Cardiac problems

- Cardiomyopathy
- Heart failure

Eye

- Corneal clouding
- Cherry-Red Spot
- Blindness

Ear

- Deafness

Bone and Muscle

- Skeletal deformities

- Short stature
- Muscle weakness

Abdomen

- Umblical and inguinal hernias
- Organomegaly (especially liver and spleen)

Skin

- Angiokeratoma

Hematological

- Anemia
- Leukopenia

The worsening of symptoms should suggest progresive disease.

1.2.3. LABORATORY TESTS

1.2.3.1. CHITOTRIOSIDASE

Recently discovered human chitotriosidase is a member of chitinase family capable to hydrolyze chitin, a polymer of N-acetylglucosamine. It is released from activated macrophages. Serum and plasma chitotriosidase activity is usually measured as the first step in diagnosis of Gaucher disease (one of LSDs). Monitoring chitotriosidase activity is widely used during treatment of this pathology by enzyme replacement therapy. Its elevated plasma level reflects gradual intralysosomal accumulation in Gaucher cells (lipid-loaded macrophages). Macrophages overloaded by the enzyme accumulated in lysosomal material (lipids) were shown to secrete chitotriosidase; its increased expression was noted in several lysosomal storage diseases. Serum chitotriosidase activity increases excessively in all Gaucher patients (100-100 fold), moderately increases in other lysosomal storage diseases (eg GM1 gangliosidosis, Nieman Pick A and C, acid lipase deficiency etc). Chitotriosidase activity is used as a screening test in lysosomal storage diseases.

Preparation of Samples:

1. Blood can be collected in tube or on dried blood paper.
2. If tube is used, 2-3 ml of blood is collected into the tube with EDTA or sodium heparin.
3. It is recommended to take the sample on the day of laboratory work.
4. If the test can not be performed immediately, the tube should be centrifuged and the plasma frozen and sen to the laboratory on dry ice.
5. Chitotriosidase activity measurements were done on tube and microplate by a spectrofluorimetric assay using plasma and 5 mm punches of the dried blood samples.

1.2.3.2.LYSOSOMAL ENZYME ACTIVITIES

Measurement of lysosomal enzyme activities; in principle, using 4-methylumbelliferyl-derived fluorescent substrates, it is based on carrying out enzymatic reactions at acidic PH and spectrofluorometric measurement of 4-methylumbelliferyl levels. Leukocyte samples are used for the measurement of enzyme activity for Gaucher, Niemann Pick A/B, Fabry, GM1 diseases. Wolman and Pompe diseases are analyzed by dry blood samples.

Low enzyme activity or lack of enzyme activity is associated with LSD. For the diagnosis of patients have suspicious signs and symptoms for LSD in our center, we need materials listed in Table 2.

Table 2. Materials Needed For Our Project

NAME OF MATERIAL	NAME OF COMPANY	QUANTITY OF MATERIAL
Microplate Reader Device	Varioskan LUX multimode microplate reader/ Thermo	1 piece

As a Result,

- The chance of treatment is so high that if lysosomal storage diseases are diagnosed early (Enzyme replacement therapy, hematopoietic stem cell transplantation, new gene therapies).
- In our country, the Ministry of Health only screening phenylketonuria and biotinidase deficiency. Lysosomal storage diseases can not be screened in our country.
- Due to the high rate of consanguineous marriages in our country, this disease group is more common than other countries.
- In our country, where consanguineous marriages are high, the definition of the spesific diagnosis is very important in terms of providing genetic counseling to the family.
- Our aim in this Project,
    1. Our aim is to investigate lysosomal diseases at neonates and other age groups at risk with a single blood sample.
    2. Our aim is to diagnose lysosomal storage diseases which are more common than our country and provide effective treatment.
- Blood analysis of approximately 3000 patients is planned within 2 years.
- Chitotriosidase activity and spesific lysosomal enzyme activities can be analyzed by spectrofluorimetric assay.

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