HETEROZYGOTE SCREENING IN TAY-SACHS DISEASE

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INTRODUCTION

In 1887, Bernard Sachs reported on the clinical features, diagnostic signs and symptoms and pathology of a patient with a condition that would someday bear his name(1). It was several decades later before it was discovered that these patients suffered from a recessive genetic trait resulting in the accumulation of a specific ganglioside, GM2, due to the deficiency of the lysosomal enzyme hexosaminidase A(2). Heterozygotes for this recessive gene are clinically unaffected but have reduced concentration of this enzyme in serum and circulating leukocytes(3), as well as the other tissues of the body. As homozygotes are afflicted with a progressive, uniformly fatal, untreated disease, a strong rationale exists for heterozygote screening.

CLINICAL

Infants with Tay-Sachs disease exhibit no abnormalities at birth. However, by the age of six months, the infant becomes unusually quiet, listless, inattentive and apathetic. Early in the course of the disease, characteristic "cherry red" spots appear in the maculas of the majority of patients. There is progressive mental and motor deterioration, and blindness occurs between the ages of twelve and eighteen months. Epileptiform seizures are common and hypothalamic involvement may lead to precocious puberty. There is an increase in head size due to the retention of GM2 ganglioside within the cells of the central nervous system. Progressive dementia and muscular flaccidity follows, and death occurs at two to four years of age.

The medical expenses of Tay Sachs Disease patients are considerable. In 1973, Kaback et. al. estimated the cost of medical care to be $52,000 per child(4). Also, since enzyme replacement and other treatment modalities have proven totally unsuccessful(5), these efforts are all in vain. Of course, the emotional cost among the families of Tay-Sachs infants are undescribable. This situation has been greatly reduced by identifying heterozygotes. Genetic counselling is made available to couples if both partners are heterozygotes. In those couples who opt to have children, amniocentesis or chorionic villous biopsy can be performed to detect
affected fetuses. In confirmed cases of Tay-Sachs disease, elective abortion can be performed.

**Biochemistry**

The cerebral degeneration in Tay-Sachs disease is a result of the accumulation of ganglioside GM2 within the tissues of the central nervous system. Normally, these compounds are catabolized by stepwise removal of sugar and sialic acid residues. In Tay-Sachs disease, hexosaminidase A, which facilitates the removal of a sugar unit from GM2 to form GM3, is deficient(6).

The enzyme hexosaminidase A is composed of an alpha chain and two beta chains. A similar enzyme, hexosaminidase B, is composed of four beta chains(7). The alpha subunit gene locus is present on chromosome 15, and the beta subunit gene is on chromosome 5(8). In Tay-Sachs disease, the alpha subunit is defective. The defective alpha subunits result from a number of underlying mutations and not a single, identical abnormality present throughout the entire affected population(9).

**Population**

Tay-Sachs disease is a recessive genetic defect. The purpose of screening is to detect the clinically unapparent heterozygous state. As with any screening test, the detection of Tay-Sachs heterozygotes must be performed upon an appropriate population. Tay-Sachs disease is one hundred times more common in individuals of Ashkenazi Jewish ancestry(which comprises 98% of the American Jewish population) than in the general population. Approximately 1/30 Jewish individuals carry the gene for Tay-Sachs disease while only 1/300 do in the general population(10). As one-quarter of the children will be affected in a marriage between two heterozygotes, this equates to 1/3600 Ashkenazi Jewish infants being born with Tay-Sachs disease as opposed to 1/360,000 in the general population. As a well defined ethnic group has a significantly elevated risk, this population is applicable to screening.

The reason for the high incidence of Tay-Sachs disease in the Ashkenazi Jewish population is unknown. It has been estimated that a 6% reproductive advantage for heterozygotes would compensate for the devastating effects upon the homozygotes(11). Although it has been theorized the heterozygotes might have an increased resistance to tuberculosis(11), no firm evidence for this exists. A function for the gene might be theorized since the defect is
heterogeneous at the DNA level rather than a single defect. However, the possibility of genetic drift (chance factors) within an isolated population cannot be definitively ruled out (12).

Methods

The techniques for identifying heterozygotes for Tay-Sachs disease take advantage of the enzymatic activity of hexosaminidase A. Although substrates specific for hexosaminidase A have now been developed, the first and most commonly utilized test used a substrate which can be degraded by either hexosaminidase A or B (3), 4-methyl-umbelliferyl-N-acetyl-B-D-glucosaminide. Both hexosaminidase A and B cleave the fluorescent 4-methylumbelliferone, which can be quantitated. First, total serum enzymatic activity is measured. A serum specimen is then heated at 50°C for four hours, which inactivates 95% or more of the heat labile hexosaminidase A while leaving the majority of the relatively heat stable hexosaminidase B intact (13). Enzymatic activity is then remeasured and percent hexosaminidase A activity is determined from the difference. This measurement allows for the identification of heterozygotes. This technique has been automated (14) and continues to be the most commonly used commercial assay today (6).

Although the determination of serum hexosaminidase A is used as an initial screening, in inconclusive cases or when confounding variables are present, serum values may not be adequate. In these cases, the hexosaminidase A assay is repeated upon extracts from the patient's leukocytes (3, 4, 15). Since hexosaminidases are of lysosomal origin, the leukocyte assay measures a primary site of the enzymes while the serum levels give an indirect (and therefore less accurate) measurement which can be affected by hormones and disease states. In this assay, the leukocytes are pelleted and washed, and the cells are sonicated. The resultant supernatant is utilized for the assay (16). The leukocyte assay is more complicated, requiring more trained technician time and is not appropriate for automation. For this reason, it is not useful as a mass screening tool. It serves as a follow-up test in those patients whose serum tests are positive or inconclusive for the heterozygous state or who have a confounding medical condition.

Although the measurement of hexosaminidase A activity upon the nonspecific substrate continues to be the most commonly utilized detection method for heterozygotes, it is not without its difficulties. Since the level of hexosaminidase A is determined by subtracting
hexosaminidase B activity from total hexosaminidase activity, errors can be compounded. For this reason, a direct assay for hexosaminidase A would be more desirable. Sulfated derivatives, such as 4-methylumbelliferyl-6-sulfo-2-acetamido-2-deoxy-B-D glucopyranoside, are specific substrates for hexosaminidase A(hexosaminidase B unreactive)(15,17). The enzymatic activity is determined from timed incubation of serum or leukocyte derived enzyme with these substrates with subsequent separation of GM2 and GM3 by DEAE column chromatography(15,18,19). This test, although conceptually more satisfying, has not yet proven to have greater clinical significance than hexosaminidase determination with nonspecific substrates and is not in widespread use commercially(6).

Results

The results of serum assays performed with 4-methylumbelliferyl-N-acetyl-B-D-glucosaminidase(nonspecific substrate) have proven the efficacy of this procedure as a screening test. Infants with Tay-Sachs disease are found to have a mean hexosaminidase A activity of 1.7±1.1% while heterozygotes have a mean activity of 36.1±5.1% and noncarriers 56.2±4.1%(4). Genotype determination are made on a statistical basis. Any subject whose serum hexosaminidase level is more than three standard deviations above the mean measurement for heterozygotes is designated as a noncarrier. Since the statistical probability of being a heterozygote at this level is 1/1000 and the probability at large in the Ashkenazi Jewish population is 1/30, this gives a 1/30,000 chance of a negative test in a heterozygote for the Tay-Sachs gene(4,6). Patients with a value of 1.7 standard deviations of less above the mean are classified as heterozygotes and, in order to minimize the number of misclassifications, a broad inconclusive range of from 1.7 to 3.0 standard deviations above the mean carrier value is arbitrarily designated. In those patients whose serum screening tests are positive or inconclusive, the hexosaminidase assay is repeated upon leukocyte extracts. After the leukocyte assay is performed, genotypic designation can be made in all but 1.4% of those inconclusive by the serum test(less than .1% of the total patients tested)(4).

Problems with the test were noted shortly after the screening was begun. Pregnant women and women on oral contraceptives have a markedly elevated number of inconclusive and carrier results by the serum assay over that which would be expected. Padeh et. al. reported a 4.88% inconclusive rate in the general population, but a
13% inconclusive rate in women on birth control pills (20). Lowden reported a 15% false positive rate when using the serum test for women on birth control pills (21). It is not clear what factors affect the serum hexosaminidases during pregnancy or with birth control pills, but total serum hexosaminidase activity is increased, and a relative decrease in percent hexosaminidase A activity (by heat inactivation) occurs (4). Although initial studies seemed to indicate that the serum test with the more specific sulfated substrate might not be effected during pregnancy (18), it too has proven unsuitable for screening purposes. Specifically, carriers with unaffected fetuses will have serum hexosaminidase A levels in the normal range due to fetal enzymatic production (19). However, the leukocyte assay has proven to be unaffected in these patients (4).

Conditions other than birth control pills and pregnancy can also result in aberrant values using the serologic test. Diabetes mellitus has been reported to give false positive results (3), although this has not been found to be true in other studies (21). The serum hexosaminidase A assay is also reported to give false positive results in cases of tissue necrosis, including acute myocardial infarction, pancreatitis, hepatitis, metastatic carcinoma, Wegener's granulomatosis, myocarditis, glomerulonephritis, gunshot wounds or subdural hematoma (3). In these patients, total serum hexosaminidase activity is elevated, but percent hexosaminidase A activity is decreased (3). These conditions are, fortunately, not common in the population being screened (reproductive age individuals). However, when these complicating conditions do occur, the leukocyte assay is best employed.

Another complication occurs as a result of variant alleles for hexosaminidase A. Patients with juvenile onset Tay-Sachs disease, adult onset Tay-Sachs disease and some phenotypically normal individuals also have little or no hexosaminidase A activity (22). Should these cases (which have a frequency of 1/67,000) be diagnosed in utero, they would have been incorrectly diagnosed as infantile onset Tay-Sachs disease. The estimated carrier rate for these variants is 1/1200. Thus, 2-3% of those individuals who test as heterozygotes are probably not carriers for infantile onset Tay-Sachs disease, resulting in an approximately 2-3% false positive rate (22).

As of June 1981, more than 350,000 people were screened. Of those people, approximately 14,000 carriers were identified, and 333 couples were found at risk (23). The heterozygote rate among Ashkenazi Jew was 1/29 in these studies, which is close to the predicted level. No proven false negative have been reported using both the serum and leukocyte assays (False positives are not clinically
detectable, i.e. the patient will be considered at risk for affected children but will not have affected children, so an experimental false positive rate cannot be determined.). Of the couples shown to be heterozygotes, 912 pregnancies have been monitored. Fetal genotypes were determined by either amniocentesis or chorionic villous biopsy(24). Of the at-risk pregnancies, 202(22%) were found to be affected, and all but thirteen were terminated(23). Some Tay-Sachs affected fetuses have been detected too late in pregnancy for abortion while other couples have opted not to abort their pregnancies(8). One fetus was misdiagnosed in utero and was found to be affected after birth(23).

Conclusions

Heterozygote testing of Tay-Sachs disease has proven effective in the screening of the at-risk population(Ashkenazi Jews). First, a serum hexosaminidase A assay is performed as a screening test. Then, a follow-up leukocyte assay is performed in those patients with inconclusive or positive tests and those with confounding factors. The sensitivity of the combined serum and leukocyte assay is estimated at greater than 97%, and the specificity is considered to be much greater than 99%(1/30,000 false negatives)(6). In practice, no known false negatives have been reported, but, as approximately 1/3600 births occur in the unscreened population, the false negative rate should be exceedingly low. False positives do not present clinically so will not be identified, but since 22% of the at risk pregnancies have resulted in affected children(statistically predicted value-25%), the false positive rate seems to be negligible as predicted. Thus, the heterozygote screening for Tay-Sachs disease has been quite effective at identifying at-risk couples.. These couples require genetic counselling. Those desiring childbearing may have their pregnancies monitored, and affected fetuses may be aborted at the discretion of the parents. Thus, Tay-Sachs disease heterozygote testing has a high sensitivity and specificity as well as clinical utility.


Addendum 1

Specimen Parameters

Although several laboratories, including Mayo Medical Laboratories, offer the screening tests for Tay-Sachs Disease, only the laboratory in Children's Hospital and Health Center in San Diego has been accredited by the National Tay-Sachs Quality Control Program. They should be contacted in advance at (619)495-7737 before any specimen is sent.

This laboratory offers both the serum and leukocyte assays for $65.00. The tests should be performed upon both parents. During pregnancy, it is advisable to perform both the serum and leukocyte assays immediately due to the need for rapid results. If there are no complications, results from specimens received by Tuesday should be ready by Friday (The serum assay is performed on Wednesdays and the leukocyte assay on Thursdays.).

An approximately 1-2 ml. serum sample should be drawn into a non-heparinized (red-top) tube and allowed to clot at room temperature for two hours. The specimen then must be centrifuged, and the serum is drawn off and frozen. The white blood cell pellet is prepared as in Addendum 2. Two controls from non-Jewish individuals in good health with no family history of Tay-Sachs Disease must also be sent. The specimens should be mailed on dry ice in a well-sealed styrofoam container to:

The California Tay-Sachs Screening Program
8101 Birmingham Drive
San Diego, Calif. 92123
Addendum 2

WHITE CELL PREPARATION FOR ENZYME ANALYSIS

SOLUTIONS: 3% Dextran (Clinical Grade, Av. Mol. Wt. 200,000 - 275,000) in 0.9% NaCl, (Store at 4 C)
1.8% NaCl, (Store at 4 C)
0.9% NaCl, normal saline, (Store at 4 C)
Dextran available at: Sigma Chemical Company
P.O. Box 14508
St. Louis, MO 63178
1-800-325-3010

MATERIALS: 50 ml plastic syringes
18G x 1.5" needles
50 ml centrifuge tubes (preferably plastic)
Polypropylene tube 12 x 75 mm
Pasteur pipettes
Graduated serologic pipettes 10 ml. (when doing large number of WBC pellets, use Oxford repipettors.)

PROCEDURE: 1) Draw 10 ml of sodium heparinized blood, mix well, place immediately in ice. WBC should be prepared within 2 hours after blood is drawn.
2) Transfer 10 ml (approx.) of well mixed heparinized whole blood to a 50 ml plastic syringe from which the plunger has been removed, and the tip firmly secured with a piece of parafilm.
3) Add 3 volumes of chilled 3% Dextran in normal saline per volume of blood.
4) Replace the syringe plunger and gently mix the blood-Dextran suspension. Keeping the syringe vertical, remove all the air bubbles and then attach an 18G needle bending it at a downward angle. Leave a small air space at the top of the blood-Dextran mixture.
5) Set the syringe in a vertical position for 45 minutes at room temperature.
6) Keep the syringe vertical, transfer the supernatant (WBC suspension) to a cold 50 ml plastic centrifuge tube through the bent needle. Stop before the first bloody-red drop.
7) Centrifuge the supernatant at 4 C for 10 minutes at 1500 rpm (500xg) (Damon/IEC Centrifuge model #CRU-5000 used in California Tay-Sachs Disease Prevention Program lab).
8) Suction the supernatant and discard.
9) To lyse the remaining red blood cells, add 7 ml ice cold H2O and mix the suspension forcefully with a Pasteur pipette for 45 seconds. Restore isotonicity by adding 7 ml chilled 1.8% NaCl.
10) Aliquot the lysed suspension into 12 x 75 mm polypropylene tubes. DO NOT USE GLASS as sonification is to be done in these tubes. The number of aliquots will depend on the original blood volume, i.e., 3 aliquots per 10 ml of whole blood.
11) Centrifuge the plastic tubes at 4 C for 5 minutes at 2000 rpm (800xg). Aspirate supernatant off and discard. Put tubes in ice. Wait for 1 minute and aspirate the remaining supernatant off.
12) Wash pellet of step 11 with 2 ml cold normal saline. Vortex to resuspend pellet. Spin at 1000 rpm (200xg) for 5 minutes. Aspirate the supernatant off. The pellet should be 4-5 mm in diameter. Cap tube and store pellet as is, in freezer.