Introduction

The Wolfram Syndrome that is also known as DIDMOAD syndrome is characterized with the presence of diabetes insipidus, diabetes mellitus, optic atrophy and deafness resulting from a degenerative process involving pancreatic β cells, supraoptic and paraventricular nuclei, the optic nerve, and cranial nerve VIII. Dilatations in uro-genital system and variable neurological and psychiatric abnormalities are seen in majority of patients. In this study, we describe a family in which two members had the main features of the syndrome while a third sibling had only sensorineural deafness. DNA analysis revealed that the fully affected siblings were homozygote for a pointmutation on chromosome 4p whereas the third sibling with deafness was a heterozygote carrier for the same mutation. The characteristics of disease and phenotypic variations that possibly related to heterozygote carrier state were discussed.

Patients and Methods

Cases

Case One: An eighteen-year-old diabetic male patient was admitted with complaints of urinary retention and incontinence starting three months prior to admission. The patient had type 2 diabetes and was treated with oral hypoglycaemic agents. He had diabetes insipidus (DI) with urine osmolality 400 mOsm/kg and serum osmolality 315 mOsm/kg. The patient had a history of anosmia and had no family history of diabetes or DI. Physical examination revealed no abnormal findings except for DI, no mental status changes, and no evidence of a CNS mass. The patient had not experienced any episodes of fever or hallucinations. The patient was discharged from the hospital with the diagnosis of type 2 diabetes and DI.

Case Two: Twenty-nine year old male patient had Type 1 diabetes mellitus for ten years and were using insulin with poor compliance to diet. He had a history of optic atrophy and deafness. The patient had a history of anosmia and had no family history of diabetes or DI. Physical examination revealed no abnormal findings except for DI, no mental status changes, and no evidence of a CNS mass. The patient was discharged from the hospital with the diagnosis of type 1 diabetes and DI.

Case Three: This sibling is a thirty-two year old female. She had only complete deafness. From her history, it was learned that she had congenital diabetes mellitus. She had no family history of diabetes or DI. Physical examination revealed no abnormal findings except for DI, no mental status changes, and no evidence of a CNS mass. The patient was discharged from the hospital with the diagnosis of congenital diabetes and DI.

Introduction

The Wolfram Syndrome is a rare neurodegenerative disorder with autosomal recessive inheritance. The main characteristic features of this disorder are diabetes mellitus and optic atrophy. However, diabetes insipidus, sensorineural deafness, renal tract and neurologic abnormalities are seen in majority of patients. In this study, we describe a family in which two members had the main features of the syndrome while a third sibling had only sensorineural deafness. DNA analysis revealed that the fully affected siblings were homozygote for a pointmutation on chromosome 4p whereas the third sibling with deafness was a heterozygote carrier for the same mutation. The characteristics of disease and phenotypic variations that possibly related to heterozygote carrier state were discussed.

Key words: Wolfram syndrome, DIDMOAD syndrome, Heterozygote carriers, Mutation

CASE REPORT

WOLFRAM SYNDROME IN A FAMILY WITH VARIABLE EXPRESSION

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Summary: Wolfram syndrome is a rare neurodegenerative disorder with autosomal recessive inheritance. The main characteristic features of this disorder are diabetes mellitus and optic atrophy. However, diabetes insipidus, sensorineural deafness, renal tract and neurologic abnormalities are seen in majority of patients. In this study, we describe a family in which two members had the main features of the syndrome while a third sibling had only sensorineural deafness. DNA analysis revealed that the fully affected siblings were homozygote for a pointmutation on chromosome 4p whereas the third sibling with deafness was a heterozygote carrier for the same mutation. The characteristics of disease and phenotypic variations that possibly related to heterozygote carrier state were discussed.

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ACTA MEDICA (Hradec Králové) 2001;44(3):115-118

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Submitted June 2001.
Accepted November 2001.
Four: Thirty-seven year old man had chest pain suggesting angina pectoris. He had not a major risk factor of coronary heart disease in history and physical examination. The other two siblings are a 22 year-old woman and 28 year-old man. The fifth sibling was no abnormality in history and physical examination. The sixth sibling has not been examined since he was living another city but it was learned from other family member that he had not a known health problem. The history and examination of affected cases and similar health problems among the siblings suggested a genetic disorder in this family that consistent with Wolfram syndrome. So, all siblings and parents underwent a detailed laboratory examination for diabetes mellitus, diabetes insipidus, hearing loss and renal tract abnormalities. It has been learned from the family history that grand-father of these patients had committed suicide. No severe mental illness was detected on the psychiatric examination of cases but a tendency to depression on the first case and some changes in character on the second case were observed.

DNA analysis
For DNA analysis, blood samples of a living grandmot- her, parents and all siblings (except only sixth sibling) were collected in tubes with EDTA. Genomic DNA was isolated from blood according to standard procedures. Mutations present in the WS patients or their parents (obligate carriers) were identified through direct sequencing of the WS locus. In order to search for additional genomic DNA. Each exon of the WS gene and its splicing sites was amplified from genomic DNA by the polymerase chain reaction (PCR) using Genome PCR system 9600 (PE Applied Biosystems, Foster City, CA). The PCR products were directly sequenced by the Big Dye Primer Cycle Sequencing Reactions using the ABI 877 Integrated Thermal Cycler and the ABI 377 Automated Sequencer (Perker-Elmer Applied Biosystems, CA). A variation from the WS gene sequence in GenBank was determined to be a true mutati- on if there was a corresponding change in the amino acid sequence. Once a specific mutation was identified in the parental DNA, the appropriate region of the gene was then amplified from DNA of the other relatives. Those relatives showing the WS mutation found in the parents were considered carriers.

Results
The following results were found in the laboratory tests of cases.

Case One: Laboratory findings revealed that blood glu- cose level 374 mg/dl, hemoglobin A1c 13 %, urinary grav- ity 1006, urinary osmolality 305 mOsm/kg. Plasma and serum creatinine 13 mg/dl and 1.7 mg/dl respectively. Urinary osmolality in this family was normal. A mild sensorineural hearing loss (30 dB on the right and 22 dB on the left) was found in audio- metric examination.

Tab. 1: The results of water deprivation test in case 1 and 2 showing diabetes insipidus.

| Hours | Psm | Uosm | Hours | Psm | Uosm |
|-------|-----|------|-------|-----|------|
| 1     | 310 | 254  | 1     | 310 | 254  |
| 2     | 315 | 166  | 2     | 315 | 166  |
| 3     | 327 | 144  | 3     | 327 | 144  |

* The samples obtained after 1 hour of 10 μg desmopres- sin nasal spray

The blood sugar of patient was regulated with appropri- ate insulin doses. The clinical findings of diabetes insipidus improved dramatically 2 weeks after starting desmopressin. Urinary osmolality started to increase after a 10 μg desmopressin by nasal spray (Table 1). Abdominal ultrasonography and intravenous pyelography showed increased echogenicity in the parenchyma of both kidneys and dilatati- on in pelvicaliect systems and ureters as well as increased residual volume in the bladder. Cranial computed tomo- graphy was normal. A mild sensorineural hearing loss (30 dB on the right and 22 dB on the left) was found in audio- metric examination.

Discussion
Wolfram syndrome is a genetic disorder inherited in an autosomal recessive mode and it occurs in siblings of unaffected parents, which both are heterozygote carriers for the same autosomal recessive gene (3.7). Autosomal recessive diseases are clinically evident only in the homozygous for the mutation found. This means both alleles at a particular genetic locus are mutant in these conditions. On the other hand the heterozygote carriers in autosomal recessive inheri- tance are unaffected showing that mutations in the gene cosegregate with the phenotype. Predispotion of Wolfram syndrome heterozygote to psychiatric illness as well as homozygote for the same gene. The WS gene in this study is very important from this aspect. The clinical presentation of the first and second cases were typical for Wolfram syndrome but the from this case it was not possible to explain exactly how some manifestations of this patient detected no cause for hearing loss. Therefore, the association of hearing loss with Wolfram is obscure in this patient. The DNA analysis detected no mu- tation in fourth case. Therefore, impaired OGTT test and early coronary heart disease in this patient does not seem related to Wolfram syndrome.

It is not possible to explain exactly how some manifesta- tions of an autosomal recessive disease occur in a hetero- zygoate carrier state. It is also likely that there is a more complex genetic alteration rather than classical mendelian inheritance in at least some of Wolfram patients. However, this hypothesis requires more complex and fur- ther analysis of these patients. Variable manifestations among patients, even between siblings suggested that mito- chondrial dysfunctions could be involved in the disease. A loss of mitochondrial function was showed in two sporadic cases (2.8). Moreover, Barrientes et al showed the pre- sence of a high proportion of deletion in mtDNA of a Wolfram patient (1). However this hypothesis has not been supported by a more recent study (4). Inove et al found mutations in a novel gene encoding a putative trans- membrane protein in Wolfram patients and this appears to function in survival of beta-cells and neurons (5). This is an important finding to explain the different manifestations of a disease phenotype but still insufficient to explain va- riable manifestations among siblings and in heterozygote carriers.

Optic atrophy is a characteristic finding of Wolfram syndrome. However aside optic atrophy, we observed unusual ophthalmic findings for Wolfram patients such as posterior atrophy of optic atrophy. Optic atrophy of some patients had committed suicide in our family may also be related to a possible heterozygote carrier for the mutant gene of Wolfram.

Although the association is not clear, this study notices that some members of Wolfram families other than homo- zygoate siblings may have some findings of this syndrome. Moreover, some unusual eye findings and cardiac abnor- malities may be related to Wolfram syndrome. However, further massive investigation would be needed to explain these phe- notypic variations of this rare syndrome.

Tab. 2: The clinical parameters of the siblings.

| Age | Clinical Findings |
|-----|------------------|
| I 18 | DM, DI, OA, hearing loss, neurological manifesta- tions, neurological manifestations, other eye fin- dings, sensory nerve deficit, retinopathy, optic atrophy, diabetes mellitus, diabetes insipidus, OA |
| II 29 | DM, DI (incomplete), aortic insufficiency, deaf- ness, abnormal eye findings, neurological mani- festations |
| III 36 | DM, DI, OA, hearing loss, neural atrophy, diabetes insipidus, deafness |
| V 36 | Pre diabetic, early coronary heart disease |

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collected in tubes with EDTA. Genomic DNA was isolated from peripheral blood mononuclear cells with standard procedures. Mutations present in the WS patients or their parents (obligate carriers) were identified through direct sequencing of the WS locus. Linkage was studied with two microsatellite marker.

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| Case   | Hours | Posm | Uosm | Posm | Uosm |
|--------|-------|------|------|------|------|
| 1      | 0     | 151  | 350  | 151  | 350  |
| 2      | 1     | 154  | 296  | 149  | 296  |
| 3      | 2     | 166  | 302  | 186  | 303  |
| 4      | 3     | 190  | 310  | 311  | 311  |

The blood sugar of patient was regulated with appropri-
ate insulin doses. The clinical findings of diabetes insipidus implied diabetes insipidus type 1. The arginine vasopres-
sin (AVP) was less than 1 pg/ml. This may be related to a small and nonfunctioning normal pituitary gland.

Tab. 2: The clinical parameters of the siblings.

| Age | Clinical Findings                     |
|-----|---------------------------------------|
| 0   | DM, DI, OA, hearing loss, renal tract abnormalities |
| 1   | DM, DI (incomplete), aortic insufficiency, deaf-
ness, abnormal eye findings, neurological mani-
festations. |
| 0   | DM, diabetes mellitus, DI, Diabetes insipidus, OA: Optic atrophy |

The DNA analysis revealed a 1885 C to T transversion in the ABCC6 gene. The DNA analysis for the other relatives showed no change. The DNA analysis showed no cause for hearing loss. Therefore, the association of hearing loss with Wolfram is very obscure in this patient. The DNA analysis detected no mu-

tion in fourth case. Therefore, impaired OGTT test and early coronary heart disease in this patient does not seem related to Wolfram syndrome.

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Opotic atrophy is a characteristic finding of Wolfram syndrome. However aside optic atrophy, we observed unsu-
onal ophthalmological findings in affected cases such as poste-
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Submitted March 2001.
Accepted November 2001.

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