Long-term follow up of carbonic anhydrase II deficiency syndrome

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ABSTRACT

Objective: To describe the long term clinical, biochemical and radiological features of 35 Saudi Arabian children with carbonic anhydrase II deficiency syndrome who have been followed at King Faisal Specialist Hospital and Research Center, Riyadh since 1979.

Methods: The records of these patients were retrospectively evaluated. The diagnosis was based on the clinical and the radiological evidence of the disease. Carbonic anhydrase II level was measured in 9 patients.

Results: Clinically, these patients had typical facial features, growth failure and varying degrees of psychomotor retardation. Biochemically, all children had renal tubular acidosis that was of distal type in the majority of them. Radiologically, this syndrome was characterized by metyphyseal osteopetrosis and intracranial calcification that was progressive in 2 patients. Five patients were blind secondary to optic nerve entrapment and 2 patients developed anemia and secondary erythropoesis due to bone marrow involvement. Nineteen patients had attained the final adult height; the mean adult height was146 cm (-3 standard deviation) in 11 females and 152 cm (-4 standard deviation) in 8 males. Two patients were married and had clinically and radiologically normal children.

Conclusion: The syndrome of carbonic anhydrase II deficiency is usually benign in nature and compatible with long term survival, however it can progress and involve the cranial nerves. Close clinical and neurological assessment of these patients is mandatory to early detect and manage potential serious complications.

Keywords: Osteopetrosis, renal tubular acidosis, carbonic anhydrase II deficiency, intracranial calcification.

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The association of osteopetrosis and renal tubular acidosis was first described in 1972 in 3 separate reports.[1-3] In 1980, Ohlsson et al described the syndrome in 3 Saudi families in association with striking facial similarity, mental retardation, stunted growth and intracranial calcification.[4] In 1983, Sly et al[5] identified carbonic anhydrase II deficiency in erythrocytes of patients with this syndrome and proposed that carbonic anhydrase II deficiency is the primary defect in these patients. In the same year, Venta et al[6] located the gene for carbonic anhydrase II to the long arm of chromosome 8. Subsequently several sporadic case reports have been published describing the disease and several carbonic anhydrase II gene mutations have been described in these patients.[7-18] In this report, we describe our long-term clinical experience with 35 patients with this autosomal recessive syndrome who have been followed in one institution over the last 2 decades.

Methods. ***Subjects.*** We retrospectively evaluated the records of 35 Saudi patients (20 females, 15 males) with the syndrome of osteopetrosis, renal tubular acidosis and cerebral calcification who have

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been followed in the Pediatric Endocrine Clinic at King Faisal Specialist Hospital and Research Center from 1979. Some of these cases have been reported as a part of collaborative studies.[4,19-21] The current age of patients ranged from 8.5 to 34 years (median 15 years), and the age of the diagnosis ranged from 23 days to 16 years (median 9 years). These patients have been followed for a mean duration of 12 years (range 2-19 years). The patients in this series are products of only 10 families. The parents were first degree cousins in 6 families, 2nd degree cousins in 3 families and far relatives in one family. Three families were originally from the same tribe. Four families had more than 4 children affected. All patients were evaluated clinically in the genetic, ophthalmology, orthopedic, neurology, psychology and nephrology clinics. Biochemical assessment included complete blood counts, renal function tests, electrolytes, bone profiles and urine analysis. Radiological evaluations included skeletal surveys, serial bone age determinations and cranial computerized tomographies (CT scan).

Carbonic anhydrase II was analyzed in 9 patients, 5 fathers and one mother. The results of carbonic anhydrase levels in these Saudi patients were previously reported by Ohlsson et al,[20] and Sly et al.[21] The levels were undetectable (carbonic anhydrase I/II ratios above 10,000) in tested patients and ranged from 8.8 to 18.4 in heterozygous parents (normal levels fall between 6.2 and 9.8). Carbonic anhydrase I and II were measured after they were separated by reverse-phase high performance-liquid chromatography. The ratios of carbonic anhydrase I to carbonic anhydrase II in each hemolysate was calculated from these results. Carbonic anhydrase I and II were also detected by protein stains and by their esterase activities on starch-gel electrophoretograms and with double-immunodiffusion studies on agar plates as previously described.[20,21]

Results. ***Facial features.*** The majority of these patients have a peculiar facial appearance, 25 patients had craniofacial disproportion with broad forehead, large cranial vault, prominent nose, thin upper lip, small mouth and delayed dentition. Ten patients had additional features such as irregular carious peg-shaped teeth, enamel hypoplsia, micrognathia, short philtrum and malocclusion. Other features were also noted such as squint in 3 patients, nystagmus in 3 patients, genu valgum in 2 patients, simian crease and long fingers in one patient.

Growth retardation. Failure to thrive and growth retardation are universal findings in these patients. Twenty-eight patients had a height of more than 2 standard deviation (SD) below the mean (mean 3.9SD, range from -4.2SD to --3.3SD). Short stature in these patients was proportionate. Nearly 80% of them showed an improvement in growth rate in response to sodium bicarbonate therapy. Bone age was performed in 25 patients and reported to be retarded (more that 2SD below the mean) in 15 patients (mean -3SD, range from -3.5 to -2.5SD). As a part of work-up for growth retardation, thyroid function tests (free thyroxine level (FT4) and thyroid stimulating horomon (TSH)) and insulin growth factor 1 were obtained in 20 patients and reported to be normal. The mean IGF1 level was 195 ug/l, range from 90ug/l to 421ug/l (normal IGF1 range for children 6-11years is 88-565ug/l). The birth lengths and weights of 15 patients were available and reported to be normal. The mean birth length was 49cm, range from 48cm to 51.5cm and the mean birth weight was 3.1kg, range from 2.8kg to 3.9kg.

Psychomotor retardation. Developmentally, 17 patients had motor and psychosocial retardation; 5 of them were legally blind secondary to optic nerve compression and had, as well, significant hearing impairment. Intelligence quotient (IQ) was assessed in these patients and showed that 11 patients had mild mental retardation, 3 had moderate mental retardation and 3 had severe mental retardation.

Renal tubular acidosis. All patients had a persistent normal anionic gap metabolic acidosis. Distal renal tubular acidosis was suspected in 23 children. In these patients, bicarbonate level ranged from 12 to 18mmol/l (mean 15mmol/l) with a mean serum pH level of 7.2 (range from 7.1 to 7.3) and accompanied by inappropriately high urine pH (mean 6.3, range 6.0-7.5), chloride level was elevated and ranged from 110 to 119 mmol/l, potassium levels were borderline low and ranged from 3.0 to 3.5 mmol/l, 2 patients had nephrocalcinosis with elevated urinary calcium to creatinine ratio. In the remaining patients, proximal renal tubular acidosis was suspected, bicarbonate level ranged from 9 to 17mmol/l (mean 14mmol/l) with a mean urine pH level of 5.5 (range from 5.0 to 6.0), 4 patients had aminoaciduria, 2 patients had glucosuria. All patients required 2-6mmol/l per kg per day of sodium bicarbonate that was adequate to correct acidosis.

Radiological findings. The radiological features in all patients were indistinguishable from other forms of osteopetrosis. At presentation, all patients had increased bone density involving the skull, axial skeleton and the long bones with abnormal modeling and transverse banding of the metaphyses. Two patients had a radiological evidence of rickets (fraying and cupping of the distal ends of the long bones). They had normal calcium, magnesium and phosphorus levels and a slightly elevated alkaline phosphatase level. No vitamin D metabolites were performed in these patients to confirm the diagnosis of rickets. Sixteen patients had repeated fractures with normal healing. The fractures frequency ranged from 1 to 3 fractures per patient per year. The frequency of fractures reduced after puberty to 0-1 fracture per year. Only 2 patients continued to have

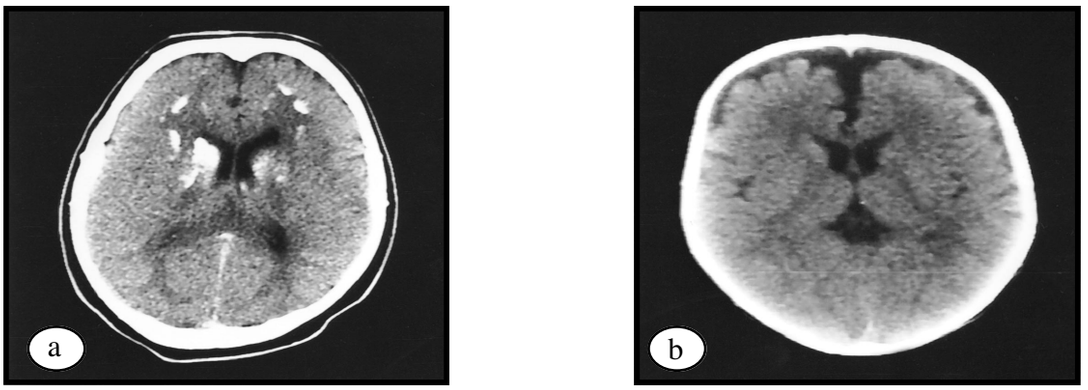

Figure 1 - Brain computerized tomography of a patient with carbonic anhydrase II deficiency **(a)** at 11 months of age with no calcification **(b)** and at 8 years with periventricular calcifications.

1-2 fractures per year. Skeletal surveys were performed for clinically normal parents and siblings of 2 families and were normal. Intracranial calcifications were present in all patients included in the study that involved the basal ganglia; caudate and putamen nuclei and periventricular white matter of the frontal, parietal and occipital lobes. The intracranial calcifications were progressive in 2 patients and became more extensive and apparent during childhood than during the neonatal period **(Figure 1).**

Other features. Restrictive lung disease was observed in 2 patients with a total lung capacity of 50% and a vital capacity of 55%. There was no radiological evidence of parenchymal lung disease in these patients. Two patients had hepatosplenomegaly, leukopenia, thrombocytopenia and anemia and needed frequent blood transfusion. Bone marrow biopsy and aspiration were not performed. There were no human leukocyte antigen (HLA) matched donors for bone marrow transplantation. Formal hearing assessment using audiograms and brain stem evoked potential, or both were performed in 21 patients. Six patients had sensorineural deafness and 2 patients had mild conductive hearing loss. Nine patients had visual impairment with varying degrees of optic nerve pallor; 5 of them were legally blind. Four patients with optic nerve atrophy and

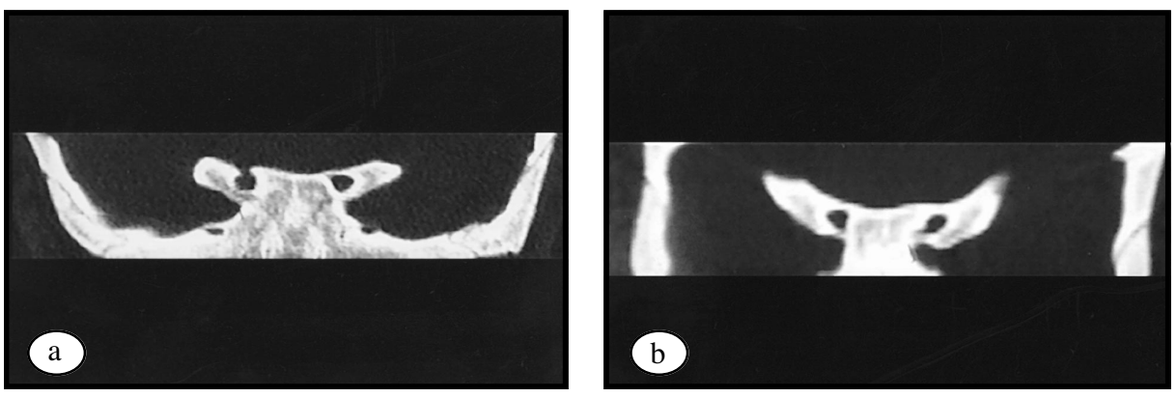

Figure 2 - Optic canal computerized tomography of a patient with carbonic anydrase II deficiency **(a)** showing optic foramina narrowing and **(b)** following optic canal decompression.

radiological evidence of optic nerve encroachment underwent decompression, 2 of them showed slight clinical improvement **(Figure 2).**

Final adult height. Nineteen patients (11 females and 8 males) have attained the final adult height. These patients were diagnosed and treated with sodium bicarbonate before the age of 4 years. At the age of 5 years, the mean height was 93cm (-3.5SD) (range from 89 to 95cm) for the female children and 89cm (-4.3SD) (range from 86 to 92cm) for male children. At the age of 10 years, the mean height was 119cm (-2.9SD) (range from 115 to 122 cm) for the female children and 111 cm (-4.2SD) (range from 108 to 113cm) for male children. As adults, the mean final adult height was146 cm (-3SD) (range from to 141 to 150cm) for the female patients and 152 cm (-4SD) (range from to 149 to 156cm) for the male patients. Two patients (one male and one female) were married and had clinically and radiologically normal children.

Discussion. The carbonic anhydrase II deficiency syndrome is an autosomal recessive disease that produces osteopetrosis, renal tubular acidosis, and intracranial calcifications. Carbonic anhydrase is a water-soluble zinc metalloenzyme that catalyses the reversible hydration of CO_2. It is distributed in many tissues including red blood cells, glial cells, bone, lungs, and proximal and distal renal tubules and its deficiency was presumed to be the primary defect in this syndrome.[4] Carbonic anhydrase II gene is 20 kb long, contains 7 exons and mapped to chromosome 8q22. Several mutations in the structural gene have been identified by polymerase chain reaction amplification of the genomic deoxyribonucleic acid from these patients. Worldwide, more than 50 patients with this syndrome have been reported,[22] however long term follow up is lacking. Thirty-Five patients with this syndrome have been followed over the last 2 decades at King Faisal Specialist Hospital and Research Center.

The majority of reported patients are from Saudi Arabia. They share the same typical facial features, which might be present in patients from other ethnic groups.[22] Stunted growth and failure to thrive are common findings that are usually evident after the neonatal period. Renal tubular acidosis is the most likely cause of growth retardation that usually improves after correction of acidosis however they usually remain short as adults.[2] Other features include mental retardation and dental malocclusion, malalignment, dental caries, and enamel hypoplsia which, were present in some of our patients. Carbonic anhydrase II enzyme plays a role in proximal renal tubular bicarbonate reclamation and distal renal tubular acidification. Most carbonic anhydrase II-deficient patients have proximal and distal components of renal tubular acidosis.[23] Some patients have predominantly proximal renal tubular acidosis, while in others the distal renal tubular acidosis predominates.[22] In our series, the majority of children had distal renal tubular acidosis that was evidenced by hyperchloremia, hypokalemia and inappropriately elevated urine pH values when the serum was acidotic. This was further supported by the presence of nephrocalcinosis in 2 patients. Medullary nephrocalcinosis and urolithiasis have also been reported in Arabic children with this syndrome.[24]

Intracranial calcification involving the basal ganglia and the periventricular and subcortical white matter is a constant manifestation of this disease. The mechanism of cerebral calcification is unclear and the function of carbonic anhydrase II enzyme in the brain is unknown.[22] Cumming and Ohlsson[20] reviewed the radiographs of 16 Saudi children with this syndrome and showed that calcification is not present at birth but appears usually after the 2nd year of life and increases in density and extent through childhood. We could not determine the rate of progression of cerebral calcification in all patients, however 6 patients, in our series, had a normal neonatal brain CT with a childhood onset of calcification which progressed over time in 2 patients. This form of osteopetrosis is thought to be a benign disease with relatively few symptoms however, cranial nerve encroachment on neuronal foramina was described in 6 patients among the 21 patients reported by Sly et al.[21] Optic nerve atrophy has been also described in patients in whom the optic foramina were of normal size with unclear underlying mechanism.[25] Deafness, anemia, hepatospelomegaly and hydrocephalus were described in Arabic patients with this syndrome from Kuwait and Saudi Arabia.[26,27] In our series, 5 patients had optic and acoustic nerves compression that underwent optic nerve decompression and had some visual improvement. Hematological complications, including anemia, leukopenia and thrombocytopenia are typically present in the recessive malignant lethal form of osteopetrosis and are usually not seen in osteopetrotic patients with carbonic anhydrase II deficiency. Two of our patients had bone marrow involvement manifested by anemia, splenomegaly and secondary erythropoisis. No bone marrow biopsy or aspirations were performed in these patients to confirm the diagnosis of bone marrow involvement, however we believe than bone marrow failure secondary to bone expansion is the most likely cause. Unfortunately, there was no HLA matched donors for these children and they were managed symptomatically. It was reported recently that bone marrow transplantation has a role in the radiological and histological resolution of the osteopetrosis in these patients; allogenic bone marrow stem cell replacement was successfully performed in 2 Irish

children with this syndrome and resulted in retardation of the development of cerebral calcification however, it had a little effect on the tubular renal acidosis and mental retardation.[28] Alkali supplementation and correction of acidosis remain the standard therapy of this syndrome, however recent animal studies have shown that gene therapy and brain glial cell progenitors transplantation can be considered as future therapies for renal tubular acidosis and demyelination.[29,30]

In conclusion, the adverse sequela associated this syndrome indicates the potential malignant nature of this supposedly benign disease and mandates close monitoring of these patients. Long term follow-up of these patients showed that some of them may live independently with no major physical or mental disabilities and get married and have normal children.

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