CLINICAL AND GENETIC DIAGNOSIS OF MULTIPLE OSTEOCHONDROMAS IN RWANDAN PATIENTS

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ABSTRACT

Multiple Osteochondromas (MO) or hereditary multiple exostoses (HME) is an autosomal dominant skeletal disorder mainly characterized by multiple osteochondromas predominantly located at the growth plates of long bones. MO is a genetically heterogeneous disorder and results from mutations in EXT1 and EXT2 genes located on chromosome 8q23-q24 and 11p11-p12.
We hereby report a case of a 23-year-old girl who presented characteristic clinical and radiological features of MO. The same clinical signs were observed in her relatives. The p.Arg340Cys mutation in the EXT1 gene was found in the proband confirming the clinical diagnosis.
A surgical management was carried out in all affected bones which consisted of excision of the bigger and pain full osteochondromas. The patient was informed of her problem and genetic counseling was offered to the family's members.

Key words: Multiple osteochondromas - p.Arg340Cys mutation - EXT1 gene - Rwandan patient

RESUME

La maladie des exostoses multiples ou ostéochondromes multiples est maladie osseuse d'origine génétique autosomique dominante principalement caractérisée par la présence de plusieurs excroissances osseuses qui se présentent comme des bosses prédominant au niveau de l'extrémite des os longs. L'exostose multiple est une maladie génétiquement hétérogene pouvant résulter des altérations (mutations) de plusieurs gènes dont les plus fréquents sont les gènes EXT1 et EXT2 localisés sur les chromosomes 8q23-q24 et 11p11-p12, respectivement.
Ici nous présentons un cas d'une jeune fille âgée de 23 ans qui présentait des signes cliniques et radiologiques caractéristiques d'exostoses multiples. Le même phénotype a été observé chez certains membres de sa famille. Une mutation p.Arg340Cys a été identifiée chez cette patiente confirmant le diagnostic.
Une prise en charge chirurgicale a été entreprise et consistant à enlever les excroissances osseuses grosses et qui entrainaient la douleur chez la patiente. En plus, toutes les informations concernant la maladies ont été donnees à la patiente et un conseil génétique a été donné aux autres membres de la famille

Mots-clés: Exostoses multiples - mutation p.Arg340Cys - gène EXT1 - patiente Rwandaise

INTRODUCTION

Multiple Osteochondromas (MO) or hereditary multiple exostosis (HME) is an autosomal dominant bone disorder characterized by the presence of multiple benign cartilage-capped tumors (osteochondromas or exostoses) [1, 2]. MO is genetically heterogeneous, and two causal genes have been identified so far: EXT1, on chromosome 8q23–q24 and EXT2, on 11p11–p12 [3-5]. Most of mutations involving EXT1 and EXT2 are undoubtedly the causing of MO. However, in 10 to 20 % of the patients, no mutation is found.
Osteochondromas predominantly arise from the metaphyses of endochondral bones in the region adjacent to the growth plate and develop during skeletal growth [6, 7]. The most frequent locations are long bones, pelvis, ribs, scapula, and vertebrae. Patients with MO are short and have bowed bones. They also have unequal growth of two paired bones, which might be related to the bowing bones. Pain is felt when osteosarcomas impinge on nerves, which can severely limit movement. Although deep seated osteochondromas, such as those of the proximal femur or pelvis, rarely cause symptoms or pain, deformities around the hip have been described with premature osteoarthritis [7].

Osteochondromas may cause complications, including osseous and cosmetic deformities, fracture, bursa formation and impingement on adjacent structures (tendons, nerves, and vessels) and malignant transformation [8]. The treatment of patients with MO depends on the complications that they cause, usually it consists of surgical removal of osteochondromas and liberation of compressed

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or elongated structures, this to reduce the pain and correction of limb's bone deformities [9]. The correction of limb's bone deformities is done generally by attaching an external fixator that gradually increases the distraction gap between the two resected ends of the bone leading to lengthening and straitening of the bone.

CASE PRESENTATION

This paper presents a 23-year-old girl that referred to the department of surgery of the Butare University Teaching Hospital/Rwanda for a recent history of 3 months of multiple and painful masses located on the limbs especially surrounding the knees, the shoulder and the hip with deformity of the lower limbs.

Clinical history showed that the disease was recognized when the patient was 12-year-old by small hard and painless masses near the right knee and progressively growth and attacked other joints.
Her family history revealed other affected relatives as it is shown in her family pedigree (Figure 1), notably her mother and her young brother who did not consult the

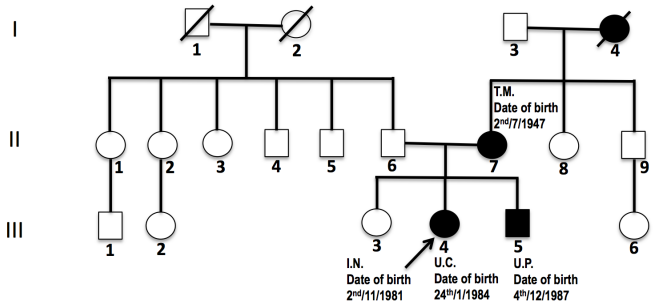

Figure 1: Family pedigree of Rwandan patients with hereditary multiple exostoses. Note the presence of the disease in three consecutive generations (affected patients I.4, II.7, and III.4, III.5). The index case is indicated by arrow.

The physical examination showed hard masses located bilaterally near the knees, on the arms around the shoulder and on the right thigh on the sub trochanteric region. A remarkable deformity of the limbs was seen. X-rays were performed and showed bilateral multiple osteochondromas near the joints on the metaphyseal and epiphyseal regions especially on the knees (Figure 2). The sites involved and seen on the plain radiography include the knees (Figure 3(a)) and the proximal humerus (Figure 3(b)). The morphology of the lesions was mostly pedunculated lesions, one sessile on the fibula was noted and some calcifications were found in different lesions. The clinical diagnosis of MO was stated.

A surgical management was carried out in all affected bones which consisted of excision of the bigger and pain full osteochondromas.

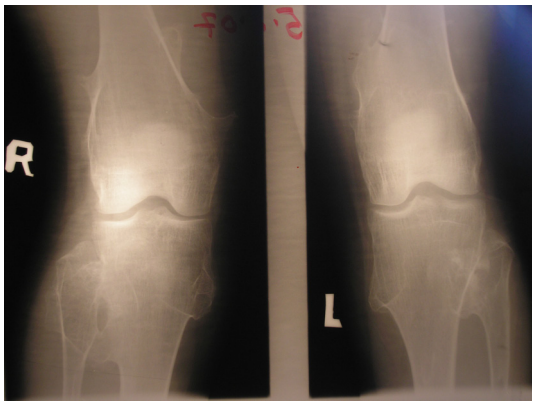

Figure 2: Knee X-ray showing the bilateral localization of exostoses.

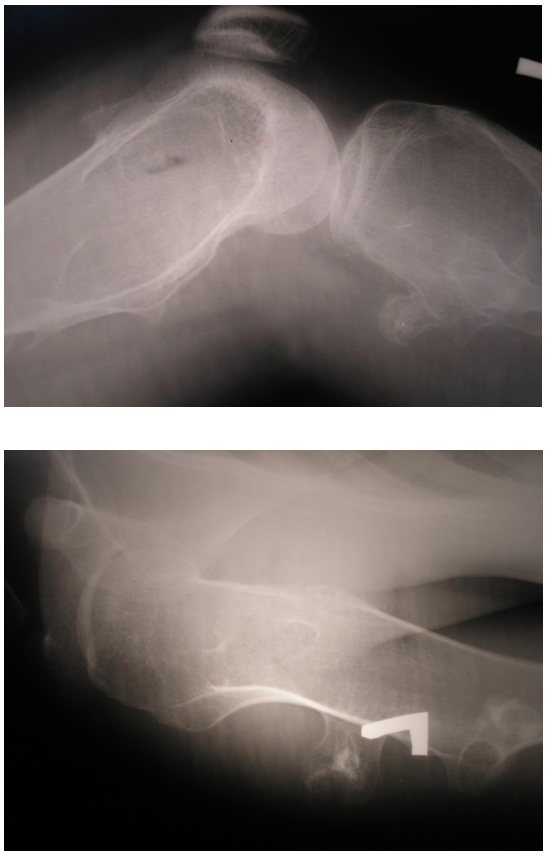

Figure 3: Localization of exostoses near the knee joint on the metaepiphyseal regions of the bone (a) and the localization of exostoses on the proximal part of the humerus (b).

After obtaining a written informed consent, 5 ml of peripheral blood were obtained from the index patient for molecular analysis. The genomic DNA was extracted and sent to the Department of Medical Genetics at the University of Antwerp in Belgium for molecular studies. DNA analysis was performed by Polymerase Chain Reaction amplification (PCR) and Denaturing High Performance Liquid Chromatography (DHPLC) / direct sequencing of all coding exons (exons 1-11) of the EXT1 gene and Multiplex

Ligation-dependent Probe Amplification (MLPA) analysis (Salsa MLPA P215). Sequence comparison and numbering were based on genbank file sequence NM_000127 with A of start ATG position + 1.

Sequence analysis revealed that the patient harbours a c.1018C>T (p.Arg340Cys) mutation in exon 2 of the EXT1 gene. Thus, this analysis confirmed that a mutation in the EXT1 gene is responsible for the development of multiple osteochodromas in this patient. The patient was informed of the genetic consequences and genetic counseling was provided to the family's members.

DISCUSSION

The basic defect in MO consists of a disturbed enchondral bone formation, with formation of multiple benign bony overgrowths (exostoses or osteochondromas) at the juxtaepiphyseal regions of bones, where the enchondral ossification occurs.

The growth of an osteochondroma results from a defect in the circumferential ring of perichondrium, known as the ring of Ranvier, covering the epiphyseal plate [10]. This perichondrial defect allows for aberrant lateral growth of cartilage resulting in the typical appearance of an osteochondroma arising at a 90° angle from the growth plate.

Interestingly, a recent study reported a mouse model of osteochondromagenesis which recapitulates the human phenotype of multiple metaphyseal osteochondromas [11]. They also confirmed homozygous disruption of Ext1 in osteochondroma chondrocytes and their origin in proliferating physeal chondrocytes.

Besides the formation of osteochondromas, there is also a defective remodeling defect at the metaphyses of the long bones, leading to mild dwarfing, metaphyseal widening, with the formation of trumpet shaped bones, and wrist deformities ('bayonnet hand'), owing to shortening of the ulna. Clinically, the major complaint is the discovery of single or multiple hard, painless masses near the joints. The lesions are usually not detected at birth but become obvious clinically and radiologically between the ages of 2 and 10 years [12]. Fortunately, some are spontaneously resorbed. They may enlarge during the growth period, but non complicated lesions usually exhibit no further growth after closure of the growth plate. Growth of individual lesions after puberty is suggestive for a complicated lesion [8]. In our patient the pathology was obvious at 12 years.

The distribution is usually bilateral in MO, but also a strong unilateral predominance has been described by other authors [13]. In the present case, the distribution of lesions was bilaterally found only on the long bones. Symptomatic lesions are due to complications, which include fracture, osseous deformity limiting range of motion, impingement of osteochondromas on vessels, nerves and tendons, bursa formation and malignant complications [8, 14].
Most MO complications include cosmetic deformity, caused by an underlying exostosis or due to growth disturbances, caused by an abnormal modeling, necessitating surgical intervention. Malignant transformation is the most feared complication of osteochondroma.

The most common tumor encountered is a low grade chondrosarcoma arising in the cartilage cap of the lesion, although rarely osteosarcoma is reported at the base of the osteochondroma stalk [7, 8].
Clinically, the possibility of malignant degeneration is elicited by local pain or growth of lesion after skeletal maturity. Centrally located osteochondromas about the pelvis, hips, and shoulders are particularly more prone to undergo malignant transformation. Malignant transformation before the age of 20 is rare. Other complications are rare, and include osteomyelitis, infarction of the cartilage cap or osseous component, muscle impingement, and hemarthrosis [8]. Non significant complications were noted except the pain which was due to an impingement of exostoses on tendons on the knees joints and arm muscles.

Radiologically, osteochondromas are characterized by a contiguity of both the cortical and medullary bone with the underlying host bone. Plain radiography is also an excellent tool to determine the number, location and morphology of the exostoses. Osteochondromas may be found in nearly every bone, with the exception of the calvaria. The most frequent sites of involvement include the knees, humerus, hips, scapula and ribs, wrist, ankles, elbows, hands, feet and pelvis. Morphologically, osteochondroma may be sessile, pedunculated or calcified [15]. Sessile osteochondromas have a broad base of insertion on the host bone (diameter of the lesion base exceeding its length), whereas pedunculated osteochondromas has a narrow stalk, with a bulbous tip. Typically pedunculated lesions point away from the joints owing to the forces of the overlying tendons and ligaments. In our patient, the morphology of the lesions was mostly pedunculated lesions and one sessile on the fibula was noted.

Sequence analysis of the EXT genes in patients with MO results in the identification of a mutation in more than 90% of the patients [16]. These mutations are inactivating mutations. Also, the p.Arg340Cys mutation, identified in our patient, was shown to results to result in loss of EXT1 function [16]. Inactivation of EXT genes leads to formation of an exostosis, with subsequent inactivation of a second EXT gene (or possibly another gene) causing malignant transformation. The loci on chromosomes 8 and 11 have been associated with malignant transformation, when loss of heterozygosity occurs, a phenomenon not described for chromosome 19 [17].

CONCLUSION

There is a large variability in expression of number, location morphology, and evolution of exostoses, and in the modeling defect in affected individuals of families with known MO. Most complications are benign, but malignant transformation occurs in 3–5% of patients with MO. Although conventional radiography is sufficient to confirm the diagnosis and to define the extent and the evolution of the disease, MRI is the imaging technique of choice to evaluate symptomatic lesions. Plain radiography is an excellent tool to determine the number, location and morphology of the exostoses, and to document complications such as cosmetic and osseous deformities and fracture. The evaluation of other painful exostoses

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