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Advances in Research, Diagnostics and Therapy

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Pokroky ve výzkumu, diagnostice a terapii

LOCOMOTOR SYSTEM 4/2000

Advances in Research, Diagnostics and Therapy

OBSAH

SOUBORNÉ REFERÁTY

Cetta G, Tenni R, Zanaboni G, Valli M, Rossi A, Forlino A, Piazza R and Dyne KM. Vrozené choroby pojivové tkáně - 25 leté zkušenosti s výzkumem v Pavii 225

PŮVODNÍ PRÁCE

Vukasinovic Z, Zivkovic Z, Zajic L. Naše zkušenosti s osteoartikulární TBC u dětí 237

Stok K, Oloyede A. Závislost biomechanických odpovědí trabekulární kosti na hustotě a poměrném namáhání při torsním zatěžování 245

Hyánek J, Mařík I, Pejznochová H, Hyánková E, Pinkasová J, Dubská L, Cabrnochová I, Jenčová H, Štancová C, Martiníková V. Diagnostický význam mírné hyperhomocysteinémie u dětí s kostními dysplaziemi 258

KASUISTIKY

CONTENTS

REVIEWS

Cetta G, Tenni R, Zanaboni G, Valli M, Rossi A, Forlino A, Piazza R and Dyne KM. Inherited connective tissue disorders - 25 years of research experience in Pavia 225

ORIGINAL PAPERS

Vukasinovic Z, Zivkovic Z, Zajic L. Our experience with osteoarticular tuberculosis in children 237

Stok K, Oloyede A. Dependence of the biomechanical responses of the trabecular boneon density and strain-rate under torsional loading 245

Hyánek J, Mařík I, Pejznochová H, Hyánková E, Pinkasová J, Dubská L, Cabrnochová I, Jenčová H, Štancová C, Martiníková V. Diagnostic significance of mild hyperhomocysteinemia in children suffering from bone dysplasias 258

CASE REPORTS

Skotáková J, Straka M. Recurrence stress

Skotáková J, Straka M. Opakovaná stress fraktura tibie u dítěte 264
Smrčka V, Svenssonová M, Mařík I. Deformace kyčelního kloubu na Langobardském pohřebišti - jižní Morava 269

ZPRÁVY
Obsah ročníků 1999 - 2000 274

ŽIVOTNÍ JUBILEUM
MUDr. Ivo Mařík, CSc. 280

fracture of the tibia in a child 264
Smrčka V, Svenssonová M, Mařík I. Hip joint deformation in Langobard´s burial-ground - South Moravia 269

NEWS
Contents Vol. 1999 and 2000 277

ANNIVERSARIES
Anniversary of Ivo Mařík, MD, PhD. 280

INHERITED CONNECTIVE TISSUE DISORDERS: 25 YEARS OF RESEARCH EXPERIENCE IN PAVIA

G. CETTA, R. TENNI, G. ZANABONI, M. VALLI, A. ROSSI, A. FORLINO, R. PIAZZA, K.M. DYNE

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This paper is the report of a lecture given at the VIth Conference on Osteoarthritis and Osteoporosis organized by the Society for Connective Tissue Research and Application, held in Prague in November 1999. It does not deal directly with common multifactorial pathologies involving the connective tissue matrix such as Osteoarthritis and Osteoporosis, but mainly deals with collagens, the most abundant proteins in the connective tissue extracellular matrix. Collagens are involved in the aforementioned disorders, not only because of disease induced alterations to the extracellular matrix, but also because collagen mutations may be at the origin of these conditions. In fact, according to Darwin Prockop (**24**) "mutations in genes for type I procollagen have been found in about 1 to 2 % of patients with osteoporosis and mutations in the gene for type II procollagen have been found in about 1 to 2 % of patients with early onset osteoarthritis". This means that some patients with osteoarthritis or osteoporosis could harbour collagen defects.

Studies of inherited connective tissue diseases performed in our laboratory also involve other non collageneous components, i.e. proteins not present in the extracellular matrix, for example fibrillin or sulfate transporters and enzymes involved in protein metabolism, such as prolidase.

Collagen molecules are able to self-assemble to form fibrils and fibers as long as certain conditions are met: the triple helix domain is characterized by the presence of tripeptide repeats $(Gly\text{-}Xaa\text{-}Yaa)_n$ with glycine residues occurring every third aminoacid in each chain. Xaa is often proline and Yaa, hydroxyproline. In osteoporosis or early onset osteoarthritis, possible mutations not involving a codon for obligate glycine residues are compatible with an apparently normal zipper-like folding of triple helix as well as a normal self-assembly of the protein into fibers. They do not significantly change the mechanical properties of collagen. However, small structural defects in the triple helix caused by such mutations which could compromise collagen-collagen or collagen-extracellular matrix interactions are enough to reduce collagen resistence and to alter the mechanical properties of tissues during an individual's lifetime.

Osteogenesis Imperfecta

At the Department of Biochemistry, University of Pavia we have carried out

extensive studies on Osteogenesis Imperfecta (OI) for a number of years. This disease is an autosomal dominant connective tissue disorder characterized by extremely high bone fragility (brittle bone disease of children). It shows a wide range of clinical phenotypes ranging from mild to lethal.

In 1979 David Sillence proposed classification of the disorder into four different types (OI types I to IV) (**29**) from a lethal prenatal form to another with mild bone fragility, with more or less severe forms in between**.** Apart from bone fragility, other characteristic symptoms include: bone deformity, precocious hearing loss, blue sclera and dentinogenesis imperfecta.

OI is generally caused by mutations in either one of the two genes (COL1A1 or COL1A2) coding, respectively, for the 1 and 2 chains of type I collagen**,** the main protein component of bone, skin and tendon.

Patients with the mildest phenotype (OI type I) typically have blue sclera and fracture their bones more easily during childhood but generally there is little or no bone deformity. Hearing loss has been reported in 50 % of the patients, whereas dentinogenesis imperfecta is extremely rare. Cultured skin fibroblasts from such patients show a decreased type I to type III collagen ratio and it has been demonstrated (**33**) that most of these patients have a non-functional or null collagen allele resulting in a reduced tissue content of type I collagen. Individuals with OI I and dentinogenesis imperfecta belong to a rare subset (type I B) of patients and it has been demonstrated that this form of OI is caused by multiexon deletions in the 5' region of the genes.

Moderately severe type IV OI, the severe type III OI and the lethal type II OI are caused by qualitative defects in collagen genes. The most common mutations are glycine substitutions in the triple helical domain, but exon-skipping, deletions and insertions have also been reported. Type IV OI presents mild to moderate skeletal deformity, normal or grayish sclera, and variable short stature. Dentinogenesis imperfecta and premature hearing loss are common.

Type III OI is a severe, non-lethal form, recognized at birth because of the patient's short stature, large head, triangular facies and deformities resulting from *in utero* fractures. X-ray at birth generally demonstrates undermineralized calvarium and Wormian bones, thin, gracile ribs, gracile long bones with evidence of fractures and healing. Short, grotesque, bent and bowed femurs, as well as generalized osteopenia, are frequent. If not present at birth, long bone deformitity, fractures with minimal trauma and kyphoscoliosis become apparent during the first years of life. Scleral hue is usually pale blue, fading with age. This form of OI is the progressive, deforming variety of OI.

Type II OI is the perinatally lethal form. Affected infants show severe deformities of long bones with short extremities and bowed legs, dwarfism with a large head. Radiographic findings reveal particularly shortened and crumpled femurs, beaded ribs and decreased calvarian mineralization. They usually die *in utero* or in the perinatal period, death generally being due to respiratory insufficiency, congestive heart failure or infection.

When we first started studying OI the cause of the disease was still unknown.

Initially, we focused our attention on investigating the changes in the tissues of affected subjects and attempted therapy that had been demonstrated to be effective in an experimental disease affecting connective tissue (**5**). Some of our patients were treated with flavonoids (**6**). The treatment appeared to work in that the frequency of fractures was reduced but objective evaluation of the results was difficult. At that time, the involvement of collagen metabolism in OI was firstly demonstrated by Penttinenn (**23**) and in subsequent years the biochemical heterogeneity of the disease was identified (**7**).

The search for mutations in collagen genes was pursued mainly by Darwin Prockop and his coworkers, although other groups were also working in this field; the first mutations were only demonstrated in the 1980s.

Analysis of collagen synthesized by skin fibroblast cultures is a suitable way to start investigating the biochemical defect present in a patient's collagen. Biochemical analysis of synthesized collagens by denaturing SDS-PAGE can provide some insight into the collagen defect, for example it can show up certain quantitative and qualitative abnormalities. If mutant 1 and 2 chains appear as a doublet or broad band, bidimensional map of the CNBr collagen peptides can help localize the defect along the chains. Sequencing the mutant allele in the suggested portion will give information concerning the exact nature of the mutation. However, in some cases the OI patient's collagen may sometimes appear normal after screening making it difficult to identify the molecular defect as otherwise the whole of COL1A1 and COL1A2 would need to be sequenced.

In spite of the large (more than 200) number of distinct mutations that have been identified in OI (**17, 3, 8**), the relationship between molecular defect and clinical outcome is still poorly understood. Two models have been proposed (**4, 20**) although few exceptions have been reported for both of them. However, some general predictions and correlations between mutations and clinical phenotype can be drawn. Mutations that only have a quantitative effect i.e. those producing a stop codon, which therefore prevent mutant chains from being incorporated into the triple helical region of the collagen molecules, generally produce the mildest phenotype. On the contrary, mutations that allow mutant chains to participate in triple helix formation and disrupt the crucial Gly-X-Y repeat domain are more harmful causing a variable degree of impairement of secretion and extracellular matrix deposition.

The severity of the phenotype depends, to some extent, on the chain involved [errors in α1(I) cause more severe symptoms than errors in α2(I)], and on the location of the mutation along the triple helical domain. In general, the degree of severity is greater the closer the defect is to the C-terminal end of the triple helix. It appears also possible that hot spots along the triple helix may exist where the likelihood of a mutation occurring is more probable or else where a mutation produces more severe consequences than in other regions.

Moreover, the extent of secretion of both mutant and normal molecules into the extracellular space and their possible subsequent incorporation into fibers certainly play important roles in determining the phenotype.

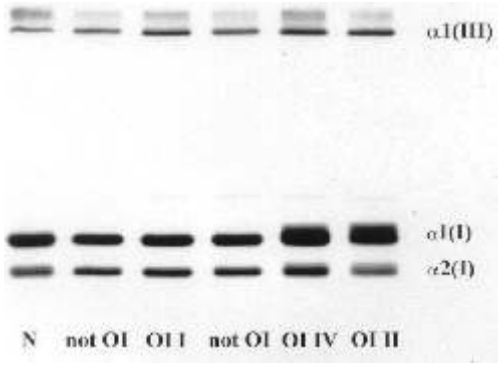

Fig.1. Typical electrophoretic pattern after SDS-PAGE analysis of collagens synthesised by cultured skin fibroblasts from normal and OI patients. Abnormal α1(I) and α2(I) chains are generally evident. A case of OI type I whose collagen appears quite normal is shown in lane 3 on the lefth.

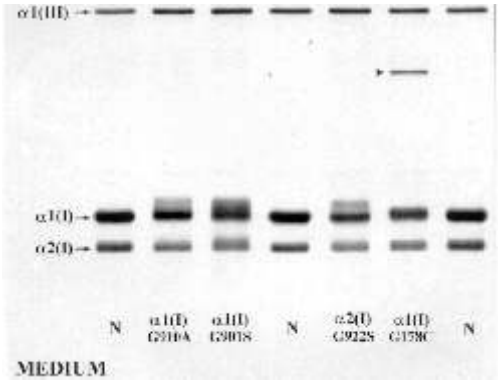

Fig.2 Skin fibroblast cultures from normal subjects and OI patients whose mutations (indicated at the bottom) have been demonstrated on the basis of SDS-PAGE followed by bidimensional map of the CNBr peptides in order to localize the mutation along the triple helix.

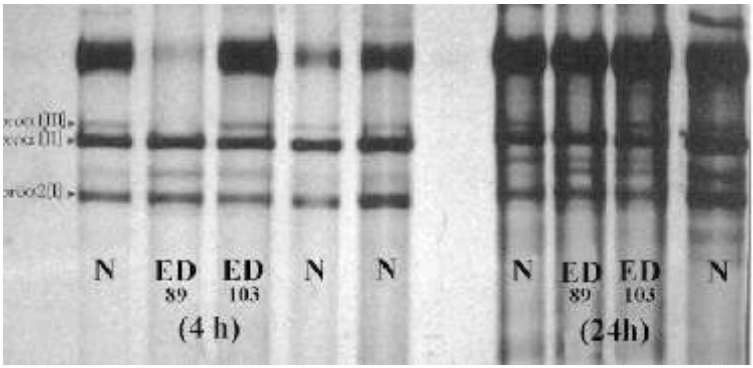

Fig.3. SDS-PAGE analysis of collagens synthesised by cultured skin fibroblasts from normal and EDS patients. Screening of collagens secreted into culture medium in pulse chase experiments shows type III collagen secretion is delayed. The type III/ type I collagen ratio appears significantly decreased in all EDS patients..

Recent data suggest that other genes, besides collagen genes, and the interaction between mutant collagen and different extracellular proteins could be responsible for the severity of the outcome, so the identification of the molecular defect is not sufficient to explain the clincal characteristic of Osteogenesis Imperfecta.

Our laboratory in Pavia has collaborated closely with a group of Molecular Biologists from the University of Verona and we have been able to demonstrate the molecular defect in about twenty OI patients (**34, 35, 36, 38**). All the mutations we found had not been previously reported, except for a Gly922Ser, which had already been demonstrated in two other unrelated patients (**11**). This mutation enabled to observe that the same molecular defect, and also a similar extent of incorporation of the mutant chains in the matrix produces a similar clinical outcome. However, it is noteworthy that very similar phenotypes may show very different biochemical pictures.

*A*t present we provide a screening service based on screening of collagens and procollagens synthesized by cultured skin fibroblasts in order to aid physicians confirm the diagnosis of Osteogenesis Imperfecta. Basic research is being carried out regarding the problem of correlating genotype to phenotype. Moreover a member of our group has been worked on a project at the National Institute of Health in Bethesda, USA, involving the generation of a Knock-in murine model for OI and in the develop of a new gene therapeutic trial through the use of hammerhead ribozymes (**12**).

Ehlers- Danlos syndrome

Ehlers-Danlos Syndrome (EDS) is

CLASSIFICATION OF EHLERS - DANLOS SYNDROME

Type	Major clinical signs	Inheritance
Classical Type	Hyperextensible skin; atrophic scar; joint hypermobility	Autosomal dominant
Hypermobility Type	Hyperextensible and velevet skin; generalized joint hypermobility	Autosomal dominant
Vascular Type	Arterial, intestinal rupture; extensive bruising; thin and translucent skin	Autosomal dominant
Kyphoskoliosis Type	Generalized joint hypermobility; scoliosis at birth; scleral fragility	Autosomal recessive
Arthrochalasia Type	Severe generalized joint hypermobility; congenital bilateral hip dislocation	Autosomal dominant
Dermatosparaxis Type	Severe skin fragility; redundant skin	Autosomal recessive

Table 1. Ehlers Danlos classifications according Beigthton et al. (1).

Diagnostic criteria of Marfan syndrome

Skeletal

Major (presence of at least four of the following)	Minor
Pectus carinatum	Joint hypermobility
Pectus excavatum requiring surgery	High arched palate
Reduced upper-to lower segment ratio or arm span-to-height ratio › 1,05	Pectus excavatum
Wrist and tumb signs	Dolichocephaly
Scoliosis of › 20ş or spondylolisthesis	
Reduced extension at the elbows (‹ 170ş)	
Pes planus	
Protusio acetabuli	

Ocular

Major	Minor
Ectopia lentis	Flat cornea
	Increased lenght of the globe

Cardiovascular

Major	Minor
Dilation of ascending aorta	Mitral valve prolapse
Dissection of ascending aorta	Dilation or dissection of the descending thoracic or abdominal aorta below age of 50 years

Dura

Lumbosacral dural ectasia

Table 2. The diagnosis of the Marfan syndrome was codified on the basis of revised clinical criteria (10). In absence of genetic/family contribution, major criteria in at least two different organ system and involment of a third organ system are necessary for a positive diagnosis.

another disease involving connective tissues. It is a heterogeneous group of heritable disorders characterized by joint hypermobility, skin extensibility and tissue fragility. A recent paper (1) has proposed classification of EDS into six major types according to the cause of each type (**table 1**) namely: 1) classical type EDS (formerly EDS types I, II), 2) hypermobility type EDS (formerly EDS III), 3) vascular type EDS (EDS IV), 4) kyphoscoliosis type EDS (formerly EDS VI), 5) arthrochalasia type EDS (EDS VIIA. VIIB), and 6) dermatosparaxis type EDS (EDS VIIC).

One subtype of EDS, the arthrochalasia type EDS, is caused by mutations of type I collagen (**17,30, 24**) leading to deficient processing of the aminoterminal end of the procollagen α1(I) (type A) or procollagen α2(I) (type B) chains of type I collagen. The mutations cause skipping of exon 6 with loss of the cleavage site of the N-terminal propeptide which is retained in one or more chains of the triple helical monomer. Typical clinical manifestations of this subtype of Ehlers-Danlos syndrome are severe generalized joint hypermobility which is severe enough to cause dislocation of the knees and congenital bilateral hip dislocation. This form of EDS is also often associated with increased ratio of bone fractures and short stature. Dermatosparaxis type EDS (formerly EDS VIIC) is caused by a deficiency in the activity of procollagen I N-terminal peptidase (**22**). Major diagnostic criteria include very severe fragility of the skin which may be redundant and sagging. The persistence of aminopropeptide dramatically alters fibril formation leading to marked irregularity in collagen fibril cross sections.

Our group has been mainly interested in biochemical analysis of collagens synthesized by fibroblasts from patients with the life-threatening form of Ehlers-Danlos, vascular type EDS (**37**). This form is characterised by easy bruising and bleeding and there is a risk of spontaneous ruptures of the arteries and internal organs. Affected individuals have no or minimal hyperelasticity of the skin in contrast to individuals affected by other subtypes of EDS. The skin may be unusually thin and transluscent and the venous pattern easily visible. Joint hypermobility is generally limited to the small joints.

EDS IV or vascular EDS is caused by mutations in type III collagen (**17, 1**) and to date more than sixty mutations have been reported. Mutations include amino acid substitutions, alterations in splice donor and acceptor sites and a range of large or small deletions. Type III collagen is present in many tissues but is a primarily a component of extensible tissues such as blood vessels, the gut, skin and uterus. In contrast with arthrochalasia EDS, it is hard to find a phenotype/genotype correlation for type III collagen mutations in vascular EDS. In general amino acid substitutions towards the C-terminal end of the collagen domain often result in more severe forms of vascular EDS.

We have been able to make or to exclude the diagnosis of vascular EDS in different patients on the basis of the screening of collagen from skin fibroblast cultures. In some cases we confirmed defects in the synthesis and metabolism of type III collagen that is slowly secreted by the cell due to a mutation in triple helix domain of the molecule. In our lab two different mutations of type III collagen in two patients affected by this form were demonstrated.

Diseases not involving collagen

Among the connective tissue disorders caused by mutations in non-collagen components of extracellular matrix we studied the *Marfan syndrome*. This condition is characterized by cardiovascular, ocular and skeletal manifestations (**table 2**) and is caused by mutations in the gene encoding the protein fibrillin-1 (FNB-1), a 350 kd glycoprotein that is the major structural component of matrix microfibrils (**13,21**). In spite of the fact that the fibrillin gene contains 65 exons and is over 110 kb in length, more than 150 different mutations, mainly aminoacid substitutions, have been reported to date. Generally a single EGF-like domain of the protein is involved, causing loss of structure following the disappearance of a disulfide bond or of an aminoacid necessary for binding calcium.

At present one correlation between the localization of the mutation and the severity of clinical phenotype has been found. In fact if the series of EGF-like domains

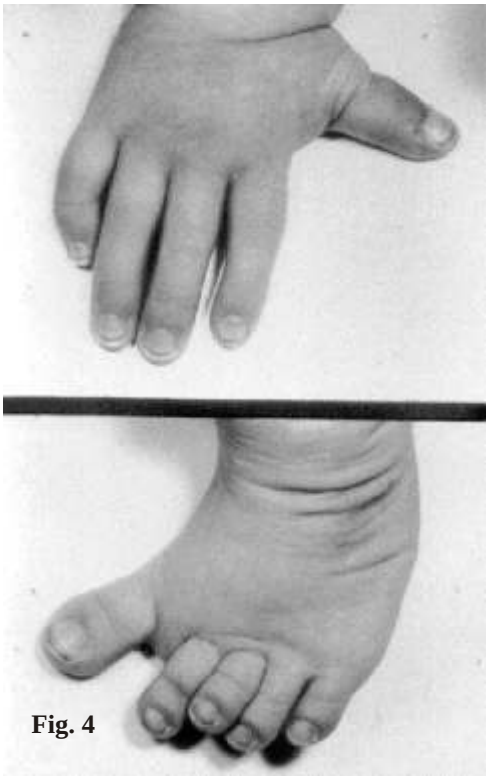

Fig. 4

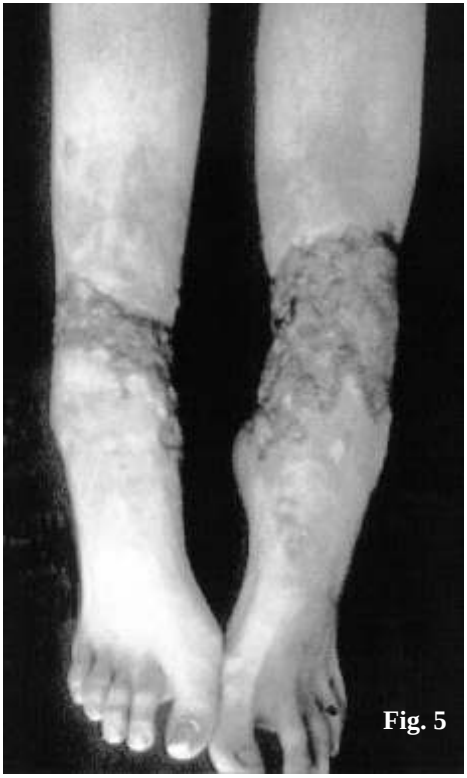

Fig. 5

Fig. 4. Typical appearance of hands and feets in a new born patient affected with Diastrophic Displasia (courtesy of Dr. A. Superti-Furga - Zurich.).
Fig. 5. Characteristic leg ulcerations in a Prolidase deficient subject (16).

between exon 23 and exon 32 is involved, the result is the most severe form of Marfan syndrome known as Neonatal Marfan Syndrome. Because of the complexity of the fibrillin gene, we have only been able, in collaboration with a Molecular Biology group, to demonstrate two new mutations in this disorders.

Finally, it is worth remembering that there are many disorders where alterations of the extracellular matrix and the resulting anomalies of tissues, organs or systems are not due to defects in extracellular matrix components, but rather to alterations in membrane proteins. The connective tissue involvement can depend on impairment of some transport or signal transduction processes resulting in the formation of anomalous molecules that can disrupt normal matrix.

For instance, some well known diseases are due to mutations in one member of the large family of Fibroblast Growth Factor Receptors (FGFR). FGFRs are members of the receptor tyrosine kinase family that bind fibroblast growth factors (FGF). The FGF family appears to play a major role in numerous aspects of embryogenesis, growth and homeostasis. FGFs also stimulate mitogenesis, chemiotaxis, and angiogenesis. The first human disorder characterized by FGFR mutations was *Achondroplasia* (**28**), by far the most common form of dwarfism. Common clinical manifestations of this chondrodysplasia include**:** macrocephaly, rhizomelia, lumbar lordosis and narrowing of the spinal column. It is caused by mutations in fibroblast growth factor receptor III (FGFR III). Genetic studies of the FGFR III locus, localised at the tip of the short arm of chromosome 4, clarified that there is a strong degree of homogeneity in mutations responsible for achondroplasia (**28**). In fact, more than 95 % of all achondroplastic alleles analyzed carried the same mutation: a G to A transition at nucleotide 1138. This results in the substitution of glycine to arginine at position 380 (Gly380Arg) in the transmembrane domain of the molecule. At present it is not clear how alterations of the FGFR III gene interfere with the clinical phenotype in achondroplasia. However, studies of the growth plates in FGFR III null mice provided evidence that FGFR III was a negative regulator of long bone growth during endochondral ossification (**9**).

Another unique membrane protein is responsible for a family of skeletal disorders with a widely phenotypic expression. *Diastrophic Dysplasia*, a non lethal disease, is caused by mutations in the DTDST gene (**15**). It is an autosomal recessive chondrodysplasia with clinical features including short-limbed stature, kyphoscoliosis, generalized dysplasia of the joints, flexion limitation of the finger joints, hitchhiker thumbs, deformity of the feet and often deformation of the ear lobes and the cleft palate. Patients are severely handicapped and need repeated corrective surgery. This disease occurs with low frequency in most populations, but with high frequency in Finland where DTD appears to be one of the most common autosomal recessive disorders. Subsequently it was demonstrated that two other very severe and lethal diseases, *Achondrogenesis 1B* and *Atelosteogenesis type II*, are due to the involvement of the same gene (**16, 31, 32**). This gene encodes a transmembrane sulfate/chloride anion exchanger expressed in most tissues (**15**). Its impairment consequent to mutations

results in undersulfation of cartilage proteoglycans leading to severe disorders in skeletal development.

Achondrogenesis type 1B is an autosomal recessive disorder; affected individuals usually dye in the pre or perinatal period. It is the most severe of the three DTDST-related disorders. Atelosteogenesis type II is an autosomal recessive disease characterized by severely shortened limbs, small chest, scoliosis, and club foot. It is lethal in the newborn period. We have contributed towards demonstrating that the disorders, classified as being distinct clinical diseases, are actually the consequence of different mutations in the same gene. Phenotypic severity is correlated to the degree of impairment of the sulfate transporter function (**26,27**).

The diseases are now under investigation in order to check the possibility of stimulating alternative pathways for sulfate supplementation.

Prolidase Deficiency

Prolidase deficiency (PD) is a very rare autosomal recessive disorder with highly variable expression. It is characterized by severe skin manifestations including chronic, recurring ulcers (**14, 19**), dermatitis with dry skin and pruritis, and recurrent respiratory tract infections and mental retardation, probably due to the presence in the tissues of undigested iminodipeptides, mainly Gly-Pro. The condition is correlated to deficient activity of prolidase, an enzyme (EC 3.4.13.9) that hydrolyzes dipeptides with a carboxy-terminal iminoacid. Impaired prolidase activity leads to massive urinary excretion of iminodipeptides.

Prolidase is a homodimeric, cytosolic, ubiquitous enzyme which requires Mn^{++}. It plays a key role in recycling of proline formed during the final stages of degradation of collagen and dietary proteins. The prolidase gene is located on chromosome 19p13.2 and the 2,2 kb mRNA encodes a polypeptide of 493 amino acids. Prolidase deficiency is generally due to the presence of two mutant alleles. Several mutations have been demonstrated (**18**). Since 1968, when the disease was first described (**14**), only about fifty patients have been reported **.** Of these ten italian patients have been diagnosed in our laboratory after detecting iminodipeptides in patients' urine and finding deficient prolidase activity in patients' erythrocyte haemolysates.

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OUR EXPERIENCE WITH OSTEOARTICULAR TUBERCULOSIS IN CHILDREN

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The final aims of the therapy of the osteoarticular tuberculosis have been currently spread out, while the holding up of the infectious process previously had been outstanding demand (by the use of antituberculotic drugs and long-lasting immobilization or by surgical radical excision of the focus), today the imperative is functional sparing of the affected joint. The ankylosis has been considered today as the last choice in a case of delayed therapy or when destruction has been rather advanced (**10,11,12,13,14**).

In attempt to achieve the best possible results, two main goals are important: 1.) Early diagnosis and treatment (before the large part of the joint has been destructed) which make anatomical and functional reparation of the affected segment possible by itself; 2.) Once when diagnosis has been established adequate therapy should be carried out (proper combination of the antituberculotic medications, time and type of the surgical procedure, adequate physiotherapy).

Our investigation enrolled 28 patients with osteoarticular tuberculosis treated in Institute for Orthopedic Surgery "Banjica", Belgrade, from 1986 to 1996. (**figures 1 and 2**)

The investigation enclosed all patients with complete documentation and two years follow-up period after the treatment. Majority of patients have been followed up to the end of bone growth.

According to the results of their own investigation the authors suggest certain guideline for prevention, early detection and therapy of the osteoarticular tuberculosis, particularly in children in attempt to gain better results in longer duration: 1.) Regular BCG vaccination at birth, tuberculin test at unprotected period of childhood and revaccination on as needed basis, fluorographic checking of the population, detection of the possible sources of the infection and their curing, improvement of the continuous health education and protection; 2.) In order to detect tuberculosis at the very beginning it is important to keep in mind this type of infection; beside this it is obligatory to conduct proper diagnostic protocol: physical examination, laboratory and radiological investigations, tuberculin test, while the best option is patho-histological confirmation of the process (by open biopsy) associated with bacteriological culture (Lowenstein ground), some of the tests for fast detection and identification of

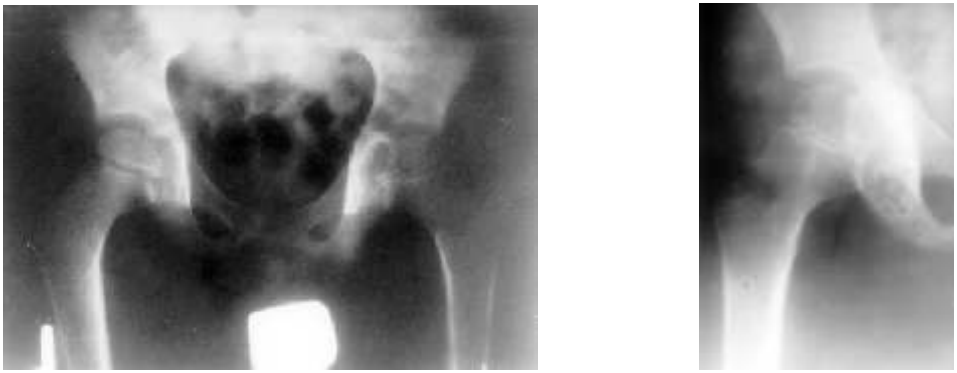

Figure 1. Right ischial bone tuberculosis (radiograph and macrograph), treated no operatively, good anatomical and functional result

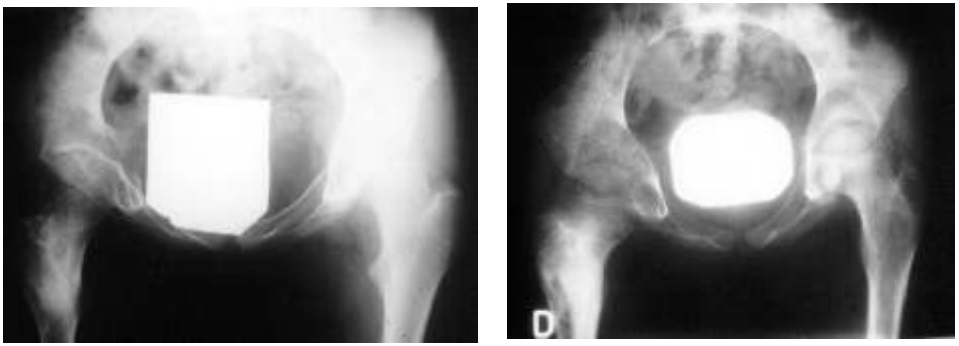

Figure 2. Right hip tuberculosis, surgically treated, good anatomical and functional result

Mycobacteria; 3.) Therapy should be consisted of: antituberculotic drugs upon the protocol of WHO for countries of former Yugoslavia, surgical elimination of the focus and early physiotherapy, without foothold until bone consolidation has been completed.

INTRODUCTION

In developed countries of the world prevalence rate of tuberculosis has been diminished recently.

Although the incidence of tuberculosis in Yugoslavia has been stable for years recently the increasing number of tuberculous patients occurred. There are several reasons for that: progressive deterioration of life standard, incomplete vaccination, improper tuberculin testing, increasing number of HIV infected patients (postprimar tuberculosis particularly affect immunodefficient patients), failure of treatment of refugees and under the war conditions. When the number of primary tuberculosis has been increasing the number of postprimar rises as well.

Since the osteoarticular tuberculosis

has been the significant cause of handicap in childhood and illness related to long-lasting treatment the social importance is evident.

Due to various diagnostic and therapeutic protocols that were applied previously there were lot of speculations and different results of therapy.

As we already stated the osteoarticular tuberculosis may be presented on the spine and joints as well. The consecutive handicap of the large joints of the lower extremities has been a reason for our special interest in this work. The other reason is that infection in early infancy could be the cause of the damaging of the epiphysis and epyphiseal growth line which may induce the occurrence of inequality, degenerative changes of the joints and spine, extreme handicap, life and work disability. All these reasons affect our decision to consider this problem exactly in childhood.

MATERIAL AND METHODS

Complete documentation means: detailed interview, functional state and radiography at the beginning and the end of treatment, and on the last consultation and as well as laboratory findings and immunological parameters of the illness activity. We speculated that two years follow-up period might be sufficient because all patients experienced certain osteoarticular restitution, normalization of the laboratory findings and functional restitution.

All patients treated with antituberculotic drugs associated with surgical elimination of the focus and early physiotherapy we considered as ***observed group***. Ten patients belong to this group and they were all treated during last years according to modern protocols.

Control group of patients consisted of 18 children, treated with antituberculotic drugs in all cases but associated with complete rest in five cases, plaster immobilization in 11 cases and extension in two cases.

Relevant data included in our questionnaire had been: sex and age of patients, localization of the illness, contact with the TB infection, BCG scar, pulmonary affection, tuberculin test reaction, previous diagnosis and treatment, assessment of the stage of disease when diagnosis has been established. The special interest has been given to the diagnostic procedures performed for establishing the diagnosis, type of treatment and preoperative antituberculotic therapy, type of surgical intervention for elimination of the focus, complication rate, treatment results and eventual need for postponed reconstructive surgery.

Tuberculin test reaction has been divided into six groups according to tissue induration: 1.) Negative; 2.) Less than 5mm; 3.) 6-9mm (+); 4.) 10-14mm (++); 5.) 15-30mm (+++); 6.) More than 30mm with necrosis (++++).

We defined three phases of illness at the moment when diagnosis has been established: acute disease (diagnosis established after one month of the occurrence of symptoms), subacute (diagnosis established 1-6 months after), chronic disease (proper diagnosis established after more than 6 months).

According to the localization of the process three groups had been made: with synovial, bone and mixed forms of the disease.

The results had been classified into five

groups: 1.) Complete restitution of the anatomy and function; 2.) Complete functional restitution with joint deformity; 3.) Diminished function of the joint (contracture); 4.) Loss of the joint function (ankylosis); and 5.) Advance of the process.

The obtained material had been elaborated by various statistical methods: distribution of the frequency of the analyzed categories, by Spearman's coefficient of correlation.

RESULTS AND DISCUSSION

It has been well known that osteoarticular tuberculosis occurred equally in sexes, all races and particularly affect groups with low socio-economic background. Primary, especially pulmonary tuberculosis occurred predominantly in susceptible categories (children and elders) that live in poor social conditions (**2**).

According to our results the sex distribution has been similar to literature data: male patients slightly predominate, 16 or 57,1%.

Speaking about the age range, our youngest patient was two years old and the oldest 10 years. The majority of patients were about five years of age (6 patients or 21,4 %) that means in the period of extensive activity and exposition of children to the infection but at the same time in the period of diminished immunity established by BCG (**7**). After six years of age the morbidity rate decreased because at the time of investigation regular tuberculin test and revaccination had been performed in all preschool children.

In our study contact with tuberculosis has been revealed in only five children (17,9 %). That is a result of insufficient heteroanamnestic data obtained from parents from socially deprivated and poorly civilized classes who were conducted only by idea to hide the presence of contagious or congenital diseases.

Proper BCG scar had been considered as solid immunity state and such children even expressed no need for tuberculin test. However, nowadays it is well known that BCG scar gives us information about the vaccination state and the immunity state could be assessed only by tuberculin test (**9**). The results of our study could serve as a confirmation for this statement because clear BCG scar had 14 (50 %) children with tuberculous infection and without the immunity.

We already noted that diagnosis of tuberculosis had been made in seven patients (25 %) prior to admission in our Hospital. All others were without clear diagnosis for months. Differential diagnosis in all these cases were aseptic necrosis, osteoid osteoma, and in majority of cases (10 patients or 35,7 %) nonspecific inflammation. Obviously, osteoarticular tuberculosis has been forgotten and from the other side the difficulties in diagnosis could be raised from the absence of pathognomonic radiographic and laboratory findings.

Misunderstanding in the very beginning of the illness leaded to inadequate treatment in 17 patients or 60,7 %. Certain number of cases started with antituberculotic treatment after the admission in our Hospital although proper diagnosis had been established at previous Institutions.

Majority of patients (23 or 82,1 %) had chest x-ray examination. The signs of active or old treated pulmonary tuberculosis have been verified in 13

patients (46,4 %) while chest x-rays were normal in 10 patients (35,7 %). Five patients (17,9 %) had no chest x-ray examination.

Among others some reasons for only few positive x-rays findings could be: 1.) Main expression of the primo infection could be tracheobronchial lymphangitis while lymph node is reservoir for Mycobacterium; 2.) As a consequence of vaccination the reaction of the lungs is weak, therefore lesions on chest x-ray are hardly visible; 3.) Careless reading of the chest x-ray; 4.) Although the site of primo infection is in the lungs in 90% we should keep in mind all other localizations (jejunum, kidneys, parotid gland, skin, etc.) (**9**).

Literature data suggest some other results and significantly lower rate of pulmonary involvement, Singh and coworkers (**7**) had only 16,2 % of positive findings in the lungs.

Tuberculin test has been performed in 22 children and strongly positive reaction (one case with necrosis included) has been revealed in 19 patients or 67,9 %. That is clear confirmation for already known statement that strongly positive reaction means undoubtfully presence of the illness whereas tuberculin test certainly is important diagnostic tool with positive result in 90 % of patients with osteoarticular tuberculosis (**2,5,9**). Since 21,4 % of our patients hadn't been tested obviously there were small percentage of highly positive reactions in our study comparing with literature data.

Correlations between previous diagnosis and tuberculin test results ($r = -0,0078$; $p > 0,05$), between previous diagnosis and radiography of the chest ($r = 0,1509$; $p > 0,05$) were evaluated by Spearman's correlation test. The same statistical method was used for correlations between presence of BCG scar and tuberculin test results ($r = -0,1677$; $p > 0,05$), presence of BCG scar and radiography of the chest ($r = -0,1846$; $p > 0,05$), between tuberculin test and radiography of the chest ($r = -0,3046$; $p > 0,05$). Obtained results confirming no statistically significant correlation between examined data seem completely reasonable. Actually, diagnosis of tuberculosis had been delayed in majority of cases; therefore, tuberculin test and radiography of the chest had been performed after long time in relation to the onset of the disease. We already stated the reason for very few positive findings on chest x-rays. Obviously, the absence of significant correlation between BCG scar and tuberculin test had been expected because the disease occurred the most frequently after the age of five when the immune response becomes more and more weak.

The most frequent localization of the osteoarticular tuberculosis on our material had been a hip (17 patients or 60,7 %), than knee and ankle (4 cases or 14,3 % each), and at last ischial bone (3 patients or 10,7 %). Very similar data could be found in the literature. Nagusse (**6**) stated 53,6 % of tuberculous coxitis in their hospitalized patients, giving the explanation that the clinical picture has been the most serious and provoke significant handicap in early stage of the disease.

Concerning the definitive diagnosis in our study it had been established in early stage of the disease in only two patients (7,1 %). Basically, the correct diagnosis had been proved mainly in subacute phase (14 patients or 50 %) and chronic phase (12

patients or 42,9 %). The time between the onset of symptoms and establishing the diagnosis had been from one to 14 months (average 6 months), which is somewhat shorter period than in literature data. Foley and coworkers cited 10,7 months, Martini and coworkers 25 months (**5**). Our opinion for the reasons for delayed diagnosis could be: very ominous onset of the disease, nonspecific clinical picture associated with mild general symptoms (result of the relative immunity after BCG vaccination), nonspecific radiographic and laboratory findings, false interpretation of tuberculin test reactions, low percentage of positive radiographic involvement of the lungs (probably, it could the leading cause) and tuberculosis has been forgotten disease for some time.

The disease had been rarely revealed in synovial form (3 patients or 10,7 %), probably because the late diagnosis frequently had been considered as mixed synovial-oseal form (8 patients or 28,6 %). Similar results had Grujic (4), stated the synovial form in 10 % of cases. In our study, bone form of the disease had been revealed in 17 patients or 60,7 %.

The involvement of the ankle and knee had been proved in the earlier stages in relation to the involvement of the hip. The exposition of these joints are evidently greater, clinical signs seem much more clear and there are only a few diseases in differential diagnosis.

At that time the osteoarticular tuberculosis in bone or mixed form had been diagnosed earlier in relation to synovial form, obviously, because the x-rays were the leading diagnostic tools.

Relation between the localization and phase of the disease analyzed by Spearman's test revealed no statistically significant correlation ($r = 0,1331$; $p > 0,05$), same as for the correlation between type and phase of the disease, and type and localization of the disease.

Correct diagnosis had been proved in 15 patients or 53,6 % without pathohistological verification and in others with biopsy, pathohistological verification and elimination of the focus. The diagnostic protocol had been strictly related to the management of the various phases of treatment but to the orientation of the orthopaedic surgeon. The diagnostic protocol hadn't been in relation to the localization and phase of the disease while diagnosis had been missed for some time in the presence of good BCG scar, negative tuberculin test or negative chest x-ray picture. Neither of these relations showed statistically significant correlation.

Diagnostic difficulties had been presented mainly prior to admission in our Hospital as a result of acute phase of illness, negative contact with TB patient, good BCG scar and negative tuberculin test, normal chest x-ray.

Proper antituberculotic drugs had treated all our patients but their sort, combination and duration of treatment depended on severity of the disease and therapeutic manner. In all cases the inflammatory process had been stopped unrelated to the associated therapeutic procedures. Among all, 18 patients or 64,3 % were treated by conservative means and others were operated. In three patients biopsy had been performed and in 10 (35,8 %) both, biopsy and elimination of the focus. In order to eliminate focus of infection we performed synoviectomy on two patients, bone focal synovialectomy on seven and osteoplasty in the same maneuver on one patient. The majority of

patients surgically treated had been treated by antituberculotic medications in preoperative period (8 patients or 61,5 %). One of our patients untreated preoperatively, had got severe complication - meningoencephalitis. Liberation of the bacillus from the focus had been the reason for dissemination so far the administration of antituberculotic drugs prior to surgical intervention must be an imperative.

Our results were excellent concerning limitation of the inflammation because it was stopped in all cases. Functional restitution we had in 60,7 % of patients, which is not satisfactory. Anatomic restitution of the joint we had in 13 patients (21,4 %). Unfortunately, seven patients finished their treatment with severe deformities and joint contractures (seven or 25 %), while 4 or 14,3 % with complete ankylosis.

Results of treatment were significantly better if it was started early associated with surgical focal synovialectomy and especially if it was followed by physiotherapy.

When results of treatment were unsatisfactory (6 patients or 21,4 %), reconstructive surgery had been necessary and it was done on five patients (one knee arthrodesis, one corrective osteotomy in knee region, one corrective osteotomy in hip region and two desinsertions of the hip).

The relation between the need for adjuvant surgical treatment and results of treatment showed high statistical significance ($r = -0,7008$; $p < 0,01$). Martini and coworkers (5) stated that conservative treatment without surgical intervention gave better results while most other authors found the results very similar to ours. Silva (**8**) published very positive results of radical surgical interventions performed on 219 patients. The main advantage they pointed had been better vascularisation and influx of the drugs as well as significant shortening of hospital stay. Chow (**1**) agreed with these statements emphasizing very frequent occurrence of pain in non-surgically treated patients.

During this period we turned on surgical treatment for achieving safe and early pathohistological verification of the process, elimination of focus, better vascularisation and distribution of the drugs. The aim was to perform decompression of the joint by muscle desinsertion in order to avoid severe epiphyseal destruction and to protect growth of the epyphysis.

Physiotherapy in the early postoperative period helped in getting complete function of the joint and to avoid postponed mutilating operations (resections and arthrodesis).

CONCLUSION

The final aims of the therapy of the osteoarticular tuberculosis have been currently spread out, while the holding up of the infectious process previously had been outstanding demand (by the use of antituberculotic drugs and long-lasting immobilization or by surgical radical excision of the focus), today the imperative is functional sparing of the affected joint. The ankylosis has been considered today as the last choice in a case of delayed therapy or when destruction has been rather advanced (**10,11,12,13,14**).

In attempt to achieve the best possible results, two main goals are important: 1) Early diagnosis and treatment (before the large part of the joint has been destructed)

which make anatomical and functional reparation of the affected segment possible by itself; 2) Once when diagnosis has been established adequate therapy should be carried out (proper combination of the antituberculotic medications, time and type of the surgical procedure, adequate physiotherapy).

According to the results of their own investigation the authors suggest certain guideline for prevention, early detection and therapy of the osteoarticular tuberculosis, particularly in children in attempt to gain better results in longer duration: 1) Regular BCG vaccination at birth, tuberculin test at unprotected period of childhood and revaccination on as needed basis, fluorographic checking of the population, detection of the possible sources of the infection and their curing, improvement of the continuous health education and protection; 2) In order to detect tuberculosis at the very beginning it is important to keep in mind this type of infection; beside this it is obligatory to conduct proper diagnostic protocol: physical examination, laboratory and radiological investigations, tuberculin test, while the best option is pathohistological confirmation of the process (by open biopsy) associated with bacteriological culture (Lowenstein ground), some of the tests for fast detection and identification of Mycobacteria; 3) Therapy should be consisted of: antituberculotic drugs upon the protocol of WHO for countries of former Yugoslavia, surgical elimination of the focus and early physiotherapy, without foothold until bone consolidation has been completed.

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DEPENDENCE OF THE BIOMECHANICAL RESPONSES OF THE TRABECULAR BONE ON DENSITY AND STRAIN-RATE UNDER TORSIONAL LOADING

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SUMMARY

Stok K, Oloyede A. Dependence of the biomechanical responses of the trabecular boneon density and strain-rate under torsional loading.

This paper investigates the dependence of trabecular bone's torsional stiffness and ultimate shear stress on both density and rate of loading. The results show that its ultimate shear stress, stiffness and shear strain energy exhibit dramatic variation with both density and torsional loading rate. A prominerit and repeatable observation is that the samples of the same density exhibited varying levels of decrease in the value of zero-strain stiffness, (i.e. the slope of the tangent to the stress-strain curve at zero strain) with increasing strain-rate. This decrease in stiffness with strain-rate is contrary to that common in viscoelastic materials in which stiffness increases with increasing strain rate, such as bone. On the other hand the slope of the tangent for the higher strain portion of the shear stress-strain curve, or tangent modulus, increased with increasing strain rate. We therefore argued that the behaviour could be a consequence of the interaction of trabecular bone porosity and speed of loading, where the degree and rate of `fracture' or collapse of the lattice structure plays a significant role in determining the stiffness.

Keywords: Trabecular Bone, Density, Torsional Loading, Strain-rate, Torsional Stiffness.

INTRODUCTION

Our tendency to slip, stumble or lose balance, during both normal and rigorous physiological activities can subject the trabecular bone at the end of long bones to twisting forces (**1**). Characteristically sporting activities are usually accompanied by a wide range of loading velocities in the human joints. With age and osteoporosis bone density can decrease with severe consequences for bone function. Such decrease in bone density is known to increase its susceptibility to fracture. Consequently this paper addresses the effect of varying density and strain-rate on

the biomechanical responses of the trabecular bone under torsional loading.

Trabecular bone is the porous lattice structure commonly found in the epiphysis of the long bones amongst other places. It serves to assist growth, and cushion, attenuate and transmit forces applied to the bone. These physiological functions can be impaired by damage such as traumatic loading induced by excessively high static or very fast, applied loads. It is this susceptibility to fracture and its deleterious consequences on physiological function that accounts for the general and clinical relevance of this present investigation. In the past research has concentrated on the effects of strain rate and density with respect to loading in tension and compression. In particular, Carter and Hayes (**2, 3**) established equations to describe the effect of density and strain rate on the strength and stiffness of trabecular bone under compressive loading. This approach was reinforced by Rice, Cowin and Bowman (**4**), who conflrmed the relationship previously proposed by Carter and Hayes (**2, 3**). This relationship was,

$$S = k.\varepsilon^{\alpha}.\rho^{\beta} \qquad (1)$$

where S is the compressive strength, e is the strain-rate, is the density and k, a, b are experimentally derived constants, (**table 4**).

In the case of shear/torsional loading, one of the more comprehensive studies has been that of Ford and Keaveny (**5**). They investigated the shear properties of trabecular bone with respect to shear failure strain and determined the effect of apparent density and trabecular orientation on shear failure stresses and strains. They also found that shear failure may be a dominant fracture mode in trabecular bone and purposed that more consideration should be given to its shear and torsional properties. We therefore extend the work of Carter and Hayes (**2, 3**) in this present study by determining the effect of density and strain-rate on both yield and ultimate shear strength, torsional stiffness and strain energy of trabecular bone.

FACTORS CONSIDERED FOR PRESENT EXPERIMENTAL MODEL PROTOCOL AND ANALYSIS

Structure and Architecture

Trabecular bone is anisotropic and inhomogeneous (**6, 7, 8**). Behrens et al (**9**), and Gibson and Ashby (**6**) observed that these structural/mechanical properties are developed relative to the type of loads normally experienced by this bone, and in agreement with Wolff´s law (**10**).

Despite its anisotropic nature it is usual to assume that it is a transversely isotropic or orthotropic material, with the consequence that the analysis is simplifed. This simplifcation has been used successfully by previous investigators with a high degree of accuracy (**5, 6, 9, 11**).

Density

This parameter determines the mass, and hence structural rigidity, of the trabecular bone. It is usual to assume that high bone density results in high bone strength with a concomitant increase in the ability to support physiological loads. This assumption seems to be the basis of the clinical treatment of osteoporosis where drugs that increase bone mineral content, and hence density, are applied. Because of this interrelationship between density, architecture and the mechanical properties

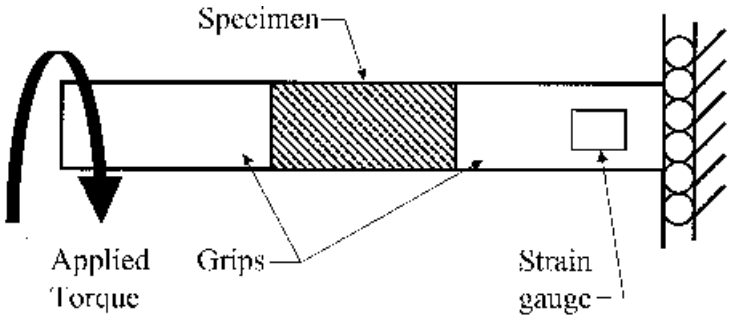

Figure l. Free body diagram of the torsion-loading device. The specimen is loaded by rotating its grip through an axial pull applied on the left-hand side. The loading effect and angular displacement is measured through the strain gauges placed on the right-hand side.

of trabecular bone (**2, 3, 12**), we seek to obtain the relationship between torsional stiffness, ultimate shear strength and elastic strain energy and the speed of torsional loading and density. This will contribute further insight into the mechanics and mechanisms of fracture of trabecular bone, and provide insight into the question of whether or not an increase in bone density should be the most important target of clinical treatment, especially in the treatment of osteoporosis.

Strain-rate

The study of the relationship between strain-rate and material properties is of relevance as it allows the modelling of near physiological conditions. Weaver and Chalmers (**13**) conducted a series of tests into age related fracture because of the high incidence of fracture in older bones. They concluded that fracture occurred under normal loading in these bones, rather than at traumatic loads, with the consequence that any representative study of strain-rate effects must include both low and high rates of loading. Carter and Hayes (2) (3), reported that normal walking is carned out at strain-rates in the order of $0.002s^{-1}$, while running involves strain-rates in the vicinity of $0.01\ s^{-1}$. They further stated that torsional impact loading fracture of trabecular bone occurs in the range $0.1\ s^{-1}$ to $0.01s^{-1}$. Cowin (14) reported similar values of 0.002 to $0{,}01s^{-1}$ for normal bone activity, and 0.1 - $1.0s^{-1}$ for trauma-induced fracture. The present work would therefore involve loading in the range of the strain-rates found in the literature under torsional loading conditions.

PERTINENT TORSION THEORY

The theoretical model adopted in this section is relevant to the design of both the specimen and the torsional loading attachment for the Hounsfeld (H5000M, capacity 5 kN) testing equipment. The attachment was designed for the purpose of loading specimens in torsion, where previously the testing facility was only capable of compression and tension. The primary torque versus angular rotation displacement would also be converted into shear stress versus shear strain curves using relationships 2 and 3 below. The twisting

attachment is shown in **figure l**, while a typical sample is presented in **figure 2**. The sample gauge length is rectangular, and so we shall consider the engineering analysis of a rectangular sample of an elastic rigid material. While it is mostly customary to use this type of geometry for axial loading (**15**), we shall adopt a similar configuration for our present study and ensure an acceptable level of accuracy by applying appropriate theoretical and experimental analyses. The analytical method for torsional loading of rectangular sections is formulated as follows (**16**).

When approximated as a linear elastic material which is subjected to a torque T, at one end and fixed at the other; and if the axis of twist runs centrally through the bar, along its horizontal or x-axis, then the classical torsion theory can be applied in its design and analysis using the following relationship (**16**).

$$t = \frac{T}{wt_g^{\,2}}\left[3 + 1.8\left(\frac{t_g}{w}\right)\right] \qquad (2)$$

Where T is applied torque, w is width and t is section thickness. Torsional stiffness G.J/L is the gradient of the torque-angular displacement curve. Alternatively, the torsional stiffness can be evaluated using the angle of twist as expressed below,

$$q = \frac{TL}{GJ} \qquad (3)$$

Where L is the length of the bar, $J = wt_g(w^2 + t_g^2) / 12$ is the polar moment of inertia for a bar with a rectangular section (**16, 17**).

It should be noted that the theoretical equation is for a homogeneous and isotropic material, which trabecular bone is not. Instead it is heterogeneous and anisotropic (**18**). However, Stone, Beaupre and Hayes (**11**), have argued that an inhomogeneous material can be modeled as homogeneous as long as its mechanical properties, eg. average elastic modulus, are established experimentally for the solution. We have therefore adopted this approach in the present work.

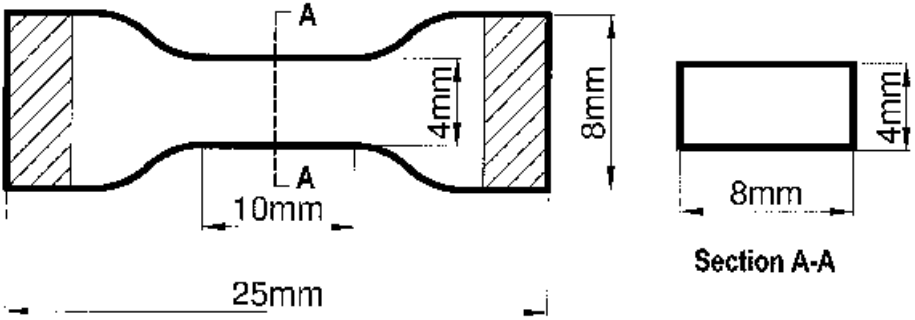

Figure 2. A typical bone specimen showing the rectangular cross-section analysed.

EXPERIMENTS

Factors Influencing Experimental Procedure

The methodology adopted in this research has gained substantially from previous studies. In particular, factors such as specimen condition (moisture content), environmental conditions (temperature and humidity), site, shape, size and machining were controlled for experimental integrity using the methods highlighted below.

In accordance with published research (**2, 12, 19, 20, 21**) the moisture content was maintained at physiological conditions (in 0.15M saline) in order to ensure that energy absorption by the loaded bone closely approximates those in-vivo. Brear et al. (**22**) studied the environmental conditions in which to measure the mechanical properties of bone. They recommend that it is better to test the specimen under conditions similar to those inside the body, while conceding that the effects of this factor on measured properties were not significant.

Also, because Linde and Hvid (**23**) have argued that both the length and cross sectional area have a significant effect on mechanical behaviour we have taken due care to conf gure our specimens in accordance with their conclusions.

Sample Preparation

Patella grooves from freshly slain 2-3 year old bovine animals were obtained from the local abattoir and stored at -20°C until needed for experiments. Before machining, the bones were taken from the freezer and allowed to thaw in cold water.

Specimens of trabecular bone were cut from the patella grooves of twelve bovine femurs using a purpose-built specimen shaper in accordance with the method recommended by Cowin (**14**). The bone was kept wet throughout the cutting process to avoid dehydration. Eight to seventeen specimens were obtained from each femur, and in total 145 specimens were prepared. They contained no articular cartilage, cortical bone or epiphysial growth plates. Specimens were cut to a length (L) of approximately 25mm and a planar cross-section(w x t) ~ 8mm x 8mm, and after measurement of specimen density the cross-section of the specimens was reduced at the centre using a specially designed specimen shaper. The dimensions for the grooves (tg x le) were 4mm x 8mm, to conform with the Linde, Hvid and Madsen analysis[15]. (**figure 2.**)

It should be noted that sawing of the bone could introduce surface cracks. However, Carter and Hayes (**2, 3**) have observed that this surface damage has minimum to negligible influence on the measured mechanical properties of trabecular bone samples. Further Linde and Hvid (**23**) observed that the samples tended to move within the grips, with undesirable consequences for the measured values of stiffness. They claimed that this was due to the trabecular bone being taken out of its natural constraint, which is provided by the surrounding bone, i.e. the "structural end phenomenon". It was recommended that a thin cyanoacrylate adhesive layer be used to constrain the ends of the specimens and thereby restore some of the support (**23**).

Experiments 1 - Verification of the Accuracy of a Rectangular Section in Torsional Loading Analysis

Because it is easier to cut and shape rectangular cross-sections, we have opted for this configuration in our experiments, while noting that the torsional analysis of

Density Value Group Tested	Wet Density (g/cm^3)	Dry Density (g/cm^3)	No. of Specimens
Group 1	0,983 - 1,128	0,533 - 0,678	32
Group 2	1,130 - 1,170	0,680 - 0,720	30
Group 3	1,172 - 1,274	0,722 - 0,824	33

Table 1: Density groups for trabecular bone samples.

this cross-section is readily formed in Engineering texts (**16, 17**). However, in acknowledgement of the fact that it is more usual to use cylindrical specimens with circular cross-section in torsional loading studies, we have carried out the experiments described in this section to verify the representative capacity of out chosen geometry in torsion.

Tasmanian oak hardwood specimens with both rectangular and circular cross-sections, and equivalent dimensions, were loaded by twisting on the same equipment used for testing the bone. Wood was chosen as an appropriate model for this test because of its non-uniform, fibrous and orthotropic material properties, which are similar to those of bone. The results of this study, presented in **figure 3**, show the difference in stress-strain profiles for the cylindrical and rectangular specimens were within acceptable engineering limits i.e. between 4 % and 12 %. This result was also confrmed using ANSYS 5.5 fnite element analysis; the results of this are not included for compactness of the paper.

Experiments 2 - Determination of Trabecular Bone Density

The measurement of wet apparent density is necessary for the current investigation as this is a parameter against which the data will be analysed. Specimen mass was measured using digital weighing scales, and specimen dimensions using the vernier calliper. The volume of the specimens could be determined from its dimensions. The wet density (mass of all material in a volume of trabecular bone) of the specimens was then calculated.

A second specimen shaper with a semi-circular groove, and a fine file were used to reduce the cross-section of the specimens at the centre. The specimens were kept in a wet state throughout the preparation.

Specimen Experimental Groups

After determining the individual density of each of the 95 specimens they were classified into three density groups as shown in **table l**. By grouping the specimens into three density groups, comparisons can be made between low, medium and high densities rather than 95 individual specimen densities.

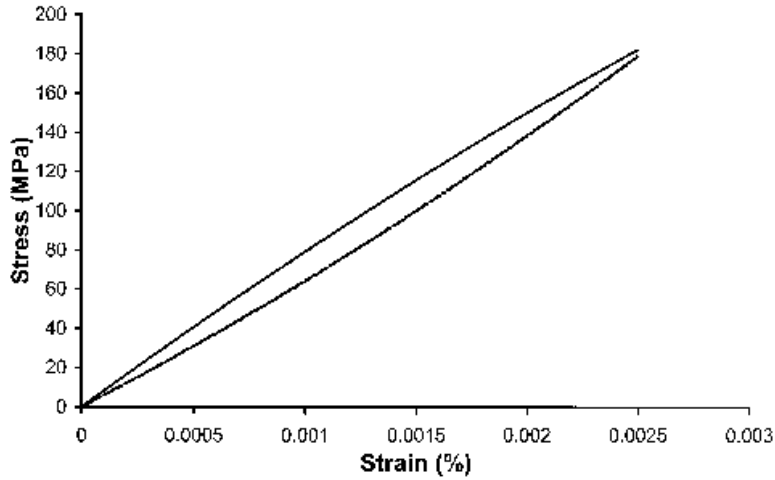

Figure 3. Comparison of the shear stress-strain curves for both rectangular continuous) and circular (dotted) cross-sections for wood specimens tested in torsion. These curves demonstrate the similarity in the stress-strain responses of the two configurations, thereby supporting the use of a rectangular cross-section in our study.

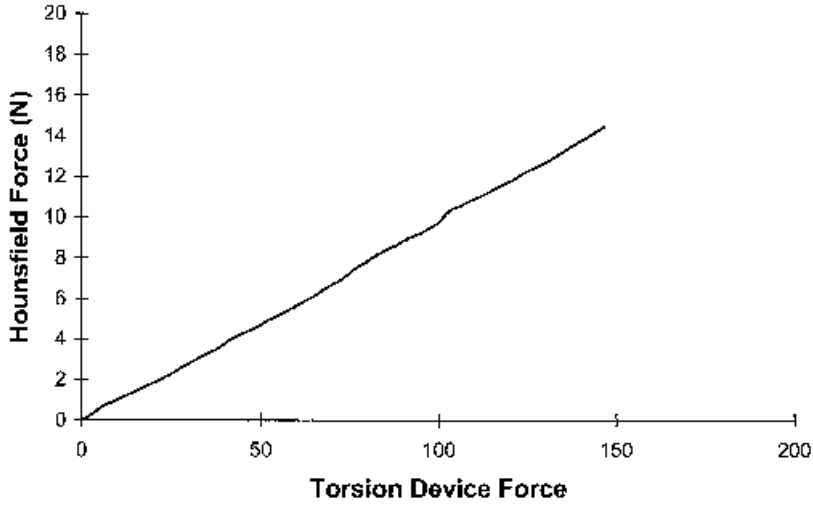

Figure 4. Calibration curve for the load cell of the torsional attachment relating the axial force-displacement of the Hounsfield testing equipment to the torque and angular twist recorded using the attachment.

Experiments 3 - Determination of Load Carriage Parameters

Torsional Testing of Samples at Different Strain-rates

The torsional loading attachrnent was secured to the Hounsfield testing facility and then calibrated using seventeen specimens. The result of this exercise is presented in **figure 4**. This calibration allows us to relate Hounsfield axial force and displacement to the torque and angular twist of the attachment.

Specimens were removed from the freezer and left in a solution until testing. The cross-section of the gauge length of each specimen was measured and then the ends were immersed in cyanoacrylate adhesive LOCTITE 454 and sprayed with LOCTITE Accelerator 5113 to increase the curing speed. They were then left in ambient air for two minutes to cure. The accelerator reduced the curing time substantially by a factor of about 15, ie. from 30 minutes to 2 minutes, and also prevented the glue from soaking through the bone. Constraining the specimen ends in a thin cyanoacrylate layer restored the natural support previously provided by the surrounding bone (**23**).

After curing, the specimen was mounted onto the torsion attachment and then assembled on to the Hounsfield.

A dial gauge was used to centralise the specimen within the clamps. By rotating the arm, a specimen can be centred along its axis of twist. All specimens in the three density groups were subjected to torsional loading at one of three different strain-rates using the Hounsfield 5 kN testing facility, ie. $0.1s^{-1}$, $0.02s^{-1}$, $0.002s^{-1}$.

The specimens were loaded until fracture, which was determined by the dip in the force-displacement graphs shown on the LABView data collection program during testing. The data was saved onto disk before the next test occurred.

RESULTS

Firstly it should be mentioned that the response of a rectangular specimen such as those used in the present work, could be modified by deplanation. However, any effect of this confgurational influence on deformation would be minimal due to relatively small specimen sizes used in our experiments.

A typical shear stress-strain curve is presented in **figure 5**. This graph shows the characteristic pattern of behaviour exhibited by all samples under torsional loading. From these curves elastic stiffness (ie. slope of graph at zero strain), ultimate shear stress and strain energy were evaluated. Fracture strength is the stress at fracture and is designed in this study as the maximum stress before fracture commences, ie. at the maximum inflexion point where the stress-strain cuives begin to

Maximum Torque	
Bensusan et al.*[1]	range 0,13 - 0,37 N.m
this study	range 0,13 - 0,38 N.m

Table 2: Comparison of torque ranges for two studies.

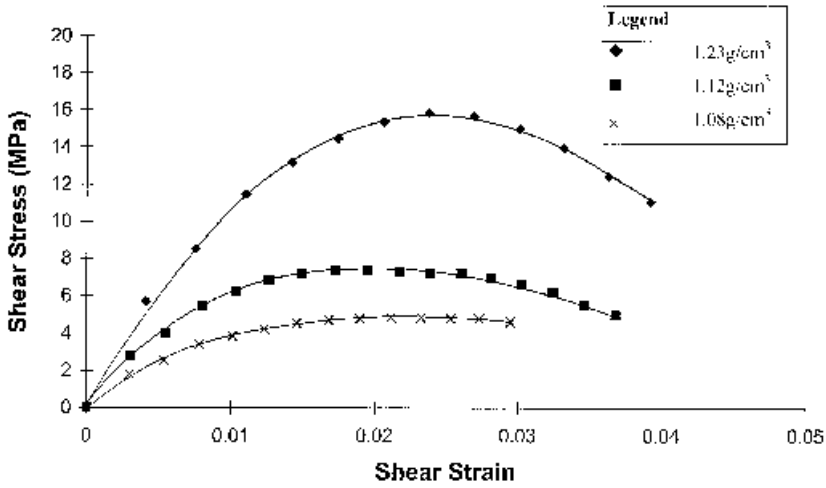

Figure 5. A typical shear stress-strain curve for specimens tested at a strain-rate of $0.02s^{-1}$, for densities, ◆=1.23g/cm^3, ■ =1.12g/cm^3, x =1.08g/cm^3.

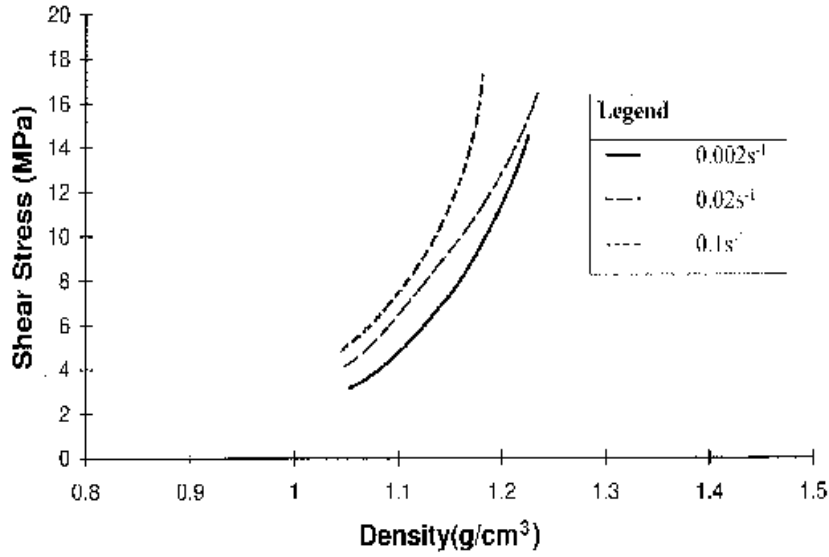

Figure 6. The effect of density on ultimate shear stress for strain-rates of $0.002s^{-1}$- $0.1s^{-1}$.

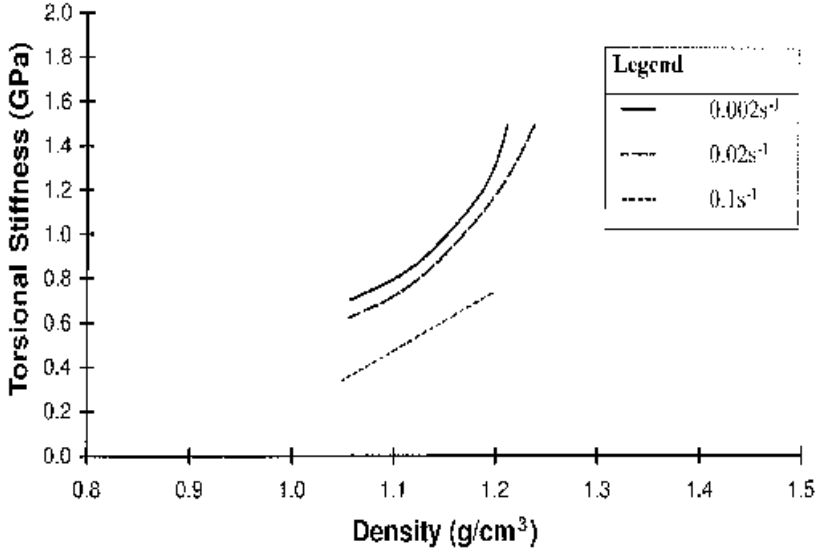

Figure 7. The effect of density on torsional stiffness for strain-rates of $0.002s^{-1}$ - $0.1s^{-1}$.

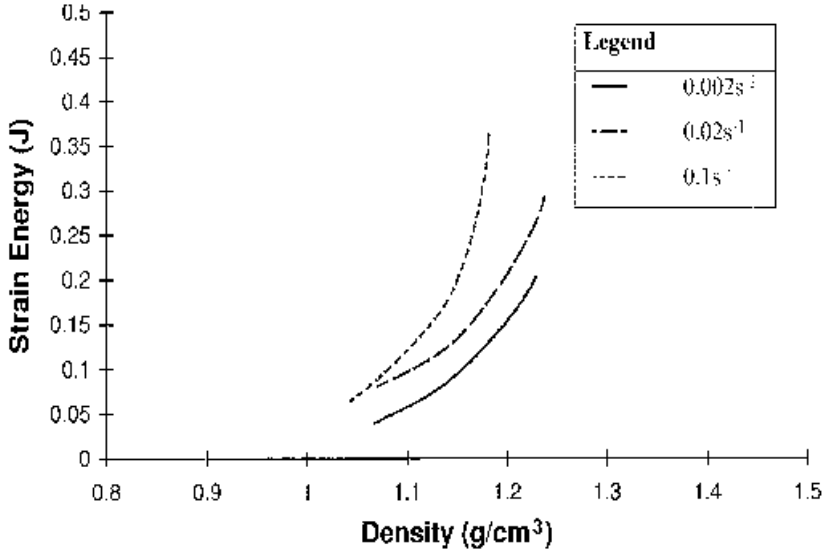

Figure 8. The effect of density on strain energy for strain-rates of $0.002s^{-1}$-$0.1s^{-1}$.

drop to zero. The level of strain energy developed before fracture is measured by the area under the shear stress-strain curve covering the period before the onset of fracture, multiplied by the volume of the specimen. Two measurements of torsional stiffness are defined here. Firstly, low-strain torsional stiffness is the measured as the slope of the shear stress-strain curve at zero strain, and high-strain torsional stiffness is measured at 1.5-2% strain

A plot of ultimate shear stress against density (**figure 6**), for the range of densities and at varying strain rates shows repeatedly that there is an increase in the shear stress-strain values for increasing strain-rate with a concomitant increase in the levels of the ultimate shear stress for each density group.

With regards to the plot of low-strain torsional stiffness versus density for the various strain rates, again it can be seen in figure 7 that the stiffness increased with increasing density. Our data also reveal that the stiffness decreased with increasing strain-rate for a given value of density. Juxtaposing these two behaviours, it can be inferred that the stress necessary for inducing shear in the trabecular bone decreases with increasing strain rate.

The non-linear relationship between low-strain torsional stiffness, density and strain-rate indicates that the behaviour of the specimen under torsional loading is affected to a signifcant extent by both parameters.

Finally, the strain energy values displayed in figure 8 show that there is an increase in the energy absorption capacity of this bone, for increasing density and strain rate. Because strain energy is indicative of the resistance of a material to fracture i.e. toughness 24, the strain energy

Maximum Shear Stress		
Ford and Keaveny	average 6,13 MPa	range 2 - 18 MPa
this study	average 9,3 MPa	range 2 - 16 MPa

Table 3: Comparison of shear stress averages and ranges for two studies.

		mode of loading	k (Dk)	a (Da)	b (Db)
Strength (MPa)	Stok & Oloyede	torsion	4,20 (0,005)	0,140 (0,01)	10,0 (0,3)
	Carter & Hayes	compression	68	0,06	2
Stiffness (GPa)	Stok & Oloyede	torsion	0,20 (0,27)	-0,145 (0,07)	6,0 (0,2)
	Carter & Hayes	compression	3970	0,06	3
Strain Energy (J)	Stok & Oloyede	torsion	0,06 (0,08)	0,190 (0,01)	12,0 (0,6)

Table 4: Experimental constants derived from the data.

characteristics seem to suggest that the trabecular bone becomes tougher as both the density and strain-rate increase.

DISCUSSION

The shear stress-strain curves are non-linear in form, similar to polymeric materials and soft metals such as aluminium. Consequently torsional yield stress values cannot be established. They are highly dependent on both density and strain-rate, increasing with an increase in these parameters. The range of maximum torque for the specimens is comparable with that of Bensusan et al. (**25**) (**table 2**). The range of maximum shear stresses and average shear stress for the specimens are comparable with those of Ford and Keaveny (**5**), (**table 3**).

Carter and Hayes (**2, 3**) proposed equations for the dependence of strength and stiffness on density and strain-rate for compressive testing of trabecular bone. Rice, Cowin and Bowman (**4**) examined this approach and proposed improvements to the methods of measurements of mechanical parameters during both experiments, but agreed with the earlier relationship between shear stress, strain-rate and density proposed by Garter and Hayes (**2, 3**) (**equation 1 and table 4**). The experimental data obtained from our investigation have been used to develop empirically based similar constitutive relationships for the torsional deformation of trabecular bone. However, it is important to note according to Rice et al (**4**) that the relationships developed presently would be specific to the species from which the data was taken. For the plots of shear stress, torsional stiffness and strain energy against density, curve fitting our present experimental data on bovine samples revealed that a similar relation to equation 1 can approximate the strength, stiffness and the strain energy characteristics. The curve of best fit was found for a straight line by transforming the equation $S = k.\ \varepsilon^{\alpha}.\rho^{\beta}$, where k, α, and β are constants, to a linear form using natural logarithms. That is, $\ln(S) = \ln(k) + \alpha\ln(\varepsilon) + \beta\ln(\rho)$. This allowed for calculation of each parameter and the associated error using regression of ln(S) on each variable. The experimental constants derived from the data are shown in **table 4**. The fitted curves are compared to the original data using the values of the constants summarised above. The curves correspond well between these graphs. In particular the results concerning the relationship between stiffness and density correlate well with the results published by Bensusan et al. (**25**).

A noteworthy relatíonship is that between low-strain stiffness and strain-rate for a given value of density. Specifically, the values of stiffness when evaluated as the slope of the tangent, from zero, of the stress-strain curve decreased with increasing strain-rate, thus suggesting that the load carnage capacity of the trabecular bone might be impaired at moderately high strain-rates. This is also apparent from our a-value evaluated in the constitutive relationship for stiffness, where strain-rate has a negative exponential value. This result is contraiy to the hypothesis that stiffness would increase with strain-rate because of the viscoelastic nature of trabecular bone. However, further examination of our results reveal that the slope of the `plastic' portion of the stress-strain curve prior to failure (high-strain stiffness) is greater for the samples tested at

higher strain-rates, thereby reversing the trend seen for zero strain. This leads us to believe that the speed of loading influences the rate of `fracture' or collapse of the trabecular structure thus influencing the determination of stiffness. Following these observations, it is plausible to argue that density alone, and hence bone mineral content, is not a good indicator of trabecular bone load-bearing capability, with possible consequence for the clinical treatment of osteoporosis.

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DIAGNOSTIC SIGNIFICANCE OF MILD HYPERHOMOCYSTEINEMIA IN CHILDREN SUFFERING FROM BONE DYSPLASIAS*)

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***)** Presented on The 4th International Anthropological Congress of Aleš Hrdlička - Symposium "Bone Dysplasias", Prague, 1999.

SUMMARY

Hyánek J, Mařík I, Pejznochová H et al. Diagnostic significance of mild hyperhomocysteinemia in children suffering from bone dysplasias.

Among 80 children patients suffering from miscellaneous bone dysplasias relatively high incidence of mild hyperhomocysteinemia was observed (l:10) and that fact will need further explanation, differentiation and confirmation regarding its ethiopathogenetic significance.

Key words:
bone dysplasia, hyperhomocysteinemia, total homocysteine

The clinical picture of very rare inherited metabolic disorder of methionine cycle called classical homocystinuria (McKusick 23620) is characterized by skeletal, ocular, vascular abnormalities and mental retardation as described by Carson in 1962 (**13**).To the most striking changes belong dolichostenomelia, osteoporosis, biconcave “fish” vertebrae, scoliosis, arachnodactylia etc.The metabolic base of these disorders is the deficiency of enzyme cystathionine-beta-synthase responsible for conversion of homocysteine to cystathionine.The vascular arterial and venous thromboembolic occlusions are the life - threatening complications that very often short the life of patients in 20-30 years of age (**9**). After this causal correlaton Mc Cully postulated in 1969 the possible role of homocysteine (Hcy) in ethiopathogenesis of atherosclerotic plates - "homocysteine theory of atherosclerosis"(**8**). In 1974 Wilckens (she is the pediatrician-specialist in inborn errors of metabolism and her husband cardiologist) hypothesied the possible toxic effect of increased levels of homocysteine on the premature development of vascular damage, thickening of intima media in large as well as in small arteries leading to premature coronary or periphery artery diseases (**16**). Hladovec et al. proved the

direct toxic effect on endothelial cells by increased level of Hcy (**4**).

The last decade has been devoted to the experimental (**14**), epidemiological (**10**), clinical (**2,14,15**) and therapeutical (**7,12**) studies on mild hyperhomocysteinemia (mHHC) as the new independent risk factor for early development of coronary heart disease, cerebral or peripheral artery occlusions. The systematic finding for hyperhomocysteinemia among patients indicated for aortocoronary or peripheral artery bypasses in our Hospital Na Homolce proved relatively high incidence of increased homocysteine and different types of hyperhomocysteinemia have been detected till now, especially the mild form of HHC in frequency 1:52 (**5**) (**tab. 1**).

Homocysteine (Hcy) is a nonesential aminoacid participating in remethylation or transsulfuration metabolic pathways of essential amino acid methionine (**fig. 1**). Under physiological conditions is quickly remethylated to methionine by enzymatic complex-methionine synthase, in presence of B_{12} vitamin as cofactor and methylenetetrahydrofolate as substrate.This substrate must be synthetized from folates by other enzyme - methylenetetrahydrofolate reductase (MTHFR). Hcy is transsulfurated with pyridoxine as cofactor to cystathionine and after some further metabolic steps eliminate as sulphates by urine. Insufficient activity of MTHFR from genetic reasons or from deficiency of critical vitamins (folate, pyridoxine, cobalamin) cause the deficiency of substrate or brakes the remethylation or transulfuration reactions. The level of Hcy increases and causes the new type of ekogenetic or ekometabolic disorders - mild or severe hyperhomocysteinemia (**11**). Toxical effect of HHC was proved outside vessel disturbances in a lot of other metabolic interferences, among them the most serious in tissues of mesenchymal origine (**tab. 2**). The frequency of the mutation 677C-T in MTHFR allele responsible for defficient enzyme acitivity in European population was detected in homozygote form 5-15 %, in heterozygous form 20 - 40 %. Therapeutical supplementation of homozygotes with folate together with other critical vitamins improves the enzymatic activity of MTHFR (**7**).

Although the direct relation between increased Hcy and thromboembolic events in homocystinuria were intensively studied and the direct relation between increased Hcy and accelerated arterial occlusions, increased intimal thickness was proved, the correlation between increased plasmatic tHcy and the risk of early development of connective tissue disorders has not yet been practically followed. Elevated Hcy interferes by its very active-SH groups with the crosslinking of collagen mediated by aldehydic groups. Since crosslinking of elastin is also mediated by aldehydic groups - this action of elevated Hcy might interfere with elastin structure, too (**6,9**).

The diagnosis of classical homocystinuria was relatively simply thanks to very high levels of Hcy and methionine in plasma. The detection of only moderate elevated levels of Hcy in plasma is methodically more complicated, needs higher sensitivity and in the last 10 years it was possible only by use of more sofisticated HPLC techniques with fluorescent detection.The aim of our present pilot study was to assess the possible incidence and role of mHHC in children patients suffering from different bone dysplasias.

MILD FORM (16-30 mol/l)	**1 : 52**
INTERMEDIATE FORM (31-100 mol/l)	**1 : 1235**
SEVERE FORM = Class. homocystinuria (> 100 mol/l)	**1 : 5766**

Tab.1. Incidence of different form of hyperhomocysteinemia in 5766 patients suffering from cardiovascular diseases.

DIRECT ON ENDOTHELIAL CELLS: destruction of elastine fibres, desquamation,increase of the total collagen synthesis and collagen cross-linking, increased NO production
ON REDOX-OXIDATIVE STATUS: increased production of H_2O_2 and OH´ radicals, increased pro-oxidative activity , production of nitrils and nitrosothiols
CHANGE OF HEMOSTATIC CONDITIONS FROM ANTITHROMBIC TO THROMBOGENIC: inhibition of thrombomodulin-thrombin cofactor, reduce endothelial protein C activator, inactivation of FVa+FVIIa, increase PAI-1, decrease of fibrinolysis due to competition of cringels of Lp(a) with tPA , inhibition of v.Willebrand factor processing, activation of Hagemann factor, reduce of antithrombin III, increase synthesis of thromboxane A_2
INCREASED ATHEROGENESIS: thiolation of LDL-cholesterol, stimulation of proliferation of smooth-muscle cells, deposition of thiolated LDL-cholesterol in atherosclerotic plate

Tab. 2. Toxic effects of increased levels of homocysteine.

	Patients	**Controls**
Total Number	80	30
Girls	46	15
Boys	34	15
Ages (yrs)	2 - 18	5 - 18

Tab. 3. Subjects Characteristic.

	Patients		Controls	
Age (yrs)	2 - 11	12 - 18	5 - 11	12 - 18
tHcy (μmol/l)	4,9+/-1,6	6,6+/-3,6	4,8+/-1,2	5,4+/-1,3
Significance	n.s.	p<0,05		

Tab.4. Average values of total homocysteine in patients with bone dysplasias.

tHcy(umol/l)	Diagnosis	Age (yrs)
24,2	Hypophosphatasemic Rickets	17
12,6	Hypochondroplasia	16
9,8	Multiple Sy of Pterygia	11
9,7	Osteoporosis-Pseudoglioma Sy	13
8,8	Rachitis Renalis	6
8,6	Algoneurodystrophy	9
7,8	Spina Bifida Lumbalis	10

Tab.5. Total homocysteine values over 95th percentile detected in patients with diagnosis.

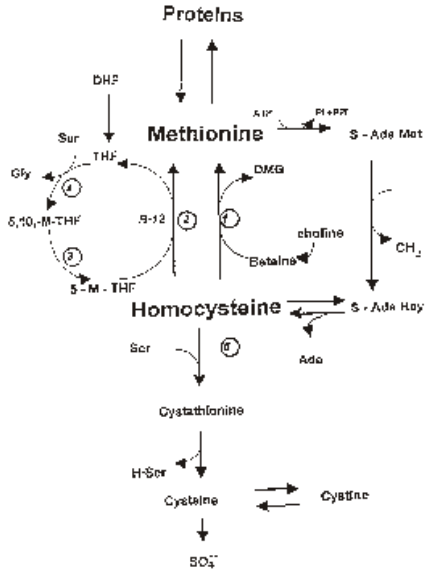

Fig. l. Metabolic pathways of the methionine cycle. 2-methionine synthase, 3-methylenetetrahydrofolate reductase, 5-cystathionine-beta-synthase.

PATIENTS and METHODS

Since 1998 the level of all types of Hcy in plasma after reduction (called total homocysteine - tHcy) has been estimated by use of chromatographic method after Araki and Sako (**1**) in 80 children (2-18 yrs) outpatients of orthopaedic department of Ambulant centre for defects of locomotor apparatus with the most frequent diagnoses as given in **tab. 3**. Because the plasma Hcy levels show the dependency on age, group of our patients was organized into an younger group (2-12 yrs) and in an older group (13-18 yrs). As a control group 30 healthy children of similar age were used.

RESULTS

Average values of tHcy in the younger patients and in the group of healthy children were without any statistical difference (4,88+/-1,65 umol/l versus 4,85+/-1,21 umol/l). In the older children only the moderate increase in tHcy but statistically significant could be observed (6,58 +/-3,61 umol/l versus 5,41 +/-1,31 umol/l) (**tab. 4**). The tHcy values overlapping the 95 percentil were detected in 3 older and 5 younger patients with the following diagnoses: 24,2 umol/l Hypophosphatemic Rickets (14 y); 12,6 umol/l in Hypochondroplasia (16 y); 9,8 umol/l in Multiple Pterygia Syndrome (11 y); 9,7 umol/l in Osteoporosis Pseudoglioma Syndrome (13 y); 8,8 umol/l in Algoneurodystrophic Syndrome (11 y); 8,6 umol/l in Spina Bifida Lumbalis (7 y); (**tab. 5**).

DISCUSSION

The first findings of our pilot study in bone dysplasias show a quite high incidence of HHC among patients suffering from miscellaneous bone dysplasias (l :10) comparing to healthy population and proved the higher incidence of HHC among patients suffering from connective tissue disorders. Our findings will need further confirmation for estimation of remethylation capacity after methionine load (100mg/kg b.w.) and after moleculargenetic estimation of mutations for deficient allele of MTHFR in children as well as in their parents. The estimation of critical vitamins must be completed in order to exclude possible nutritional deficiency.

The Framingham Study performed few years ago proved in older population suffering from cardiovascular diseases the positive therapeutical effect of supplementation by critical vitamins especially folate and resulted in the supplementation of cereal foods introduced in USA since 1.st January 1999 (**3,7**). This fact stimulates us to try the possible therapeutical effect of "critical vitamins" especially folate must be in our patients carefully followed.

We can only conclude that metabolic disturbances of homocysteine in bone dysplasias could play a similarly important role in connective tissue disorders like it was postulated in cardiovascular diseases and need further ethiopathogenetic confirmation if they are specific only for these metabolic or clinical entities.

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OPAKOVANÁ STRESS FRAKTURA TIBIE U DÍTĚTE

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SOUHRN

Autoři referují o vyšetření opakované stress fraktury tibie magnetickou rezonancí (MR) u dětského pacienta. Literární odkazy potvrzují oprávněnost indikace tohoto vyšetření v některých případech.
Klíčová slova: opakovaná stress fraktura tibie - indikace k vyšetření MR.

SUMMARY

Skotáková J, Straka M. Recurrence stress fracture of the tibia in a child.

The authors reffer about recurrence stress fracture of the tibia in a child and indication of magnetic resonance imaging (MRI) in some cases.
Key words: Recurrence stress fracture of the tibia- indications for MRI.

ÚVOD

Fraktury z přetěžování - "stress" fraktury a mediální tibiální stress syndrome (1), se vyskytují u zdravých adolescentů a mladých dospělých, mohou doprovázet osteoporózu u starších jedinců. Je dobře známo, že se často vyskytují u sportovců (8) při opakovaném přetěžování. Pro stressové fraktury je typickým příznakem bolestivost a klidová bolest. U dětí se vyskytují na tibii, tarzálních kostech, os cuboideum, kalkaneu. Nalezneme je dokonce již u kojenců, kteří přestanou chodit, nebo kulhají. V iniciální fázi je skiagram v 72% negativní (5). Poranění z přetěžování se u sportujících dětí v poslední době objevují stále častěji (9). Poranění měkkých tkání je u dětí méně časté než u dospělých. Pro stanovení správné diagnózy je někdy požadováno vyšetření pomocí magnetické rezonance (MR). MR je indikována u pacientů v rámci diferenciální diagnostiky: stress fraktura, kostní nádor (Ewingův tumor, osteosarkom), osteomyelitida. Při MR vyšetření je u stress fraktur typický nález: linie lomu, edém kostní dřeně a zesílení signálu (enhancement) extraosálně po podání kontrastní látky. V kazuistice autoři referují o výskytu neobvyklé opakované stress fraktury v oblasti proximální diafýzometafýzy tibie u pěti letého, respektive sedmi letého chlapce. K vyloučení suspektního maligního procesu bylo skiagrafické vyšetření doplněno vyšetřením magnetickou rezonancí.

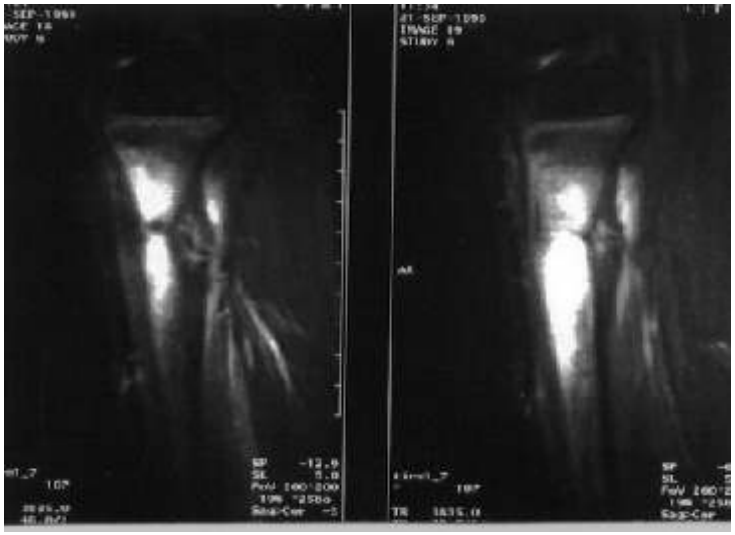
KAZUISTIKA

Chlapec, který aktivně nesportuje, s příčnou frakturou z přetěžování v oblasti proximální diafýzometafýzy levé tibie ve věku 5 roků, se v 7 letech dostavil s

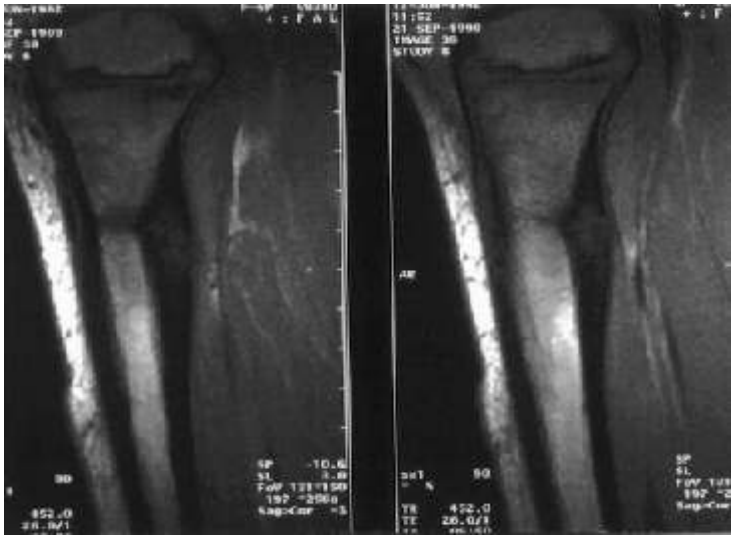
bolestivostí v oblasti levého kolenního kloubu. Na zhotovených skiagramech byla vysoce suspektní fraktura z přetěžování ve stejné lokalizaci jako v předchozím případě, s velmi výrazným periostálním svalkem. Vzhledem k anamnéze a ne zcela typickému obrazu na skiagramech bylo vyšetření doplněno magnetickou rezonancí k vyloučení tumoru. Byl použit přístroj Magnetom Open Viva 0,2 Tesla.Ve všech sekvencích byly prokázány změny typické pro stress frakturu: ve STIR (**obr.1**) a T2 zvýšení signálu odpovídající edému, v T1 vážených obrazech hypointenzní linie lomu (**obr.2**). Nitrodřeňová dutina nebyla postižena žádným patologickým procesem. Na kontrolních skiagramech za 2 měsíce (**obr.3,4**) a za 4 měsíce (**obr.5,6**) bylo hojení a regrese patologického nálezu. STIR sekvence-short tau inversion recovery-sekvence s potlačením signálu tuku. Je mimořádně citlivá pro detekci tekutinových kolekcí a edematózních změn. T1 sekvence má krátký repetiční čas (TR), T2 sekvence má dlouhý TR, tekutina v ní má vysoký signál.

DISKUSE

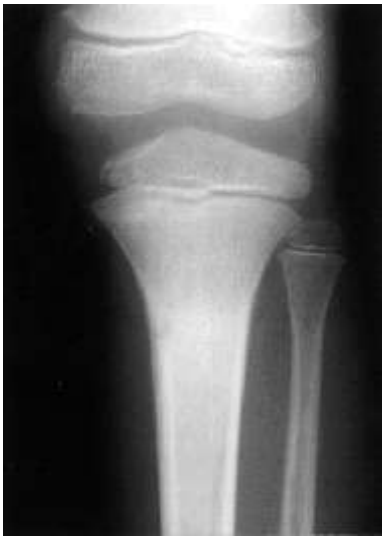
Stress fraktury se vyskytují u dětí od raného věku (**2**). Mitchell (**11**) referuje o vzácné dvojité stress fraktuře tibie u deseti leté dívky, u které byly skiagramy doplněny MR vyšetřením k vyloučení maligního procesu. Také Cuadra (**4**) publikoval práci týkající se stress fraktur u dětí. U jednoho pacienta prokázal 3 stress fraktury, z toho na téže tibii nepřetržitě, či následně.V rámci diferenciální diagnostiky bylo provedeno vyšetření MR, kostní scintigrafie a biopsie. V literatuře byly popsány longitudinální stress fraktury v oblasti střední a distální diafýzy tibie. K jejich diagnostikování bylo využito vyšetření MR (**13**). MR jednoznačně prokáže linii lomu a abnormální signál, jak ve dřeňové dutině, tak v okolních měkkých tkáních. Daunt (**3**) uvádí, že tibie je u dětí jednou z nejčastěji postižených kostí při stress frakturách. Lomná linie bývá transverzální, méně často šikmá, velmi neobvykle podélná (referuje o 4 případech podélné stress fraktury tibie). Mnoho autorů se shoduje v tom, že ve většině případů jsou skiagramy v iniciální fázi u stress fraktur negativní. Například Umans (**13**) uvádí takový soubor 6 dětí s podélnou stress frakturou tibie. Rentgenové vyšetření bylo doplněno MR, které prokázalo přítomnost stress fraktury ve všech případech. Autor tuto vyšetřovací metodu považuje za nejpřínosnější, neboť MR ukáže: linii lomu, edém v okolí, svalek, abnormality kostní dřeně i měkkých tkání. Je-li nález z vyšetření MR jasný, jsou možné kontroly pouze skiagramy. Doporučují se za 7 až 14 dnů, kdy se objeví svalek. Dostupné jsou i komparativní práce, které srovnávají přínos CT a MR vyšetření. MR má vyšší senzitivitu než CT při detekci edému kostní dřeně a postižení měkkých tkání. Podle některých autorů (**7**) však mohou obrazy MR vést k mylné interpretaci ve smyslu hodnocení nálezu jako agresivního tedy maligního procesu. CT zůstává podle stejného autora nejlepší zobrazovací metodou pro diagnostiku longitudinálních stressových fraktur tibie, avšak správné hodnocení obrazů MR může na druhé straně zabránit zbytečné biopsii nebo CT vyšetření. DiFiori (**6**) popisuje případ neobvyklé stress fraktury v oblasti proximálního konce fibuly u čtrnácti letého chlapce s bolestivostí trvající několik týdnů, bez úrazové anamnézy. Také u tohoto pacienta se vyšetření nativními



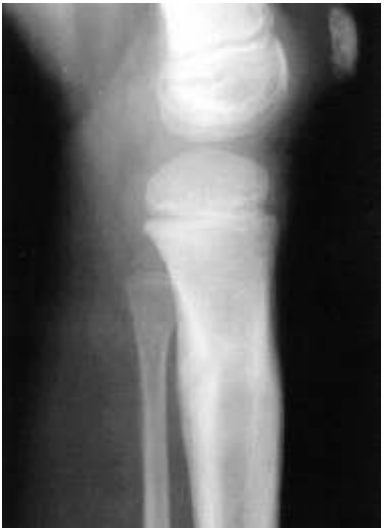
Obr. 1 STIR sekvence
Fig. 1 STIR of the tibia



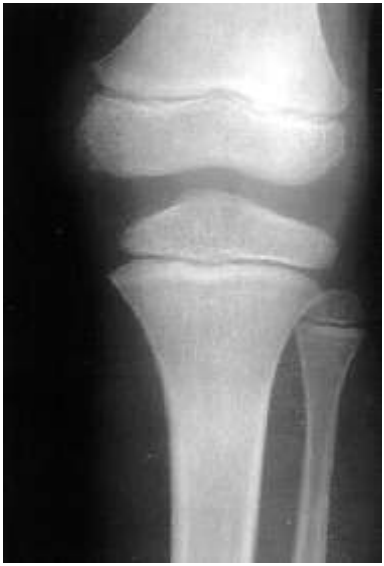
Obr. 2 T1 vážený obraz
Fig. 2 T1 SE



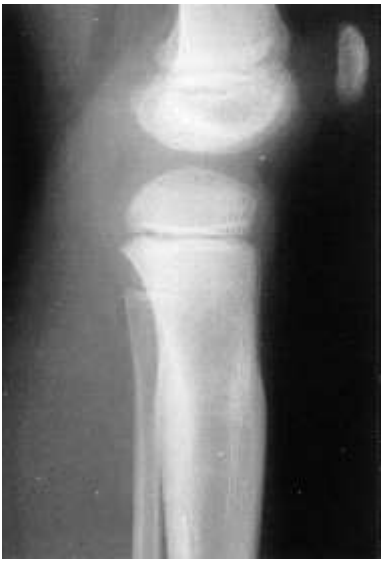
Obr. 3 Skiagram bérce - AP projekce
Fig. 3 X-ray of shank - AP projection



Obr. 4 Skiagram bérce - bočná projekce
Fig. 4 X-ray of shank - side projection



Obr. 5 Skiagram bérce - AP projekce
Fig. 5 X-ray of shank - AP projection



Obr. 4 Skiagram bérce - bočná projekce
Fig. 4 X-ray of shank - side projection

snímky doplnilo MR k vyloučení tumoru. Na MR má vlastní stress fraktura nízký signál,v okolí je přidružený intermediární signál v Tl vážených obrazech, v T2 a STIR je zóna vysokého signálu, která obsahuje vlastní frakturu a zónu okolního edému.

V poslední době se objevuje výraz medial tibial stress syndrom (**12**), který byl přiřazen ke stress frakturám. Předpokládá se, že je způsoben stress reakcí fascie, periostu a kosti podél posteromediální části tibie. Bolestivost a citlivost je přítomna podél mediální hrany střední a distální tibie. Příznaky exacerbují při pohybu a ustupují v klidu (**9**). Časné změny při medial tibial stress syndromu nejsou diferencovatelné na konvenčním skiagramu a vyžadují scintigrafii nebo MR.
I. stupeň prokáže edém periostu - zvýšení signálu v T2, dřeň je v T1 a T2 normální.
II. stupeň poranění ukáže výrazný periostální edém a přidružený edém dřeně, obojí hyperintenzní v T2.
III. stupeň má edém kostní dřeně v T1 i T2.
IV. stupeň je doprovázen již linií lomu. K tomuto rozdělení autoři dospěli na základě komparace nálezů na scintigrafii a MR.

Vyšetření MR se u stressových fraktur doporučuje tam, kde není diagnóza jasná z nativního skiagramu. Vyloučí v diferenciální diagnostice malignitu, jestliže ji stress fraktura imituje, nebo osteomyelitidu.V poslední době se proto stále častěji objevují práce zabývající se touto problematikou.

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DEFORMACE KYČELNÍHO KLOUBU NA LANGOBARDSKÉM POHŘEBIŠTI - JIŽNÍ MORAVA

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SOUHRN

V langobardském pohřebišti Lužice (jižní Morava) byla na skeletu muže (hrob číslo 2) pozorována deformita hlavice a krčku femuru vzniklá velmi pravděpodobně jako následek Perthesovy choroby nebo coxa vara adolescentium (epifyzeolýza proximální epifýzy femuru). Tvar a velikost ostatních neúplných, ale dobře zachovalých kostí vylučuje postižení kostry systémovou kostní chorobou (metabolickou nebo kostní dysplazií). Levé acetabulum je velmi široké a mělké, pravé je normálního tvaru. Střed hlavice levého femuru je posunut distálně, hlavice je plochá zobákovitě protažená, krček krátký a široký. Na mediálním okraji je hlavice porotická. Nelze určit depresi hlavice v oblasti ligamentum capitis femoris (fovea capitis femoris). Krček je krátký a široký. RTG snímek ukazuje dobře vytvořenou trabekulární strukturu. Ortnerova kriteria suchých kostí svědčí pro diagnózu coxa vara adolescentium. Na základě klinických zkušeností se přikláníme k diagnóze morbus Legg-Calvé-Perthes.
Klíčová slova: langobardské pohřebiště Lužice (jižní Morava, CZ), Perthesova choroba, coxa vara adolescentium

SUMMARY

Smrčka V, Svenssonová M, Mařík I. Hip joint deformation in Langobard´s burial-ground - South Moravia

Perthes´disease or slipped femoral capital epiphysis (epiphyseolysis upper femoral epiphysis) was diagnosed in the left femur of a male skeleton (grave 2) from the Langobard burial - ground in Lužice (South Moravia) in Czech Republic. The shape and size of other incomplete but well-preserved bones exclude the observed deformity of the femoral head from systemic bone diseases (metabolic bone diseases and bone dysplasias).

The left-hand side acetabulum is very large and shallow, the right-hand side one is normal. The central part of left femoral head is displaced inferiorly. There is porosity on medial view of the femoral head. The depression for the ligamentum capitis femoris cannot be defined. The femoral neck is short and thick. The X-ray film shows well-organised trabecular

structure.

The Ortner´s criteria with dry bones speaks for slipped femoral capital epiphysis but on the other hand our clinical view for Perthes´disease.

Key words: Langobard´s burial-ground in Lužice (South Moravia, CZ), Perthes´ disease, slipped femoral capital epiphysis

ÚVOD

Langobardské pohřebiště Lužice se nachází 5 km jižně od Hodonína na Jižní Moravě.

Jde o doposud největší objevené pohřebiště z doby stěhování národů na našem území datované do přelomu 5. a 6. století našeho letopočtu (**5**).V 80. letech zde bylo odkryto pracovníky Archeologického ústavu AV v Brně pod vedením Z. Klanicy 120 kostrových hrobů. Antropologický materiál je deponován v Národním muzeu v Praze.

V hrobu číslo 2 byl uložen muž starší 50 let (**2, 3**) s deformovaným levým kyčelním kloubem, jak v oblasti hlavice stehenní kosti, tak i acetabula. Dalšími chorobnými změnami na skeletu je pseudoatróza žebra a intravitální ztráty zubů v horní čelisti.

Skelet muže není robustní, svalový reliéf je dobře vyznačen, lebka je značně neúplná, z postkraniálního skeletu chybí především horní část hrudníku. Zelené zbarvení na levé tibii ukazuje na kontakt s bronzovým či měděným předmětem (**4**).

Patologické nálezy v hrobu číslo 2

Deformace levého kyčelního kloubu.

Hlavice levého femuru je oploštělá, s okraji rozšířenými do hřibovitého tvaru. Mediální třetina hlavice je oddělena žlábkem, hlubším na přední straně a mělčím dorzálně. Při mediálním okraji zobákovitě zahnuté hlavice jsou osteoartrotické osteofyty. Z velkého trochanteru zbyla jen jeho část a zřejmě převyšoval hlavici. Krček femoru je zkrácený a ztluštěný. Na pravém femuru se hlavice nezachovala. Diafýzy obou femurů mají stejný tvar i šířku. Acetabulum vlevo je ve srovnání s pravostranným zřetelně plošší, v průměru i v šíři facies lunata je o l cm větší. Ve střední a přední části facies lunata jsou tři mělké jamky v průměru l cm. Zadní okraj acetabula je oproti pravé straně zaoblen, nejsou známky neoacetabula proximálně ani dorsálně - **obr. 1.**

Obr. 1. Kyčelní klouby skeletu z hrobu číslo 2 v Lužicích - dorzální pohled.

Na RTG snímku levého kyčelního kloubu je sklerotická struktura kostní tkáně v přední části facies lunata jako následek funkčního přetěžování. Hlavice je plochá, mediálně zobákovitě protažená, krček je krátký a široký, velký trochanter je v pozici C. Kostní struktura Adamsova oblouku a trámčina ventromedilního kvadrantu hlavice je sklerotická - **obr. 2** a **3.**

Pseudoartróza žebra

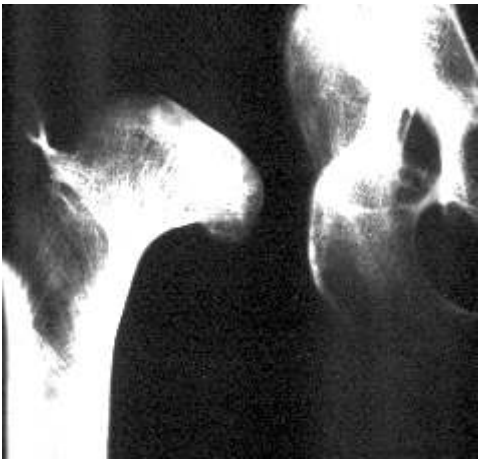
V oblasti hrudníku byl nalezen úlomek žebra s pseudoartrózou obtížně lokalizovatelný. Lomná linie je zvlněná, prochází pouze přední stranou žebra. Zadní strana není porušena a ve směru lomné linie je pohmatem zjistitelné vyklenutí. To je i viditelné na horním okraji žebra. Na RTG snímku je zřetelná kondenzace sklerotické kosti v celém okolí lomné linie.

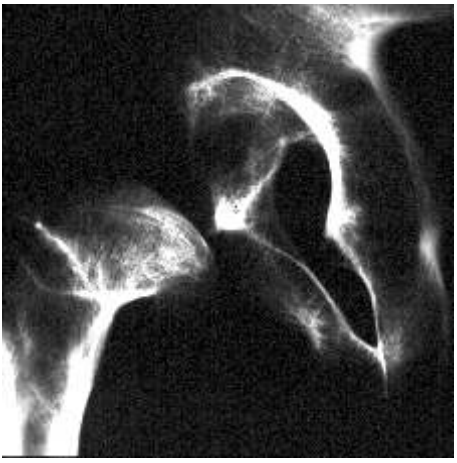
Intravitální ztráta zubů v horní čelisti

V horní čelisti vlevo je intravitální ztráta C1, P1, P2 a M1 a současně i M2 vpravo. Důkazem, že ke ztrátě došlo za života jedince je rozdílný obrus v dolní čelisti vlevo na P1 a P2 . Tyto zuby nejsou obroušeny, kdežto na všech ostatních vyjma stoliček M3 je zřetelný silný obrus.

DISKUSE

Jednostranná deformace hlavice femuru, rozšíření a varozita krčku vzniká nejčastěji jako následek Legg-Calvé-Perthesovy choroby (osteochondritis of the upper femoral epiphysis, coxa plana) - **obr. 4,** nebo jako důsledek epifýzeolýzy akutní (traumatické) či spontánní (poškození růstové epifýzy zánětem, hypothyreoidismem aj.), která se vyskytuje vzácněji (1 - 3 : 100 000 živě narozených dětí /**1**/)- **obr. 5**. V některých případech je příčina vrozená (např. coxa vara congenita - **obr. 6**, mnohočetná epifyzární dysplazie - **obr. 7**, dysplazie kyčelního kloubu apod.). Tvar hlavice a acetabula v dospělosti je výsledkem adaptačních funkčních změn (remodelace), které vznikají v růstovém období v závislosti na závažnosti





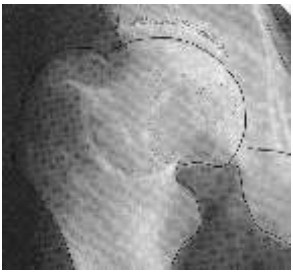
Obr. 2 a 3. RTG snímky proximálního konce levého femuru a acetabula v zadopřední projekci - různá tvrdost RTG záření.

primárního poškození cévního zásobení hlavice a na věku, ve kterém afekce vznikla. Obecně platí, že čím je dítě mladší, tím se více uplatní remodelace, která může být příznivě ovlivněna konzervativním anebo operačním léčením. V opačných případech, kde osteonekróza hlavice z jakýchkoliv příčin vznikne u staršího dítěte nebo dokonce po skončení růstu, dochází k biomechanicky závažné destrukci hlavice a rychlému rozvoji deformační koxartrózy.

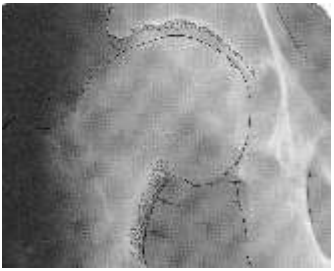
Žlábek na hlavici levého femuru je velmi pravděpodně způsoben tlakem okraje acetabula na subluxovanou deformovanou hlavici. Oploštěné a rozšířené acetabulum, hřibovitý tvar hlavice mediálně zobákovitě protažené, nemožnost určení deprese pro ligamentum capitis femoris a částečná poróza hlavice nás vede k závěru, že deformace kyčelního kloubu vznikla jako důsledek neléčené jednostranné aseptické nekrózy hlavice femuru (morbus Legg-Calvé-Perthes).

Při hodnocení dle kriterií vypracovaných Ortnerem a Putscharem (**4**) nemůžeme vyloučit ani epifyzeolýzu proximálního konce femuru. Levý femur byl uložen v rozšířeném, zvětšeném acetabulu, ale bez neoacetabula. Centrum hlavice je posunuto distálně pod vrchol velkého trochanteru. Krček hlavice je zkrácený a rozšířený. Na RTG snímku je zřetelná trabekulární struktura.

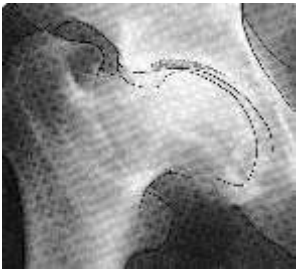
U pseudoartrózy žebra jde



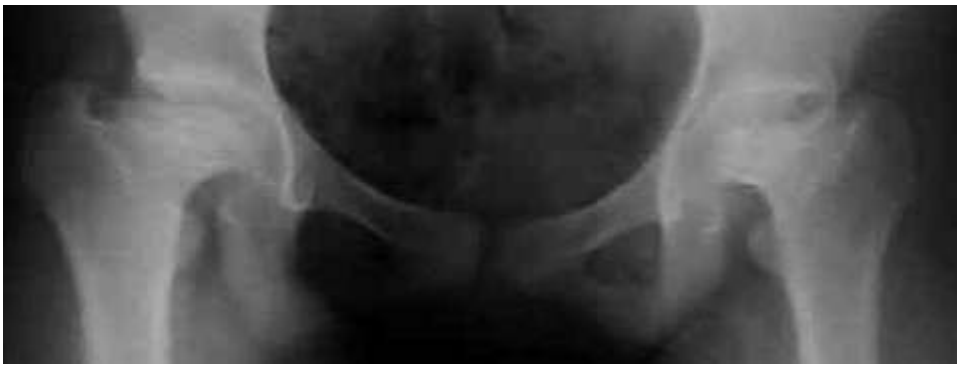
Obr. 4. M.Perthes, 18 let.



Obr. 5. Coxa vara adolescentium, 17 let.



Obr. 6. Coxa vara congenita, (38 let).



Obr. 7. Mnohočetná epifyzární dysplazie - typ Fairbank, 45 let.

pravděpodobně o 4. - 9. žebro, kde nejčastěji vznikají zlomeniny a to v jeho zadní části vzhledem k tomu, že jde o nepřímou zlomeninu. Nepřímým mechanismem, stlačením hrudníku zepředu i zezadu vzniká zlomenina přední strany a to v axiální či vertebrální části, jako v tomto případě (**2**). Jde o pseudoartrózu se zduření v horním okraji žebra a kondenzací okrajů lomných ploch na RTG snímku.

ZÁVĚR

Předpokládáme, že deformace levého kyčelního kloubu u hrobu číslo 2 na langobardském pohřebišti v Lužici vznikla velmi pravděpodobně jako následek aseptické nekrózy hlavice femuru - morbus Legg-Calvé-Perthes. Nelze vyloučit epifyseolýzu proximální epifýzy femuru, ale ani jiné vrozené a vývojové vady s asymetrickým postižením kyčelních kloubů.

Poděkování

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Doc. MUDr. Václav Smrčka, CSc.
Nádražní 113
264 01 Sedlčany

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Mazurová F, Mazura I, Mařík I. Možnosti diagnostiky achondroplazie a neurofibromatózy (NF-1) molekulárně genetickými metodami 1/99, s.6
Strouhal E, Kuklík M. Paleopatologie - historie a současnost 1/99, s.11
Janíček P. Struktura a vymezení bioinženýrských oborů 1/99, s.16
Procházková J. Dentinogenesis imperfecta jako jeden ze symptomů osteogenesis imperfecta a vrozené vady dentinu 1/99, s.24
PetrtýlM. Biomechanické a biotermodynamické zákonitosti remodelace kostní tkáně 1/99, s.28
Adam M. Osteoporosa - diagnosa a léčba (dopis předsedy Společnosti pro výzkum a využití pojivových tkání ministrovi zdravotnictví) 1/99, s.73
Janíček P.Biomechanické problémy ortopedie,možnosti a bariéry jejich řešení .2/99, s.84
Poul J. Indikace ortopedických vložek v dětském věku .....3+4/99, s.164
Kuklík M. Genetika končetinových vad.....3+4/99, s.168
Dylevský I. Morfogeneze končetin.....3+4/99, s.180
Prokopec M. Hlavní vědecké problémy, řešené Alešem Hrdličkou .....1/00, s. 6
Cetta G, Tenni R, Zanaboni G, Valli M, Rossi A, Forlino A, Piazza R and Dyne KM. Vrozené choroby pojivové tkáně - 25 leté zkušenosti s výzkumem v Pavii ..... 4/00, s. 225

MONOGRAFIE

Mařík I. Systémové, končetinové a kombinované vady skeletu – 1. část: diagnostické, terapeutické a biomechanické aspekty ..... 2+3/00, s. 83

PŮVODNÍ PRÁCE

Vukasinovic Z, Zajic L, Slavkovic S, Vukadin 0, Milickovic S.První zkušenosti s trojí pánevní osteotomií v Jugoslávii 1/99, s.49
Sochor M, Balík K, Cabrnoch B, Vilímek M, Křena J. Kompozitní dlahy pro osteosyntézu dlouhých kostí - srovnání experimentálních a FEM (početních) výsledků 2/99, s.106
Čulík J, Petrtýl M. Materiálové charakteristiky osteonů kortikální kosti 2/99, s.114
Straus J. Geometrické a dynamické znaky v podogramu dětské nohy 2/99, s.124
Mařík I, Smrčka V, Kuklík M, Kříž V, Hadraba I. Klasifikace, biomechanické aspekty a komplexní léčení vrozených končetinových anomálií u generalizovaných skeletálních vad.....3+4/99, s.187
Zemková D, Mařík I. Predikce růstu vrozených zkratů segmentů končetin.....3+4/99, s.224
Petrtýl M, Danešová J. Obecná teorie remodalce kostní tkáně.....3+4/99, s.244
Roth M. Neuroadaptivní změny vypadající jako primární kostní afekce ..... 1/00, s. 15
Hulejová H, Martínek J, Adam M. Vliv kolagenního hydrolyzátu na expresi některých cytokinů při adjuvantní artritidě a histologické sledování jejího průběhu..... 1/00, s. 24
Pešáková V, Klézl Z, Čech O, Pohunková H, Adam M. Transplantace autologních chondrocytů do defektů kloubní chrupavky miniprasat ..... 1/00, s. 34

Čulík J. Mechanika ruky ..... 1/00, s. 42
Vukasinovic Z, Zivkovic Z, Zajic L. Naše zkušenosti s osteoartikulární TBC u dětí ..... 4/00, s. 237
Stok K, Oloyede A. Závislost biomechanických odpovědí trabekulární kosti na hustotě a poměrném namáhání při torsním zatěžování ..... 4/00, s. 245
Hyánek J, Mařík I, Pejznochová H, Hyánková E, Pinkasová J, Dubská L, Cabrnochová I, Jenčová H, Štancová C, Martiníková V. Diagnostický význam mírné hyperhomocysteinémie u dětí s kostními dysplaziemi ..... 4/00, s. 258

KASUISTIKY

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Kuklík M, Mařík I. Syndrom Hallermanův-Streiffův-Francoisův (dysmorphia mandibulo-oculo-facialis) 2/99, s.131
Skotáková J, Straka M. Opakovaná stress fraktura tibie u dítěte ..... 4/00, s. 264
Smrčka V, Svenssonová M, Mařík I. Deformace kyčelního kloubu na Langobardském pohřebišti - jižní Morava ..... 4/00, s. 269

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IV. Setkání evropské skupiny pro denzitometrii v pediatrii, Praha, 25. září 1998 .....1/99, s.62
Adam M. 6. Mezinárodní konference o chemii a biologii mineralizovaných tkání, Vittel - Francie, 1.- 6. Listopadu 1998 ..... 2/99, s.146
Mařík I. 4. Seminář o podologii, Praha, 20. 2. 1999 ..... 2/99, s.157
Mařík I. Seminář Vrozené končetinové vady, 5.6. 1999 ..... 2/99, s.158
Bláha P, Šabík D. 4. mezinárodní antropologický kongres Aleše Hrdličky, Praha a Humpolec, 31.srpna - 4. září 1999, vybraná abstrakta.....3+4/99, s.264
Pavelka K. Tegzová D. Muskuloskeletální onemocnění, jejich výskyt, závažnost, léčba a prevence, Symposium "Dekáda kostí a kloubů" 25. 2. 2000, Praha ..... 1/00, s. 59

ZPRÁVY

Slovo čtenářům 1/99, s.5
Charta práv dětí v nemocnici 1/99, s.59
Zpráva o činnosti Společnosti pro výzkum a využití pojivových tkání v roce 1998 (Praha,CZ) 1/99, s.70
Zpráva o činnosti Odborné společnosti ortopedicko-protetické České lékařské společnosti J.E. Purkyně v roce 1998 ..... 1/99, s.72
Odborné služby Národní lékařské knihovny 2/99, s.159
Obsah ročníků 1998 a 1999.....3+4/99, s. 286

Slovo čtenářům ..... 1/00, s. 5
Zpráva o činnosti Společnosti pro výzkum a využití pojivových tkání (Praha, CZ) v roce 1999..... 1/00, s. 63
Zpráva o činnosti Odborné společnosti ortopedicko-protetické České lékařské společnosti J.E.Purkyně v roce 1999 ..... 1/00, s. 68
Obsah ročníků 1999 - 2000 ..... 4/00, s. 274

RECENZE

Straus J. Forenzní biomechanika (1999) .....2/99, s. 143
Smrčka V, Dylevský I. Flexory ruky. Brno, Institut pro další vzdělávání pracovníků ve zdravotnictví, 1999, 162 s. ..... 1/00, s. 69

ŽIVOTNÍ JUBILEA

Životní jubileum doc. MUDr. Ivana Hadraby, CSc. .....1/00, s. 70
Prof.MUDr. Jaroslav Blahoš, DrSc. ..... 2+3/00, s. 216
MUDr. Ivo Mařík, CSc. ..... 4/00, s. 280

CONTENTS VOL. 1999 & 2000

REVIEWS

Mazurová F, Mazura I, Mařík I. The possibilities of diagnostics of achondro-plasia and neurofibromatosis (NF-1) with the use of molecular genetic methods..... 1/99, p.6
Strouhal E. Paleopathology - history and present..... 1/99, p.11
Janíček P. The structure and delineation of bioengineering branches..... 1/99, p.16
Procházková J. Dentinogenesis imperfecta as a one symptom of osteogenesis imperfecta and an inborn errors of dentine..... 1/99, p.24
Petrtýl M. The biomechanical and biothermodynamical laws of bone tissue remodelation..... 1/99, p.28
Osteoporosis - diagnosis and treatment (a letter of head of the Society for Connective Tissue Research and Biological Use to Minister of Health)..... 1/99, p.73
Janíček P. Biomechanical problems of orthopaedics, possibilities and barriers of their solution..... 2/99, p.84
Poul J. Indication of arch supporters in childhood.....3+4/99, p.164
Kuklík M. Genetics of congenital limb defects .....3+4/99, p.168
DylevskýI. Morphogenesis of extremities.....3+4/99, p.180
Prokopec M. Major scientific problems worked on by Aleš Hrdlička ..... 1/00, p. 6
Cetta G, Tenni R, Zanaboni G, Valli M, Rossi A, Forlino A, Piazza R and Dyne KM.
Inherited connective tissue disorders
- 25 years of research experience in Pavia ..... 4/00, p. 225

MONOGRAPH

Mařík I. Systemic, limb and combined defects of the skeleton – part 1: diagnostic, therapeutical and biomechanical aspects ..... 2+3/00, p. 83

ORIGINAL PAPERS

Vukasinovic Z, Zajic L, Slavkovic S, Vukadin O, Milickovic S. The first experience with triple pelvic osteotomy in Yugoslavia..... 1/99, p.49
Sochor M, Balík K, Cabrnoch B,Vilímek M, Křena J. Composite plates for osteosynthesis of long bones - comparison of experimental and FEM results..... 2/99, p.106
Čulík J, Petrtýl M. Material characteristics of cortical bone osteons..... 2/99, p.114
Straus J. Geometric and dynamic features of child foot podogram 2/99, p.124
Mařík I, Smrčka V, Kuklík M, Hadraba I. Classification, biomechanical aspects and comprehensive treatment of congenital limb anomalies
at generalised skeletal deformities .....3+4/99, p.187
Zemková D, Mařík I. Prediction of growth and segmental shortenings of limb defects.....3+4/99, p.224
Petrtýl M, Danešová J. General Theory of Bone Remodeling.....3+4/99, p.244
Roth M. Neuroadaptive changes of seemingly primary bone affections ..... 1/00, p. 15
Hulejová H, Martínek J, Adam M.
The effect of collagen hydrolysate on some cytokines expression in the adjuvans arthritis

and on histological picture of its course ..... 1/00, p. 24
Pešáková V, Klézl Z, Čech O, Pohunková H, Adam M. Transplantation of autologous chondrocytes into cartilage defects of minipigs ..... 1/00, p. 34
Čulík J. Mechanics of the hand ..... 1/00, p. 42
Vukasinovic Z, Zivkovic Z, Zajic L. Our experience with osteoarticular tuberculosis in children ..... 4/00, p. 237
Stok K, Oloyede A. Dependence of the biomechanical responses of the trabecular boneon density and strain-rate under torsional loading ..... 4/00, p. 245
Hyánek J, Mařík I, Pejznochová H, Hyánková E, Pinkasová J, Dubská L, Cabrnochová I, Jenčová H, Štancová C, Martiníková V. Diagnostic significance of mild hyperhomocysteinemia in children suffering from bone dysplasias ..... 4/00, p. 258

CASE REPORTS

Hasegawa T, Kozlowski K, Nishimura G, Hara H, Hasegawa Y, Aso T, Koto S, Nagai T, Tsuchiya Y: Spondylo-metaphyseal Dysplasia with Regressive Platyspondyly..... 1/99, p.53
Kuklík M, Mařík I. Hallermann - Streff - Francois syndrome (Oculomandibulo - facial syndrome) 2/99, p.131
Skotáková J, Straka M. Recurrence stress fracture of the tibia in a child ..... 4/00, p. 264
Smrčka V, Svenssonová M, Mařík I. Hip joint deformation in Langobard´s burial-ground - South Moravia ..... 4/00, p. 269

CONFERENCES

XIIth European Meeting of the Paleopatho-logy Association, Prague - Pilsen, Czech Republic, August 26 - 29, 1998..... 1/99, p.60
IVth Meeting European Group for Densitometry Measurement in Paediatrics, Prague, September 25, 1998..... 1/99, p.62
Adam M. 6th International Conference about Chemistry and Biology of Mineralized Tissues, Vittel - France, 1st -6th November 1998 .....2/99, p.146
Mařík I. The 4th Conference on Podology, Prague, 20th February 1999.....2/99, p.157
Mařík I. Conference on Congenital Limb Defects, Prague, 5th June 1999 .....2/99, p.158
Bláha P, Šabík D. IVth International Congress of Aleš Hrdlička, Prague and Humpolec, Czech Republic,August 31-September 4, 1999:
Selected abstracts.....3+4/99, p.264
Pavelka K. Tegzová D. Musculoskeletal diseases, their incidence, severity, treatment and prevention. Symposium “Bone and Joint Decade”
25. 2. 2000, Prague ..... 1/00, p. 59

RECENSION

Straus J. Forensic Biomechanics (1999)..... 2/99, p.143

NEWS

A word to readers..... 1/99, p.5

The Charter of Children`s Rights in Hospital..... 1/99, p.59
Activities of the Society for Connective Tissue Research and Biological Use, (Prague, Czech Republic) in 1998..... 1/99, p.70
Activities of the Czech Society for Prosthetics and Orthotics of the Czech Medical Society J. E. Purkyně in 1998..... 1/99, p.72
Professional Services of National Medical Library..... 2/99, p.9
Contents Vol. 1998, 1999.....3+4/99, p. 286
A word to readers ..... 1/00, p. 5
Activities of the Society for Connective Tissue Research and Biological Use (Prague, Czech Republic) in 1999 ..... 1/00, p. 63
Activities of the Czech Society for Prosthetics and Orthotics of the Czech Medical Society J.E.Purkyně in 1999 ..... 1/00, p. 68
Contents Vol. 1999 and 2000 ..... 4/00, p. 277

NEW BOOKS

Smrčka V, Dylevský I. Hand flexors. Brno, Institute for postgraduate education of workers in health care, 1999, 162 p..... 1/00, p. 69

ANNIVERSARIES

Anniversary of Assoc. Prof. Ivan Hadraba, MD, PhD. ..... 1/00, p. 70
Anniversary of Jaroslav Blahoš, MD, DSc, Prof. ..... 2+3/00, p. 216
Anniversary of Ivo Mařík, MD, PhD. ..... 4/00, p. 280

MUDr. Ivo Mařík, CSc. - laudatio k 50. narozeninám

Je zvykem přiblížit oslavence čtenářům údaji životopisnými, a proto připojujeme jeho curriculum vitae.

MUDr. Ivo M a ř í k , CSc. se narodil v rodině lékaře a učitelky 6. února 1950 v Praze.

Základní škola, Praha 10, 1956 - 1965
Střední škola, Praha 10, 1965 - 1968
Maturitní zkouška květen 1968
Lékařská Fakulta KU v Praze, 1969 - 1975
Odborná kvalifikace: Lékařská Fakulta v Praze - získání diplomu - červen 1975
Atestace z pediatrie 1. st. - květen 1979
Atestace z ortopedie a traumatologie 1. st. - listopad 1982
Kandidát lékařských věd - téze "Kostní dysplazie" - květen 1987
Atestace z ortopedie 2. st. - prosinec 1987
Získání Licence ČLK pro obor ortopedie 1995, pediatrie 1995, ortopedická protetika 1997

POSTGRADUÁLNÍ PRAXE
Léčebna TRN v Janově, KÚNZ Plzeň - 6 týdnů, útvarový lékař VÚ Prostějov - 1 rok
Sekundární lékař pediatrického oddělení NsP v Berouně a obvodní pediatr -1976-1979. Obvodní pediatr OÚNZ v Praze 3 - 1979 - 1980
Sekundární lékař ortopedické kliniky 2. LF UK v Praze - 1980-82
Vědecký aspirant na ortopedické klinice 2. LF UK v Praze - 1982-86
Samostatný vědecký pracovník na ortopedické klinice 2.LF UK v Praze - 1988 - 1991
Získání vědeckého stupně II.a ČSAV - prosinec 1991
Odborný asistent ortopedické kliniky 2.LF UK v Praze 1991-1994
Zakladatel a vedoucí Ambulantního centra pro vady pohybového aparátu v Praze 3 - 1994 až dosud, 1.1.1998 afiliace ke Katedře antropologie a genetiky člověka, PřF UK v Praze
Ordinář pro dětskou ortopedii rehabilitačního oddělení NsP Kostelec n. C. l. - 1991 - 1996
Ordinář pro dětskou ortopedii ortopedického oddělení NsP Příbram - 1994 - 2000

DALŠÍ ČINNOST
- zakládající člen České společnosti chirurgie a rehabilitace ruky, 1988
- zakládající člen České společnosti pro výzkum a využití pojivových tkání, 1993 (člen výboru od 1993 -)
- zakládající člen Odborné společnosti ortopedicko-protetické České lékařské společnosti J.E. Purkyně, 1994
- zakládající člen pracovní skupiny pro osteologii a fosfokalciový metabolismus České pediatrické společnosti J.E. Purkyně, 1995 (člen výboru od roku 1997-)
- členství ve společnostech České lékařské společnosti J.E. Purkyně - Česká pediatrická. Česká revmatologická, dále v České společnosti pro ortopedii a traumatologii, České společnosti pro biomechaniku při ČSAV a v České společnosti pro úrazovou chirurgii
- zakladatel a vedoucí redaktor vědeckého časopisu

"Pohybové ústrojí - pokroky ve výzkumu, diagnostice a terapii", EMBASE / Excerpta Medica (1994 -)
- zakládající člen Maříkovy nadace pro děti s vadami pohybového ústrojí - 21. října 1992, ukončení činnosti říjen 1994
- externí člen a učitel Katedry antropologie a genetiky člověka PřF UK v Praze, obor *"Biomechanika a patobiomechanika pohybového aparátu"* (patobiomechanika kostních deformit u kostních dysplazií, končetinových a kombinovaných defektů a získaných kostních deformit -1.3. 1998.

PUBLIKACE, PŘEDNÁŠKY, MONOGRAFIE

Uveřejnil přes 70 odborných publikací v periodicích doma i v zahraničí (27 původních prací, 18 kasuistických a 21 souborných prací, 6 proceedings, 17 abstrakt bylo publikováno v časopisech). Přednášky nebo postery - okolo 170, z toho polovina na kongresech s mezinárodní účastí či v zahraničí. Abstrakta - více než 100, více než polovina z nich byla uveřejněna ve sbornících ze zahraničních kongresů a sjezdů nebo ve sbornících s mezinárodní účastí.

Napsal 2 kapitoly ve skriptech pro mediky LF (Kubát R et al. Ortopedie a traumatologie pohybového ústrojí. Skripta, Praha: SPN, 1985).

Mařík I. Kostní dysplázie. Kandidátská disertační práce, 1. a 2. svazek, Praha: FDL UK, 1986, 470 s.

Vytvořil s týmem spolupracovníků jeden výukový videofilm " Complex care for patients with osteogenesis imperfecta" (Praha: IPVZ, 1991).

Je spoluautorem monografie: Smrčka V, Dylevský I, Mařík I. Extenzory ruky. Brno: Institut pro další vzdělávání pracovníků ve zdravotnictví v Brně, 1998, 130 s.

Předložil habilitační práci: Mařík I. Systémové, končetinové a kombinované vady skeletu: diagnostické, terapeutické a biomechanické aspekty - 1. část. Monografie. In: Pohybové ústrojí, 7, 2000, 2+3, 137 s. - v tisku.

STUDIJNÍ A PŘEDNÁŠKOVÉ POBYTY v zahraničí (invited speaker)

Klinikum Der Johannes Gutenberg - Univesitat, Kinderklinik, Mainz, SRN - 9.10 - 26.10. 1989 (Head Prof. J. Spranger)

Glostrup County Hospital, orthopedic department A (head Dr. Ole Mogens Hansen), Glostrup, Denmark - 31.1. - 30. 3. 1991 - během pobytu 2 přednášky. Rigshospitalet - orthopaedic clinic (Head Dr. Steen Back Christensen), Copenhagen, Denmark - přednáška 7.3.1991.

Orthopaedic clinic Wien, Austria (Head Prof. Dr. Kotz) - 10.6.1991

Werner-Wicker-Klinik, Bad Wildungen - Reinhardshausen, Orthopaedisches Schwerpunktklinikum (Head Doz. Dr. Mätz), SRN - 18.8. - 23.8.1991

The Princess Margaret Rose Orthpaedic Hospital, Edinburgh and The Hospital for Sick Children (Head Mr. M. Malcolm), London, UK - 1.11. - 17.12. 1993

The New Children´s Hospital, Royal Alexandra Hospital for Children, Parramatta NSW 2124, Orthopaedic paediatric department (Head Dr. M. Bellemore) Sydney, Australia - 19.11. 1997 a 1.12.1999

ZÁLIBY

veslování, cyklistika, terénní běh, lyžování na běžkách
veslování - zlatá medaile, čtyřka párová (kategorie C - 43 let a výše), Fisa Masters Regatta, Adelaide 1997

UZNÁNÍ

Diploma of Fellowship of the American Biographical Institute (F.A.B.I), August 27, 1999
Honorary Member of the International Biographical Centre (IBC) Advisory Council, 1st October 1999

ORGANIZACE KONFERENCÍ A SYMPOZIÍ

1. Seminář firmy Euroortopedi AB Sweden, 20.1.1996, sál VIA na Újezdě, Praha. **2.** Seminář o podologii, 22.2.1997, sál VIA na Újezdě, Praha 1. **3.** Seminář o spondylologii, 14. 6. 1997, sál VIA na Újezdě, Praha 1. **4.** Seminář „Osteochondrodysplazie", 25.4. 1998, sál VIA na Újezdě, Praha 1. **5.** 3. Seminář o podologii, 16.5.1998, sál VIA na Újezdě, Praha 1. **6.** 4. Seminář „PODOLOGIE", 20.2.1999, sál VIA na Újezdě, Praha 1. **7.** Seminář „Vrozené končetinové vady", 5.6. 1999, sál VIA na Újezdě, Praha 1. **8.** The Symposium "Bone Dysplasias" v rámci Ivth International Conress of Aleš Hrdlička, Hotel Krystal, Praha 3. 9. 1999. **9.** 5. doškolovací podologický seminář, 19.2. 2000, sál VIA na Újezdě, Praha 1. **10.** The 1st Prague-Sydney Symposium: Kostní dysplazie diagnostika, genetické poradenství, léčení, 14.11. 2000, Katedra antropologie a genetiky člověka, PřF UK Praha.

Osobnost MUDr. Maříka a jeho odborný růst v letech 1980 - 1990 charakterizoval jeho bývalý učitel pan

profesor MUDr. Rudolf Kubát, DrSc. (emeritní přednosta ortopedické kliniky 2. LF UK a FN v Motole).

Od samého začátku působení na klinice se MUDr. Mařík věnoval vrozeným vadám pohybového ústrojí a také jeho celá vědecká práce se nesla tímto směrem. Pracoval jako samostatný vědecký pracovník pověřený specielně problematikou systémových chorob skeletu. V této problematice byl pověřen zpracováním kostních dysplazií jako téma kandidátské práce. Jeho školitelem byl i pan prof. MUDr. Stanislav Popelka, DrSc., který se této problematice na klinice věnoval v letech minulých. Kandidátskou disertační práci "Kostní dysplazie" Dr. Mařík obhájil v roce 1987. Byla většinou členů komise hodnocena jako práce přesahující rámec práce kandidátské. V rámci svých povinností se Dr. Mařík věnoval těm nejtíže postiženým dětem a jeho péče o ně byla vždy příkladná. Tyto děti, které až dosud postrádaly systematickou péči, nalezly v Dr. Maříkovi zaníceného lékaře, který se jim věnoval na úkor všech svých zálib a zcela bez jakýchkoli požadavků. Tato práce je velmi nevděčná, dlouhodobá a výsledky může mít jen ten pracovník, který se systematicky této problematice věnuje.

Od roku 1994 MUDr. Ivo Mařík, CSc. působí v Ambulantním centru pro vady pohybového aparátu v Praze 3 a zabývá se nejen vrozenými, ale i získanými ortopedickými a neuro-ortopedickými vadami a chorobami. Od ČLK získal licenci v oboru ortopedie, pediatrie a ortopedická protetika a s týmem svých spolupracovníků poskytuje komplexní léčení a péči postiženým dětem z celé České republiky. Jeho prioritou zůstala nesmírně složitá rozsáhlá problematika vrozených vad pohybového aparátu systémových, kombinovaných a končetinových, kterou řeší (ve spolupráci s klinickým a molekulárním genetikem) i biomechanický výzkum. Na tuto málo prozkoumanou oblast biomedicíny jej přivedl jeho další učitel pan doc. Ing. Zdeněk Sobotka, DrSc., s kterým publikoval významné práce v oblasti patobiomechaniky rostoucího skeletu a spolupůsobení nitrodřeňové a zevní fixace. Nové poznatky aplikuje ve své klinické praxi při konzervativním a operačním léčení (je u nás průkopníkem v nitrodřeňové fixaci používané při léčení dětí s osteogenesis imperfecta a v prolongačních operacích u disproporcionálních dětí s kostními dysplaziemi). Řeší výzkumný projekt *"Funkční adaptace a patobiomechanika končetinového a axiálního skeletu při silových účincích"* (grant GAČR č. 106/00/0006), jehož výsledky nacházejí využití při ortotickém léčení (navrhl např. končetinové ortézy s vysoko-účinným ohybovým předpětím, modifikované Beckerovy ortézy pro korekci abnormální torze bérců aj.) a jsou podkladem pro další původní práce prezentované doma i v zahraničí. Spolupracuje a podporuje i Společnost lidí malého vzrůstu PALEČEK, u jehož vzniku byl přítomen. V posledních letech se aktivně zúčastnil významných mezinárodních kongresů ve Francii, Německu, Švédsku, Rakousku a dokonce na Novém Zélandu. Významná je jeho mnohaletá spolupráce s odborníkem v diagnostice kostních vad a syndromů panem prof. Dr. K. Kozlowskim, M.R.A.C.R. ze Sydney, který se zúčastňuje diagnostických seminářů "Case Presentation", pořádaných každoročně Ambulantním centrem v Praze 3. Od roku

1998 se zásluhou Dr. Mařík vyučuje nový předmět *"Biomechanika a patobiomechanika pohybového aparátu"* na Katedře antropologie a genetiky člověka PřF UK v Praze, kde je externím členem a učitelem. Jeho široké zájmy zasahují i do genetiky klinické a molekulární a do paleopatologie.

Tělesnou kondici si pěstuje veslováním, jako veterán se zúčastňuje se svými partnery mezinárodních regat - World FISA Masters.

Jedná s vytříbeným citem pro přátelství. Jeho ušlechtilá povaha se projevuje značnou velkorysostí a neobyčejnou pracovitostí. Ve všech jeho činnostech se projevuje schopnost integrovat lidi se stejným zaměřením a nadšením pro věc a vytváří v této souvislosti ryzí přátelské vztahy. Patří do skupiny "běžců na dlouhé tratě" (dle doc. Dr. I. Mazury, CSc.) stejně jako jeho nejbližší spolupracovníci.

Rádi bychom připomněli, že MUDr. Mařík vypracoval projekt na využití bývalého plicního sanatoria v Kostelci nad Č.l., který byl opuštěn sovětskými vojsky v roce 1991. Cílem projektu bylo vybudování specializovaného pracoviště pro postižené s vadami pohybového aparátu, kde by se poskytovala komplexní péče "pod jednou střechou". Na podporu nově vzniklé kostelecké nemocnice byla 32 zřizovateli založena Nadace pro děti s vadami pohybového aparátu (Maříkova nadace). Projekt Nadace byl přijat a podpořen odbornými lékařskými společnostmi a významnými odborníky. Přesto v NsP Kostelec nad Č.l. vzniklo pouze rehabilitační oddělení pro postižené děti.

Je naší milou povinností přát jubilantovi do dalších let pevné zdraví, dostatek sil a spokojenost v kruhu rodiny, která je mu oporou v jeho bohulibé záslužné práci.

Za redakční radu:

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prof. Ing. Miroslav Petrtýl, DrSc.

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