UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE DA CRIANÇA E DO ADOLESCENTE

ESTUDO CLÍNICO E MOLECULAR EM INDIVÍDUOS COM OSTEOGÊNESE IMPERFEITA E ANÁLISE DO TRATAMENTO COM BIFOSFONADOS

TESE DE DOUTORADO

EVELISE SILVA BRIZOLA

PORTO ALEGRE, BRASIL

2015

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE DA CRIANÇA E DO ADOLESCENTE

ESTUDO CLÍNICO E MOLECULAR EM INDIVÍDUOS COM OSTEOGÊNESE IMPERFEITA E ANÁLISE DO TRATAMENTO COM BIFOSFONADOS

EVELISE SILVA BRIZOLA

ORIENTADORA: TÊMIS MARIA FÉLIX

A apresentação desta tese é um requisito do Programa de Pós-graduação em Saúde da Criança e do Adolescente da Universidade Federal do Rio Grande do Sul para a obtenção do título de doutor.

Porto Alegre, Brasil

2015

CIP - Catalogação na Publicação

Silva Brizola, Evelise
ESTUDO CLÍNICO E MOLECULAR EM INDIVÍDUOS COM OSTEOGÊNESE IMPERFEITA E ANÁLISE DO TRATAMENTO COM BIFOSFONADOS / Evelise Silva Brizola. -- 2015.
195 f.

Orientador: Têmis Maria Félix.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Saúde da Criança e do Adolescente, Porto Alegre, BR-RS, 2015.

1. Osteogênese Imperfeita. 2. Fraturas. 3. OI tipo V. 4. Biomarcadores ósseos. 5. Bifosfonados. I. Félix, Têmis Maria, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE DA CRIANÇA E DO ADOLESCENTE

ESTA TESE FOI DEFENDIDA PUBLICAMENTE EM:

04/11/2015

E, FOI AVALIADA PELA BANCA EXAMINADORA COMPOSTA POR:

Prof. Drª Marise Lazaretti Castro

Escola Paulista de Medicina

Universidade Federal de São Paulo – UNIFESP

Prof. Drª Lavínia Schüler Faccini

Programa de Pós-graduação em Saúde da Criança e do Adolescente

Universidade Federal do Rio Grande do Sul - UFRGS

Drª Liliane Todeschini de Souza

Serviço de Genética Médica

Hospital de Clínicas de Porto Alegre - HCPA

DEDICATÓRIA

Ao meu pai, **José Antônio**, que enquanto eu estive em outro país, trabalhando e aprendendo para contribuir na melhoria do tratamento e da vida de tantas pessoas, lutou pela própria vida.

À minha mãe e meus irmãos, **Vera, Paula e Lucas**, que enfrentaram essa batalha como uma família, unidos e com amor.

A vocês, que mesmo nesse momento, me encorajaram a continuar esse trabalho e seguir em frente.

Eu Amo Vocês!

AGRADECIMENTOS

Aos pacientes e familiares que participaram deste trabalho e dão sentido a todas as pesquisas e estudos desenvolvidos, sempre nos incentivando a buscar melhorias no atendimento à saúde.

O meu mais profundo agradecimento a minha orientadora **Drª. Têmis Maria Félix** pela confiança e dedicação de sempre, pelo ensinamento ao longo dos anos e pelo incentivo em todos os momentos dessa caminhada.

Ao **Dr. Jay Robert Shapiro** pela oportunidade e confiança, por me receber de portas abertas e compartilhar comigo seu conhecimento. Essa experiência foi única e com certeza "divisora de águas" na minha vida pessoal e profissional.

As minhas amigas **Luciane Schmitt, Dariene Schumanski e Renata Corá** pelo apoio e presenças física ou virtual constantes e à **Mariane Berthier** por ser essa amiga querida para todas as horas.

À minha amiga e colega **Marina Zambrano** sempre presente e pronta para tudo... discutir novos projetos, revisar análises estatísticas ou tomar um café.

Aos meus amigos e colegas do Serviço de Genética Médica em especial à **Tássia Tonon, Flávia Romariz, André dos Anjos, Ana Paula Vanz, Karina Donis** e **Bruna Pinheiro**.

Ao **Daniele Mandrioli** pelo carinho, incentivo e centenas de bons conselhos.

À **Rosane Blanguer** e à **Ceres Oliveira,** pela assistência fundamental na concretização deste trabalho.

A toda equipe do programa de doenças ósseas do Instituto Kennedy Krieger, Johns Hopkins University School of Medicine, pelos momentos e ensinamentos compartilhados.

À Universidade Federal do Rio Grande do Sul e ao Programa de Pós-graduação em Saúde da Criança e do Adolescente pela oportunidade e excelência no ensino.

Ao Governo Brasileiro, à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e ao Fundo de Incentivo à Pesquisa e Eventos (FIPE) pela oportunidade e investimento no meu aperfeiçoamento profissional e neste trabalho.

A **DEUS**, sempre.

“Estudar o fenômeno da doença sem livros é como navegar sem mapa,

mas estudar em livros sem ver pacientes é como não navegar.”

William Osler

RESUMO

A Osteogênese Imperfeita (OI) é uma doença genética do tecido conjuntivo caracterizada por fragilidade óssea e susceptibilidade à fratura sob mínimo ou nenhum trauma. O objetivo deste trabalho foi estudar características clínicas e moleculares de crianças e adultos com Osteogênese Imperfeita e analisar o efeito do tratamento medicamentoso com bifosfonados em relação aos biomarcadores metabólicos e ósseos em pacientes adultos. Esta tese se dividiu em dois capítulos onde 1) foi realizado um estudo retrospectivo sobre as características clínicas no momento do diagnóstico de OI, com ênfase nas características clínicas, especialmente em relação às fraturas ósseas; 2) avaliação clínica e análise da mutação c.-14C>T no gene *IFITM5* foi estuda em uma população com características sugestivas de OI tipo V; e 3) estudo retrospectivo em adultos com OI divididos em 2 grupos tratados com bifosfonados e não tratados. Em relação ao tratamento com bifosfonados foram avaliados os seguintes parâmetros: tipo de droga e duração do tratamento, valores de biomarcadores metabólicos e ósseos por um período de 5 anos, incidência de fraturas num de período de 5 ou 10 anos e densidade mineral óssea da coluna lombar, quadril total e colo femural no início e no final do tratamento. Nossos resultados mostraram que 1) no momento do diagnósico de OI características como escleras azuladas, dentinogênese imperfeita, ossos wormianos e fraturas de membros inferiores e superiores podem ser observadas. Pacientes com formas mais graves de OI foram diagnosticados mais precocemente quando comparados com pacientes com formas leves. Nenhuma criança com OI apresentou fraturas posteromediais das costelas, fratura de escápula ou lesões metafisárias. Essas informações associadas a história da saúde da criança são relevantes para a realização do diagnóstico diferencial. 2) OI tipo V correspondeu a 4% dos casos de OI atendidos no Centro de Referência para OI do HCPA. Indivíduos com OI V associada a mutação c.-14C> T no gene

IFITM5 apresentaram características clínicas distintas como formação de calo hiperplásico, calcificação das membranas interósseas, deslocamento da cabeça radial e deformidade de coluna, porém a expressão da doença é variável. 3) Observamos que o tratamento de adultos com OI a longo prazo não foi associado com redução na incidência das fraturas e não se refletiu de forma significativa nos níveis de biomarcadores metabólicos e ósseos, porém houve uma melhora significativa na densidade mineral óssea da coluna lombar associada à terapia. Por ser uma doença rara com prevalência variável e ampla variabilidade fenotípica e genotípica, estudos clínicos e moleculares bem como estudos sobre o efeito do tratamento medicamentoso são imprescindíveis, contribuindo no melhor entendimento da doença, aconselhamento genético acurado e propiciando melhores estratégias de prevenção e tratamento para esta população.

Palavras-chaves: Osteogênese Imperfeita, fraturas, OI tipo V, biomarcadores ósseos, bifosfonados, tratamento

ABSTRACT

Osteogenesis Imperfecta (OI) is a genetic connective tissue disease characterized by bone fragility and susceptibility to fracture under minimal or no trauma. The aim of this study was to evaluate clinical and molecular features of children and adults with OI and analyze the effect of the drug treatment with bisphosphonates in regarding to metabolic and bone biomarkers in adult patients. This thesis was divided by two chapters: 1) a retrospective study was performed where the clinical characteristic at the moment of diagnosis of OI, the clinical characteristics specially related to bone fractures was evaluated; 2) clinical evaluation and mutation analysis of c.-14C>T in the *IFITM5*gene was studied in a population with clinical charcteristics suggestive of OI type V; and 3) retrospective study in adults with OI divided in two groups treated with biphosphonates and not treated. Bisphosphonate treatment was evaluated according to the parameters: type of drug and duration of treatment, metabolic and bone biomarkers values for a period of 5 years, incidence of fractures in a period of 5 or 10 years and bone mineral density of the lumbar spine, total hip and femoral neck at baseline and at the end of treatment. Our results showed that 1) at the time of OI diagnosis features such as bluish slerae, dentinogenis imperfecta, wormian bones, and fractures of upper and lower limbs can be observed. Patients with more severe forms of OI were diagnosed earlier when compared with patients with mild forms. No OI children presented posteromedial fractures of the ribs, scapula fracture or metaphyseal lesions. This information associated with the child's health history are relevant for carrying out the differential diagnosis. This information is relevant for carrying out the differential diagnosis. 2) OI type V corresponds to 4% of OI cases at the Reference Center for OI at HCPA. Subjects with OI V associated to the mutation c.-14C> T in the *IFITM5* gene presented distinctives clinical features as hyperplastic callus formation, calcification of interosseous membranes,

dislocation of the radial head and spinal deformity, but the expression of the disease is variable. 3) We observed that long-term treatment with bisphosphonates (BP) for adults with OI was not associated with reduced incidence of fractures and was not reflected significantly in the levels of metabolic and bone biomarkers, but there was a significant improvement in bone mineral density of the lumbar spine associated to the therapy. Because it is a rare disease with a prevalence variable and wide phenotypic and genotypic variability, clinical and molecular studies and studies of the effect of drug treatment are essential, contributing to the better understanding of the disease, accurate genetic counseling and providing better strategies for prevention and treatment for this population.

Keywords: Osteogenesis Imperfecta, fractures, OI type V, bone biomarkers, bisphosphonates, treatment

LISTA DE ILUSTRAÇÕES

ARTIGO 1

Versão em Inglês

Figure 1. *Age at time of diagnosis according to OI type* ……..…..……...**63**

Figure 2. *Female patient with OI-4. Radiographic exam of thoracolumbar spine showed fracture of multiple vertebral bodies* ….....……...**64**

Versão em Português

Figura 1. Idade no momento do diagnóstico de acordo com o tipo de OI.....**80**

Figura 2. Paciente do sexo feminino com OI-4. O exame radiográfico da coluna vertebral tóraco-lombar mostrou fratura de múltiplos corpos vertebrais.....**81**

ARTIGO 2

Versão em Inglês

Figure 1. *Sanger sequencing showing c.–14C>T mutation in the IFITM5 gene*……**104**

Figure 2. *Patients and radiological findings*.....**105**

LISTA DE TABELAS

REVISÃO DA LITERATURA

Tabela 1. Classificação de OI de acordo com o gene responsável.....**27**

ARTIGO 1

Versão em Inglês

Table 1. *Clinical features at the time of diagnosis by OI type*……...…….....**65**

Versão em Português

Tabela 1. Características clínicas no momento do diagnóstico por tipo de OI.....**82**

ARTIGO 2

Versão em Inglês

Table 1. *Clinical and radiographic characteristics of patients with OI-V (mutation c.–14C>T in the IFITM5 gene) and a comparison to the literature*……**106**

ARTIGO 3

Versão em Inglês

Table 1. *Comparison of bone and metabolic biomarkers: A) after 5 years of follow-up between non-treated and BP-treated groups B) between periods under treatment versus after 5 years of treatment interruption for BP-treated group*……**125**

Table 2. *Fracture rate in BP treated group comparing intervals of 5 or 10 years pre- and post-treatment with BP started* ……...**126**

Table 3. *Comparison of BMD pre- and post-treatment for BP treated group and between BP treated and non-treated* groups……...…**127**

Versão em Português

Tabela 1. Comparação de biomarcadores metabólicos e ósseos: A) após 5 anos de observação entre os grupos não-tratado e BP-tratado; B) durante o tratamento com BP e após 5 anos de interrupção no grupo BP-tratado.....**144**

Tabela 2. Taxa de Fratura no grupo BP-tratado comparando intervalos de 5 ou 10 anos antes a após o início do tratamento com BP.....**145**

Tabela 3. Comparação da DMO pré e pós-tratamento para o grupo tratado com BP e entre os grupos BP-tratado e não-tratado.....**146**

LISTA DE SIGLAS E ABREVIATURAS

BMB	*Bone and metabolic biomarkers*
BMD	*Bone mineral density*
BP(s)	Bifosfonado (s) – *bisphosphonate (s)*
CIM	*Calcification of interosseous membrane*
CPA	*Child physical abuse*
CROI	Centro de Referência em Osteogênese Imperfeita
CTX	C-telopeptideo
DHR	*Dislocation of radial head*
DI	Dentinogênese imperfeita - *dentinogenesis imperfecta*
DMO	Densidade mineral óssea
DXA	*Dual Energy X-ray Absorptiometry*
HPC	*Hyperplastic callus*
INCDS	*International Nomenclature Group for Constitutional Disorders of the Skeleton*
IQR	*Interquartile range*
IV	*Intravenous*
LS-aBMD	*Lumbar spine areal BMD*
MSCs	*Mesenchymal stem cells*
MTF	Maus-tratos físicos infantis
NTX	N-telopeptideo
OI	Osteogênese Imperfeita
OMIM	*Online Mendelian Inheritance in Man database*
OPG	*Osteoprotegerin*

PEDF	*Pigment Epithelium Derived Factor*
RX	Raio-x
Scl-Ab	*Sclerostin antibody*

SUMÁRIO

1. INTRODUÇÃO **20**
2. REVISÃO DA LITERATURA **23**
2.1. Osteogênese Imperfeita..... 23
2.2. Epidemiologia da OI..... 25
2.3. Classificação da OI..... 25
2.4. Genética da OI..... 28
2.5.Metabolismo Ósseo e Biomarcadores 29
2.6. Fragilidade Óssea e Fraturas..... 32
2.7. Tratamento da OI..... 33
2.7.1. Tratamento Clínico e Cirúrgico …..... 33
2.7.2. Tratamento Medicamentoso..... 35
3. JUSTIFICATIVA..... **38**
4. OBJETIVOS **38**
4.1. Objetivos Gerais..... 39
4.2. Objetivos Específicos..... 40
5. REFERÊNCIAS BIBLIOGRÁFICAS **40**
6. CAPÍTULO I. CARACTERIZAÇÃO CLÍNICA E MOLECULAR **50**
6.1. Metodologia I..... **51**
6.1.1. Delineamento..... 51
6.1.2. Amostra..... 51
6.1.3. Dados Clínicos..... 51
6.1.4. Aspectos Éticos..... 52
6.2. Análise Estatística..... 52
6.3. Resultados..... 52

6.4. Metodologia II **83**

6.4.1. Delineamento.....83

6.4.2. Amostra.....83

6.4.3. Dados Clínicos.....83

6.4.4. Análise Molecular.....84

6.4.5. Aspectos Éticos..... 84

6.5. Resultados.....85

7. CAPÍTULO II. TRATAMENTO COM BIFOSFONADOS **107**

7.1. Metodologia III..... 108

7.1.1. Delineamento.....108

7.1.2. Amostra.....108

7.1.3. Dados Clínicos.....109

7.1.3.1. Biomarcadores Metabólicos e Ósseos.....109

7.1.3.2. Incidência de Fraturas 109

7.1.3.3. Densidade Mineral Óssea.....110

7.1.4. Aspectos Éticos.....110

7.1.5. Análise Estatística 111

7.2. Resultados.....111

8. CONSIDERAÇÕES FINAIS.....**147**

9. PUBLICAÇÕES ADICIONAIS.....**149**

9.1. Artigo de Revisão…….…….....149

9.2. Editorial…….....……..…….186

10. ANEXOS 194

10.1. Termo de Consentimento Livre e Esclarecido..... 194

1. INTRODUÇÃO

A Osteogênese Imperfeita (OI) é uma rara doença sistêmica que afeta os tecidos ósseo e conjuntivo. A incidência da OI nas populações mundiais é de 6-7/100.000 nascimentos (Van Dijk et al., 2011; Van Dijk & Sillence, 2014). Na América Latina a OI corresponde a 33% das Osteocondrodisplasias (Barbosa-Bruck et al., 2012), no entanto, não existem dados epidemiológicos brasileiros oficiais em relação à doença. A apresentação clínica da doença é amplamente variável, bem como a gravidade. Indivíduos com OI apresentam fragilidade óssea e alto risco de fraturas que ocorrem espontaneamente ou sob mínimo trauma. Diversas características compõem o quadro clínico da doença como escleras azuladas ou acinzentadas, dentinogênese imperfeita, hiperextensibilidade articular, baixa estatura, perda auditiva progressiva, deformidades de coluna, complicações cardíacas e oculares, entre outras (Martin & Shapiro, 2007; Basel & Steiner, 2009; Van Dijk & Sillence, 2014).

A grande maioria dos casos são causados por mutações nos genes *COL1A1* e *COL1A2,* genes codificadores das cadeias pró-colágenas α1 e α2 afetando a síntese normal do colágeno tipo 1 (Ben Amor et al., 2011; Marini et al., 2014). No entanto, com o avanço da tecnologia e dos estudos moleculares na última década, diversos outros genes foram descobertos também como causadores da doença. A OI pode resultar de herança autossômica dominante ou recessiva, e ainda há um novo tipo descrito com padrão de herança ligado ao X (Marini et al., 2014; Van Dijk & Sillence, 2014).

Em 1979, baseados na gravidade clínica, características radiológicas e padrão de herança, Sillence e colegas categorizaram quatro fenótipos de OI: leve - OI tipo I; grave com letalidade perinatal - OI tipo II; gravemente deformante - OI tipo III e moderadamente deformante - OI tipo IV (Sillence et al., 1979). A classificação de Sillence para OI é utilizada

mundialmente e considerada a forma clássica para subdividir a doença de acordo com a gravidade clínica. Porém, com a descoberta dos outros genes, alguns com características clínico-radiológicas distintas e outros com sobreposição fenotípica aos quatros tipos prévios, uma revisão da classificação original de Sillence foi realizada (Warman et al., 2011). Até o momento, outros 17 genes relacionados a doença e classificados entre OI tipos I à XVII estão descritos no banco de dados online para doenças mendelianas "*Online Mendelian Inheritance in Man database* (OMIM)" (OMIM, 2015).

Contudo, a doença ainda é diagnosticada clinicamente com base em critérios clínicos e radiológicos e história familiar. Fraturas ou sinais clínicos isolados não são suficientes para confirmação do diagnóstico, uma vez que diversas outras doenças osteometabólicas, esqueléticas ou doenças que levam a osteoporose secundária podem mimetizar casos de OI. Do mesmo modo, casos de suspeita de maus-tratos infantis devem ser cuidadosamente avaliados, uma vez que são frequentes e a criança também pode apresentar diversas fraturas (Glorieux, 2008; Harrington et al., 2014).

Ainda não existe cura para a OI e o tratamento é baseado no manejo medicamentoso, cuidados ortopédicos, reabilitação e nutrição (Glorieux, 2008; Van Dijk & Sillence, 2014). O tratamento medicamentoso com bifosfonados nitrogenados tem sido a opção de preferência tanto para crianças quanto para adultos com OI. Indivíduos com formas moderadas a graves ou com formas leves que apresentam fraturas frequentes têm se beneficiado com o uso dessa terapia por mais de vinte anos (Glorieux, 2008; Shapiro & Sponseller, 2009; Hoyer-Kuhn et al., 2015).

Administrados por via oral ou intravenosa, os benefícios a curto e médio prazos têm sido extensamente descritos na literatura para esta população, mesmo com diferenças entre protocolos de dosagem, duração e tempo de administração (Bachrach & Ward, 2009; Russel,

2015). Aumento da densidade óssea, diminuição da incidência de fraturas, melhora da mobilidade e da dor óssea são alguns dos diversos efeitos positivos associados ao tratamento com bifosfonados. A resposta ao tratamento difere entre crianças e adultos, uma vez que após a puberdade há uma diminuição do remodelamento ósseo limitando a eficácia da droga que possui forte afinidade por osso mineral, principalmente em locais de elevado *turnover* ósseo (Martin & Shapiro, 2007; Hoyer-Kuhn et al., 2015; Russel, 2015). No entanto, após anos de amplo uso destes fármacos nessa população, diversas questões quanto a segurança e eficácia estão em discussão como duração do tratamento, efeito a longo prazo e efeitos adversos (Dwan et al, 2014; Rijks et al., 2015; Hald et al., 2015).

O tratamento ortopédico busca otimizar a funcionalidade e independência do indivíduo com OI. A escolha entre tratamento conservador ou cirúrgico é dependente de cada caso especificamente, podendo ser requeridos após fraturas, casos de não-união crônica de fraturas, deformidades de ossos longos graves e incapacitantes e deformidades de coluna que comprometam a mobilidade e a função pulmonar (Shapiro & Sponseller, 2009; Nicolaou et al., 2011; Hoyer-Kuhn et al., 2015).

A reabilitação é fundamental para indivíduos com OI, uma vez que a doença afeta o sistema musculoesquelético levando a fraturas, hiperextensibilidade articular, fraqueza muscular, diminuição do tônus muscular e dor musculoesquelética prejudicando a funcionalidade e a qualidade de vida do indivíduo (Engelbert et al., 1998; Glorieux, 2008; Hoyer-Kuhn et al., 2015). Conjuntamente, crianças com OI podem apresentar atraso na aquisição de marcos motores incluindo alteração na marcha e em diversas outras atividades anti-gravitacionais (Engelbert et al., 1998; Brizola et al., 2014). A orientação nutricional propicia o consumo de uma dieta saudável, adequada para a idade e com ingesta adequada de cálcio e vitamina D (Chagas et al., 2012).

Pesquisas nas áreas clínica e de biologia molecular expandiram rapidamente nos últimos anos. Diversas outras abordagens terapêuticas estão em investigação, incluindo novas drogas (Harrington et al., 2015), diferentes técnicas cirúrgicas (Puvanesarajah et al., 2015) e transplante de células troncos mesenquimais (Westgren & Götherström, 2015). Além disso, estudos para desenvolvimento de novos agentes farmacogenéticos estão em foco (Marini & Smith, 2015), bem como estudos na área de engenharia genética e terapia gênica (Niyibizi & Li, 2009; Tarnowski et al., 2010; Evans, 2012; Marini & Smith, 2015).

2. REVISÃO DA LITERATURA

2.1. Osteogênese Imperfeita

A Osteogênese Imperfeita (OI) é uma doença genética do tecido conjuntivo caracterizada por fragilidade óssea e susceptibilidade a fraturas sob mínimo ou nenhum trauma. É considerada a osteocondrodisplasia letal mais comum (Basel & Steiner, 2009; Van Dijk & Sillence, 2014) e corresponde a 33% das osteocondrodisplasias na América Latina (Barbosa-Bruck et al., 2012). A OI afeta todos os tecidos que contém colágeno em sua quantidade e/ou qualidade incluindo ossos, pele, tendões, dentina, tecidos conectivos, pulmões, escleras e córneas. O colágeno tipo 1 corresponde a quase 90% do colágeno total do organismo e mutações afetando esta proteína causam diversas doenças associadas à fragilidade óssea (Marini et al., 2007; Nassa, 2012).

Além da fragilidade óssea e das fraturas de repetição, outros sinais compõem o quadro clínico da doença como diminuição generalizada da massa óssea (osteopenia ou

osteoporose), baixa estatura, escleras azuladas ou acinzentadas, dentinogênese imperfeita, hiperextensibilidade articular, hipoacusia, presença de ossos wormianos, deformidades ósseas, complicações oculares como ruptura espontânea escleral e comprometimento cardíaco valvular e vascular (Ben Amor et al., 2011; Shapiro & Germain-lee, 2012; Soma et al., 2012; Van Dijk & Sillence, 2014; Marini & Smith, 2015). Deformidades da coluna vertebral como escoliose, cifose, espondilolistese, invaginação basilar na junção crânio-cervical e ainda colapso vertebral também podem ser observados tanto em crianças quanto em adultos com OI (Hatz et al, 2011; Anissipour et al, 2014).

Com o avanço nas pesquisas moleculares diversos novos genes foram descobertos relacionados à OI, porém o diagnóstico ainda é realizado com base em critérios clínicos e radiológicos e história familiar (Warman et al., 2011). A história das fraturas associada aos sinais clínicos e à história familiar positiva para a doença são usualmente suficientes para concluir o diagnóstico. Através da ultrassonografia pré-natal formas moderada a graves da doença podem ser diagnosticadas ainda intraútero a partir do segundo semestre gestacional pela detecção de baixa ecogenicidade do esqueleto, forma anormal do crânio e dos arcos costais, deformidades de ossos longos, fraturas plenas, fraturas em fase de consolidação com calo ósseo, micromelia, ventriculomegalia e atraso do crescimento fetal (Ulla et al., 2011; Krakow, 2013).

No entanto, outras causas de fragilidade óssea e fraturas patológicas devem ser excluídas, dentre as quais deficiências nutricionais, neoplasias, osteoporose idiopática juvenil, doença celíaca, síndrome de Ehlers-Danlos, hipofosfatasia e ainda outras osteocondrodisplasias (Santili et al., 2005; Basel & Steiner, 2009; Harrington et al., 2014). Casos suspeitos de maus-tratos infantis também devem ser cuidadosamente investigados, uma vez que a criança também pode apresentar múltiplas fraturas e diminuição da massa óssea secundária a deficiências nutricionais (Harrington et al., 2014).

2.2. Epidemiologia

A incidência de OI nas populações mundiais é variável. A prevalência geral é de de 1: 15.000 à 1: 20.000 nascimentos, sem predisposição de etnia ou sexo (Roughley et al., 2003; Van Dijk & Sillence, 2014). Não existem dados epidemiológicos oficiais para a população brasileira, porém de acordo com a Associação Brasileira de Osteogênese Imperfeita existem mais de 12.000 brasileiros com a doença (ABOI, 2012). Nos Estados Unidos estima-se que existam aproximadamente 25.000 a 50.000 indivíduos com OI (Martin & Shapiro, 2007) com uma frequência de nascimentos na ordem de 1: 30.000 nascidos vivos para OI tipo I (Shapiro & Germain-Lee, 2012). Na Dinamarca, um estudo epidemiólogico indicou uma uma prevalência de OI de 21,8: 100.000 nascimentos (Martin & Shapiro, 2007).

2.3. Classificação da OI

Devido a grande variabilidade fenótipica da OI, Sillence e colegas em 1979 delinearam quatro fenótipos distintos de OI com base em critérios clínicos, radiológicos e padrão de herança. Os quatro fenótipos foram classicados como OI tipos I, II, III e IV. A classificação de Sillence é considerada clássica e ainda é aplicada mundialmente dividindo a OI em formas leve - OI tipo I (OI-I), letal - OI tipo II (OI-II), gravemente deformante - OI tipo III (OI-III) e progressivamente deformante - OI tipo IV (OI tipo IV) (Sillence et al., 1979).

No entanto, com a descoberta recente de diversos novos genes a classificação de OI tornou-se confusa, uma vez que a maioria desses casos se sobrepõem fenotipicamente aos tipos clássicos de Sillence. Van Dijk e colegas (2010) propuseram a utilização da classificação de Sillence revisada incluindo os tipos I ao VI de OI, mas excluindo os tipos VII e VIII que são causados por mutações nos genes C*RTAP* e *LEPRE1*, respectivamente,

mas são indistinguíveis clínicamente dos tipos II e IV (Van Dijk et al., 2010). Uma classificação baseada no genótipo foi proposta por Forlino e colaboradores (2011), mantendo os tipos I a IV de Sillence e incluindo um novo tipo para cada novo gene descrito como causador da doença (Forlino et al., 2011).

Contudo, em 2010, o Grupo Internacional de Nomenclatura para Transtornos Constitucionais do Esqueleto optou pela exclusão de referências moleculares diretas e propôs a expansão da classificação de Sillence com a inclusão da OI tipo V, mantendo a classificação baseada nos sinais clínicos e radiológicos da gravidade da doença e agrupando os novos genes descobertos de acordo com os 5 fenótipos distinguíveis entre si previamente descritos (Warman et al., 2011).

Contudo, na prática clínica a classificação expandida de Sillence (OI tipos I ao V) segue sendo amplamente utilizada, porém uma classificação baseada no genótipo está disponível no banco de dados de doenças mendelianas (*Online Mendelian Inheritance in Man database* - OMIM) dividindo a OI em 17 tipos classificados como OI tipos I à XII (**tabela 1**) (Van Dijk et al., 2010; Van Dijk & Sillence, 2014; OMIM, 2015).

Tabela 1. Classificação de OI de acordo com o gene responsável

Tipo de OI	Gene	Fenótipo	Herança
I	*COL1A1; COL1A2*	leve	AD
II	*COL1A1; COL1A2*	letal	AD; AR
III	*COL1A1; COL1A2*	deformidade progressiva	AD; AR
IV	*COL1A1; COL1A2*	moderado	AD
V	*IFITM5*	moderado, com calo hipertrófico e ossificação da membrana interóssea	AD
VI	*SERPINF1*	moderado a grave	AR
VII	*CRTAP*	grave a letal	AR
VIII	*LEPRE1*	grave a letal	AR
IX	*PPIB*	grave a letal	AR
X	*SERPINH1*	grave	AR
XI	*FKBP10*	deformidade progressiva com contraturas (Síndrome de Bruck)	AR
XII	*SP7*	moderado	AR
XIII	*BMP1*	grave	AR
XIV	*TMEM38B*	gravidade variável	AR
XV	*WNT1*	gravidade variável	AR
XVI	*CREB3L1*	grave	AR
XVII	*SPARC*	grave	AR
Não classificados			
	PLOD2	grave com contraturas articulares congênitas (Síndrome de Bruck)	AR
	P4HB	grave (Síndrome Cole-Carpenter-1)	AR
	SEC24D	grave (Síndrome Cole-Carpenter-2)	AR
	PLS3	leve	Ligado ao X

AD: autossômico dominante; AR: autossômico recessivo; Adaptado de Van Dijk & Sillence, 2014

2.4. Genética da OI

A grande maioria (85-90%) dos casos de OI são causados por mutações nos genes *COL1A1* e *COL1A2* correspondendo aos tipos de OI I, II, III e IV. *COL1A1* e *COL1A2* são genes codificadores das cadeias procolágenas α 1 e α 2. Os procolágenos são transportados para dentro do retículo endoplasmático onde são montados em tripla hélice contendo duas cadeias de procolágeno α 1 e uma cadeia α 2. O mecanismo de montagem do procolágeno se inicia na região C-terminal após a síntese completa da proteína, diferentemente da maioria das outras proteínas cuja montagem inicia a partir da região N-terminal durante o processo de tradução. Cada cadeia α contém 338 aminoácidos repetitivos (Gly-Xaa-Yaa). Os resíduos X e Y são em geral constituídos por prolina e hidroxiprolina, que correspondem a 25% dos aminoácidos presentes no colágeno. Durante a síntese de colágeno, cada cadeia α sofre modificações pós-tradução que são essenciais para a formação da hélice e estabilidade da molécula (Marini et al., 2010; Makareeva et al., 2011). Mais de 800 mutações já foram descritas relacionadas à OI no *Human Collagen Mutation Database* (http://www.le.ac.uk/genetics/collagen) levando a alterações quantitativas e qualitativas do colágeno tipo 1. Alterações quantitativas causam a OI formas leve a moderada, enquanto alterações qualitativas, OI formas grave ou letal. Em torno de 75-80% dos defeitos estruturais do colágeno são causados por mutações que resultam na substituição da glicina por outro aminoácido. O padrão de herança nestes casos é autossômico dominante (Makareeva et al., 2011;Van Dijk & Sillence, 2014).

Em torno de 4-5% dos casos de OI são causados por uma única mutação (c.-14C>T) na região 5’ UTR do gene *IFITM5* levando à um fenótipo específico classificado como OI tipo V. Esta mutação c.-14C>T cria um novo sítio de iniciação levando a adição de 5 aminoácidos na porção N terminal da proteína (Cho et al., 2012; Semler et al., 2012). A OI tipo V é uma forma moderada de OI herdada de forma autossômica dominante e com grande

variabilidade na apresentação clínica, porém com caracterísiticas clínicas e radiológicas distintas como ausência de dentinogênese imperfeita, calcificação da membrana interóssea entre os ossos dos antebraços (rádio-ulna) e das pernas (tíbia-fibula), deslocamento da cabeça radial e formação de calo hiperplásico após fratura ou osteotomia (Shapiro et al., 2013; Rauch et al., 2013).

Os demais 5-10% dos casos apresentam padrão de herança autossômico recessivo e são causados por mutações nos genes *SERPINF1, CRTAP, LEPRE1, PPIB, SERPINH1, FKBP10, SP7, BMP1, TMEM38B, WNT1, CREB3L1 e SPARC* correspondendo aos tipos VI à XII de OI, respectivamente. Esses novos tipos de OI apresentam gravidade moderada a letal sendo alguns indistinguíveis dos fenótipos descritos por Sillence (Van Dijk et al., 2014; Marini et al., 2015; OMIM, 2015). Mutações nos genes *PLOD2, P4HB e SEC24D* herdadas de forma autossômica recessiva também foram descritas como causadoras da doença, porém esses genes ainda não foram oficialmente classificados como um tipo específico de OI. O gene *PLS3* é o único gene relacionado à OI que apresenta herança ligada ao cromossomo X e foi descrito em individuos com ocorrência precoce de fratura associada a diminuição da densidade mineral óssea (DMO) (Van Dijk et al., 2013).

2.5. Metabolismo Ósseo e Biomarcadores

A formação óssea inicia na fase intraútero e segue pela adolescência até a vida adulta. Mais de 90% da massa óssea é adquirida no período entre a infância e a adolescência, sendo estes os únicos períodos de crescimento físico longitudinal. Nessa fase observa-se altos índices de mineralização da matriz óssea sendo 25% da massa óssea incorporada nos 2 anos que circundam o pico máximo de velocidade da estatura (Jones & Boon, 2008; Silva et al., 2011).

O desenvolvimento esquelético acontece em um ritmo próprio; embora haja um padrão similar para mudanças no tamanho, geometria e aquisição mineral dos ossos, esse padrão não é o mesmo para crianças e adolescentes (Bachrach & Ward, 2009; Jones & Boon, 2008). A resistência a forças externas ou a fraturas depende da quantidade e da qualidade do tecido ósseo; e a qualidade é dependente da forma dos ossos, da microarquitetura trabecular e cortical e das remodelações, tanto minerais quanto do colágeno. O processo de remodelamento ósseo baseia-se em dois processos antagônicos: a formação e a reabsorção ósseas, consequentemente, a modelação e a remodelação ósseas (Bachrach & Ward, 2009).

Diversos fatores locais e sistêmicos que atuam sobre células osteoprogenitoras, osteoblastos e osteócitos estão envolvidos na regulação da formação óssea; já a atividade dos osteoclastos promove a reabsorção do tecido ósseo (Wu et al., 2008). A manutenção e a qualidade da massa óssea são dependentes do equilíbrio homeostático entre a formação e a reabsorção ósseas (Baron, 2003). Dessa forma, alterações ou desequilíbrios metabólicos, fisiológicos ou patológicos podem afetar a estrutura esquelética.

Todo o interesse acerca da avaliação dinâmica e acurada do tecido ósseo ocorre devido as mudanças vistas ao longo dos períodos de vida. Sabe-se que a puberdade é um período sensível para o incremento das reservas ósseas e para a redução de futuras perdas, porém a partir dos 30 anos ocorre uma diminuição fisiológica da massa óssea de 1 a 2% nas mulheres e de 0,3 a 1% nos homens. A massa óssea é maior nos homens do que nas mulheres, pois eles possuem esqueletos maiores; além disso, o período de perda óssea se inicia mais tardiamente nos homens do que nas mulheres, cerca de uma década depois (Lacativa e Farias, 2006; Silva et al., 2011). Diferentes técnicas para avaliação do esqueleto estão disponíveis incluindo histomorfometria, densitometria e marcadores bioquímicos.

A utilização de biomarcadores do metabolismo ósseo como um método dinâmico de avaliação do *turnover* ósseo tem sido sugerido na literatura (Silva et al, 2011; Wekre et al., 2011; Biver, 2012). A importância da avaliação clínica dos biomarcadores deve-se a sua rápida produção durante a remodelação óssea, em comparação com avaliações da DMO por métodos tradicionais. A atividade dos biomarcadores representa os processos de formação e reabsorção ósseas. Osteocalcina intacta, total e fragmento, fosfatase alcalina total e óssea, peptídeo carboxi-terminal do procolágeno I (PICP) e peptídeo amino-terminal do procolágeno pró-peptídeo do colágeno tipo I (PINP) são marcadores do processo de formação óssea. Cálcio urinário, fosfatase ácida tartarato-resistente, hidroxiprolina urinária, piridinolina, deoxipiridinolina, telopeptídeos aminoterminais (NTX) e carboxiterminais (CTX) do colágeno tipo I são alguns dos marcadores do processo de reabsorção óssea (Saraiva & Lazaretti-Castro, 2002; Hlaing & Compston, 2014).

Diversos fatores influenciam a atividade dos biomarcadores gerando flutuações nos valores como ritmo circadiano, idade, período menstrual, gravidez, fratura recente, metabolismo do indivíduo, imobilidade prolongada, desnutrição, artrite reumatóide, neoplasias e uso de algumas drogas incluindo anticonvulsivantes e corticóides (Saraiva & Lazaretti-Castro, 2002; Hlaing & Compston, 2014).

A literatura científica tem sugerido o uso dos biomarcadores associado ao exame de densitometria óssea, especialmente em casos de pacientes com osteoporose, sendo esta uma das principais causas de fraturas por fragilidade (Silva et al., 2011; Wreke et al., 2011). O exame de densitometria óssea mede estaticamente a DMO, porém a avaliação dos biomarcadores reflete dinamicamente o processo de remodelação óssea no momento do exame (Saraiva & Lazaretti-Castro, 2002). Os biomarcadores ósseos são uma importante ferramenta na avaliação de pacientes com baixa massa mineral óssea e no acompanhamento dos efeitos dos medicamentos utilizados para o tratamento (Wreke et al., 2011).

2.6. Fragilidade Óssea e Fraturas

A fragilidade óssea característica da OI leva ao risco aumentado de ocorrência de fraturas ao longo da vida dos pacientes. A maioria das fraturas ocorre ainda na infância e após a puberdade existe uma tendência a diminuição da incidência dessas fraturas devido ao declínio fisiológico do *turnover* ósseo (Shapiro & Sponseller, 2009; Harrington et al., 2014).

Mediante a ocorrência de uma fratura, uma rápida formação de novos osteoblastos a partir das células progenitoras é iniciada levando ao aumento do tecido osteoblástico e da nova matriz orgânica entre as extremidades da fratura, culminando na formação do calo ósseo no local fraturado (Karsenty, 2003; Wu et al., 2008). O osso é composto por material adaptativo e encontra-se em constante troca de propriedades devido a estímulos intrínsecos e extrínsecos ao seu funcionamento como influência hormonal, terapia medicamentosa e cargas externas (Webber, 2009). O objetivo do tratamento da fratura é propiciar a consolidação restaurando a função biomecânica do osso e funcionalidade do membro (Staheli, 2008; Watzl et al., 2009).

As fraturas de ossos longos, principalmente em membros inferiores, são as mais frequentes em pacientes com OI. Em crianças, as fraturas isoladas dos ossos longos podem ser tratadas de forma conservadora ou cirúrgica dependendo da gravidade (Shapiro & Sponseller, 2009; Lin et al., 2013). Quando as fraturas são tratadas cirurgicamente os danos secundários ao período de restrição da mobilidade devem ser considerados incluindo a piora da osteopenia aumentando o risco de novas fraturas, fraqueza muscular e perda de amplitude de movimento (Santili et al., 2005; Watzl et al., 2009; Shapiro & Sponseller, 2009).

Colapso vertebral ou fratura vertebral por compressão é a forma mais comum de fratura na coluna em indivíduos com OI e a ocorrência de múltiplas fraturas vertebrais é frequentemente observada, principalmente, nas regiões torácica e lombar (Diacinti et al,

2015). Este tipo de fratura pode ocorrer em todos os tipos de OI, porém o número de fraturas e a gravidade da deformidade são dependentes do tipo de OI (Sposito & Arlet, 2013). Fraturas vertebrais secundárias a deformidade da vértebra e lesão da placa de crescimento são consideradas uma das maiores causas de escoliose em indivíduos com OI (Anissipour et al., 2014). A hiperextensibilidade do tecido conectivo característico da OI afeta ligamentos, tendões e músculos alterando a estrutura dinâmica de sustentação da coluna também influenciando no desenvolvimento de escoliose (Staheli, 2008; Sposito & Arlet, 2013). A ocorrência de vértebras colapsadas em múltiplos níveis é mais comum em pacientes com os tipos mais graves de OI. Em geral, fraturas vertebrais múltiplas em indivíduos com a forma leve de OI ocorrem quando há um trauma significativo (Sposito & Arlet, 2013).

2.7. Tratamento da OI

2.7.1. *Tratamento Clínico e Cirúrgico*

O tratamento da OI é dependente do grau de gravidade da doença, da capacidade funcional e da idade do indivíduo (Rauch & Glorieux, 2004; Monti et al., 2010). O tratamento medicamentoso associado à atuação de uma equipe multidisciplinar tem sido valioso para o melhor prognóstico da doença, visando melhorar a qualidade óssea e propiciar a máxima independência funcional desses pacientes (Engelbert et al., 1998; Zeitlin et al., 2003; Glorieux, 2008; Shapiro & Germain-lee, 2012).

Para os indivíduos com OI a fisioterapia, a reabilitação e a cirurgia ortopédica são considerados os pilares do tratamento. Exercícios regulares de baixo impacto, como caminhadas e natação, propiciam fortalecimento muscular, estabilidade articular e potencializam o crescimento longitudinal em crianças (Monti et al., 2010). Tratamento cirúrgico ortopédico pode ser necessário em casos de fraturas de repetição, importante

deformidade óssea levando à prejuízo nas habilidades motoras, não-união crônica de fraturas, escoliose grave e invaginação basilar (Basel & Steiner, 2009; Lin et al., 2013; Lee et al., 2015).

Aproximadamente 20% dos pacientes com OI apresentam não-união crônica de fraturas, também conhecidas como "pseudoartrose", sendo requerido tratamento cirúrgico. Embora o reparo dessas pseudoartroses seja difícil devido a pobre qualidade óssea, novas técnica cirúrgicas tem demonstrado sucesso na promoção da consolidação da fratura com recuperação da funcionalidade do membro (Puvanesarajah et al., 2015)

O uso de hastes intramedulares fixas ou extensíveis tem sido o tratamento de escolha em casos de deformidades graves de ossos longos ou fraturas importantes propiciando maior suporte e resistência aos ossos (Sofield & Millar, 1959; Santili et al., 2005; Watzl et al., 2009). As hastes extensíveis são indicadas para crianças onde há uma expectativa de crescimento ósseo maior que três centímetros, uma vez que este tipo de haste acompanha o crescimento do osso e assim a necessidade de reintervenções cirúrgicas é reduzida. Já as hastes fixas ou não extensíveis são indicadas para pacientes já na fase de maturidade esquelética ou com expectativa de crescimento ósseo menor que três centímetros, considerando o menor risco de recidiva da deformidade ou de fratura nesses casos (Santili et al., 2005; Staheli, 2008; Watzl et al., 2009).

A terapia nutricional propicia uma ingesta adequada de cálcio e de alimentos ricos em vitamina D oriundos de uma alimentação balanceada. A ingestão regular de cálcio durante a infância e a adolescência é essencial na manutenção da massa óssea e a vitamina D desempenha um papel essencial na homeostase do cálcio e na saúde óssea (Monti et al., 2010; Edouard et al., 2011). Em alguns casos a suplementação exógena desses componentes é necessária para a acurada manutenção da massa óssea (Edouard et al., 2011).

Por ser uma doença crônica, a OI interfere nas atividades diárias dos pacientes e a atenção ao aspecto psicossocial é essencial (Basel e Steiner, 2009). Acompanhamento psicológico e assistencial aos pacientes e familiares proporciona suporte emocional auxiliando no entendimento e aceitação da doença e em casos de alterações comportamentais (Lianza, 2007).

2.7.2. *Tratamento Medicamentoso*

Diversos agentes foram utilizados na tentativa de reduzir a incidência de fraturas e melhorar a massa óssea como fluoreto de sódio, óxido de magnésio, calcitonina, esteróides anabolizantes e vitaminas A, C e D, porém sem sucesso (Glorieux, 2008).

No entanto, em 1987 Devogelaer e colaboradores trataram com pamidronato de sódio um paciente com OI de 12 anos de idade e observaram significativa melhora clínica e radiológica (Devogelaer et al., 1987). Esse relato gerou uma nova perspectiva em relação ao tratamento da doença. Em 1998, Glorieux e colegas descreveram os resultados de um ensaio clínico com pamidronato de sódio administrado ciclicamente por um ano em 30 crianças com formas moderada a grave de OI. Os resultados foram expressivos e diversos efeitos benéficos foram relatados como: diminuição acentuada e rápida da dor óssea crônica e da fadiga, maior sensação de bem-estar, aumento da massa mineral óssea e dos corpos vertebrais, diminuição na incidência média de fraturas por ano e melhora na mobilidade (Glorieux et al., 1998). A partir deste estudo, o tratamento com bifosfonados (BPs) passou a ser amplamente difundido e tornou-se o tratamento medicamentoso padrão-ouro para pacientes com OI (Basel e Steiner, 2009; Phillipi et al., 2008).

Seguindo estes achados iniciais, outros estudos provaram a eficácia do tratamento com BPs (orais e intravenosos) a curto e médio prazos e diversos efeitos benéficos foram

descritos. Dentre esses efeitos: incremento da DMO, recuperação de corpos vertebrais colapsados e redução de fraturas por compressão vertebral, diminuição na incidência de fraturas ósseas, ganho na estatura, alívio da dor musculoesquelética crônica e da fadiga, melhora da força muscular e mobilidade e impacto positivo sobre as atividades de vida diária e, ainda, diminuição no número de consultas ambulatoriais e na frequência de cirurgias (Plotkin et al, 2000; Åström e Söderhäll, 2002; Adami et al, 2003; Zeitlin et al., 2003; Land et al., 2006; Glorieux, 2009; Shapiro & Sponseller, 2009).

Contudo, a grande maioria dos estudos são desenvovidos na população pediátrica e muitos dos benefícios secundários ao tratamento com BPs não são observados na população adulta com OI (Rauch & Glorieux, 2004; Basel & Steiner, 2009; Bishop et al., 2010). BPs são potentes inibidores da função osteoclástica, consequentemente, inibindo a reabsorção óssea e aumentando a massa óssea. Análogos de pirofosfato são caracterizados pela forte ligação aos cristais de hidroxiapatita se fixando ao osso (Rauch e Glorieux, 2004; Russel, 2007; Russel, 2015). Diferenças na resposta ao tratamento podem ser observadas entre crianças e adultos, uma vez que os BPs apresentam melhor ação em estados de elevado *turnover* ósseo (Martin & Shapiro, 2007; Hoyer-Kuhn et al., 2015; Russel, 2015).

BPs são armazenados no esqueleto por muitos anos e após vinte anos de ampla administração desse tratamento para crianças e adultos com OI, os efeitos a longo prazo e a segurança da droga estão em discussão (Dwan et al, 2014; Rijks et al., 2015; Hald et al., 2015). Num estudo histomorfométrico ósseo que incluiu 45 pacientes pediátricos com OI tratados com pamidronato por 2,4 ± 0.6 anos (variando de 1 a 4 anos), Rauch e colegas (2002) relataram aumento na área e volume da DMO através do exame de desitometria. Em conjunto, o autores observaram que o tratamento com pamidronato levou a uma diminuição relativamente maior nos parâmetros de formação óssea do que nos de reabsorção óssea (Rauch et al., 2002).

Através de biópsia óssea e densitometria, Rauch e colegas (2006) descreveram os efeitos histomorfométricos do tratamento a longo prazo com pamidronato (9 mg/kg/ano) em 25 pacientes pediátricos que receberam a droga por 4 anos ou mais. Um aumento de 72% da área da DMO foi observada nos primeiros 2,7 ± 0.5 anos do tratamento, mas na segunda metade do tratamento (5,5 ± 0.7 anos de tratamento) esse aumento foi de apenas 27% e a taxa média de formação óssea em superfícies ósseas trabecular diminuiu em 70% após o início do tratamento. Os autores concluíram com esse estudo que os maiores ganhos parecem ser alcançados nos primeiros 2 a 4 anos de tratamento com pamidronato (Rauch et al., 2006).

Recentemente, Palomo e colaboradores (2015) publicaram os efeitos a longo prazo do tratamento com pamidronato em 37 crianças com OI. Os pacientes avaliados iniciaram o tratamento com BP antes dos 5 anos de idade e foram acompanhados por pelo menos 10 anos. Recuperação da altura dos corpos vertebrais foi descrita, dos 35% de vértebras com fraturas por compressão observadas no início do tratamento apenas 6% ainda apresentavam compressão no final do tratamento. Em conjunto, o tratamento com pamidronato foi associado com aumento na área da DMO da coluna lombar. No entanto, a taxa de fratura de ossos longos permaneceu elevada e a maioria dos pacientes desenvolveu escoliose, não havendo um efeito protetor do tratamento em relação a essas variáveis (Palomo et al, 2015).

Meta-análises e estudos de revisão da Cochrane investigaram os efeitos do tratamento com BPs em crianças e adultos com OI (Phillipi et al., 2008; Dwan et al., 2014, Hald et al., 2015). Aumento da DMO foi reportada, porém os estudos não puderam confirmar a existência de um efeito benéfico do tratamento com BPs na redução da incidência de fraturas, esse efeito foi considerado "inconclusivo" (Phillipi et al., 2008; Dwan et al., 2014, Hald et al., 2015). Entretanto, a meta-análise desenvolvida por Shi et al. (2015) mostrou que em crianças o tratamento com BPs foi eficaz em reduzir a incidência de fraturas, enquanto em adultos os resultados foram similares aos resultados observados nos pacientes controles.

Diversos efeitos adversos associados ao tratamento com BPs estão descritos na literatura. Dor e úlcera gastrointestinal associada ao uso de BP oral, febre e dor muscular associada a primeira infusão intravenosa de BP, não-união crônica de fraturas, atraso na consolidação de locais de osteotomia e hipocalcemia assintomática transitória (Munns et al., 2004; Basel & Steiner, 2009; Harrington et al., 2014). Fraturas atípicas de fêmur foram descritas em pacientes adultos e pediátricos que receberam tratamento com BPs a longo prazo (Carpintero et al., 2015; Hegazi et al., 2015).

Outra condição importante e pouco investigada é o efeito da droga no embrião e no feto durante a gestação. Um estudo mostrou que em ratas grávidas tratadas com alendronato a droga foi capaz de ultrapassar a barreira placentária, diminuindo o peso fetal e afetando o esqueleto (Patlas et al., 1999), entretanto em humanos não há confirmação de efeito teratogênico da droga. Três estudos que avaliaram gestantes tratadas com alendronato antes ou durante a gestação não encontraram malformações ou anomalias congênitas nos fetos (Rutgers-Verhage et al., 2003; Levy et al., 2004; Ornoy et al, 2006).

3. JUSTIFICATIVA

A Osteogênese Imperfeita é uma doença rara que apresenta ampla variabilidade tanto fenotípica quanto genotípica tornando a correlação genótipo-fenótipo complexa. A descoberta de diversos genes, bem como novas características clínicas e radiológicas tem gerado novas discussões, como por exemplo, a variabilidade na expressividade da doença entre indivíduos de uma mesma família, características clínicas raras observadas apenas em alguns indivíduos e diferenças na resposta ao tratamento medicamentoso entre os pacientes.

No Brasil, não há dados oficiais sobre a incidência da doença e poucos estudos clínicos e genéticos foram desenvolvidos na população brasileira. No entanto, com o desenvolvimento dos Centros de Referência em Osteogênese Imperfeita distribuídos pelo país, um atendimento clínico adequado e atualizado tem sido disponibilizado para esses pacientes, bem como o desenvolvimento de pesquisas relevantes para o meio científico.

Após vinte anos de tratamento com bifosfonados questões relacionadas à eficácia e segurança da droga são questões relevantes e em plena investigação. A eficácia do tratamento com bifosfonados no curto e médio prazo para crianças com OI é conhecida e amplamente divulgada, porém os efeitos a longo prazo ainda não são claros. Conjuntamente, em adultos com OI não há estudos provando sustentada redução de fraturas com o uso da terapia e diversos efeitos adversos têm sido descritos associados ao uso prolongado da medicação.

O estudo clínico e genético nessa população contribuirá para um maior conhecimento da doença e auxiliará no adequado aconselhamento genético dessas famílias, bem como esclarecimentos quanto a resposta ao tratamento medicamentoso propiciará melhores estratégias de prevenção e tratamento.

4. OBJETIVOS

4.1. Objetivo Geral

Estudar características clínicas e moleculares de crianças e adultos com Osteogênese Imperfeita e analisar o efeito do tratamento medicamentoso com bifosfonados em relação aos biomarcadores metabólicos e ósseos em pacientes adultos.

4.2. Objetivos Específicos

a. Descrever características clínicas e radiológicas no momento do diagnóstico de OI em crianças.
b. Avaliar a frequência da mutação c.-14C> T no gene *IFITM5* numa coorte de indivíduos com OI e descrever as características clínicas dos indivíduos com resultado positivo para a mutação.
c. Analisar níveis de biomarcadores metabólicos e ósseos e incidência de fraturas em adultos com OI tratados com bifosfonados.

5. REFERÊNCIAS BIBLIOGRÁFICAS

ABOI - Associação Brasileira de Osteogênese Imperfeita. 2012. Disponível em: www.aboi.org.br Acesso: 09/03/2012.

Adami S, Gatti D, Colapietro F, Fracassi E, Braga V, Rossini M & Tato L. Intravenous neridronate in adults with osteogenesis imperfecta. Journal of Bone and Mineral Research. 2003; 18:126–130.

Anissipour AK, Hammerberg KW, Caudill A, Kostiuk T, Tarima S, Zhao HS, Krzak JJ, Smith PA. Behavior of scoliosis during growth in children with osteogenesis imperfecta. J Bone Joint Surg Am. 2014; 96(3):237-43.

Åström E, Söderhäll S. Beneficial effect of long term intravenous bisphosphonate treatment of osteogenesis imperfect. Arch Dis Child. 2002; 86:356–364.

Bachrach LK, Ward LM. Clinical review 1: Bisphosphonate use in childhood osteoporosis. J Clin Endocrinol Metab. 2009; 94(2):400-9.

Barbosa-Buck CO, Orioli IM, da Graça Dutra M, Lopez-Camelo J, Castilla EE, Cavalcanti DP. Clinical epidemiology of skeletal dysplasias in South America. Am J Med Genet A. 2012; 158A(5):1038-45.

Baron R. General Principles of Bone Biology. In: Favus MJ, ed. Primer on the bone metabolic bone disease and disorders of mineral metabolism. 5th ed. Washington ASBMR 2003: 1-8.

Basel D, Steiner RD. Osteogenesis imperfecta: recent findings shed new light on this once well-understood condition. Genet Med. 2009; 11(6):375-85.

Ben Amor IM, Glorieux FH, Rauch F. Genotype-phenotype correlations in autosomal dominant osteogenesis imperfecta. J Osteoporos. 2011; 2011:540178.

Bishop N, Harrison R, Ahmed F, et al. A randomized, controlled dose-ranging study of risedronate in children with moderate and severe osteogenesis imperfecta. J Bone Miner Res. 2010; 25(1):32-44.

Biver E. Use of bone turnover markers in clinical practice. Curr Opin Endocrinol Diabetes Obes. 2012;19(6):468-73.

Brizola E, Staub AL, Félix TM. Muscle strength, joint range of motion, and gait in children and adolescents with osteogenesis imperfecta. Pediatr Phys Ther. 2014; 26(2):245-52.

Carpintero P, Del Fresno JA, Ruiz-Sanz J, Jaenal P. Atypical fracture in a child with osteogenesisimperfecta. Joint Bone Spine. 2015;82(4):287-8.

Chagas CE, Roque JP, Santarosa Emo Peters B, Lazaretti-Castro M, Martini LA. Do patients with osteogenesis imperfecta need individualized nutritional support? Nutrition. 2012; 28(2):138-42.

Cho TJ, Lee KE, Lee SK, et al. A single recurrent mutation in the 5'-UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Hum Genet. 2012; 91(2):343–348.

Devogelaer JP, Malghem J, Maldague B, Nagant de Deuxchaisnes C. Radiological manifestations of bisphosphonate treatment with APD in a child suffering from osteogenesis imperfecta. Skeletal Radiol. 1987; 16:360–363.

Devogelaer JP, Malghem J, Maldague B, Nagant de Deuxchaisnes C. Radiological manifestations of bisphosphonate treatment with APD in a child suffering from osteogenesis imperfecta. Skeletal Radiol. 1987;16:360

Diacinti D, Pisani D, D'Avanzo M, Celli M, Zambrano A, Stoppo M, Diacinti D, Roggini M, Todde F, D'Eufemia P, Pepe J, Minisola S. Reliability of vertebral fractures

assessment (VFA) in children with osteogenesis imperfecta. Calcif Tissue Int. 2015;96(4):307-12

Dwan K, Phillipi CA, Steiner RD, Basel D. Bisphosphonate therapy for osteogenesisimperfecta.Cochrane Database Syst Rev. 2014;7:CD005088.

Dwan K, Phillipi CA, Steiner RD, Basel D. Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev. 2014; 7:CD005088.

Edouard T, Glorieux FH, Rauch F. Relationship between Vitamin D Status and Bone Mineralization, Mass and Metabolism in Children with Osteogenesis Imperfecta: Histomorphometric Study. J Bone Miner Res. 2011; 26(9):2245-51.

Engelbert RHH, Pruijs HEH, Beemer FA, Helders PJM. Osteogenesis imperfecta in childhood: Treatment strategies. Arch Phys Med Rehabil. 1998; 79(12):1590–1594.

Evans CH. Gene delivery to bone. Adv Drug Deliv Rev. 2012; 64(12):1331-40.

Forlino A, Cabral WA, Barnes AM, et al. New perspectives on osteogenesis imperfecta. Nat Rev Endocrinol. 2011; 7:540-557.

Glorieux FH, Bishop N, Plotkin H, Chabot G, Lanoue G, Travers RT. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. N Engl J Med. 1998;339:947–952.

Glorieux FH. Osteogenesis imperfecta. Best Pract Res Clin Rheumatol. 2008; 22(1):85-100.

Hald JD, Evangelou E, Langdahl BL, Ralston SH. Bisphosphonates for the prevention of fractures in osteogenesis imperfecta: meta-analysis of placebo-controlled trials.J Bone Miner Res. 2015;30(5):929-33.

Hald JD, Evangelou E, Langdahl BL, Ralston SH. Bisphosphonates for the prevention of fractures in osteogenesis imperfecta: meta-analysis of placebo-controlled trials. J Bone Miner Res. 2015; 30(5):929-33.

Harrington J, Sochett E, Howard A. Update on the evaluation and treatment of osteogenesis imperfecta. Pediatr Clin North Am. 2014; 61(6):1243-57.

Hatz D, Esposito PW, Schroeder B, Burke B, Lutz R, Hasley BP. The incidence of spondylolysis and spondylolisthesis in children with osteogenesis imperfecta. J Pediatr Orthop. 2011;31(6):655-60.

Hegazy A, Kenawey M, Sochett E, Tile L, Cheung AM, Howard AW. Unusual Femur Stress Fractures in Children with Osteogenesis Imperfecta and Intramedullary Rods on Long-term Intravenous Pamidronate Therapy. J PediatrOrthop. 2015. Epub 2015 Jun 8.

Hoyer-Kuhn H, Netzer C, Semler O. Osteogenesis imperfecta: pathophysiology and treatment. Wien Med Wochenschr. 2015; 165(13-14):278-284.

Hlaing TT, Compston JE. Biochemical markers of bone turnover - uses and limitations. Ann Clin Biochem. 2014; 51(Pt 2):189-202.

Jones G, Boon P. Which bone mass measures discriminate adolescents who have fractured from those who have not? Osteoporosis International. 2009; 19(2): 251-255.

Karsenty G. The complexities of skeletal biology. Nature. 2003; 423(6937): 316-8.

Krakow D. Skeletal Dysplasias. ClinPerinatol. 2015; 42(2):301-319.

Lacativa PGS; Farias MLF. Office practice of osteoporosis evaluation. Arq Bras Endocrinol Metab. 2006; 50(4):674-684.

Land C, Rauch F, Montpetit K, Ruck-Gibis J, Glorieux FH. Effect of intravenous pamidronate therapy on functional abilities and level of ambulation in children with osteogenesis imperfect. J Pediatr. 2006; 148:456-60.

Lee RJ, Paloski MD, Sponseller PD, Leet AI. Bent Telescopic Rods in Patients With Osteogenesis Imperfecta. J Pediatr Orthop. 2015 Apr 25. [Epub ahead]

Levy S, Fayez I, Han JY, Aeillo J, Matsui D, Koren G, et al. Fetal outcome after intrauterine exposure to bisphosphonates. Birth Defects Res Part A: Clin Mol Teratol. 2004;70:359–60.

Lianza S, Pavan K, Rossetto R, et al. Avaliação da Incapacidade In: Medicina de Reabilitação. Rio de Janeiro: Guanabara Koogan, 2007.

Lin D, Zhai W, Lian K, Ding Z. Results of a bone splint technique for the treatment of lower limb deformities in children with type I osteogenesis imperfecta. Indian J Orthop. 2013; 47(4):377-81.

Makareeva E, Aviles NY, Leikin S. Chaperoning osteogenesis: new protein-folding disease paradigms. Trends in Cell Biology. 2011; 21:168-176.

Marini J, Smith SM. Osteogenesis Imperfecta. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, editors. SourceEndotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-2015.

Marini JC, Forlino A, Cabral WA, et al. Consortium for osteogenesis imperfecta mutations in the helical domain of type I collagen: regions rich in lethal mutations align with collagen binding sites for integrins and proteoglycans. Hum Mutat. 2007; 28(3):209-21.

Marini JC, Reich A, Smith SM. Osteogenesis imperfecta due to mutations in non-collagenous genes: lessons in the biology of bone formation. Curr Opin Pediatr. 2014; 26(4):500-7.

Marini, J.C., Cabral, W.A., and Barnes, A.M. Null mutations in LEPRE1 and CRTAP cause severe recessive osteogenesis imperfecta. Cell and tissue research. 2010; 339:59-70.

Martin E, Shapiro JR. Osteogenesis imperfecta: epidemiology and pathophysiology. Curr Osteoporos Rep. 2007; 5(3):91-7.

Monti E, Mottes M, Fraschini, P, et al. Current and emerging treatments for the management of osteogenesis imperfecta. Therapeutics and Clinical Risk Management. 2010; 6:367–381.

Munns CF, Rauch F, Zeitlin L, Fassier F, Glorieux FH. Delayed osteotomy but not fracture healing in pediatric osteogenesisimperfecta patients receiving pamidronate. J Bone Miner Res. 2004;19(11):1779-86

Nassa M, Anand P, Jain A, et al. Analysis of human collagen sequences. Bioinformation. 2012; 8(1):26-33.

Nicolaou N, Bowe JD, Wilkinson JM, Fernandes JA, Bell MJ. Use of the Sheffield telescopic intramedullary rod system for the management of osteogenesis imperfecta: clinical outcomes at an average follow-up of nineteen years. J Bone Joint Surg Am. 2011; 93:1994–2000.

Niyibizi C, Li F. Potential implications of cell therapy for osteogenesis imperfecta. Int J Clin Rheumtol. 2009; 4(1):57-66.

Online Mendelian Inheritance in Man (OMIM), from http://www.omim. org/ acesso: 20 setembro de 2015.

Ornoy A, Wajnberg R, Diav-Citrin O. The outcome of pregnancy following pre-pregnancy or early pregnancy alendronate treatment. Reproductive Toxicology. 2006; 22:578–579.

Palomo T, Fassier F, Ouellet J, et al. Intravenous Bisphosphonate Therapy of Young Children with Osteogenesis Imperfecta: Skeletal Findings During Follow Up Throughout the Growing Years. J Bone Miner Res. 2015 Jun 8. doi: 10.1002/jbmr.2567. Epub 2015 jun 30.

Patlas N, Golomb G, Yaffe P, Pinto T, Breuer E, Ornoy A. Transplacental effects of bisphosphonates on fetal skeletal ossification and mineralization in rats. Teratology. 1999;60:68–73.

Phillipi CA, Remmington T, Steiner RD. Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev. 2008;(4):CD005088.

Plotkin H, Rauch F, Bishop NJ, et al. Pamidronate treatment of severe osteogenesis imperfecta in children under 3 years of age. J Clin Endocrinol Metab. 2000; 85:1846–50.

Puvanesarajah V, Shapiro JR, Sponseller PD. Sandwich allografts for long-bone nonunions in patients with osteogenesis imperfecta: a retrospective study. J Bone Joint Surg Am. 2015; 97(4):318-25.

Puvanesarajah V, Shapiro JR, Sponseller PD. Sandwich allografts for long-bone nonunions in patients with osteogenesis imperfecta: a retrospective study. J Bone Joint Surg Am. 2015;97(4):318-25.

Rauch F, Glorieux FH. Osteogenesis Imperfecta. Lancet. 2004; 363:1377-85.

Rauch F, Moffatt P, Cheung M, Roughley P, Lalic L, Lund AM, Ramirez N, Fahiminiya S, Majewski J, Glorieux FH. Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.-14C>T mutation in all patients. J Med Genet. 2013; 50(1):21-4.

Rauch F, Travers R, Glorieux FH. Pamidronate in children with osteogenesis imperfecta: histomorphometric effects of long-term therapy. J Clin Endocrinol Metab. 2006; 91(2):511-6

Rauch F, Travers R, Plotkin H, Glorieux FH. The effects of intravenous pamidronate on the bone tissue of children and adolescents with osteogenesis imperfecta. J Clin Invest. 2002; 110(9):1293-9

Rijks EB, Bongers BC, Vlemmix MJ, et al. Efficacy and Safety of Bisphosphonate Therapy in Children with Osteogenesis Imperfecta: A Systematic Review. Horm Res Paediatr. 2015;84(1):26-42.

Rijks EB, Bongers BC, Vlemmix MJ, et al. Efficacy and Safety of Bisphosphonate Therapy in Children with Osteogenesis Imperfecta: A Systematic Review. Horm Res Paediatr. 2015; 84(1):26-42.

Roughley PJ, Rauch F, Glourieux FH. Osteogenesis Imperfecta - Clinical and Molecular Diversity. Eur Cell Mater. 2003; 5:41-7.

Russel RG. Bisphosphonates: Mode of Action and Pharmacology. Pediatrics. 2007; 119:150-162.

Russell RG. Pharmacological diversity among drugs that inhibit bone resorption. Curr Opin Pharmacol. 2015; 22:115-130

Rutgers-Verhage AR, deVries TW, Torringa MJ. No effects of bisphosphonates on the human fetus. Birth Defects Res Part A: Clin Mol Teratol. 2003;67:203–4.

Santili C, Akkari M, Waisberg G, Júnior JOCB, Ferreira WM. Clinical, radiographic and laboratory evaluation of patients with osteogenesis imperfecta. Rev Assoc Med Bras. 2005; 51:214-20.

Saraiva GL, Lazaretti-Castro M. Marcadores Bioquímicos da Remodelação Óssea na Prática Clínica. Arq Bras Endocrinol Metab. 2002; 46(1): 72-78.

Semler O, Garbes L, Keupp K, et al. A Mutation in the 5'-UTR of IFITM5 Creates an In-Frame Start Codon and Causes Autosomal-Dominant Osteogenesis Imperfecta Type V with Hyperplastic Callus. Am J Hum Genet. 2012; 91(2):349–357.

Shapiro JR, Germain-Lee EL. Osteogenesis imperfecta: Effecting the transition from adolescent to adult medical care. J Musculoskelet Neuronal Interact. 2012; 12(1):24-27.

Shapiro JR, Lietman C, Grover M, Lu JT, Nagamani SCS, Dawson BC, Baldridge DM, Bainbridge MN, Cohn DH, Blazo M, Roberts TT, Brennen FS, Wu Y, Gibbs RA,

Melvin P, Campeau PM, Lee BH. Phenotypic Variability of Osteogenesis Imperfecta Type V Caused by an IFITM5 Mutation. J Bone Miner Res. 2013; 28(7): 1523–1530.

Shapiro JR, Sponseller PD. Osteogenesis imperfecta: questions and answers. Curr Opin Pediatr. 2009; 21(6):709–716.

Shi CG, Zhang Y, Yuan W. Efficacy of Bisphosphonates on Bone Mineral Density and Fracture Rate in Patients With Osteogenesis Imperfecta: A Systematic Review and Meta-analysis. Am J Ther. 2015. Epub 2015 Apr 3.

Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet. 1979; 16:101–116.

Silva ECC, Terreri MTR, Castro TCM, et al. Linhas escleróticas metafisárias em crianças e adolescentes em uso de alendronato. Rev Bras Reumatol. 2010; 50(3):283-90.

Sofield HA, Millar EA. Fragmentation, realignment, and intramedullary rod fixation of deformities of the long bones in children. J Bone Joint Surg Am. 1959; 41:1371-1391.

Soma K, Abe H, Takeda N, et al. Myocardial involvement in patients with osteogenesis imperfecta. Int Heart J. 2012; 53(1):75-7.

Sposito, PJ; Arlet V. Spinal Fractures, Spondylolysis and Spondylolysthesis. In: Shapiro JR, Byers PH, Glorieux F, Sponseller PD (eds). Osteogenesis imperfecta: a translational approach to brittle bone disease. Elsevier Academic Press: USA, 2013; pp. 407-419.

Staheli LT. Ortopedia pediátrica na prática. 2ª ed. Porto Alegre: Artmed. 2008. 464p.

Tarnowski M, Szydło A, Anioł J, Koryciak-Komarska H, Lesiak M, Gutmajster E, Sieroń AL, Kusz D. Optimization of genetic engineering and homologous recombination of collagen type I genes in rat bone marrow mesenchymal stem cells (MSC). Cell Reprogram. 2010; 12(3):275-82.

Ulla M, Aiello H, Cobos MP, et al. Prenatal diagnosis of skeletal dysplasias: contribution of three-dimensional computed tomography. Fetal Diagn Ther. 2011; 29(3):238-47.

Van Dijk FS, Pals G, Van Rijn RR, Nikkels PG, Cobben JM. Classification of Osteogenesis Imperfecta revisited. Eur J Med Genet. 2010; 53(1):1-5.

Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014; 164A(6):1470-81.

Van Dijk FS, Zillikens MC, Micha D, et al. PLS3 mutations in X-linked osteoporosis with fractures. N Engl J Med. 2013; 369(16):1529-36.

Warman ML, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet Part A. 2011; 155A:943–968.

Watzl MTP, Abreu AV, Kruse R. Surgical treatment of deformities and fractures on lower limbs with osteogenesis imperfecta. Acta Ortop Bras. 2009; 17(4):202-6.

Webber CE, Sala A, Barr RD. Accounting for body size deviations when reporting bone mineral density variables in children. Osteoporos Int. 2009; 20:113-121.

Wekre LL, Eriksen EF, Falch JA. Bone mass, bone markers and prevalence of fractures in adults with osteogenesis imperfecta. Arch Osteoporos. 2011; 6:31–38.

Westgren M, Götherström C. Stem cell transplantation before birth - a realistic option for treatment of osteogenesis imperfecta? PrenatDiagn. 2015. Epub ahead 2015 May 11.

Wu Y, Humphrey MB, Nakamura MC. Osteoclasts – the innate immune cells of the bone. Autoimmunity. 2008; 41(3):183-94.

Zeitlin L, Rauch F, Plotkin H, Glorieux F. Height and weight development during long-term therapy with cyclical intravenous pamidronate in children and adolescents with osteogenesis imperfect types I, III and IV. Pediatr. 2003;111:1030–36.

Esta tese foi divida em capítulos de acordo com a característica de cada estudo e será apresentada na forma de artigos científicos:

Capítulo I. Caracterização Clínica e Molecular

Capítulo II. Tratamento com Bifosfonados

Seguindo as normas do Programa de Pós-Graduação da Saúde da Criança e do Adolescente, artigos aceitos para publicação ou já publicados serão apresentados no idioma no qual o artigo foi redigido, os demais artigos serão apresentados nos idiomas português e inglês.

CAPÍTULO I

CARACTERIZAÇÃO CLÍNICA E MOLECULAR

6.1. METODOLOGIA I

6.1.1 Delineamento

Estudo retrospectivo.

6.1.2. Amostra

Prontuários de pacientes com OI, ambos os sexos, idades entre 0 e 18 anos e em acompanhamento no Centro de Referência em Osteogênese Imperfeita do Hospital de Clínicas de Porto Alegre, entre janeiro de 2002 e janeiro 2014 foram revisados.

Foram excluídos pacientes com outras causas primárias e secundárias de osteoporose como hipofosfatasia, deficiência de cálcio e uso de glucocorticóides.

6.1.3. Dados Clínicos

O diagnóstico de OI foi baseado em características clínicas e radiológicas de acordo com critérios de Sillence (Sillence et al., 1979). Os dados coletados incluíram dados clínicos no momento do diagnóstico de OI como idade, história familiar positiva para a doença e características clínicas da OI (esclera azulada, dentinogênese imperfeita, número de fraturas e local das fraturas).

Exames radiográficos de rotina foram revisados com atenção especial quanto à presença de ossos wormianos. As fraturas relatadas foram classificadas como ocorrendo nos membros inferiores (fêmur, tíbia ou fíbula) ou superiores (rádio, ulna ou úmero) e/ou outro local específico.

Este estudo foi limitado aos resultados presentes no momento do diagnóstico de OI e não pretende representar o curso natural da doença em crianças.

6.1.4. Aspectos Éticos

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (nº. 13-0079) e todos os pacientes ou responsáveis assinaram um termo de consentimento livre e esclarecido.

6.2. Análise Estatística

As variáveis contínuas foram expressas como medianas e intervalos interquartis devido à distribuição assimétrica de dados. Variáveis categóricas foram descritas por meio de frequências absolutas e relativas e comparadas entre os tipos de OI através do teste Chi-square. O nível de significância adotado foi de 5% ($p \leq 0,05$).

As análises estatísticas foram realizadas utilizando o software SPSS (versão 18.0; SPSS Inc., Chicago, IL, EUA).

6.3. Resultados

Os resultados serão apresentados no formato de artigo científico. O artigo intitulado "*Clinical Features and Pattern of Fractures at the Time of Diagnosis of Osteogenesis Imperfecta in Children*" será submetido para publicação no periódico "*Clinical Pediatrics*".

VERSÃO EM INGLÊS

Clinical features and pattern of fractures at the time of diagnosis of Osteogenesis Imperfecta in children

Evelise Brizola[1]; Marina B. Zambrano[1]; Bruna S. Pinheiro[1];

Ana Paula Vanz[1]; Têmis M. Félix[1,2]

[1] Postgraduate Program in Child and Adolescent Health, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

[2] Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil

ABSTRACT

Objective: To characterize the fracture pattern and clinical history at the time of osteogenesis imperfecta (OI) diagnosis. **Methods:** In this retrospective study were included all patients, both genders, aged 0–18 years with OI who were under treatment at the Reference Center for OI, Porto Alegre, Brazil, between 2002 and 2014. Medical records were assessed and were collected clinical data including presence of blue sclerae, dentinogenesis imperfecta, positive familial OI history and site of fractures, and radiographic findings such as wormian bones at the time of OI diagnosis. **Results:** Seventy-six patients (42 females) with OI diagnosis were included in the study. Individuals' age at the time of diagnosis ranged from 0 to 114 months, with a median (interquartile range [IQR]) age of 38

(6 - 96) months. Blue sclerae were observed in 93.4% of patients; dentinogenesis imperfecta was observed in 27.6% and wormian bones were observed in 29.4% of patients. The number of fractures at diagnosis ranged from 0 to 17, with a median (IQR) of 3 (2–8) fractures. Forty (57%) patients showed fractures of the upper and lower extremities, and nine patients also had spinal fractures. OI was diagnosed at birth in 85.7% of patients with OI-3 and 39.3% of those with OI-4/5. **Conclusion:** OI is a genetic disorder with distinctive clinical features such as bone fragility, recurrent fractures, blue sclerae and dentinogenesis imperfecta. It is important to know how to identify these characteristics facilitating the diagnosis, optimizing the treatment and differentiating of other disorders that also can lead to fractures.

Keywords: osteogenesis imperfecta, bone fracture, clinical features, clinical diagnosis, differential diagnosis

INTRODUCTION

Osteogenesis imperfecta (OI) is a systemic genetic disorder of connective tissue with prevalence 6-7/100.000 births (Van Dijk & Sillence, 2014). OI affects all tissues containing collagen, mainly bone tissue; its main feature is low bone mass, which renders bone fragile and susceptive to deformities and repeated fracture (Van Dijk et al., 2011; Marini et al., 2013).

The majority of OI cases are characterized by autosomal dominant inheritance caused by mutations in *COL1A1* or *COL1A2* genes; nonetheless, recent studies have showed that OI can also be caused by mutations in other 19 genes involved in collagen biosynthesis or osteoblasts function with dominant, recessive or X-linked inheritance (Van Dijk & Sillence, 2014; OMIM 2015). Due to wide genotypic and phenotypic heterogeneity, OI has been classified into several types according to clinical features, radiological aspects, and

responsible genes (Van Dijk et al., 2014; Marini et al., 2013). The Nosology Group of the International Skeletal Dysplasia Society redefined the classical clinical OI classification (Sillence et al., 1979) by adding OI type 5 (OI-5) to the four originally described groups (Warman et al., 2011).

OI type 1 (OI-1) is a mild form characterized by none or few fractures and discrete deformities. OI type 2 is the most severe type, characterized by extreme bone fragility leading to death in the neonatal period. OI type 3 (OI-3) is severe; patients present multiple fractures, significant bone deformities, and short stature. OI type 4 (OI-4) is a moderate form with high clinical variability in which patients can show few or many fractures associated with bone deformities (Sillence et al., 1979; Van Dijk & Sillence, 2014; Marini et al., 2013). Individuals with OI-5 had a moderate form of OI and distinct clinical and radiological features such as interosseous membrane calcification between the radius and ulna and/or tibia and fibula, hyperplastic callus formation in the long bones, dislocation of the radial head, and absence of dentinogenesis imperfecta (Van Dijk & Sillence, 2014).

Fractures can occur during lifetime in patients with OI, but the majority occur during childhood. The clinical features observed in individuals with OI can also be observed in other genetic and metabolic disorders (Harrington et al., 2014). The diagnosis of OI is still made based in clinical and radiological features (Harrington et al., 2014; Warman et al., 2011). Genetic test is not available in many countries as a routine test or is not covered by insurance or health system. For this reason, it is important to professionals that deal with general pediatric population to know how to differentiate and identify these specific cases and be aware about the clinical conditions at the diagnosis of this genetic disorder.

This study was conducted to characterize fracture pattern and clinical history at the time of OI diagnosis in pediatric patients.

METHODS

Study Population

In this retrospective study, medical charts of male and female patients with OI aged 0–18 years who were under treatment at the Reference Center for Osteogenesis Imperfecta (CROI-HCPA), Porto Alegre, Brazil, between January 2002 and January 2014 were reviewed. Were excluded patients with other primary and secondary causes of osteoporosis such as hypophosphatasia, calcium deficiency and treatment with glucocorticoids. This research was performed approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (no. 13-0079) and all patients or guardians signed an informed consent**.**

Data Collection

The diagnosis of OI was based on clinical and radiological features according Sillence criteria (Sillence et al., 1979). Data collected included clinical data at the time of OI diagnosis such as age, family history for OI and clinical features of OI (blue sclerae, dentinogenesis imperfecta, number of fractures and site of fractures). Routine radiographic exams were reviewed with special attention to the presence of wormian bones in the skull. Reported fractures were classified as occurring in the lower (femur, tibia, or fibula) or upper (radius, ulna, or humerus) extremities and/or other specific site.

Our study was limited to the findings present at the time of OI diagnosis and it does not intended to represent the natural course of children with OI.

Statistical Analysis

Continuous variables were expressed as medians and interquartile ranges (IQRs) due to asymmetric distribution of data. Categorical variables were described by absolute and relative frequencies and compared among OI type groups using the chi-squared test. The level of significance was set to 5% ($p \leq 0.05$). Statistical analyses were performed using SPSS software (version 18.0; SPSS Inc., Chicago, IL, USA).

RESULTS

The study sample comprised 76 patients (42 female) with OI. All cases of OI were diagnosed clinically and radiologically according to classic Sillence OI classification (Sillence et al., 1979); only the single case of OI-5 was confirmed by molecular analysis. For statistical analysis, the patient with OI-5 was grouped with those with OI-4 considering that both types representing moderate forms of OI. **Table 1** shows patients' age at diagnosis and clinical features by OI type. Forty-one (54%) patients had OI-1, 7 (9%) had OI-3, and 28 (37%) had OI-4/5. Individuals' age at the time of diagnosis ranged from 0 to 114 months, with a median (IQR) age of 38(6 -96) months. Most (85.7%; n=7) patients with OI-3 and 39.3% (n=28) with OI-4/5 were diagnosed in the perinatal period, and 68.3% (n=41) of subjects with OI-1 were diagnosed between the ages of 1 and 5 years (**Figure 1**).

Thirty-three (44.6%) subjects had positive family histories of OI. In two cases, family history was unknown because the children were adopted and data were not available. Of all patients, 71 (93.4%) had blue sclerae, 21 (27.6%) had dentinogenesis imperfecta (DI), and 15 (29.4%) had wormian bones. The number of fractures at the time of diagnosis ranged from 0 to 17, with a median (IQR) of 3 (2–8) fractures. Only six patients (7.8%) showed no fracture at the time of diagnosis, four of them had OI-1. The majority (57.1%; n = 40) of patients had fractures in the extremities; 6 (23.1%) patients with OI-4/5 and 3 (8.1%) with

OI-1 additionally presented with spine fractures (**Figure 2**). One (1.4%) patient with OI-1 also had a history of skull fracture at the time of OI diagnosis followed by an accidental cradle fall.

DISCUSSION

Wide phenotypic variability is observed in individuals with OI; nonetheless, there are a pattern of fractures and clinical features that help to characterize clinically the disorder (Greeley et al., 2013). Clinical and radiological characteristics are the base for identification of cases and OI diagnosis. Genetic test is helpful and provides a better understand of the disease but it is not available as a routine test in many centers of treatment. As a rare disease, just few professionals have experience to recognize the specific clinical characteristics of the disease, and be able to distinguish this features can help professionals involved with health childcare.

In our study sample, more severe types of OI were diagnosed at an earlier age when compared to mild forms of OI. These results corroborate previous reports that initial fractures tend to occur *in utero* or during the perinatal period, and that a high incidence of fractures during growth often causes progressive deformity, in individuals with more severe forms of OI (Van Dijk & Sillence, 2014; Marini et al., 2013). Nonlethal forms of OI are very often not detected in the prenatal period and the prenatal ultrasonographic diagnosis is not totally reliable (Van Dijk et al., 2011; Krakow, 2013). Severe and lethal forms of OI can be diagnosed by ultrasound during the second trimester of pregnancy based on the detection of abnormalities in the skull and ribs, bowing of long bones, decreased bone echogenicity, micromelia, delayed fetal growth, ventriculomegaly, polyhydramnios, and even bone callus formation secondary to fracture (Van Dijk et al., 2011; Krakow, 2013).

Postnatal diagnosis is based on positive family history of OI, clinical signs, radiological findings, and bone densitometry; further investigation may include biochemical and molecular analysis (Renaud et al., 2013; Van Dijk et al., 2011). Characteristics considerate "classic" in OI such as blue sclerae, wormian bones, DI and fractures may be or not present in these patients. In addition, other findings may be observed as triangular face, short stature, capsular-ligamentous laxity, cardiovascular and eye abnormalities, hearing loss, platybasia and basilar invagination (Van Dijk et al., 2011; Van Dijk & Sillence, 2014; Marini et al., 2013). Restriction of the forearm movement, progressive joint contractures and craniosynostosis can also be found in OI secondary to rare mutations in the new OI-related genes (Shapiro et al., 2013; Van Dijk & Sillence, 2014; OMIM, 2015).

Nevertheless, these features are not OI exclusive and can be present in other diseases. The majority of our sample (93.4%) had blue sclerae. However, blue sclerae is also reported in health pediatric patients (Marti et al., 2013; Harrington et al., 2014), in individuals with iron deficiency (Beghetti et al., 1993) and in other syndromes including Loyes-Dietz (Drera et al., 2009), De Barsy (Kivuva et al., 2009), Marshall-Smith (Adam et al., 2005), Ehler-Danlos (Kosho et al., 2010) and Russel-Silver (Martinez Alogueiras et al., 2001; Parker et al., 2011). In addition, it is described in cases of Alkaptonuric ochronosis (Yancovitz et al., 2010), Brittle cornea syndrome (Burkitt et al., 2013) and in a report on two siblings with VATER-like defects and multiple malformations (Braddock, 2003).

Wormian bones, small supernumerary bones found within the sutures and fontanelles of the skull, commonly present in OI can also be seem in the normal pediatric population and in other conditions such as cleidocranial dysostosis, pycnodysostosis, hypophostasia, hydrocephalia, congenital hypothyroidism and Down Syndrome (Bellary et al., 2013; Marti et al., 2013). We observed wormian bones in around 30% of the OI children analyzed. Marti and colleagues analyzes CT brain scans assessing the frequency of wormian bones in a

"normal" pediatric population aged 0 to 3 years and found wormian bones in 53% of the children (n = 320). Sixty children (10%) had four or more wormian bone (Marti et al., 2013).

DI was present in less than one-third of patients in this study. However, many of these patients were diagnosed with OI in an earlier age before the time of first teething. Although, around 50% of children and adults with OI may have dental involvement (Santili et al., 2005), DI is not pathognomonic for OI and is the most common dental genetic disease (Abukabbos & Al-Sineedi, 2013; Wieczorek et al., 2013). DI causes structural defects in dentin formation in the deciduous or both the deciduous and permanent teeth (Abukabbos & Al-Sineedi, 2013). There are three types of DI and the type I is associated to OI secondary to defect in type I collagen, characterized by typical amber color and translucency of teeth (Abukabbos & Al-Sineedi, 2013; Wieczorek et al., 2013).

Bone fragility and susceptibility to fractures with none or minimal trauma are classic features of OI. In this study, initial fractures occurred later in childhood in patients with OI-1 than in those with OI-3 and OI-4/5, and the former group had fewer fractures at the time of diagnosis. An estimated 10% of children with OI-1, the mildest form of the disease and that least frequently associated with long-bone deformities, experience no fracture during childhood (Van Dijk et al., 2011; Marini et al., 2013). In a previous study, we observed that initial fractures tend to occur in the period during which children begin to walk because the upright posture promotes increased weight loading on the lower limbs, leading to secondary fractures (Brizola et al., 2014).

In a retrospective study, Greeley and colleagues described the pattern of fractures and clinical characteristics in 68 infants and children aged under 18 years at the time of OI diagnosis (Greeley et al., 2013). Twenty-six (38%) participants had no fracture, 22 (32%) had fractures of extremities and 15 (22%) had rib fractures most of those diagnosed prenatally or perinatally. The number of fractures at diagnosis ranged from 1 to >37 with 7

(10%) of the subjects having more than 2 fractures. Blue sclerae was reported in 51 (75%) subjects and DI in only 11(16%). The authors concluded that number of fractures, age at diagnosis and location of fractures are clinical features that may aid in the diagnosis of OI (Greeley et al., 2013).

Complete or incomplete long-bone shaft fractures and compression fractures in the thoracolumbar spine are observed more frequently in patients with OI (Renaud et al., 2013). The prevalence of fractures is high in children with OI, but the fracture pattern differs from that seen in victims of CPA (Renaud et al., 2013; Greeley et al., 2013). Cases of CPA are much more common than those of OI (Gahagan & Rimsza, 1993; Paterson et al., 1993), and parents of children with OI have frequently reported dealing with the suspicion of MTF by the health care teams during initial treatment of fractures, before the diagnosis is established (Dogba et al., 2014). Nevertheless, OI and CPA are not mutually exclusive (Greeley & Donnaruma-Kwoh, 2015).

For differential diagnosis should be considered several conditions that leads to higher risk of fractures, as well as disorders that resembling OI overlapping clinical features such as metabolic bone disease of prematurity, idiopathic juvenile osteoporosis, Ehlers-Danlos syndrome, hypophostasia, idiopathic hyperphosphatasia, Osteoporosis pseudoglioma syndrome, vitamin D and calcium deficiency (Viswanathan et al., 2014; Harrington et al., 2014). In addition, CPA cases and secondary causes of osteoporosis including hormone deficiencies, glucocorticoid-induced osteoporosis, and acute lymphoblastic leukemia might be investigated (Greeley et al., 2013; Harrington et al., 2014).

The present study has limitations. It was retrospective in nature, based on data recorded in medical charts. Some data, mainly the number of fractures at the time of diagnosis, were not documented radiologically because medical records of each fracture occurrence were not available and data were collected based on clinical histories. Our data

only reflects clinical and radiological OI features at the time of OI diagnosis; it does not represent the lifetime fracture pattern for these individuals.

Conclusions

Our data suggest that OI related fractures occur at similar sites, and that the number of fracture occurrences varies according to the clinical severity of the disease. Fractures of the lower limbs in association with those of the upper limbs were most frequent in all types of OI, and the number of fractures was larger in patients with more severe forms of OI. The pattern of bone fractures and clinical features are helpful measures to identify OI cases. Health care professionals must be aware of the clinical features and typical fracture patterns of OI to enable distinction of such cases from other diseases that leads to bone fragility or cases of CPA.

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

All the authors were involved in study design and data interpretation. Evelise Brizola and Têmis M. Félix drafted the manuscript. All the authors approved the final version of the manuscript.

Acknowledgements

The authors thank the patients and their families for their participation in this study. The study was supported by Fundo de Incentivo à Pesquisa e Eventos/Hospital de Clínicas de Porto Alegre and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul. Ms. Brizola

was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Proc. número 3770/14-1.

Figure 1. Age at time of diagnosis according to OI type

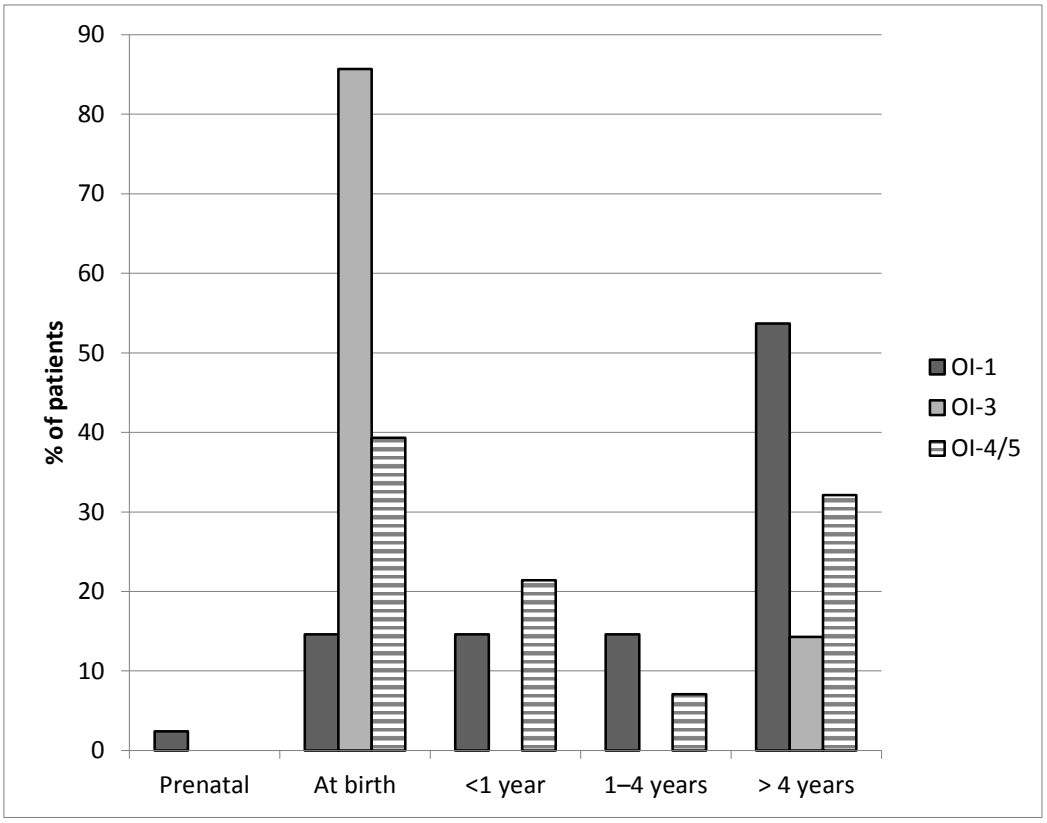

Figure 2. Female patient with OI-4. Radiographic exam of thoracolumbar spine showed fracture of multiple vertebral bodies.

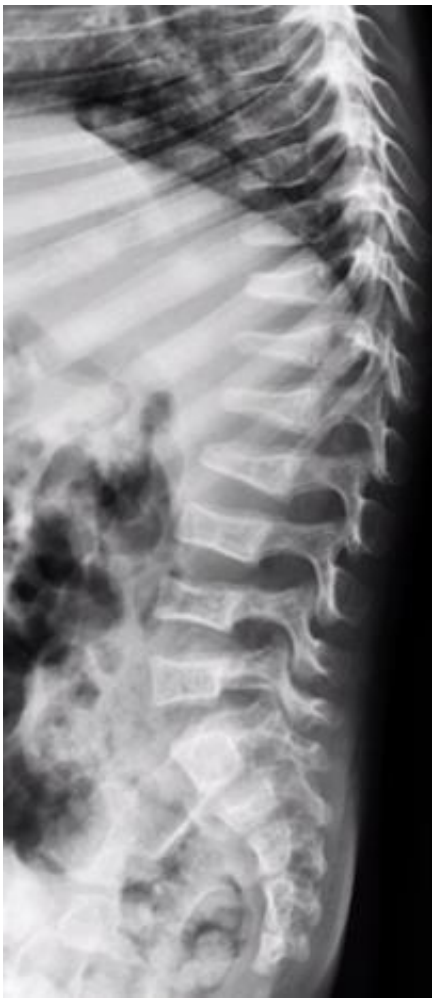

Table 1: Clinical features at the time of diagnosis by OI type

Variable	**Total**	**OI type 1**	**OI type 3**	**OI type 4/5**	***P***
Number of patients n(%)	76 (100)	41 (51.3)	7 (9.2)	27/1 (36.8)	...
Sex (male/female)	34/42	22/19	3/4	9/19	0.209
Positive family history for OI* n(%)	33 (44.6)	26 (66.7)	0	7 (25)	<0.001
Blue sclerae n(%)	71 (93.4)	38 (92.7)	7 (100)	26 (92.9)	0.762
Dentinogenesis imperfecta n(%)	21 (27.6)	6 (14.6)	3 (42.9)	12 (42.9)	0.023
Wormian bones n(%)	15 (29.4)	6 (21.4)	2 (33.3)	7 (41.2)	0.361
Age at diagnosis (months)	38 (6–96)	67 (23–114)	3 (0–3)	16 (4–68)	0.001
Number of fractures at diagnosis	3 (2–8)	3 (1.5–7)	4 (2–17)	6 (3–8.5)	0.76
Sites of fractures at diagnosis[+] n(%)					0.676
UL	9 (12.9)	6 (16.2)	1 (14.3)	2 (7.7)	
LL	11 (15.7)	6 (16.2)	1 (14.3)	4 (15.4)	
UL + LL	40 (57.1)	21 (56.8)	5 (71.4)	14 (53.8)	
UL + LL + spine	9 (12.9)	3 (8.1)	0	6 (23.1)	
UL + LL + skull	1 (1.4)	1 (2.7)	0	0	

OI: osteogenesis imperfecta; UL: upper limbs; LL: lower limbs.
Data are presented as *n* (%) or median (interquartile range).
The percentages are described according to the type of OI for each variable.
*Family history unknown in two cases.
+Six children had no fracture at the time of diagnosis.

REFERENCES

1. Van Dijk FS, Sillence DO. Osteogenesis Imperfecta: Clinical diagnosis, nomenclature and severity assessment. Am J Med Genet Part A. 2014;164A:1470–1481.
2. Van Dijk FS, Cobben JM, Kariminejad A, Maugeri A, Nikkels PG, van Rijn RR, Pals G. Osteogenesis Imperfecta: A Review with Clinical Examples. Mol Syndromol. 2011;2(1):1-20.

3. Marini JC, Blissett ARJ. New genes in bone development: what's new in osteogenesis imperfecta. Clin Endocrinol Metab. 2013;98:3095-103.
4. Online Mendelian Inheritance in Man (OMIM), from http://www.omim. org/ acessed 19 july 2015.
5. Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. J Med Genet. 1979;16:101-116.
6. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M, Mortier G, Mundlos S, Nishimura G, Rimoin DL, Robertson S, Savarirayan R, Sillence D, Spranger J, Unger S, Zabel B, Superti-Furga A: Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet Part A. 2011;155A:943–968.
7. Harrington J, Sochett E, Howard A. Update on the evaluation and treatment of osteogenesis imperfecta. Pediatr Clin North Am. 2014;61(6):1243-57.
8. Greeley CH, Donaruma-Kwoh M, Vettimattam M, Lobo C, Williard C, Mazur L. Fractures at Diagnosis in Infants and Children with Osteogenesis Imperfecta. J Pediatr Orthop. 2013;33:32–36.
9. Krakow D. Osteogenesis Imperfecta and Pregnancy. In: Shapiro JR, Byers PH, Glorieux F, Sponseller PD, editors. Osteogenesis Imperfecta: A Translational Approach to Brittle Bone Disease. USA: Elsevier. Academic Press 2013:243-250.
10. Renaud A, Aucourt J, Weill J, Bigot J, Dieux A, Devisme L, Moraux A, Boutry N: Radiographic features of osteogenesis imperfecta. Insights Imaging.2013;4:417–429.
11. Shapiro JR, Lietman C, Grover M, Lu JT, Nagamani SC, Dawson BC, Baldridge DM, Bainbridge MN, Cohn DH, Blazo M, Roberts TT, Brennen FS, Wu Y, Gibbs RA, Melvin P, Campeau PM, Lee BH. Phenotypic variability of osteogenesis imperfecta type V caused by an IFITM5 mutation. J Bone Miner Res. 2013; 28:1523-30.
12. Drera B, Ritelli M, Zoppi N, Wischmeijer A, Gnoli M, Fattori R, Calzavara-Pinton PG, Barlati S, Colombi M. Loeys-Dietz syndrome type I and type II: clinical findings and novel mutations in two Italian patients. Orphanet J Rare Dis. 2009;4:24.
13. Kivuva EC, Parker MJ, Cohen MC, Wagner BE, Sobey G. De Barsy syndrome: a review of the phenotype. Clin Dysmorphol. 2008;17(2):99-107.
14. Adam MP, Hennekam RC, Keppen LD, Bull MJ, Clericuzio CL, Burke LW, Ormond KE, Hoyme EH. Marshall-Smith syndrome: natural history and evidence of an osteochondrodysplasia with connective tissue abnormalities. Am J Med Genet A. 2005;137(2):117-24.

15. Kosho T, Miyake N, Hatamochi A, Takahashi J, Kato H, Miyahara T, Igawa Y, Yasui H, Ishida T, Ono K, Kosuda T, Inoue A, Kohyama M, Hattori T, Ohashi H, Nishimura G, Kawamura R, Wakui K, Fukushima Y, Matsumoto N. A new Ehlers-Danlos syndrome with craniofacial characteristics, multiple congenital contractures, progressive joint and skin laxity, and multisystem fragility-related manifestations. Am J Med Genet A. 2010;152A(6):1333-46.
16. Martínez Nogueiras A, Teixeira Costeira M, Saraiva Moreira H, Araujo Antunes H. Russel-Silver syndrome. An Esp Pediatr. 2001;54(6):591-4.
17. Parker MJ, Deshpande C, Rankin J, Wilson LC, Balasubramanian M, Hall CM, Wagner BE, Pollitt R, Dalton A, Bishop NJ. Type 1 collagenopathy presenting with a Russell-Silver phenotype. Am J Med Genet A. 2011;155A(6):1414-8.
18. Yancovitz M, Anolik R, Pomeranz MK. Alkaptonuria. Dermatol Online J. 2010;16(11):6.
19. Burkitt Wright EM1, Porter LF, Spencer HL, Clayton-Smith J, Au L, Munier FL, Smithson S, Suri M, Rohrbach M, Manson FD, Black GC. Brittle cornea syndrome: recognition, molecular diagnosis and management. Orphanet J Rare Dis. 2013;8:68.
20. Braddock SR. A new recessive syndrome with VATER-like defects, pulmonary hypertension, abnormal ears, blue sclera, laryngeal webs, and persistent growth deficiency. Am J Med Genet A. 2003;123A(1):95-9.
21. Bellary SS, Steinberg A, Mirzayan N, Shirak M, Tubbs RS, Cohen-Gadol AA, Loukas M. Wormian bones: a review. Clin Anat. 2013;26(8):922-7.
22. Marti B, Sirinelli D, Maurin L, Carpentier E. Wormian bones in a general paediatric population. Diagn Interv Imaging. 2013;94(4):428-32.
23. Santili C., Akkari M., Waisberg G., Bastos Junior J.O., Ferreira W.M. Clinical, radiographic and laboratory evaluation of patients with osteogenesis imperfecta. Rev. Assoc. Med. Bras. 2005;51(4):214–220.
24. Abukabbos H, Al-Sineedi F. Clinical manifestations and dental management of dentinogenesis imperfecta associated with osteogenesis imperfecta: Case report. Saudi Dent J. 2013;25(4):159-65.
25. Wieczorek A, Loster J, Ryniewicz W, Ryniewicz AM. Dentinogenesis imperfecta - hardness and Young's modulus of teeth. Acta Bioeng Biomech. 2013;15(3):65-9.
26. Brizola E, Staub AL, Félix TM. Muscle strength, joint range of motion, and gait in children and adolescents with osteogenesis imperfecta. Pediatr Phys Ther. 2014; 26:245-52.

27. Gahagan S, Rimsza ME: Child abuse or osteogenesis imperfecta: how can we tell? Pediatrics.1991;88:987–992.
28. Paterson CR, Burns J, McAllion SJ: Osteogenesis imperfecta: the distinction from child abuse and the recognition of a variant form. Am J Med Genet 1993;45:187-92.
29. Dogba MJ, Rauch F, Tre G, Glorieux FH, Bedos C. Shaping and managing the course of a child's disease: Parental experiences with osteogenesis imperfecta. Disabil Health J. 2014;7:343-349.
30. Greeley CS, Donnaruma-Kwoh M. Diagnosis in Infants and Children with Osteogenesis Imperfecta. J Pediatr Orthop. 2015;35(7):e82.
31. Viswanathan S, Khasawneh W, McNelis K, Dykstra C, Amstadt R, Super DM, Groh-Wargo S, Kumar D. Metabolic bone disease: a continued challenge in extremely low birth weight infants. JPEN J Parenter Enteral Nutr. 2014;38(8):982-90.

VERSÃO EM PORTUGUÊS

Características Clínicas e Padrão de Fraturas no Momento do Diagnóstico de Osteogênese Imperfeita em Crianças

RESUMO

Objetivo: Caracterizar o padrão de fraturas e a história clínica no momento do diagnóstico de Osteogênese Imperfeita (OI). **Métodos:** Neste estudo retrospectivo foram incluídos pacientes com diagnóstico de OI, ambos os sexos, idades entre 0 e 18 anos, que estavam em tratamento no Centro de Referência para OI, Porto Alegre, Brasil, entre 2002 e 2014. Os prontuários médicos foram revisados para coleta de dados clínicos no momento do diagnóstico de OI incluindo presença de escleras azuladas, dentinogênese imperfeita, história familiar positiva para OI e local das fraturas, e ainda exames radiográficos para confirmação de presença de ossos wormianos. **Resultados:** Setenta e seis pacientes (42 do sexo feminino) com diagnóstico de OI foram incluídos no estudo. A idade dos indivíduos no momento do diagnóstico variou entre 0 e 114 meses, com uma idade mediana (percentil 25-75) de 38 (6-96) meses. Escleras azuladas foram observadas em 93,4% dos pacientes; dentinogênese imperfeita em 27,6% e ossos wormianos foram observados em 29,4% dos pacientes. O número de fraturas no momento do diagnóstico variou entre 0 e 17, com uma mediana (percentil 25-75) de 3 (2-8) fraturas. Quarenta (57%) pacientes apresentaram fraturas dos membros superiores e inferiores, e nove pacientes também tiveram fraturas da coluna vertebral. OI foi diagnosticada ao nascimento em 85,7% dos pacientes com OI-3 e em 39,3% dos pacientes com OI-4/5. **Conclusão:** OI é uma doença genética com características clínicas distintas, tais como fragilidade óssea, fraturas recorrentes, escleras

azuladas e dentinogênese imperfeita. É importante saber como identificar essas características facilitando o diagnóstico, otimizando o tratamento e auxiliando na diferenciação de outras doenças que também podem causar fraturas.

Palavras-chave: Osteogênese Imperfeita, fratura óssea, caracterísiticas clínicas, diagnóstico clínico, diagnóstico diferencial

INTRODUÇÃO

Osteogênese Imperfeita (OI) é uma doença genética sistêmica do tecido conjuntivo com uma prevalência de 6-7/100.000 nascimentos (Van Dijk & Sillence, 2014). OI afeta todos os tecidos contendo colagéno, principalmente o tecido ósseo; sua principal característica é diminuição da massa óssea, o que torna o osso frágil e susceptível a deformidades e fraturas de repetição (Van Dijk et al., 2011;. Marini et al., 2013).

A maioria dos casos de OI apresenta herança autossômica dominante causados por mutações nos genes *COL1A1* ou *COL1A2*; entretanto, estudos recentes mostraram que a OI também pode ser causada por mutações em outros 19 genes envolvidos na biossíntese de colágeno ou função de osteoblastos com padrões de herança dominante, recessivo ou ligado ao X (van Dijk & Sillence, 2014; OMIM 2015). Devido à ampla heterogeneidade genotípica e fenotípica a OI tem sido classificada em diversos tipos de acordo com as características clínicas, os aspectos radiológicos e os genes responsáveis (Van Dijk et al., 2014; Marini et al., 2013). O Grupo Internacional de Nomenclatura para Transtornos Constitucionais do Esqueleto redefiniu a classificação clínica clássica de OI (Sillence et al., 1979) adicionando a OI tipo 5 (OI-5) aos quatro grupos originalmente descritos (Warman et al., 2011).

OI tipo 1 (OI-1) é uma forma leve caracterizada por nenhuma ou poucas fraturas e deformidades discretas. OI tipo 2 é o tipo mais grave, caracterizada por fragilidade óssea extrema levando à morte no período neonatal. OI tipo 3 (OI-3) é grave; os pacientes

apresentam múltiplas fraturas, deformidades ósseas significativas e baixa estatura. OI tipo 4 (OI-4) é uma forma moderada com grande variabilidade clínica na qual os pacientes podem apresentar poucas ou muitas fraturas associadas a deformidades ósseas (Sillence et al., 1979; Van Dijk & Sillence, 2014; Marini et al., 2013). Os indivíduos com OI-5 apresentam uma forma moderada com características clínicas e radiológicas distintas, como calcificação da membrana interóssea entre o rádio e a ulna e/ou a tíbia e a fíbula, formação de calo hiperplásico nos ossos longos, deslocamento da cabeça do rádio, e ausência de dentinogênese imperfeita (Van Dijk & Sillence, 2014).

Fraturas podem ocorrer durante toda a vida em pacientes com OI, mas a maioria destas fraturas ocorrem durante a infância. As características clínicas observadas em indivíduos com OI também podem ser observadas em outras doenças genéticas e metabólicas (Harrington et al., 2014). O diagnóstico de OI ainda é baseado em aspectos clínicos e radiológicos (Harrington et al., 2014; Warman et al, 2011). Testes genéticos não estão disponíveis em muitos países como exame de rotina ou não são cobertos pelo seguro ou sistema de saúde. Por esta razão, é importante para os profissionais que lidam com a população pediátrica em geral saber diferenciar e identificar esses casos específicos e estar ciente sobre as condições clínicas ao diagnóstico desta doença genética.

Este estudo foi conduzido para caracterizar o padrão de fraturas e a história clínica no momento do diagnóstico de OI em pacientes pediátricos.

MÉTODOS

Amostra

Neste estudo retrospectivo, foram revisados os prontuários de pacientes do sexo masculino e feminino com OI e idades entre 0-18 anos que realizavam tratamento no Centro de Referência em Osteogênese Imperfeita, Porto Alegre, Brasil, entre janeiro de 2002 e

janeiro 2014. Foram excluídos pacientes com outras causas primárias e secundárias de osteoporose como hipofosfatasia, deficiência de cálcio e uso de glucocorticóides.

Esta pesquisa foi aprovada pelo Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (nº. 13-0079) e todos os pacientes ou responsáveis assinaram um termo de consentimento livre e esclarecido.

Coleta de Dados

O diagnóstico de OI foi baseado em características clínicas e radiológicas de acordo com critérios de Sillence (Sillence et al., 1979). Foram coletados dados clínicos no momento do diagnóstico de OI como idade, história familiar positiva para OI e características clínicas de OI (escleras azuladas, dentinogênese imperfeita, número de fraturas e local das fraturas).

Exames radiográficos de rotina foram revisados com atenção especial para presença de ossos wormianos no crânio. As fraturas foram classificadas como ocorrendo nas extremidades inferiores (fêmur, tibia, fíbula) ou superiores (rádio, ulna, úmero) e/ou outro local específico.

O nosso estudo foi limitado aos achados presentes no momento do diagnóstico de OI e não pretende representar o curso natural da doença em crianças.

Análise Estatística

As variáveis contínuas foram expressas como medianas e mediana e percentil (25-75) devido à distribuição assimétrica de dados. As variáveis categóricas foram descritas por meio de frequências absolutas e relativas e comparadas entre os tipos de OI tipo usando o teste Chi-square. O nível de significância adotado foi de 5% ($p \leq 0,05$). As análises estatísticas foram realizadas utilizando o software SPSS (versão 18.0; SPSS Inc., Chicago, IL, EUA).

RESULTADOS

A amostra foi composta por 76 pacientes (42 do sexo feminino) com OI. Todos os casos de OI foram diagnosticados clínica e radiologicamente de acordo com a classificação de Sillence (Sillence et al., 1979); apenas o único caso de OI-5 foi confirmado por análise molecular. Para a análise estatística, o paciente com OI-5 foi agrupado com os indivíduos com OI-4, considerando que ambos os tipos representam formas moderadas de OI. A **tabela 1** mostra a idade dos pacientes no momento do diagnóstico e as características clínicas por tipo de OI. Quarenta e um pacientes (54%) apresentavam OI-1, 7 (9%) tinham OI-3, e 28 (37%) tinham OI-4/5. A idade dos indivíduos no momento do diagnóstico variou entre 0 e 114 meses, com uma mediana de idade (percentil 25-75) de 38 (6 -96) meses. A maioria (85,7%; n=7) dos pacientes com OI-3 e 39,3% (n=28) com OI-4/5 foram diagnosticados no período perinatal e 68,3% (n=41) dos indivíduos com OI-1 foram diagnosticados entre 1 e 5 anos de idade (**Figura 1**).

Trinta e três indivíduos (44,6%) tinham história familiar positiva para OI. Em dois casos, a história familiar era desconhecida, pois as crianças foram adotadas e os dados não estavam disponíveis. Do total de pacientes, 71 (93,4%) apresentavam escleras azuladas, 21 (27,6%) dentinogênese imperfeita (DI) e 15 (29,4%) ossos wormianos. O número de fraturas no momento do diagnóstico variou entre 0 e 17 com uma mediana (percentil 25-75) de 3 (2-8) fraturas. Apenas seis pacientes (7,8%) não apresentaram nenhuma fratura no momento do diagnóstico, quatro destes classificados com OI-1. A maioria (57,1%; n = 40) dos pacientes tiveram fraturas nas extremidades inferiores e superiores; 6 (23,1%) pacientes com OI-4/5 e 3 (8,1%) com OI-1 adicionalmente apresentaram fraturas da coluna (**Figura 2**). Um (1,4%) paciente com OI-1 apresentou fratura de crânio no momento do diagnóstico de OI seguido por uma queda acidental do berço.

DISCUSSÃO

Grande variabilidade fenotípica é observada em indivíduos com OI; no entanto, há um padrão de fraturas e de características clínicas que ajudam a caracterizar clinicamente a doença (Greeley et al., 2013). As características clínicas e radiológicas são a base para a identificação de casos de OI e para o diagnóstico. O estudo genético é útil e proporciona uma melhor compreensão da doença, mas não está disponível como exame de rotina em muitos centros de tratamento. Como uma doença rara, poucos profissionais têm experiência em reconhecer as características clínicas específicas da doença e ser capaz de distinguir estas características pode ajudar os profissionais envolvidos na assistência à saúde da criança.

Na amostra estudada, os tipos mais graves de OI foram diagnosticados em idade mais precoce quando comparados com formas leves de OI. Estes resultados corroboram com relatos anteriores de que fraturas tendem a ocorrer intraútero ou durante o período perinatal em indivíduos com formas mais graves de OI, e ainda que uma alta incidência de fraturas durante o crescimento pode levar ao desenvolvimento de deformidades progressivas nestes indivíduos (Van Dijk & Sillence, 2014; Marini et al., 2013). Formas não-letais de OI muitas vezes não são detectadas no período pré-natal e o diagnóstico ultrassonográfico pré-natal não é totalmente confiável para estes casos (Van Dijk et al., 2011; Cracóvia, 2013). Formas graves e letais de OI podem ser diagnosticadas por ultrassonografia durante o segundo trimestre da gravidez com base na detecção de anormalidades no crânio e nas costelas, curvatura dos ossos longos, diminuição da ecogenicidade óssea, micromelia, retardo do crescimento fetal, ventriculomegalia, polidrâmnios e ainda formação de calo ósseo secundário à fratura (Van Dijk et al., 2011; Krakow, 2013).

O diagnóstico pós-natal é baseado na história familiar positiva de OI, sinais clínicos, achados radiológicos e densitometria óssea; investigação adicional pode incluir análise bioquímica e molecular (Renaud et al., 2013; Van Dijk et al., 2011). Características

consideradas "clássicas" em OI como escleras azuladas, ossos wormianos, DI e fraturas podem estar ou não presentes nestes pacientes. Além disso, outros resultados podem ser observados como face triangular, baixa estatura, hiperextensibilidade articular, anormalidades cardiovasculares e oculares, perda auditiva, platibasia e invaginação basilar (Van Dijk et al., 2011; Van Dijk & Sillence, 2014; Marini et al., 2013). Restrição do movimento do antebraço, contraturas articulares progressivas e craniossinostose também podem ser observados em OI secundários a raras mutações nos novos genes relacionados a doença (Shapiro et al., 2013; Van Dijk & Sillence, 2014; OMIM, 2015).

Contudo, estas características não são exclusivas de OI e podem estar presentes em outras doenças. A maioria da nossa amostra (93,4%) apresentou escleras azuladas. No entanto, escleras azuladas também são relatadas em pacientes pediátricos “saudáveis” (Marti et al., 2013; Harrington et al., 2014), em indivíduos com deficiência de ferro (Beghetti et al., 1993) e em outras síndromes incluindo Loyes-Dietz (Drera et al., 2009), De Barsy (Kivuva et al., 2009), Marshall-Smith (Adam et al., 2005), Ehler-Danlos (Kosho et al., 2010) e Russel-Silver (Martinez Alogueiras et al., 2001; Parker et al., 2011). Além disso, escleras azuladas também são observadas em casos de alcaptonúria ocronose (Yancovitz et al., 2010), síndrome da córnea frágil (Burkitt et al., 2013) e em um estudo sobre dois irmãos com defeitos similares a sequência de VATER (VATER-like) e malformações múltiplas (Braddock de 2003).

Ossos wormianos, pequenos ossos supranumerários encontrados nas suturas e fontanelas do crânio, comumente presentes em OI também podem ser observados na população pediátrica normal e em outras condições, como disostose cleidocranial, picnodisostose, hipofosfatasia, hidrocefalia, hipotireoidismo congênito e síndrome de Down (Bellary et al., 2013; Marti et al., 2013). Nós observamos ossos wormianos em cerca de 30% das crianças com OI analisadas. Marti e colegas analisaram tomografias cerebrais para

avaliar a freqüência de ossos wormianos em uma população pediátrica "normal" com idades entre 0 e 3 anos e descobriram ossos wormianos em 53% destas crianças (n = 320). Sessenta crianças (10%) tiveram quatro ou mais osso wormianos (Marti et al., 2013).

DI estava presente em menos de um terço dos pacientes neste estudo. No entanto, muitos destes pacientes foram diagnosticados com OI previamente a idade de aparecimento da primeira dentição (Santili et al., 2005). Embora, em torno de 50% das crianças e adultos com OI talvez apresentem comprometimento dentário ao longo da vida, DI não é patognomônica para OI e é considerada a doença dentária com causa genética mais comum (Abukabbos & Al-Sineedi, 2013; Wieczorek et al., 2013). DI causa defeitos estruturais na formação da dentina nos dentes decíduos ou em ambas as dentições, decídua e permanente (Abukabbos & Al-Sineedi, 2013). Existem três tipos de DI, o tipo 1 está associado à OI secundário ao defeito no colágeno tipo 1, caracterizado pela cor âmbar típica e a translucidez dos dentes (Abukabbos & Al-Sineedi, 2013; Wieczorek et al., 2013).

Fragilidade óssea e suscetibilidade a fraturas sob nenhum ou mínimo trauma são características clássicas de OI. Neste estudo, as fraturas iniciais ocorreram mais tardiamente durante a infância em pacientes com OI-1 do que em pacientes com OI-3 e OI-4/5, e pacientes com OI-1 apresentaram menor número de fraturas no momento do diagnóstico. Estima-se que 10% das crianças com OI-1, a forma mais leve da doença e menos frequentemente associada a deformidades de ossos longos, não apresentam fraturas durante a infância (Van Dijk et al., 2011; Marini et al., 2013). Em um estudo anterior, observou-se que as fraturas iniciais tendem a ocorrer durante o período que as crianças começam a caminhar, pois a postura ereta promove aumento da descarga de peso nos membros inferiores, levando a fraturas secundárias (Brizola et al., 2014)

Em um estudo retrospectivo, Greeley e colegas descreveram o padrão de fraturas e as características clínicas em 68 recém-nascidos e crianças com idade inferior a 18 anos no

momento do diagnóstico de OI (Greeley et al., 2013). Vinte e seis (38%) participantes não apresentaram nenhuma fratura, 22 (32%) tiveram fraturas de extremidades e 15 (22%) tiveram fraturas de costela tendo sido a maioria destas fraturas diagnosticadas nas fases pré-natal e perinatal. O número de fraturas ao diagnóstico variaram entre 1 e >37 sendo que 7 (10%) indivíduos apresentaram mais de 2 fraturas. Escleras azuladas foram relatadas em 51 (75%) indivíduos e DI em apenas 11 (16%). Os autores concluíram que o número de fraturas, idade ao diagnóstico e localização das fraturas são características clínicas que podem auxiliar no diagnóstico de OI (Greeley et al., 2013).

Fratura completa ou incompleta da diáfise dos ossos longos e fratura por compressão da coluna toracolombar são observadas mais frequentemente em pacientes com OI (Renaud et al., 2013). A prevalência de fraturas é alta em crianças com OI, mas o padrão de fraturas difere do padrão de fraturas observado em vítimas de MTF (Renaud et al., 2013; Greeley et al., 2013). Casos de MTF são muito mais comuns do que casos de OI (Gahagan & Rimsza, 1993; Paterson et al., 1993), e pais de crianças com OI têm frequentemente relatado lidar com a suspeita de MTF por parte das equipes de saúde durante o tratamento inicial de fraturas, antes do diagnóstico de OI ser estabelecido (Dogba et al., 2014). OI e MTF não são mutuamente excludentes (Greeley e Donnaruma-Kwoh, 2015).

Em relação ao diagnóstico diferencial devem ser considerados diversos fatores que levam a um maior risco de fraturas, bem como transtornos que se assemelham à OI mimetizando as características clínicas da doença. Alguns exemplos são a doença óssea metabólica da prematuridade, osteoporose juvenil idiopática, síndrome de Ehlers-Danlos, hipofosfatasia, hiperfosfatasia idiopática, síndrome de osteoporose-pseudoglioma e deficiência de vitamina D e cálcio (Viswanathan et al, 2014; Harrington et al, 2014). Além disso, casos de suspeitos de MTF e causas secundárias de osteoporose, incluindo deficiências

hormonais, osteoporose induzida por glucocorticóides e leucemia linfoblástica aguda devem ser investigados (Greeley et al., 2013; Harrington et al., 2014).

O presente estudo apresenta limitações. É um estudo de natureza retrospectiva com base em dados registrados em prontuário. Alguns dados, principalmente o número de fraturas no momento do diagnóstico, não foram confirmados radiologicamente pois não havia documentação disponível para cada ocorrência de fratura nos prontuários. Desta forma os dados foram coletados com base na história clínica. Nossos dados refletem apenas achados clínicos e radiológicos no momento do diagnóstico de OI; eles não representam o padrão de fratura ao longo da vida para estes indivíduos.

CONCLUSÕES

Os nossos dados sugerem que as fraturas relacionadas à OI ocorrem em locais similares e que o número de fraturas varia de acordo com a gravidade clínica da doença. Fraturas de membros inferiores associadas a fraturas de membros superiores ocorrem com maior frequência em todos os tipos de OI e o número de fraturas foi maior em pacientes com as formas mais graves da doença. O padrão de fraturas ósseas e as características clínicas são parâmetros úteis para identificar casos de OI. Os profissionais de saúde devem estar cientes das características clínicas e típico padrão de fratura em pacientes com OI podendo assim diferenciar casos de OI de outros casos como doenças que causam fragilidade óssea ou casos de MTF.

CONFLITO DE INTERESSES

Os autores declaram não ter conflito de interesses.

CONTRIBUIÇÕES DOS AUTORES

Todos os autores participaram do desenho do estudo e da interpretação dos dados. Evelise Brizola e Têmis M. Félix redigiram o manuscrito. Todos os autores aprovaram a versão final do manuscrito.

AGRADECIMENTOS

Os autores agradecem aos pacientes e suas famílias pela participação neste estudo. Este estudo foi apoiado pelo Fundo de Incentivo à Pesquisa e Eventos/Hospital de Clínicas de Porto Alegre e pela Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul. Evelise Brizola foi apoiada pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Proc. Número 3770/14-1.

REFERÊNCIAS

Idem as referências na versão em inglês.

Figura 1. Idade no momento do diagnóstico de acordo com o tipo de OI

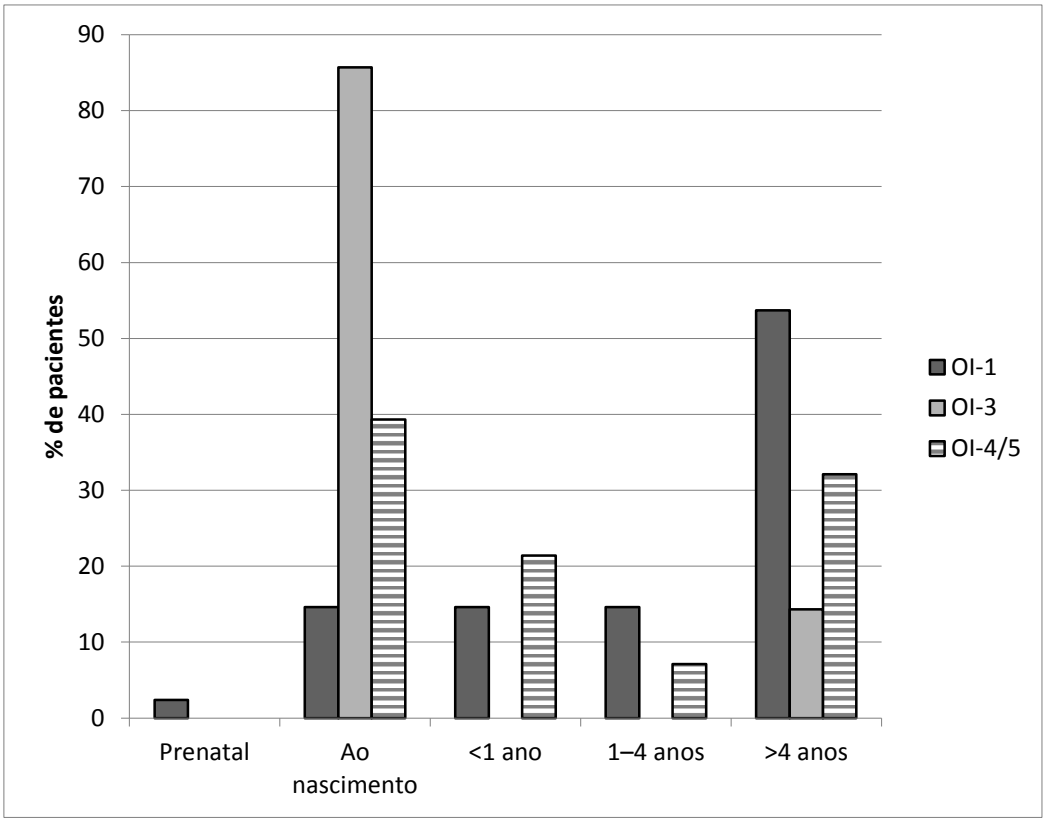

Figura 2. Paciente do sexo feminino com OI-4. O exame radiográfico da coluna vertebral tóraco-lombar mostrou fratura de múltiplos corpos vertebrais.

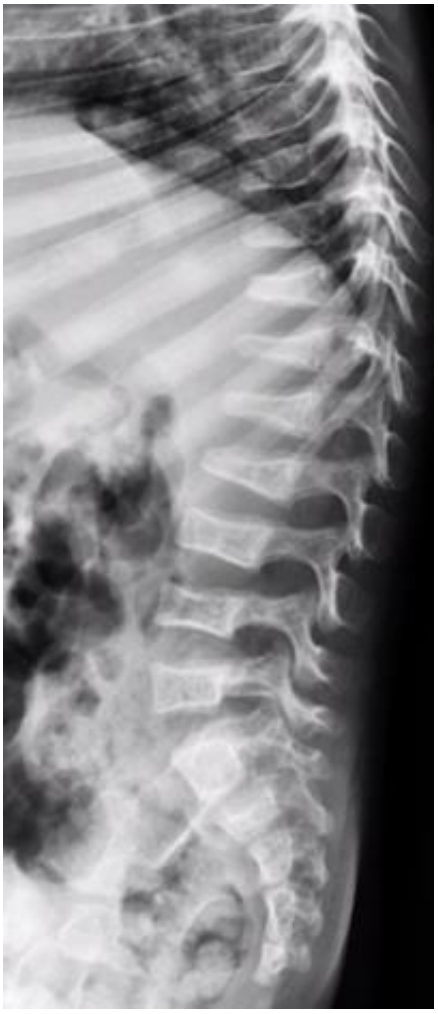

Tabela 1. Características clínicas no momento do diagnóstico por tipo de OI

Variável	Total	OI tipo 1	OI tipo 3	OI tipo 4/5	*P*
Número de pacientes n(%)	76 (100)	41 (51.3)	7 (9.2)	27/1 (36.8)	...
Sexo (masculino/feminino)	34/42	22/19	3/4	9/19	0.209
História familiar para OI positiva* n(%)	33 (44.6)	26 (66.7)	0	7 (25)	<0.001
Escleras Azuladas n(%)	71 (93.4)	38 (92.7)	7 (100)	26 (92.9)	0.762
Dentinogênese imperfeita n(%)	21 (27.6)	6 (14.6)	3 (42.9)	12 (42.9)	0.023
Ossos wormianos n(%)	15 (29.4)	6 (21.4)	2 (33.3)	7 (41.2)	0.361
Idade ao diagnóstico (meses)	38 (6–96)	67 (23–114)	3 (0–3)	16 (4–68)	0.001
Número de Fraturas ao Diagnóstico	3 (2–8)	3 (1.5–7)	4 (2–17)	6 (3–8.5)	0.76
Locais das fraturas ao diagnóstico+ n(%)					0.676
MsSs	9 (12.9)	6 (16.2)	1 (14.3)	2 (7.7)	
MsIs	11 (15.7)	6 (16.2)	1 (14.3)	4 (15.4)	
MsSs + MsIs	40 (57.1)	21 (56.8)	5 (71.4)	14 (53.8)	
MsSs + MsIs + coluna	9 (12.9)	3 (8.1)	0	6 (23.1)	
MsSs + MsIs + crânio	1 (1.4)	1 (2.7)	0	0	

OI: Osteogênese Imperfeita; MsSs: membros superiores; MsIs: membros inferiores.
Os dados são apresentados como n (%) ou mediana (percentil 25-75).
As porcentagens estão descritas de acordo com o tipo de OI para cada variável.
* História familiar desconhecida em dois casos.
+ Seis crianças não apresentaram nenhuma fratura no momento do diagnóstico.

6.4. METODOLOGIA II

6.4.1. Delineamento

Série de casos.

6.4.2. Amostra

Dentre 125 pacientes com OI registrados no Centro de Referência de Osteogênese Imperfeita do Hospital de Clínicas de Porto Alegre (CROI-HCPA), 35 pacientes de ambos os sexos, foram incluídos neste estudo.

Os casos foram selecionados de acordo com as características clínicas e radiológicas sugestivas de OI-V, e variou em idade de 1 a 52 anos. Dois casos adicionais, com características sugestivas, foram encaminhados por outros centros (Recife, PE, e Rio de Janeiro, RJ) para análise molecular.

6.4.3 Dados Clínicos

Os dados de prontuários e radiografias foram revisados para confirmação dos dados clínicos e presença de calcificação da membrana interóssea entre rádio-ulna e/ou tibia-fibula, bem como deslocamento da cabeça radial, formação ou remodelamento de calo hiperplásico, escoliose e fraturas de coluna.

A densidade mineral óssea foi mensurada através de DXA (*Dual Energy X-ray Absorptiometry*/ HOLOGICQDR-4500, versão 8,26: 3, Waltham, Massachusetts, EUA). Z-score ou T-score para massa óssea igual ou menor que 2 desvios padrão para a idade média do paciente foi definido como baixa densidade mineral óssea, seguindo o consenso oficial do brasileiro Sociedade de Densitometria Clínica em 2006 (Zerbini et al., 2007).

6.4.4. Análise Molecular

Para realização da análise molecular, 5 ml de sangue periférico foram coletados de cada paciente, e o DNA foi extraído de acordo com métodos padrão. Um fragmento de 370 pb, englobando a região 5'UTR do gene *IFITM5*, foi amplificado por PCR através do Termociclador Veriti® 96-Well (Applied Biosystems, Foster City, Calif., EUA). Os primers utilizados foram IFITM5_F 5′ CCGCAGGCTGTAATTTGTG 3′ (forward) e IFITM5_R 5′ CCACCTTGATGGAGTAGTGG 3′ (reverse), numa concentração final de 0,2 μM de cada.

As reações de PCR foram desenhadas com um volume final de 25 ul, incluindo 200 μM dNTPs, 1.5 mM Mg 2+, and 1 U Platinum Taq DNA polimerase (Life Technologies, Foster City, Calif., USA). Os produtos da amplificação foram purificados seguindo o protocolo Exo/SAP e submetidos à Sequenciamento por Sanger utilizando BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) num volume final de 10 μl em termociclador com uma fase inicial de desnaturação de 95° C durante 5 min seguido por 35 ciclos de 95° C por 30 segundos, 57° C por 30 segundos, 72° C por 30 segundos, e na fase final de extensão 72° C por 5 minutos. As amostras foram submetidas a eletroforese capilar no ABI 3130XL Genetic Analyzer (Applied Biosystems). Os eletroferogramas resultantes foram vistos e analisados utilizando-se o Software Chromas Lite (Technelysium Pty, Tewantin, Qld., Austrália).

6.4.5. Aspectos Éticos

Este estudo foi aprovado pelo Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre (nº. 13-0079) e todos os pacientes ou responsáveis assinaram um termo de consentimento informado.

6.5. Resultados

Os resultados serão apresentados no formato de artigo científico. O estudo intitulado "*Clinical and Molecular Characterization of Osteogenesis Imperfecta Type V*" foi publicado no periódico "*Molecular Syndromology*".

Clinical and molecular characterization of Osteogenesis Imperfecta type V

Evelise Brizola [1], Eduardo P. Mattos [2], Jessica Ferrari [3], Patricia O.A. Freire [4], Raquel Germer [5], Juan C. Llerena Jr [5], Têmis M. Félix [1,3]

[1] Post Graduate Program in Child and Adolescent Health, Faculty of Medicine, Universidade Federal of Rio Grande do Sul, Porto Alegre, RS, Brazil

[2] Post Graduate Program in Genetics, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

[3] Medical Genetics Service, Hospital de Clinicas de Porto Alegre, Porto Alegre, RS, Brazil

[4] Hospital da Polícia Militar de Pernambuco, Recife, PE, Brazil

[5] Medical Genetics Center, Instituto Nacional de Saúde da Mulher, da Criança e do Adolescente, Fernandes Figueira/FIOCRUZ, Rio de Janeiro, RJ, Brazil

ABSTRACT

Osteogenesis Imperfecta (OI) type V (OI-V) has a wide clinical variability, with distinct clinical/radiological features such as interosseous membrane calcification (CIM) between the radius-ulna and/or tibia-fibula, hyperplastic callus formation (HPC), dislocation of the radial head (DRH), and absence of dentinogenesis imperfecta (DI). Recently, a single heterozygous mutation (c.-14C > T) in the 5'-UTR of the *IFITM5* gene was identified to be causative for OI-V. Here, we describe seven individuals from five unrelated families that carry the c.-14C> T *IFITM5* mutation. The clinical findings from these cases are: absence of DI in all patients, presence of blue sclera in two cases, and four patients with DRH. Radiographic findings revealed HPC in three cases. All patients presented CIM between the radius and ulna, while two patients presented additional CIM between the tibia and fibula. Spinal fractures by vertebral compression were observed in all subjects. The proportion of cases identified with this mutation represents 4% of OI cases at our institution. The clinical identification of OI-V is crucial, as this mutation has an autosomal dominant inheritance with variable expressivity.

Key Words: Osteogenesis Imperfecta, *IFITM5* gene, Autosomal dominant

INTRODUCTION

Osteogenesis imperfecta (OI) is a collective term for a set of connective tissue syndromes with heterogeneous etiology characterized by bone fragility, susceptibility to bone deformity, and the occurrence of fractures. OI is considered to be the most common skeletal dysplasia, with estimated prevalence of around 6-7 per 100,000 births [Van Dijk et al., 2010; Van Dijk and Sillence, 2014].

Several molecular studies identified 17 genes, primarily involved in the biosynthesis of collagen, in which mutations can be causative for OI. However, the International Nomenclature Group for Constitutional Disorders ICHG of the Skeleton (INCDS) standardized the classification of OI by keeping the four types that were originally described by Sillence (1979), while adding OI type V [Warman et al., 2011; Van Dijk and Sillence, 2014]. This current classification is based on the severity and clinical features of the disorder and is comprised of: mild (OI type I/ OI-I), lethal (OI type II/ OI-II), severely deforming (OI type III/ OI-III), moderately deforming (OI type IV/ OI-IV), and OI type V (OI-V).

OI-V (OMIM #610967) is a non-lethal form that presents with wide variability in its severity, distinct clinical characteristics, and autosomal dominant inheritance pattern [Shapiro et al., 2013; Van Dijk and Sillence, 2014]. It was originally described in 1908 by Battle and Shattock as a specific type of OI in which calcification of the interosseous membrane was observed in the forearms and legs. Further studies complement this description, adding other specifically clinical characteristics and suggesting that OI-V correspond to 4-5% of all OI cases [Bauze et al., 1975; Glorieux et al., 2000; Van Dijk and Sillence, 2014].

Among the main features of OI-V, the formation of hyperplastic callus (HPC) after corrective surgery or fracture is often observed, as well as the absence of dentinogenesis imperfecta (DI), progressive calcification (ossification) of the interosseous membrane (CIM) between radius-ulna and/or tibia-fibula, and dislocation of the radial head (DRH) that causes restriction in the pronation and supination movement of the forearm [Glorieux et al., 2000; Cho et al., 2012; Shapiro et al., 2013].

In 2012, a single heterozygous mutation (c.-14C> T) in the 5' untranslated region (5’UTR) of the *IFITM5* gene encoding the interferon induced transmembrane protein 5 was

described to be causative for OI-V. This mutation adds five amino acids (Met-Ala-Leu-Glu-Pro) to the N-terminus of the protein. The product of the *IFITM5* gene, located on chromosome region 11p15.5, is also known as Bone Restricted IFITM-like protein (BRIL), a protein with 132 amino acids that has two transmembrane domains and an intracellular loop [Semler et al., 2012; Cho et al., 2012]. After this specific mutation was published, several studies reported cases of individuals with the same mutation and phenotype of OI-V (Semler et al., 2012; Cho et al., 2012; Takagi et al., 2012; Zhang et al., 2013; Kim et al., 2013; Balasubramanian et al., 2013; Shapiro et al., 2013; Rauch et al., 2013; Grover et al., 2013; Guillén-Navarro et al., 2014; Lazarus et al., 2014; Reich et al., 2015).

The aim of this study was to describe the clinical and radiological characteristics of OI type V, in addition to analyzing the c.-14C> T mutation in *IFITM5* gene and compare to cases already reported in the literature.

METHODS

Patients

This study was approved by the Ethics Committee of the Hospital de Clínicas de Porto Alegre (number: 13-0187) and all patients or guardians signed an informed consent**.**

Of the 125 registered OI patients at the Reference Center of Osteogenesis Imperfecta at Hospital de Clínicas de Porto Alegre (CROI-HCPA), 35 patients, both male and female, were included in this study. The cases were selected according to clinical and radiological features of OI-V, and ranged in age from 1 to 52 years old. Two additional cases, with suggestive features, were referred from other centers (Recife, PE and Rio de Janeiro, RJ) for molecular analysis.

Data from medical records and radiographic images were reviewed for confirmation of clinical data and for the presence of CIM between the radius-ulna and/or the tibia-fibula, as well as dislocation of the radial head, HPC formation or remodeling, scoliosis, and spine fractures. Bone mineral density was measured by DEXA (Dual Energy X-ray Absorptiometry/ HOLOGICQDR-4500, version 8.26:3, Waltham, Massachusetts, USA). A Z-score for bone mass that was 2 standard deviations or more below the mean for the age of the patient was defined as low bone mineral density, following the official consensus of the Brazilian Society for Clinical Densitometry in 2006 [Zerbini et al., 2006].

Molecular Analyses

To conduct the molecular analyses, 5 ml of peripheral blood were collected from each patient and DNA was extracted according to standard methods. A 370-base pair fragment, encompassing the 5´UTR of *IFITM5* gene, was amplified by polymerase chain reaction (PCR) using a Veriti® 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The primers used were IFITM5_F 5' CCGCAGGCTGTAATTTGTG 3' (forward) and IFITM5_R 5' CCACCTTGATGGAGTAGTGG 3' (reverse), in a final concentration of 0.2 μM each. PCR reactions were designed to a final volume of 25 μL, with 200 μM dNTPs, 1.5 mM Mg2+, and 1 U Platinum Taq DNA polymerase (Life Technologies, Foster City, CA, USA). Amplification products were purified by the Exo/SAP protocol and subjected to Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) in a final volume of 10 μL. Labeling reactions were performed in a thermocycler with an initial denaturing step of 95 ºC for 5 min followed by 35 cycles of 95 ºC for 30 sec, 57 ºC for 30 sec 72 °C for 30 sec and final extension step of 72ºC for 5 min. Samples were submitted to capillary electrophoresis in an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The resulting

electropherograms were viewed and analyzed using Chromas Lite software (Technelysium Pty, Tewantin, Australia).

RESULTS

Among the 35 individuals analyzed at CROI-HCPA, five cases were found to harbor the OI-V-associated c.-14C> T mutation in the *IFITM5* gene. Additionally, two individuals referred from others centers were also positive for this mutation (**Figure 1**). Clinical data and radiographic findings are described in **Table 1**. Of the seven patients harboring the *IFITM5* mutation, there were four males and three females. Three cases were isolated, while the other four had a positive family history of OI. Absence of DI was observed in all patients; four patients had DRH, and one had a bilateral displacement. **Figure 2** shows patient and radiological images. HPC was confirmed via X-ray in three cases, and was suspected in one additional case. All patients had CIM between the radius-ulna, and four individuals had additional CIM between the fibula-tibia. Spine fractures, by vertebral compression, as well as a characteristic pyramidal shape of the chest and ribs, were observed in all subjects.

CLINICAL REPORTS

All cases reported here had a confirmed diagnosed of the c.-14 C>T mutation in the *IFITM5* gene.

Case 1

Male, 14 years old, third child of non-consanguineous parents (**Figure 2A,B**). His father (Case 2), aunt (Case 3), and paternal grandfather had a history of multiple fractures. He was born by cesarean section with a birth weight of 2430g (2^{nd} percentile) and a length of 46 cm

(2nd percentile). A large fontanel was observed at birth. At three months old, upon admission to the hospital for pneumonia, rib fractures were observed on x-ray. This patient had approximately 27 fractures distributed over the arms, forearms, femurs, legs, ribs, and spine (**Figure 2C**). The latest fracture identified was at spine at 13 years of age, and was treated with a brace. The patient has an intramedullary rod in the right femur and possesses an independent gait. A physical examination at 14 years old showed a height of 158.5 cm (25 th-50th percentile) and a weight of 62.8 kg (75th - 90th percentile), with a fronto-occipital circumference (OFC) of 56.5 cm (50th - 98th percentile). Ligamentous laxity, restriction of pronation and supination of the forearms, deformities of long bones, flat and pronated feet, absence of blue sclera, and dentinogenesis imperfecta were observed. This patient has been on intravenous pamidronate treatment since two years of age. Radiographic examination showed deformities of the long bones and ribs, CIM between the forearm bones (**Figure 2D**) and leg bones, and proximal dislocation of the radius head. At three years of age, bone mineral density was 0.450g (Z-score: -5.8) at the spine and 0.509 g/cm² (Z-score: -3.5) for the whole body. At age 13 years old, the spinal bone mineral density score was 0.955g/cm² (Z-score: 0.7) and the whole body score was 0.981g / cm² (Z-score: 1.1).

Case 2

Male, 52 years old, father of Case 1 (**Figure 2 E-F**). His first fracture, in the humerus, occurred at 12 months of age and he reports a history of 16 fractures distributed among the clavicle, patella, forearms, arms, feet, and hands. He has a constant complaint of back pain. His height was 164.5 cm (5th percentile) with a weight of 59.3kg (10th - 25th percentile). Upon physical examination, a slightly blue sclera, limitation of pronation and supination movements of the forearms, no DI, and independent gait were observed. The patient had used an orthopedic brace for several years due to scoliosis, and had received treatment with oral bisphosphonate. Radiographic examination revealed deformity of the long bones and

ribs, scoliosis associated with fracture by compression in several thoracolumbar vertebrae (**Figure 2G**), dislocation of the radial head and CIM of the bones of the forearms (**Figure 2H**) and legs. A protuberant remodeled callus on humerus suggestive of HPC was noted. Bone mineral densitometry showed 0.747g/cm², (T-score: -3.9) in the spine and 0.847g/cm² (T- score: -1.8) for the total femur.

Case 3

Female, 52 years old, is the paternal aunt of Case 1 and sister to Case 2 (**Figure 2I,J**). Her first fracture occurred at 13 years of age in the right tibia and fibula. She had approximately seven fractures distributed among the femur, tibia, fibula, elbows, toes, and hands. On physical exam, her height was 127.5 cm (<2nd percentile) and weight 47.7 kg (10th percentile). Bone deformities, particularly an anterior bowing of the tibias, limitation of pronation and supination movements of the forearms, and the lack of blue sclera and DI were observed. She was able to walk with support of an auxiliary device. She has received treatment with oral bisphosphonate. At 51 years old, she was diagnosed with melanoma in the neck and underwent surgical intervention. She presented with joint degeneration in the right hip, and was determined to be a candidate for hip replacement surgery. The bone densitometry showed osteoporosis in all spine (BMD: 0.654g/cm² /T-score: -4.4 SD) and distal radius (0.268g/cm²/T-score: -4.3 SD), with proximal femoral osteopenia (BMD: 0.722g/cm²/T-score: -1.8 SD). X-ray showed deformities of the long bones and ribs, with scoliosis associated with vertebral fractures (**Figure 2K**), dislocation of the radial head and CIM between the bones of the forearms (**Figure 2L**).

Case 4

This patient is an 18 years old female with no available family history due to her being adopted at 12 months of age. Her birth weight was 2900 g (10th - 25th percentile) with a

length of 47 cm (3rd - 5th percentile) and an OFC was 34 cm (25th percentile). A large fontanel and fracture of the left clavicle was noted. She had multiple fractures distributed between the spine, pelvis, upper, and lower limbs. The latest fracture was in the pelvis at age 17. Her height was 137 cm (<3rd percentile) and her weight 36 kg (<3rd percentile). On clinical examination, blue sclera, bone deformities in the arms and legs, and a lack of DI were noted. She had intramedullary rodding in the right femur and left tibia. Up until 14 years of age, she was able to walk at home with aid; however, upon the occurrence of a new fracture, she became dependent on a wheelchair. She was treated with cyclic pamidronate between 7 and 12 years of age, and had received 14 cycles before the pamidronate was replaced by oral bisphosphonate (alendronate). X-ray showed bone deformities in the long bones of the forearms and legs, vertebral fractures, and CIM between the bones of the forearms and legs.

Case 5

Male, 30 years old, second son of non-consanguineous parents with no family history of OI (**Figure 2M,N**). His first fracture occurred between three and six months of age, and he was diagnosed with OI at the age of 5 years. He presented with approximately 30 fractures distributed among the spine, humerus, radius, ulna, femur, tibia, ribs, hands, and skull. The latest fracture occurred in the 5th metacarpal when he was 29 years old. Clinical evaluation showed a height of 146.6 cm (<3rd percentile), a weight of 49 kg (<3rd percentile), laxity of ligaments, lack of blue sclera and DI, bone deformities such as bowing of the long bones in the upper and lower limbs, limitation of pronation and supination movements of the forearms, and various deformities of the feet. He was currently taking oral bisphosphonate (alendronate) and has an independent gait. X-rays at age 29 years showed levoconvex scoliosis with a Cobb's angle of 10 degrees, severe reduction of vertebrae height due fractures by compression of virtually all vertebral bodies (**Figure 2O**), and deformities of the ribs and long bones, with DRH on the forearms, CIM between radius and ulna (**Figure**

2P), and HPC in the left humerus. Bone mineral density measurements showed 0.741g/cm² (T-score: -1.9) in the femoral neck and 0.610g/cm² (T- score: -4.4) in the spine.

Case 6

Female, three-year-old, was referred from Recife, PE, Brazil (**Figure 2R,S**). She is the first daughter of non-consanguineous parents with no family history of OI. Diagnosis of OI was made after the first year of life, at which time she presented with four fractures that were distributed among the femur, humerus, and spine. Upon physical examination, her height was 95.5 cm (75^{th} - 90^{th} percentile), her weight was 15.2 kg (75^{th} - 90^{th} percentile) and she presented with joint laxity, scoliosis, and the absence of blue sclera and DI. She was treated with cyclic pamidronate and has an independent gait. Radiographic exam revealed the presence of wormian bones in the skull, dextroconvex scoliosis, and deformity of the ribs (**Figure 2T**) as well as microfractures that reduced the height of several vertebral bodies with greater involvement between the thoracic and lumbar spine transition at age 2 years. A femur fracture under consolidation with hyperplastic callus formation in the site of the fracture was also observed (**Figure 2U**), and CIM between the forearms bones. Lower limb X-rays showed a difference of 1.01 cm between the limbs, with the right limb being shorter.

Case 7

Male, 10 years old, was referred from Rio de Janeiro, RJ, Brazil (**Figure 2V-X**). He is the second son of non-consanguineous parents; his mother and brother have OI. His first fracture occurred at 2 years of age, with a total of 6 fractures distributed between the right and left radius and the right tibia. Following the first fracture, surgical intervention for the placement of intramedullary rodding was performed. His height was 1.41 cm (75^{th} - 90^{th} percentile) and weight 53kg (>97^{th} percentile). He was observed to have dorsal kyphosis, bone deformities of the forearms, and the absence of blue sclera and DI. He had an independent gait, and had

been on oral bisphosphonate for 1 year. X-ray revealed a fracture by compression at the 3rd lumbar vertebra (**Figure 2Z**) at age 10 years, bone deformities in the radius, ulna and ribs, and CIM between both forearm and leg bones. Bone mineral density for the right hip and lumbar spine was 0.888 g/cm2 (Z-score: 1.7) and 0.734 g/cm2 (Z-score: 0.5), respectively.

DISCUSSION

We describe seven previously unreported cases with the diagnosis of OI-V with the c.-14C>T mutation in the 5' UTR of the *IFITM5* gene. This number corresponds to approximately 4% of all OI cases at CROI-HCPA. Although these individuals have been identified to harbor the same mutation, the severity of the disease phenotype varies significantly. *IFITM5* is a member of the interferon-induced transmembrane gene family, and this specific heterozygous mutation has been described to be causative for OI-V [Semler et al., 2012; Cho et al., 2012]. The specific function of *IFITM5* in bone development has not been well elucidated, but it is known to increase ectopic ossification and appears to be involved in
the process of bone formation and osteoblast maturation [Reich et al., 2015]. The expression of wild *IFITM5* has been shown to be limited to osteoblasts, and the expression of mutant *IFITM5* is restricted to bone and cartilage [Semler et al., 2012; Cho et al., 2012].

In vitro studies using mouse models have showed that the product of the *IFITM5* gene plays a role in mineralization during bone growth in the early mineralization stage and in pre-natal development [Moffatt et al., 2008; Hanagata et al., 2011]. A neomorphic effect of *Ifitm5* mutation in the bone was observed in a recent study of mutant *iftim5* transgeni*c* mice. The OI type V mutation (c.-14C>T) in *Ifitm*5 led to low bone mass, abnormal osteoblast differentiation, delayed skeletal development, and skeletal defects in these mice

that included abnormal rib cage formation, long bone deformities, and fractures [Lietman et al., 2015].

The clinical presentation of OI-V associated to this mutation ranges from moderate to severe and the literature has described specific clinical features of this type of OI. The main features are absence of DI and, HPC, CIM, and DRH with frequencies around 64%, 88%, and 78%, respectivelyx [Semler et al., 2012; Cho et al., 2012; Takagi et al., 2012; Zhang et al., 2013; Kim et al., 2013; Balasubramanian et al., 2013; Shapiro et al., 2013; Rauch et al., 2013; Grover et al., 2013; Guillén-Navarro et al., 2014; Lazarus et al., 2014; Reich et al., 2015]. However, some studies including patients with clinical diagnosis of OI-V were published before this specific related mutation was found in 2012 [Glorieux et al., 2000; Lee et al., 2006; Zeitlin et al., 2006].

In general, these patients do not have discoloration of the sclera or wormian bones in the skull, but do present with frequent fractures that lead to bone deformity, short stature, scoliosis, vertebral compression fractures, and joint hypermobility [Glorieux et al., 2000; Cho et al., 2012; Shapiro et al., 2013].

In the current study, we observed only two patients with blue sclera. Consistent with our findings, Shapiro et al. (2013) reported two out of 17 patients with blue sclera, while only two other studies have cited two or more cases of patients with OI-V presenting with blue sclera [Grover et al, 2013; Guillén-Navarro et al, 2014]. Regarding DI, we observed no impairments of dentin, which is consistent with several previous studies [Glorieux et al., 2000; Lee et al., 2006; Zeitlin et al., 2006; Semler et al., 2012; Cho et al., 2012; Takagi et al., 2013; Zhang et al., 2013; Shapiro et al., 2013; Guillén-Navarro et al., 2014].

Short stature seems to be frequent among individuals with OI-V; nine previous studies in which 85 individuals were described included 57 who presented with reduced stature [Glorieux et al., 2000; Lee et al., 2006; Cho et al., 2012; Takagi et al., 2012; Semler et al., 2012;

Hoyer-Kuhn et al., 2013; Rauch et al., 2013; Balasubramanian et al., 2013; Guillén-Navarro et al., 2014]. Only one of the individuals in our study was dependent on a wheelchair for mobility, although this person had been able to walk independently through adolescence. Cho et al. (2012) observed, among a cohort of 19 individuals, only eight with fully independent walking. In another study, among 16 subjects observed with this criterion, two presented gait with support, and four patients were dependent on a wheelchair for mobility [Shapiro et al., 2013].

Bone fragility is the main feature of OI, and OI type V with the *IFITM5* mutation appears to be no exception. A wide variability in the age of the first fracture, as well as the total number of fractures, was seen in the patients in the current study. This is consistent with previous reports that included patients with the same mutation, which find that the number of fractures is indeed highly variable; frequencies from zero to 30 fractures have been reported [Cho et al., 2013; Kim et al., 2013; Lazarus et al., 2014; Reich et al., 2015]. Zhang et al (2013) described four Chinese families with individuals aged between four and 29 years with the occurrence of three to 11 fractures. In our sample, two subjects had fractures at birth, and one had the first fracture at 13 years of age. This criterion also was analyzed in two previous studies; in the first study, 42% of patients had fractures at birth [Rauch et al., 2013]. In the second, three subjects experienced the first fracture before were born, still in utero [Reich et al., 2015].

Furthermore, we observed that all the patients had vertebral fractures by compression, and only one individual did not have scoliosis. Two previous studies have described the occurrence of vertebral fractures in 87% and 100% of the cases [Lee et al., 2006; Shapiro et al., 2013] and scoliosis was observed at a frequency between 50% and 76% in five studies involving 98 subjects [Glorieux et al., 2000; Zeitlin et al., 2006; Kim et al., 2013; Rauch et al., 2013]. Also relevant, the investigators in all these cases observed the patients to exhibit a pyramidal shape of the chest and ribs with unusual format and downward slanting [Kim et al., 2013]. The

cause for this alteration of the rib position and shape is unknown, and this phenotype has not been described in other types of OI.

Changes in bone mineralization arising from an increase in ectopic ossification lead to a characteristic osteoporotic phenotype in all these patients; however, they also present with exuberant bone formation, reflected by the HPC development, which is unique to this type of OI [Cheung et al, 2007; Cho et al, 2012; Shapiro et al., 2013]. Histomorphometrically, there are also differences in OI-V, as these individuals exhibit a lower rate of bone remodeling activation due lower bone formation and resorption parameters when compared to patients with OI type IV [Glorieux et al., 2000].

In addition, we observed the presence of HPC only in the long bones and after fractures had occurred. HPC is a soft tissue anomaly characterized by exuberant bone formation with extensive expansion beyond the site of fracture, which occurs following fracture or surgery, and whose etiology remains unclear [Cheung et al., 2007; Shapiro et al., 2013]. In general, during the formation of HPC, biochemical changes are observed that include increased of serum alkaline phosphatase and urinary excretion of N-telopeptide [Glorieux et al., 2000]. According to Cheung et al. (2007), fractures are the most common complication resulting from HPC, and may lead to bone deformation. Additionally, a differential diagnosis with osteosarcoma needs to be carefully investigated.

All subjects presented CIM in the upper limbs and 58% had dislocation of the radial head. CIM has often been observed in subjects with OI-V; similar to our findings, a high frequency of CIM around 80% and 100% have been reported in different studies [Cho et al., 2012; Kim et al., 2013; Shapiro et al., 2013; Rauch et al., 2013; Lazarus et al., 2014]. However, a greater variability in the presentation of DRH has been reported in the literature. In general, the frequency of DRH described varies between 60% and 100% in the OI-V individuals;

meanwhile, Semler et al. (2012) did not observed DRH in either of two individuals analyzed [Cho et al., 2012; Kim et al., 2013; Shapiro et al., 2013; Rauch et al., 2013;Lazarus et al., 2014].

Clinical intrafamilial variability had been reported in OI type V [Shapiro et al., 2013; Rauch et al, 2013] as was observed in this study. Bone deformities, lack of DI and white sclerae were common clinical features between the described family members (cases 1, 2 and 3); however, case 3 had a severe short stature and "saber shin" deformity of the tibias. Also, case 1 rapidly developed a severe scoliosis (Cobb's angle > 45 degrees) when he started puberty, differently from other family cases that also had spine deformity but in a milder presentation. On the family of case 7, mother and brother also had OI and experienced only a few fractures throughout life (brother: 9 fractures; mother: 2 fractures). None member of the family had DI, blue sclerae or DRH; nonetheless, both siblings had CIM between radius-ulna and, and only the brother had HPC. Molecular analysis for OI-V was performed just for the case presented.

In our study, all patients had received treatment with oral or intravenous bisphosphonate; however, the efficacy of this drug was not evaluated since this was not the goal of this study. The bone mineral density (BMD) appears to be lower in the adults compared to the children, but we could not interpret this data due to influence of bisphosphonate effect, different type of bisphosphonates, and varying schedules of treatment.

Among individuals with negative result to *IFITM5* mutation analysis, the severity of OI ranged from mild to severe (OI-I: 3/ OI-III: 10/ OI-IV: 17). White and blue sclerae were observed and 21 individuals had DI. In addition, the majority of these patients experienced multiples fractures since childhood and had some degree of join mobility restriction at forearm but followed by deformity secondary to fracture or surgery intervention at the site. Some of these patients had scoliosis or vertebrae fracture but none had the alteration of thorax shape similar to the individuals with positive mutation result. One patient had a very severe scoliosis

going through surgery when she was only 11 years old and a second one had the first vertebral fracture at early age of 6 years old.

The present study has limitations; some of the data were retrospective, based on data recorded in medical charts. We did not have radiographic confirmation for all fractures; therefore, we used also clinical histories according to family information. Bisphosphonate treatment and bone densitometry results were not evaluated, because of the many variables involved that were not within the scope of this study.

OI-V corresponds to 4% of OI cases in Brazil, which is similar to the overall incidence in other populations. All subjects described on this report had the c.-14C> T *IFITM5* mutation and OI-V phenotype similar to other populations previous described. CIM seems to be the most frequent feature present in this type of OI, as well as the absence of DI. Confirmation of the specific *IFITM5* mutation for diagnostic confirmation purposes is essential, as this will contribute to more specific genetic counseling and clinical management in these families.

ACKNOWLEDGEMENTS

We are grateful to the patients for their participation in this study and their families. The study was supported by Fundo de Incentivo à Pesquisa e Eventos/Hospital de Clínicas de Porto Alegre and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul. Ms. Brizola was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Proc. Número 3770/14-1.

DISCLOSURES

All the authors were involved in study design and data interpretation. Evelise Brizola and Têmis M. Félix drafted the manuscript. All the authors read and approved the final version of the manuscript.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

REFERENCES

Balasubramanian M, Parker MJ, Dalton A, Giunta C, Lindert U, et al: Genotype-phenotype study in type V osteogenesis imperfecta. Clin Dysmorphology 22(3): 93-101 (2013).

Battle WH, Shattock SG: A remarkable case of diffuse cancellous osteoma of the femur following a fracture, in which similar growths afterwards developed in connection with other bones. Proc R Soc Med 1:83–114 (1908).

Bauze RV, Smith R, Francis MJO: A new look at osteogenesis imperfecta. A clinical, radiological and biochemical study of forty-two patients. J Bone Joint Surg 57B:2 (1975).

Cheung MS, Glorieux FH, Rauch F: Natural history of hyperplastic callus formation in osteogenesis imperfecta type V. J Bone Miner Res 22:1181–1186 (2007).

Cho TJ, Lee KE, Lee SK, Song SJ, Kim KJ, et al: A single recurrent mutation in the 5'UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Hum Genet 91(2):343–8 (2012).

De Onis M, Onyango A, Borghi E, Siyam A, Blössner M, et al: Worldwide implementation of the WHO Child Growth. Public Health Nutr 15(9):1603-10 (2012).

Glorieux FH, Rauch F, Plotkin H, Ward L,Travers R, et al: Type V osteogenesis imperfecta: a new form of brittle bone disease. J Bone Miner Res 15(9):1650– 8 (2000).

Grover M, Campeau PM, Lietman CD, Lu JT, Gibbs RA, et al:. Osteogenesis imperfecta without features of type V caused by a mutation in the IFITM5 gene. J Bone Miner Res28:2333–7 (2013).

Guillén-Navarro E, Ballesta-Martinez MJ, Valencia M, Bueno AM, Martinez-Glez V, et al: Two mutations in IFITM5 causing distinct forms of osteogenesis imperfecta. Am J Med Genet A164(5): 1136–1142 (2014).

Hanagata N, Li X, Morita H, Takemura T, Li J, et al: Characterization of the osteoblast-specific transmembrane protein IFITM5 and analysis of IFITM5-deficient mice. J Bone Miner Metab 29:279–290 (2011).

Hoyer-Kuhn H, Semler O, Garbes L, Zimmermann K, Becker J, et al: A Non-Classical IFITM5 Mutation Located in the Coding Region Causes Severe Osteogenesis Imperfecta with Prenatal Onset. J Bone Miner Res10.1002/ jbmr. 2156 (2013).

Kim OH, Jin DK, Kosaki K, Kim JW, Cho SY, et al:. Osteogenesis imperfecta type V: Clinical and radiographic manifestations in mutation confirmed patients. Am J Med Genet A161A:1972–9 (2013).

Lazarus S, McInerney-Leo AM, McKenzie FA, Baynam G, Broley S, et al: The IFITM5 mutation c.-14C > T results in an elongated transcript expressed in human bone; and causes varying phenotypic severity of osteogenesis imperfecta type V. BMC Musculoskelet Disord 15:107 (2014).

Lee DY, Cho TJ, Choi IH, Chung CY, Yoo WJ, et al: Clinical and radiological manifestations of osteogenesis imperfecta type V. J Korean Med Sci 21(4):709-14 (2006).

Lietman CD, Marom R, Munivez E, Bertin TK, Jiang MM, et al: A transgenic mouse model of OI type V supports a neomorphic mechanism of the IFITM5 mutation. J Bone Miner Res 30(3):489-98 (2015).

Moffatt P, Gaumond MH, Salois P, Sellin K, Bessette MC, et al: Bril: a novel bone-specific modulator of mineralization. J Bone Miner Res 23(9):1497-508 (2008).

Rauch F, Moffatt P, Cheung M, Roughley P, Lalic L, et al: Osteogenesis imperfecta type V: marked phenotypic variability despite the presence of the IFITM5 c.-14C.T mutation in all patients. J Med Genet 50:21–24 (2013).

Reich A, Bae AS, Barnes AM, Cabral WA, Hinek A, et al: Type V OI primary osteoblasts display increased mineralization despite decreased COL1A1 expression. J Clin Endocrinol Metab 100(2):E325-32 (2015).

Semler O, Garbes L, Keupp K, Swan D, Zimmermann K, et al: A Mutation in the 5'-UTR of IFITM5 Creates an In-Frame Start Codon and Causes Autosomal-Dominant Osteogenesis Imperfecta Type V with Hyperplastic Callus. Am J Hum Genet 91:349–357 (2012).

Shapiro JR, Lietman C, Grover M, Lu JT, Nagamani SC, et al: Phenotypic variability of osteogenesis imperfecta type V caused by an IFITM5 mutation. J Bone Miner Res 28: 1519-1522 (2013).

Sillence DO, Senn A, Danks DM: Genetic heterogeneity in osteogenesis imperfecta. J Med Genet 16:101–116 (1979).

Takagi M, Sato S, Hara K, Tani C, Miyazaki O, et al: A recurrent mutation in the 5'-UTR of IFITM5 causes osteogenesis imperfecta type V. Am J Med Genet A 161A(8):1980-2 (2013).

Van Dijk FS, Pals G, van Rijn RR, Nikkels PG, Cobben JM: Classification of osteogenesis imperfecta revisited. Eur J Med Genet 53:1–5 (2010).

Van Dijk FS, Sillence DO: Osteogenesis imperfecta: Clinical diagnosis, nomenclature and severity assessment. Am J Med Genet Part A 164A:1470–1481 (2014).

Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, et al: Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet Part A 155A:943–968 (2011).

Zeitlin L, Rauch F, Travers R, Munns C, Glorieux FH: The effect of cyclical intravenous pamidronate in children and adolescents with osteogenesis imperfecta type V. Bone 38:13–20 (2006).

Zerbini CA, Pippa MG, Eis SR, Lazaretti-Castro M, Mota Neto H, et al: Clinical Densitometry – Official Positions 2006. Rev Bras Reumatol 47:25-33 (2007).

Zhang Z, Li M, He J-W, Fu W-Z, Zhang C-Q, et al: Phenotype and Genotype Analysis of Chinese Patients with Osteogenesis Imperfecta Type V. PLoS ONE 8(8): e72337 (2013).

Figure 1. Sanger sequencing showing c.–14C>T mutation in the *IFITM5* gene

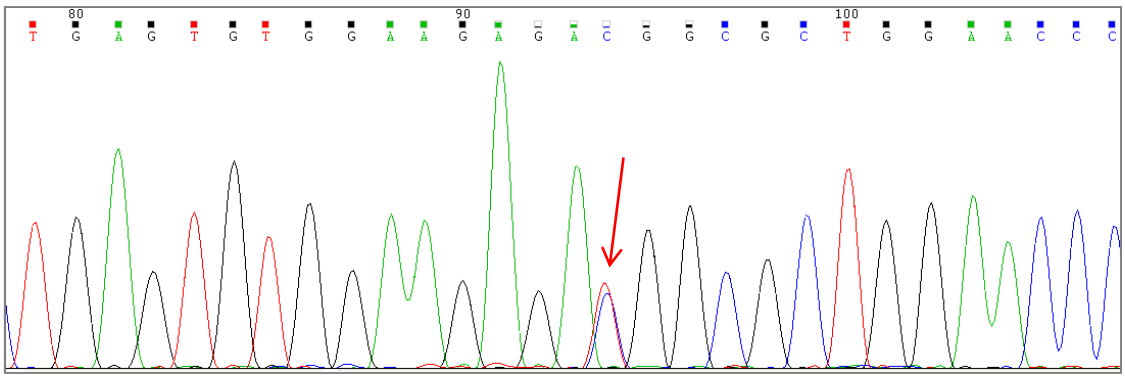

Figure 2. Patients and radiological findings. **A-D Case 1**: (A- B) Patient images; (C) Throracolumbar scoliosis and irregular ribs position; (D) Interosseous membrane calcification. **E-H Case 2**: (E -F) Patient images; (G) Thoracic scoliosis and irregular ribs position; (H) Interosseous membrane calcification. **I-L Case 3**: (I- J) Patient images; (K) Severe cervicothoracolumbar scoliosis; (L) Interosseous membrane calcification. **M-Q Case 5**: (M and N) Patient images; (O) Thoracic scoliosis and ribs deformities; (P -Q) Interosseous membrane calcification and dislocation of the radius head. **R-U Case 6**: (R–S) Patient images; (T) Reduction of height of vertebral bodies, scoliosis and ribs deformities; (U) Hyperplastic callus after femur fracture. **V-Z Case 7**: (V–W) Patient Images; (X–Y) Reduction of height of vertebral body and ribs irregularities; (Z) Interosseous membrane calcification.

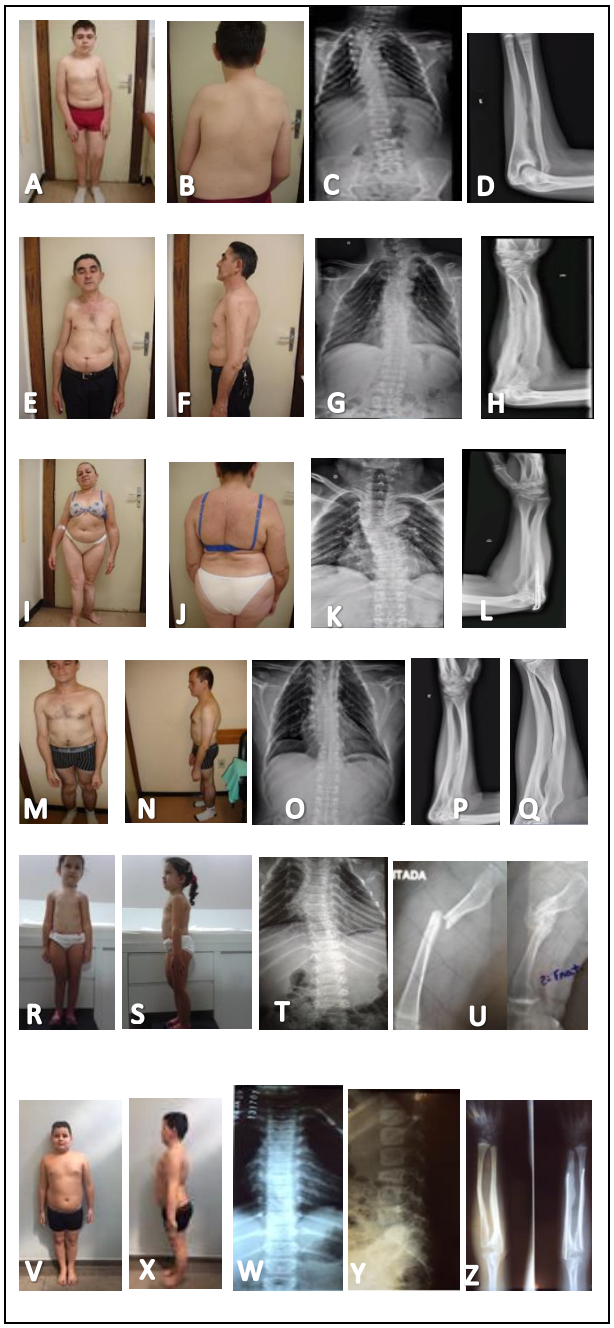

Table 1: Clinical and radiographic characteristics of patients with OI type V (mutation c.-14C> T in the *IFITM5* gene) and a comparison to the literature.

Cases	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Literature***
Gender	male	Male	female	female	male	female	male	
Family history	+	+	+	Unknown	-	-	+	
Age at clinical exam (years)	14	51	52	18	30	3	10	
Mobility	Independent	Independent	Independent	Wheelchair dependent	Independent	Independent	Independent	
Height (cm)	158.5	164.5	127.5	137	146.6	95.5	141	
Short stature	-	+	+	+	+	-	-	38%
DI	-	-	-	-	-	-	-	0%
BS	-	+	-	+	-	-	-	9.5%
BMD	Spine-Z:0.7 Total body-Z:1.1	Spine-T: -3.9 Total femur-T: -1.8	Spine-T: -4.4 femur-T:-1.8 distal radius-T:-4.3	Spine-Z:-2.0 Total body-Z:-1.6	Spine-Z:-4.4 Femur-Z:-1.9		Spine-Z:0.5 Right hip-Z: 1.7	
Age and local of first fracture	At birth rib	12 months Humerus	13 years tibia and fibula	At birth clavicula	3-6months	20 months femur	24 months	
Number of fractures	27	16	7	33	30	4	6	
BP	pamidronate	alendronate e pamidronate	alendronate	alendronate and pamidronate	alendronate	pamidronate	alendronate	28.5%
HPC	+	-	-	-	+	+	-	64%
CIM	+ R-U/ T-F	+ R-U/T-F	+ R-U	+ R-U/T-F	+ R-U	+ R-U	+ R-U/ T-F	88%
DRH	+	+	+	-	+	-	-	78%
Scoliosis	+	+	+	+	+	+	-	51%
Spine fracture by compression	+	+	+	+	+	+	+	42%
c.–14C>T mutation in *IFITM5*	+	+	+	+	+	+	+	126 subjects identified

DI: dentinogenesis imperfecta; BS: blue sclera; BMD: bone mineral density; BP: bisphosphonate; HPC: hyperplastic callus; CIM: calcification of the interosseous membrane; DRH: dislocation of the radial head; R-U: radius and ulna; T-F: tibia and fibula

***** Twelve studies were included describing 126 individuals with the phenotype of OI V and c.-14C>T mutation analyzed (Semler et al., 2012; Cho et al., 2012; Takagi et al., 2012; Zhang et al., 2013; Kim et al., 2013; Balasubramanian et al., 2013; Shapiro et al., 2013; Rauch et al., 2013; Grover et al., 2013; Guillén-Navarro et al., 2014; Lazarus et al., 2014; Reich et al., 2015).

CAPÍTULO II

TRATAMENTO COM BIFOSFONADOS

7.1. METODOLOGIA III

7.1.1. Delineamento

Estudo retrospectivo.

7.1.2. Amostra

Neste estudo foram acessados dados de prontuários médicos e imagens radiográficas de pacientes da Clínica de Osteogênese Imperfeita – Instituto Kennedy Krieger (*Osteogenesis Imperfecta Clinic of Kennedy Krieger Institute* - Baltimore - USA)**.**

Foram incluídos indivíduos de ambos os sexos, todos os tipos de OI, 18 anos ou mais, que receberam tratamento com bifosfonados (BP; oral ou intravenoso) durante pelo menos 2 anos contínuos. Para o grupo controle foram selecionados pacientes com OI que não haviam recebido qualquer medicamento com ação osteometabólica durante um período de 10 anos. Os pacientes foram agrupados em grupo BP-tratado ou não-tratado.

Foram excluídos pacientes: 1) que iniciaram o tratamento com bifosfonados antes dos 18 anos de idade 2) com outras doenças associadas como atrofia cerebral, paralisia, hematomas subdurais cerebrais, HIV, síndrome de Tourette, 3) com fratura recente e 4) que receberam tratamento com Teriparatide concomitante ao tratamento com BP ou entre intervalos de tratamento com BPs.

7.1.3. Dados Clínicos

Pamidronato e zolendronato foram agrupados como BP intravenoso e alendronato e risedronato como BP oral. O tratamento com BP foi analisado em relação ao tipo de droga, via de administração e duração do tratamento. Também foi avaliado o efeito do tratamento com BP em relação aos biomarcadores metabólicos e ósseos, incidência de fraturas e densidade mineral óssea.

7.1.3.1. *Biomarcadores Metabólicos e Ósseos*

Para análise dos biomarcadores metabólicos e ósseos foram revisados exames laboratorias que incluiam cálcio e fósforo séricos, fosfatase alcalina total, osteocalcina, 25 (OH) vitamina D e C-telopeptideo (CTX). Os exames laboratoriais foram avaliados 5 anos antes e após iniciar o tratamento com BP para o grupo BP-tratado e para o grupo não-tratado foram revisados exames dos últimos 5 anos de acompanhamento clínico. Os biomarcadores foram coletadaos em jejum e os exames laboratoriais foram realizados em laboratórios diferentes, de acordo com a preferência dos pacientes, local de procedência e cobertura do seguro de saúde.

7.1.3.2. *Incidência de Fraturas*

Para o grupo BP-tratado, a incidência de fratura foi determinada por um período de 5 anos ou de 10 anos anterior ao inicio do tratamento com BPs e por um período de 5 anos ou 10 anos após o tratamento ter sido iniciado.

Numa tentativa de evitar inconsistência na incidência de fraturas com base apenas na auto-relato, o prontuário médico de cada paciente foi examinado a procura de raio-X ou

relatórios ortopédicos do evento. Quando necessário, os pacientes ou centro de tratamento local foram contatados para solitação de raio-X confirmando a fratura. Foram incluídas fraturas de ossos longos dos membros superiores e inferiores, metacarpos, metatarsos e coluna vertebral. Fraturas periféricas, como dedos da mãos e pés e costelas foram excluídas.

7.1.3.3. *Densidade Mineral Óssea*

DMO da coluna lombar, quadril total e colo femural foram mensurados por *dual energy X-ray absorptiometry* (DXA). Foram comparados o primeiro e o último exames de densitometria sob tratamento com BPs para o grupo BP-tratado. Para o grupo não-tratado foi selecionado um exame de densitometria dos últimos 5 anos de acompanhamento clínico. A maioria dos exames de densitometria óssea foram realizados no Instituto Kennedy Krieger ou no Hospital Johns Hopkins - Departamento de Medicina Nuclear no equipamento *Hologic* (Horizon A, S / N 100164 ou 4500; Hologic Corp, Bedford, MA). No entanto, alguns exames foram obtidos nos centros de tratamento locais dos pacientes. Locais com material metálico cirúrgico, deformidade óssea grave ou escoliose grave foram excluídos da análise.

7.1.4. Aspectos Éticos

Este estudo foi realizado em conformidade com os critérios do Conselho de Revisão Institucional Johns Hopkins (Johns Hopkins Institutional Review Board – IRB).

7.1.5. Análise Estatística

As variáveis quantitativas foram descritas por média e desvio padrão/erro padrão ou mediana e intervalo interquartil ou percentil 25-75. As variáveis categóricas foram descritas por meio de frequências absolutas e relativas. Para comparar medias entre os grupos, the independent-samples t test foi aplicado. Na comparação de proporções, os testes Pearson's chi-square or Fisher's Exact test foram utilizados. Para comparações de parâmetros intra e inter-grupos simultaneamente, Generalized Estimating Equations com ajuste por Bonferroni foi aplicada. O nível de significância adotado foi de 5% ($\alpha \leq 0,05$) e as análises foram realizadas através do software SPSS (versão 21.0. SPSS Inc, Chicago, Illinois).

7.2. Resultados

Os resultados serão apresentados no formato de artigo científico. O estudo intitulado "*The Effectiveness of Bisphosphonate Treatment in Adults with Osteogenesis Imperfecta on Bone and Metabolic Biomarkers and Fracture Rate*" será submetido para publicação.

VERSÃO EM INGLÊS

The Effectiveness of Bisphosphonate Treatment in Adults with Osteogenesis Imperfecta on Bone and Metabolic Biomarkers and Fracture Rate

Brizola E[1,2], Félix TM[2,3], Shapiro JR[1]

[1] Bone and Osteogenesis Imperfecta Program, Kennedy Krieger Institute, Johns Hopkins School of Medicine, Baltimore, MD, USA

[2] Postgraduate Program in Child and Adolescent Health, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

[3] Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

ABSTRACT

Introduction: Osteogenesis Imperfecta is an inherited systemic disorder with widely expressivity affecting bone and connective tissue. The main features are bone fragility and high risk of fractures. Bisphosphonates (BP) are the first-line drug on standard of care for children and adults with OI for over twenty years. BPs act suppressing bone turnover therefore bone and metabolic biomarkers can be helpful to assist to predict fracture risk associated to bone mineral density evaluation. Reduction of fracture incidence is a goal of the BPs therapy for patients with OI, however the literature is not homogeneous about this positive effect for this specific population. **Objectives:** The present study examines the relationship of BP treatment to bone and metabolic biomarkers and fracture rate in adults with OI. **Methods:** We assessed data from medical records and radiographic images at the

Osteogenesis Imperfecta Clinic of Kennedy Krieger Institute. Individuals of any gender with all types of OI ages 18 years or older were included. They were divided into non-treated and BP treated groups. We analyzed BP treatment in relation to type of drug, route of administration and duration of the treatment. Levels of bone and mineral biomarkers (BMB) for a 5-year period of treatment and fracture incidence for a 5-year or 10-year period before and after treatment starts were analyzed. Bone mineral density (BMD) of lumbar spine, total hip and femoral neck were measured by DXA. **Results:** Forty seven individuals (24 female: 23 male) with mean age of 51.5±13.2 years were included. The median and 25^{th}-75^{th} percentile of total duration of BP treatment was 5 (4-8) years, treatment with oral BP showed longer time of duration 3 (0-5) years when compared to intravenous BP treatment (2 [0-4] years). The only bone biomarker that showed significant difference between BP treated and non-treated groups was osteocalcin. There was no difference in fracture rate comparing a period of 5 or 10 years pre- and post-treatment for treated group. BMD at lumbar spine was significantly increased from 0.730 to 0.780 g/cm2 (p=0.002) comparing baseline and last exam in the treatment group. **Conclusions:** Long-term treatment with BPs for adults with OI was not associated with sustained decrease on fracture rate and it was not significantly reflected on bone and metabolic biomarkers levels. As reported in several studies, we observed an improvement at lumbar spine BMD associated to BP therapy.

INTRODUCTION

Osteogenesis Imperfecta is an inherited systemic disorder that affects bone and connective tissue leading to bone fragility and high risk of fractures. The expressivity of the disorder is highly variable, may be observed cases of very mild impairment until perinatal lethal cases (Van Dijk & Sillence, 2014). Bisphosphonates (BPs) have been considered

standard of care for children and adults with OI for over twenty years. Most children with moderate and severe OI and some with mild OI associated to frequent fractures are treated with BPs for several years, frequently until cessation of growth (Bachrach & Ward, 2009). Adults with OI also are treated with BPs in an effort to decrease musculoskeletal pain, improve bone mineral density and reduce fracture rate.

BPs are potent inhibitors of bone resorption and have strong affinity by mineral bone in sites of high bone turnover (Russel, 2007; Russel, 2015; Hoyer-Kuhn et al., 2015). The mechanism of action differs between children and adults, once children are growing and have a high bone turnover when compared to individuals after puberty where the bone turnover is physiologically decreased limiting the effectiveness of BPs (Russel, 2007; Russel, 2015; Hoyer-Kuhn et al., 2015). Although BPs are the first-line drug option for treatment of children with OI, there is no consensus on their benefit for adults.

Bone turnover markers reflect changes in bone metabolism and may be useful for fracture risk prediction in association with bone mineral density (BMD) measurements (Biver, 2012). For patients with OI, levels of bone turnover markers were already reported as low-normal and increased (Biver, 2012; Braga et al., 2004; Wekre et al., 2011; Bradbury et al., 2012). In adults with OI just a few studies reported the effect of BPs on bone and metabolic biomarkers showing no homogenous results, however it is known that BPs act by suppressing bone turnover (Braga et al., 2004; Russel, 2007; Pávon de Paz et al., 2010; Bradbury et al., 2012).

Effect of BPs on fracture incidence has been intensely discussed considering the difficulty to prove the benefits of the BP therapy on keeping a sustained decrease of fractures rate for adults with OI (Dwan et al., 2014; Hald et al., 2015; Shi et al., 2015). Several studies reported no effect of oral or IV BPs therapy on fracture incidence (Chevrel et al., 2006;

Shapiro et al., 2010; Bradbury et al., 2012) and a few studies reported positive effect on fracture rate following intravenous BP therapy (Adami et al., 2003; Pavón de Paz et al., 2010).

In an effort to assist to define characteristics that would permit prediction of treatment response, this study examines the relationship of BP treatment to bone and metabolic biomarkers and fracture rate in adults with OI.

METHODS

Subjects

This retrospective study assessed data from medical records and radiographic images at the Osteogenesis Imperfecta Clinic of Kennedy Krieger Institute (Baltimore, MD, USA) and it is in accordance with requirements of the Johns Hopkins Institutional Review Board.

The diagnosis of OI was based on OI classification established by Sillence (Sillence et al, 1979). Individuals of any gender with all types of OI from 18 years or older were included. They were divided into non-treated and BP treated groups.

The BP treated group included individuals having started BP (oral or intravenous) in adulthood and received a minimum of 2 years of continuous BP treatment. Non-treated group included patients with OI that did not receive any bone active drug for an interval of at least 10 years.

It were excluded from the study: 1) patients that started BP treatment before 18 years old, 2) patients with other associated diseases such as cerebral atrophy, cerebral palsy, multiple subdural hematomas, HIV, Tourette syndrome, 3) with recent fracture, and 4) patients that received Teriparatide concomitant to BP treatment or between drug holidays.

Data Collection

The intravenous dose of pamidronate was 1.5 mg/kg body weight to a maximum of 60 mg/infusion every 3, 4 or 6 months depending on patient adherence to schedule and duration of treatment. Alendronate and risedronate were administered in standard weekly oral doses of 70 and 35 mg, respectively, with instructions as provided by the manufacturers. For zoledronic acid the dose of 0.05 mg/kg was administered every 6 months of interval. Pamidronate and zolendronate were grouped as intravenous BP and alendronate and risedronate as oral BP. BP treatment was analyzed in relation to type of drug, route of administration and duration.

Bone and metabolic biomarkers (BMB) analyzed were serum calcium and phosphorus, total alkaline phosphatase, osteocalcin, 25(OH) vitamin D and C-telopeptide (CTX). All BMB were obtained by fasting blood samples. Laboratory reports were assessed for a 5-year period preceding initial BP treatment and for a 5-year period of treatment for BP treated group and for non-treated group exams from the last 5 years of follow up were considered. Laboratory exams were performed in different laboratories according patients' preference, local facility, and health insurance coverage.

For BP treated group, fracture incidence was determined for a 5-year or 10- year period preceding initial BP treatment and for a 5-year or 10-year period after treatment was started.

In an attempt to try to avoid inconsistence on fracture incidence based only in self-report, each patient's medical record was examined for X-ray or orthopedic reports of the event. When required, patients or their local facilities were contacted for X-ray confirmation

of a fracture. Fractures of long bones of upper and lower limbs, metacarpal, metatarsal and spine were included. Peripheral fractures such as fingers, toes, and ribs were excluded.

Bone mineral density (BMD) of lumbar spine, total hip and femoral neck were measured by dual energy X-ray absorptiometry (DXA). Baseline DXA study was compared to the last study under treatment for BP treated group. For non-treated group one DXA study of the last 5 years of clinical follow up was selected. Most DXA studies were performed at Kennedy Krieger Institute or at Johns Hopkins Hospital Department of Nuclear Medicine on Hologic equipment (Horizon A, S/N 100164 or 4500; Hologic Corp, Bedford, MA). Nonetheless, some DXA were obtained at patients' local facility. Sites with surgical hardware or with severe bone deformity or scoliosis were excluded from the analysis.

Statistical Analysis

Statistical analysis was performed with SPSS software (ver. 21.0; SPSS Inc, Chicago, Illinois) and the level of significance was set at 5% ($\alpha \leq 0.05$). Quantitative variables were described by mean and standard deviation/standard error or median and interquartile range or 25^{th}-75^{th} percentile. The categorical variables were described by absolute and relative frequencies. To compare averages among groups, the independent-samples t-test was applied. On the comparison of proportions, Pearson's chi-square or Fisher's exact tests were used. For comparisons of parameters intra- and inter-groups simultaneously, Generalized Estimating Equations analysis with the adjustment by Bonferroni was applied.

RESULTS

Among two hundred and six patients' records reviewed, 47 matched the criteria having been included in the study. The total mean age was 51.5±13.2 years and 24 (51.1%) individuals were female. Non-treated group was composed by 12 individuals (10 OI-I, 2 OI-IV), and BP treated group by 35 individuals (17 OI-I, 14 OI-III, 4 OI-IV). There was no significant difference between both groups when adjusted by gender (p= 0.674), age (p= 0.088) and age > 50 years (p= 0.105). However, there was significant difference when adjusted by OI type (p=0.032).

Forty-two per cent of the patients received treatment with oral BP, 25.7% with IV BP and 31.4% had treatment with both types of BP, oral and IV. The median and 25^{th}-75^{th} percentile of total duration of BP treatment was 5 (4-8) years, treatment with oral BP showed longer time of duration 3 (0-5) years when compared to intravenous BP treatment (2 [0-4] years).

Among all BMB levels analyzed, only osteocalcin showed a significant difference between BP treated and non-treated groups. However, levels of 25(OH) vitamin D were low-normal for both groups. In addition, for BP treated group there was no significant difference when we compared the mean levels of BMB during period of BP treatment and 5 years after BP treatment interruption (**table 1**).

There was no difference in fracture rate comparing a period of 5 or 10 years pre- and post-treatment for treated group (**table 2**). Table 3 summarizes the BMD comparison pre- and post-treatment for BP treated group where a significant difference for BMD at lumbar spine was observed increasing from 0.730 to 0.780 g/cm2 (p=0.002). In addition, there was no significant difference comparing BMD values between non-treated group and BP treated

group, considering the last DXA study during clinical follow-up for non-treated group and the last DXA study available before the interruption of BP treatment for BP treated group.

DISCUSSION

There is limited data in the OI literature about the effects of BPs on BMB, fracture incidence and bone density for adults. The majority of the studies proving the benefits of BPs treatment were developed on children and adolescent and several of the outcomes have been difficult to transpose to OI adult population (Lindahl et al., 2014). BPs act inhibiting osteoclasts function leading to their apoptosis and consequently to decrease of the bone resorption; and it is known BPs are more effective in states of high bone turnover instead to low bone turnover (Russel, 2007; Shapiro et al., 2010; Russel, 2015). This may explain the differences on the response to the BP therapy between children with a growing skeleton with high bone turnover and the mature skeleton of adults with OI (Rauch et al., 2002; Shapiro et al., 2010; Bradbury et al., 2012; Eghbali-Fatourechi, 2014).

In this study 42% of the individuals received oral BPs and the duration of the treatment with oral BPs was longer when compared to individuals treated with IV BPs. Usually, oral route is the preferred option for treatment of adults with OI considering the low rate of fractures in this age and the facility of the administration compared to IV BPs (Martin & Shapiro, 2007; Bachrach & Ward, 2009). A significant difference was found between groups when adjusted to OI type, non-treated group was composed by the majority of type I OI. It can be explained by the fact that BPs treatment is more frequently given to patients with moderate to severe OI then to patients with mild OI in an effort to decrease musculoskeletal pain, improve BMD and reduce fracture rate (Glorieux, 2008).

In our sample of adults with OI, long-term treatment with oral or IV BP treatment was not reflected in the majority of BMB except for higher osteocalcin levels in those not treated with BP. In addition, although we have found no significant difference comparing BP treated and non-treated, both groups showed low-normal levels of 25(OH) vitamin D. Garnero et al. (2009) also found higher osteocalcin values in patients with OI not treated with BPs comparing to healthy controls. A histological study in children with OI showed increased osteoblast number and overall bone turnover (Rauch et al., 2004). Followed by BP treatment there is a decline around of 70% of bone formation from the start of treatment which may be related to the higher value of osteocalcin observed in the non-treated patients (Rauch et al., 2006). In addition, Braga et al. (2004) also observed bone markers levels from 50 to 200% higher in non-treated OI adults than in controls including serum total and bone alkaline phosphatase, serum osteocalcin, NTX and urinary free-deoxypyridinoline (ufDPD).

A prospective non-randomized study assessing data from 10 patients with OI treated with zoledronic acid reported no changes of serum calcium, phosphate, bone alkaline phosphatase or CTX and a decrease on vitamin D concentrations after 36 months of treatment (Pavón de Paz et al., 2010). In an observational study including adults with type I OI treated with risedronate for 24 months it was observed a decrease on P1NP (serum procollagen type I aminopropeptide) by 37% but not on bone specific alkaline phosphatase. The study also reported a deficiency of 25(OH) vitamin D among the patients and no effectof the oral BP treatment on fracture rate (Bradbury et al., 2012).

Following BP treatment we observed an increase on lumbar spine BMD but not on total hip or femoral neck. In addition, in our sample long-term treatment with oral or IV BP was not associated with a decrease in fracture rate either for a period of 5 or 10 years of observation. Several studies have been reported increase of BMD associated to either oral or IV BP treatment in adults with OI (Adami et al., 2003; Chevrel et al., 2006; Pavón de Paz et

al., 2010; Shapiro et al., 2010; Bradbury et al., 2012; O'Sullivan et al., 2014). In according with our findings, improve of lumbar spine BMD is the most reported gain following BP therapy between adults with OI (Adami et al., 2003; Chevrel et al., 2006; Pavón de Paz et al., 2010; Shapiro et al., 2010; Bradbury et al., 2012; O'Sullivan et al., 2014) while effect on femoral neck and total hip is reported as unchanged and as slightly improved (Adami et al., 2003; Chevrel et al., 2006; Bradbury et al., 2012; Pavón de Paz et al., 2010; Shapiro et al., 2010).

Although still unexplained, in adults with OI the bone density appears to plateau over 2 to 3 years of BP treatment (Martin & Shapiro, 2007). A histomorphometric study demonstrated significantly increase of BMD associated to BPs therapy, with the most gain achieved within the first 2 to 4 years of treatment (Rauch et al., 2006). The severity of the disease also influences the results on effectiveness of BP treatment. A large retrospective non-randomized study including 90 adults with OI treated either with oral or IV BP, showed a significant increase in lumbar spine BMD following treatment with IV BPs for individuals with types I or III/IV OI, however oral BP increased lumbar spine BMD just for individuals with mild OI. Fracture rate was only marginally reduced by IV BP treatment in patients with type III/IV OI (Shapiro et al., 2010) and there was no effect for patients with type I OI with either oral or IV BPs.

Fracture incidence is an important outcome to be measured because it reflects the bone quality different from DXA that reveals the bone quantity. In adults with OI, the relationship between fracture incidence and BP treatment have been shown no consistency. Few studies previously reported an unchanged fracture rate (Bradbury et al., 2012), a trend toward statistical significance to decrease fracture incidence in favor of treatment (Adami et al., 2003), effect on reduction of fracture incidence followed by IV BP treatment (Pavón de

Paz et al., 2010; Shapiro et al., 2010) and some could not demonstrate an effect upon fracture incidence due lack of statistical power (Chevrel et al., 2006; O'Sullivan et al., 2014).

Recent Cochrane reports assessed the results of BP treatment from meta-analysis of randomized and quasi-randomized controlled clinical trials where it was found an increase of BMD for children and adults with OI following either oral or IV BP treatment, but the effect on decreasing fracture rate was inconclusive (Phillipi et al., 2008; Dwan et al., 2014). Nonetheless, two recent meta-analysis conclude that the effect of BPs on sustaining decreased fracture incidence in adults with OI is "inconclusive" or "seemed equivalent to placebo" (Hald et al., 2015; Shi et al., 2015).

Potential benefits of the BP treatment need to be carefully investigated in adults with OI considering all adverse events reported related to this therapy such as gastrointestinal pain, muscle and bone pain, transient asymptomatic hypocalcemia, fracture non-union, delay of healing osteotomy and atypical fragility fractures (Basel & Steiner, 2009; Abrahamsen, 2010; Uihlein & Leder, 2012; Hegazi et al., 2015).

The present study has several limitations. First, this is an observational nonrandomized study however it encompassed several years of patient follow-up. There was a lack of information about different bone and metabolic biomarkers such as CTX. Laboratory exams were executed in different places according patient locality and facility, likewise it was observed for a small number of DXA studies. Due to the small sample size, men and women were not considered separately. Fracture incidence was based on self-reports what may permits missing events, documentation of fractures in adult OI patients is difficult because of inadequate X-ray confirmation and recall of dates and fracture sites. Also, this study did not address secondary treatment outcomes such as the relief of

musculoskeletal discomfort or improvements in functionality or the quality of daily activities.

CONCLUSIONS

Long-term treatment with BPs for adults with OI was not associated with sustained decrease on fracture rate and it was not significantly reflected on bone and metabolic biomarkers levels. As reported in several studies, we observed an improvement at lumbar spine BMD associated to BP therapy. Our findings are in accordance with current literature that indicates that long-term treatment with BP may not be associated with a sustained decrease in fracture rate treatment in adults with OI.

Data addressing the effectiveness of oral and IV BP in adults patients with OI is conflicting, ongoing critical assessment of the BP therapy is urgently required addressing several outcomes including fracture incidence, bone and metabolic levels, adverse effects and long-term treatment safety.

CONFLICT OF INTEREST

The authors report no conflict of interest.

CONTRIBUTIONS OF AUTHORS

All authors participated in the study design, data interpretation and writing of the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGEMENTS

The authors thank the patients and their families for their participation in this study. Evelise Brizola was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Proc. Number 3770/14-1.

Table 1. Comparison of bone and metabolic biomarkers: A) after 5 years of follow-up between non-treated and BP-treated groups B) under treatment and after 5 years of treatment interruption for BP-treated group

Variable		N	Mean	Std. error	P
Calcium	Non-treated group	11	9.41	0.07	0.625*
	BP-treated group - under treatment	27	9.47	0.08	
	BP-treated group- after treatment interruption	25	9.46	0.08	0.897^{+}
25 (OH) VitaminD	Non-treated group	12	33.4	2.4	0.774*
	BP-treated group - under treatment	28	34.5	1.99	
	BP-treated group - after treatment interruption	24	40.4	3.19	0.070^{+}
Alkaline Phosphatase	Non-treated group	12	83.2	7.22	0.617*
	BP-treated group - under treatment	28	94.4	13.9	
	BP-treated group - after treatment interruption	26	85.2	7.57	0.300^{+}
Phosphorus	Non-treated group	12	3.69	0.16	0.253*
	BP-treated group - under treatment	25	3.49	0.09	
	BP-treated group - after treatment interruption	22	3.47	0.09	0.785^{+}
Osteocalcin	Non-treated group	12	31.6	3.44	<0.001*
	BP-treated group - under treatment	26	13.7	1.52	
	BP-treated group - after treatment interruption	24	15.2	1.61	0.510^{+}
CTX	Non-treated group	11	229.3	21.7	0.371*
	BP-treated group - under treatment	15	165.5	55.6	
	BP-treated group - after treatment interruption	19	211.5	59.4	0.558^{+}

* p value for comparison between non-treated group and BP-treated group under treatment

$^{+}$ p value for comparison between periods under and after interruption of treatment for BP-treated group

Table 2. Fracture rate in BP treated group comparing intervals of 5 or 10 years pre- and post-treatment with BP started

Time of treatment		**Fracture Rate**		
		Mean	**Std. Error**	**P**
Pre-treatment	10 years	1.23	0.262	0.109^{+}
	5 years	0.86	0.181	0.380*
Post-treatment	5 years	1.06	0.156	0.380*
	10 years	1.71	0.220	0.109^{+}

***P= comparison of 5 years pre and post-treatment

$^{+}$P=comparison of 10 years pre and post-treatment

Table 3. Comparison of BMD pre- and post-treatment for BP treated group and between BP treated and non-treated groups

Variable		BP Treated				BP treated x Non-treated				
		N	Mean	Std. error	P		N	Mean	Std. error	P
Hip	pre-treatment	15	0.720	0.06	0.461	**non-treated**	11	0.740	0.05	0.864
	post- treatment	17	0.760	0.07		**BP treated**	17	0.760	0.07	
Spine	pre-treatment	25	0.730	0.03	**0.002**	**non-treated**	12	0.750	0.05	0.528
	post- treatment	26	0.780	0.03		**BP treated**	26	0.780	0.03	
FN	pre-treatment	16	0.700	0.04	0.877	**non-treated**	10	0.640	0.06	0.537
	post- treatment	18	0.690	0.05		**BP treated**	18	0.690	0.05	

FN: femoral neck

REFERENCES

Abrahamsen Bo. Adverse Effects of Bisphosphonates. Calcif Tissue Int. 2010; 86:421–435.

Adami S, Gatti D, Colapietro F, Fracassi E, Braga V, Rossini M & Tato L. Intravenous neridronate in adults with osteogenesis imperfecta. Journal of Bone and Mineral Research. 2003; 18:126–130.

Bachrach LK, Ward LM. Clinical review: Bisphosphonate use in childhood osteoporosis. J Clin Endocrinol Metab. 2009; 94(2):400-9.

Basel D, Steiner RD. Osteogenesis imperfecta: recent findings shed new light on this once well-understood condition. Genet Med. 2009; 11(6):375-85.

Biver E. Use of bone turnover markers in clinical practice. Curr Opin Endocrinol Diabetes Obes. 2012;19(6):468-73.

Bradbury LA, Barlow S, Geoghegan F, Hannon RA, Stuckey SL, Wass JA, Russell RG, Brown MA, Duncan EL. Risedronate in adults with osteogenesis imperfecta type I: increased bone mineral density and decreased bone turnover, but high fracture rate persists. Osteoporos Int. 2012; 23(1):285-94.

Braga V, Gatti D, Rossini M, Colapietro F, Battaglia E, Viapiana O, Adami S. Bone turnover markers in patients with osteogenesis imperfecta. Bone. 2004; 34:1013–1016.

Chevrel G, Schott AM, Fontanges E, Charrin JE, Lina-Granade G, Duboeuf F, Garnero P, Arlot M, Raynal C, Meunier PJ. Effects of oral alendronate on BMD in adult patients with osteogenesis imperfecta: a 3-year randomized placebo-controlled trial. Journal of Bone and Mineral Research. 2006; 21:300–306.

Dwan K, Phillipi CA, Steiner RD, Basel D. Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev. 2014; 7:CD005088.

Eghbali-Fatourechi G. Bisphosphonate therapy in pediatric patients. J Diabetes Metab Disord. 2014. 17; 13(1):109.

Garnero P, Schott AM, Prockop D, Chevrel G. Bone turnover and type I collagen C-telopeptide isomerization in adult osteogenesis imperfecta: associations with collagen gene mutations. Bone. 2009; 44(3):461-6.

Glorieux FH. Osteogenesis imperfecta. Best Pract Res Clin Rheumatol. 2008; 22(1):85-100.

Hald JD, Evangelou E, Langdahl BL, Ralston SH. Bisphosphonates for the prevention of fractures in osteogenesis imperfecta: meta-analysis of placebo-controlled trials. J Bone Miner Res. 2015; 30(5):929-33.

Hegazy A, Kenawey M, Sochett E, Tile L, Cheung AM, Howard AW. Unusual Femur Stress Fractures in Children with Osteogenesis Imperfecta and Intramedullary Rods on Long-term Intravenous Pamidronate Therapy. J Pediatr Orthop. 2015. Epub 2015 Jun 8.

Hoyer-Kuhn H, Netzer C, Semler O. Osteogenesis imperfecta: pathophysiology and treatment. Wien Med Wochenschr. 2015; 165(13-14):278-284.

Lindahl K, Langdahl B, Ljunggren Ö, Kindmark A. Treatment of osteogenesis imperfecta in adults. Eur J Endocrinol. 2014; 171(2):R79-90.

Martin E, Shapiro JR. Osteogenesis imperfecta: epidemiology and pathophysiology. Curr Osteoporos Rep. 2007; 5(3):91-7.

O'Sullivan ES, van der Kamp S, Kilbane M, McKenna M. Osteogenesis imperfecta in adults: phenotypic characteristics and response to treatment in an Irish cohort. Ir J Med Sci. 2014; 183(2):225-30.

Pavón de Paz I, Iglesias Bolaños P, Durán Martínez M, Olivar Roldán J, Guijarro De Armas G, Parra García JI. Effects of zoledronic acid in adults with osteogenesis imperfecta. Endocrinol Nutr. 2010; 57(6):245-50.

Phillipi CA, Remmington T, Steiner RD. Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev. 2008; (4):CD005088.

Rauch F, Travers R, Glorieux FH. Pamidronate in children with osteogenesis imperfecta: histomorphometric effects of long-term therapy. J Clin Endocrinol Metab. 2006; 91(2):511-6.

Rauch F, Travers R, Parfitt AM, Glorieux FH. Static and dynamic bone histomorphometry in children with osteogenesis imperfecta. Bone. 2004; 35:224–30.

Rauch F, Travers R, Plotkin H, Glorieux FH. The effects of intravenous pamidronate on the bone tissue of children and adolescents with osteogenesis imperfecta. J Clin Invest. 2002; 110(9):1293-9.

Russell RG. Bisphosphonates: mode of action and pharmacology. Pediatrics. 2007; 119(2):S150-62.

Russell RG. Pharmacological diversity among drugs that inhibit bone resorption. Curr Opin Pharmacol. 2015; 22:115-130

Shapiro JR, Thompson CB, Wu Y, Nunes M & Gillen C. Bone mineral density and fracture rate in response to intravenous and oral bisphosphonates in adult osteogenesis imperfecta. Calcified Tissue International. 2010; 87:120–129.

Shi CG, Zhang Y, Yuan W. Efficacy of Bisphosphonates on Bone Mineral Density and Fracture Rate in Patients With Osteogenesis Imperfecta: A Systematic Review and Meta-analysis. Am J Ther. 2015. Epub 2015 Apr 3.

Uihlein AV, Leder BZ. Anabolic therapies for osteoporosis. Endocrinol Metab Clin North Am. 2012; 41(3):507–525.

Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. Am J Med Genet A. 2014; 164A(6):1470-81.

VERSÃO EM PORTUGUÊS

Eficácia do Tratamento com Bifosfonatos em Adultos com Osteogênese Imperfeita em relação aos biomarcadores metabólicos e ósseos e taxa de fraturas

RESUMO

Introdução: Osteogenêse Imperfeita é uma doença sistêmica herdada com ampla expressividade que afeta os ossos e o tecido conjuntivo. As principais características da doença são a fragilidade óssea e o alto risco de fraturas. Os bifosfonatos (BPs) são considerados "padrão ouro" no tratamento medicamentoso de crianças e adultos com OI há mais de vinte anos. BPs atuam inibindo o *turnover* ósseo, portanto, biomarcadores ósseos e metabólicos podem auxliar a predizer risco de fratura associados à avaliação da densidade mineral óssea. Redução da incidência de fraturas é um objetivo da terapia com BPs para pacientes com OI, no entanto, a literatura não é homogênea sobre o efeito positivo do tratamento nesta população específica. **Objetivos:** O presente estudo analisa a relação entre o tratamento com BP e os biomacadores metabólicos e ósseos e a incidência de fraturas em adultos com OI. **Métodos:** Foram avaliados dados de prontuários médicos e imagens radiográficas de pacientes da Clínica de Osteogênese Imperfeita – Instituto Kennedy Krieger. Indivíduos de ambos os sexos, todos os tipos de OI e idade igual ou maior a 18 anos foram incluídos. Os pacientes foram divididos em dois grupos: não-tratados e BP tratados. Analisamos tratamento com BP em relação ao tipo de droga, via de administração e duração do tratamento. Valores de biomarcadores metabólicos e ósseos para um período de 5 anos de tratamento e incidência de fraturas por um período de 5 anos ou 10 anos antes e após o ínicio do tratamento foram analisados. A densidade mineral óssea (DMO) da coluna lombar,

quadril total e colo femural foram mensurados por DXA. **Resultados:** Quarenta e sete indivíduos (24 do sexo feminino: 23 do sexo masculino) com idade média de 51,5 ± 13,2 anos foram incluídos. A mediana e intervalo interquartil (IQR) de duração total do tratamento com BP foi de 5 ± 4 anos, o tratamento com BP por via oral mostrou maior tempo de duração (3 ± 5 anos: mediana ± IQR), quando comparado ao tratamento com BP intravenoso (2 ± 4 anos). O único biomarcador que apresentou diferença significativa entre os grupos BP-tratado e não-tratado foi a osteocalcina. Não foi observada diferença na taxa de fraturas comparando um período de 5 ou 10 anos pré e pós-tratamento para o grupo tratado. Densidade mineral óssea (DMO) da coluna lombar apresentou aumento significativo de 0.730 para 0,780 g / cm2 (p = 0,002) comparando o primeiro e o último exames de densitometris óssea (DXA) exame durante o tratamento no grupo BP-tratado. **Conclusões:** O tratamento a longo prazo com BPs para adultos com OI não foi associado com redução sustentada da taxa de fraturas e não se refletiu de forma significativa nos níveis de biomarcadores metabólicos e ósseos. Conforme reportado em diversos estudos prévios, observou-se uma melhora na DMO da coluna lombar associada à terapia com BP.

INTRODUÇÃO

Osteogênese Imperfeita é uma doença sistêmica hereditária que afeta os ossos e o tecido conjuntivo levando à fragilidade óssea e alto risco de fraturas. A expressividade da doença é amplamente variável, podem ser observados casos com comprometimento muito leve até casos letais ainda na fase perinatal (Van Dijk & Sillence, 2014). O tratamento com bifosfonatos (BPs) têm sido considerado padrão ouro para crianças e adultos com OI há mais de vinte anos. A maioria das crianças com moderada e grave OI e alguns com OI leve associada à fraturas frequentes são tratados com BPs por vários anos, com freqüência até o

final do crescimento (Bachrach & Ward, 2009). Adultos com OI também são tratados com BPs no intuito de diminuir dor músculo-esquelética, melhorar a densidade mineral óssea (DMO) e reduzir a incidência de fraturas.

BPs são potentes inibidores da reabsorção óssea e tem forte afinidade por osso mineral em locais de elevado *turnover* ósseo (Russel, 2007; Russel, 2015; Hoyer-Kuhn et al., 2015). O mecanismo de ação difere entre crianças e adultos, uma vez que as crianças estão em crescimento apresentando um elevado *turnover* ósseo quando comparado a indivíduos após a puberdade quando o *turnover* osso é fisiologicamente diminuido limitando a eficácia dos BPs (Russel, 2007; Russel, 2015; Hoyer-Kuhn et al., 2015). Embora BPs sejam a primeira opção para tratamento medicamentoso de crianças com OI, não há consenso sobre seus benefícios para adultos.

Biomarcadores metabólicos e ósseos (BMO) refletem as alterações no metabolismo ósseo e podem ser auxiliar a predizer risco de fratura em associação à avaliação da densidade mineral óssea (DMO) (Biver, 2012). Para os pacientes com OI, os valores de biomarcadores do *turnover* ósseo já foram descritos como baixo-normal e aumentado (Biver, 2012; Braga et al., 2004; Wekre et al., 2011; Bradbury et al., 2012). Em adultos com OI apenas alguns estudos relataram o efeito do tratamento com BP em relação aos BMO sem a apresentação de resultados homogêneos, porém sabe-se que os BPs atuam suprimindo o *turnover* ósseo (Braga et al., 2004; Russel, 2007; Pavón de Paz et al., 2010; Bradbury et al., 2012).

O efeito dos BPs sobre a incidência de fraturas tem sido intensamente discutido considerando a dificuldade de provar o benefício da terapia em manter uma reduzida taxa de fratura em adultos com OI (Dwan et al., 2014; Hald et al., 2015;. Shi et al., 2015). Em diversos estudos descritos na literatura não houve efeito da terapia com BP oral ou intravenoso sobre a incidência de fraturas (Chevrel et al., 2006;. Shapiro et al., 2010;

Bradbury et al., 2012) e alguns estudos relataram um efeito positivo na redução da taxa de fraturas após a terapia com BP IV (Adami et al., 2003; Pavón de Paz et al., 2010).

Em um esforço para ajudar a definir características que permitam a predizer resposta ao tratamento, este estudo examina a relação entre o tratamento com BPs e os biomarcadores metabólicos e ósseos e a incidência de fraturas em pacientes adultos com OI.

MÉTODOS

Sujeitos

Neste estudo retrospectivo foram avaliados dados de prontuários médicos e imagens radiográficas na Clínica de Osteogênse Imperfeita do Instituto Kennedy Krieger (Baltimore, MD, EUA). Este estudo foi realizado em conformidade com os critérios do Conselho de Revisão Institucional Johns Hopkins (Johns Hopkins Institutional Review Board – IRB).

O diagnóstico de OI foi baseado na classificação de OI estabelecida por Sillence (Sillence et al., 1979). Indivíduos de ambos os sexos com todos os tipos de OI e idade igual ou maior a 18 anos foram incluídos. Os pacientes foram divididos em dois grupos: não-tratado e BP-tratado.

O grupo BP-tratado incluiu indivíduos que iniciaram o tratamento com BP (oral ou intravenoso) na idade adulta e receberam no mínimo de 2 anos de tratamento contínuo. O grupo não-tratado incluiu pacientes com OI que não receberam qualquer droga com ação no metabolismo ósseo num intervalo de pelo menos 10 anos.

Foram excluídos do estudo: 1) pacientes que iniciaram o tratamento com BP antes dos 18 anos de idade, 2) pacientes com outras doenças associadas, tais como atrofia cerebral, paralisia cerebral, hematoma subdural múltiplo, HIV, síndrome de Tourette, e 3) pacientes

que receberam Teriparatide concomitante ao tratamento com BP ou entre intervalos de tratamento com BPs.

Coleta de Dados

A dose de pamidronato intravenoso foi de 1,5 mg/kg de peso corporal com uma dose máxima de 60 mg/infusão a cada 3, 4 ou 6 meses, dependendo da adesão do paciente ao protocolo e duração do tratamento. Alendronato e risedronato foram administrados em doses orais semanais de 70 e 35 mg, respectivamente, seguindo as instruções fornecidas pelos fabricantes. Para o ácido zoledrônico, uma dose de 0,05 mg/kg foi administrada com intervalos de 6 meses. Pamidronato e zolendronato foram agrupados como BP intravenoso e alendronato e risedronato como BP oral. O tratamento com BP foi analisado em relação ao tipo de droga, via de administração e duração do tratamento.

BMO analisados incluiram cálcio e fósforo séricos, fosfatase alcalina total, osteocalcina, 25(OH) vitamina D e C-telopeptideo (CTX). BMO foram obtidos por amostras de sangue coletadas em jejum. Laudos de exames laboratoriais foram avaliados por um período de cinco anos antes e após o início do tratamento com BP no grupo BP-tratado e para o grupo não-tratado foram considerados laudos dos últimos 5 anos de acompanhamento clínico. Os exames laboratoriais foram realizados em diferentes laboratórios, de acordo com a preferência e procedência dos pacientes e cobertura do seguro de saúde.

Para o grupo BP-tratado, a incidência de fratura foi determinada por um período de 5 anos ou de 10 anos anterior ao inicio do tratamento com BPs e por um período de 5 anos ou 10 anos após o tratamento ter sido iniciado. Para o grupo não-tratado, foi avaliado o número de fraturas durante um período de observação de 10 anos.

Numa tentativa de evitar inconsistência na incidência de fraturas com base apenas na auto-relato, o prontuário médico de cada paciente foi examinado a procrura de raio-X ou relatórios ortopédicos do evento. Quando necessário, os pacientes ou centro de tratamento local foram contatados para solitação de raio-X confirmando a fratura. Foram incluídas fraturas de ossos longos dos membros superiores e inferiores, metacarpos, metatarsos e coluna vertebral. Fraturas periféricas, como dedos da mãos e pés e costelas foram excluídas.

DMO da coluna lombar, quadril total e colo femural foram mensurados por *dual energy X-ray absorptiometry* (DXA). Foram comparados o primeiro e o último exames de densitometria sob tratamento com BPs para o grupo BP-tratado. Para o grupo não-tratado foi selecionado um exame de densitometria dos últimos 5 anos de acompanhamento clínico. A maioria dos exames de densitometria óssea foram realizados no Instituto Kennedy Krieger ou no Hospital Johns Hopkins - Departamento de Medicina Nuclear no equipamento *Hologic* (Horizon A, S / N 100164 ou 4500; Hologic Corp, Bedford, MA). No entanto, alguns exames foram obtidos nos centros de tratamento locais dos pacientes. Locais com material metálico cirúrgico, deformidade óssea grave ou escoliose grave foram excluídos da análise.

ANÁLISE ESTATÍSTICA

As variáveis quantitativas foram descritas por média e desvio padrão/erro padrão ou mediana e intervalo interquartil. As variáveis categóricas foram descritas por meio de frequências absolutas e relativas. Para comparer medias entre os grupos, the independent-samples t test foi aplicado. Na comparação de proporções, os testes Pearson's chi-square or Fisher's Exact test foram utilizados. Para comparações de parâmetros intra e inter-grupos simultaneamente, Generalized Estimating Equations com ajuste por Bonferroni foi aplicada.

O nível de significância adotado foi de 5% ($\alpha \leq 0,05$) e as análises foram realizadas através do software SPSS (versão 21.0. SPSS Inc, Chicago, Illinois).

RESULTADOS

Duzentos e seis prontuários de pacientes foram revisados e 47 patientes foram incluídos no estudo. A idade média total dos pacientes foi de 51,5 ± 13,2 anos e 24 (51,1%) indivíduos eram do sexo feminino. O grupo não-tratado foi composto por 12 indivíduos (10 OI-I, 2 OI-IV), e o grupo BP-tratado por 35 indivíduos (17 OI-I, 14 OI-III, 4 OI-IV). Não houve diferença significativa entre os dois grupos quando as variáveis foram ajustadas por sexo (p = 0,674), idade (p = 0,088) e idade > 50 anos (p = 0,105). No entanto, houve diferença significativa quando ajustado por tipo de OI (p = 0,032).

Quarenta e dois por cento dos pacientes receberam tratamento com BP oral, 25,7% com BP IV e 31,4% receberam tratamento com ambos os tipos de BP, oral e IV. A mediana e percentil 25-75 de duração total do tratamento com BP foi de 5 (4-8), o tratamento com BP por via oral mostrou maior tempo de duração (3 [0-5]: mediana [percentil 25-75]) quando comparado ao tratamento com BP IV (2 [0-4]).

Entre todos BMO analisados, apenas a osteocalcina mostrou diferença significativa entre os grupos BP-tratado e não-tratado. No entanto, os níveis de 25 (OH) vitamina D foram classificados como baixo-normal pois estavam no limite inferior do valor de referência para ambos os grupos. Além disso, para o grupo BP-tratado, não houve diferença significativa quando foram comparados os valores médios dos BMO durante o período de tratamento e 5 anos após a interrupção do tratamento (**tabela 1**).

Não foi observada diferença na taxa de incidência de fraturas comparando um período de 5 ou 10 anos antes e após o incio do tratamento com BP para o grupo BP-tratado

(**tabela 2**). A Tabela 3 sumariza a comparação da DMO pré e pós-tratamento para o grupo BP-tratado onde foi observada uma diferença significativa para a DMO da coluna lombar que apresentou aumento de 0.730 para 0,780 g / cm2 (p = 0,002). Além disso, não houve diferença significativa comparando valores de DMO entre os grupos não-tratado e BP-tratado, considerando o último estudo DXA durante o acompanhamento clínico para o grupo não tratado e o último DXA estudo disponível antes da interrupção do tratamento para o grupo BP-tratado.

DISCUSSÃO

Existe limitada literatura sobre o efeito dos BPs sobre BMO, incidência de fraturas e DMO em adultos com OI. A maioria dos estudos que comprovam os benefícios do tratamento com BPs foram desenvolvidos em crianças e adolescentes e diversos desses postivos efeitos têm sido difíceis de transpor para a população adulta com OI (Lindahl et al., 2014). BPs atuam inibindo a função dos osteoclastos e levando a sua apoptose e à diminuição da reabsorção óssea consequentemente. BPs são mais eficazes em estados de elevado *turnover* ósseo (Russel, 2007; Shapiro et al., 2010; Russel, 2015). Isso pode explicar as diferenças na resposta à terapia com BP entre crianças com o sistema esquelético em crescimento com elevado *turnover* ósseo e o esqueleto maduro de adultos com OI (Rauch et al., 2002; Shapiro et al., 2010; Bradbury et al., 2012; Eghbali-Fatourechi, 2014).

Neste estudo, 42% dos indivíduos receberam BPs orais e a duração do tratamento com BP oral foi maior quando comparado com indivíduos tratados com BPs IV. Normalmente, o tratamento por via oral é a opção preferida para adultos com OI, considerando a baixa taxa de fraturas nessa idade e a facilidade de administração comparado ao BP IV (Martin & Shapiro, 2007; Bachrach & Ward, 2009). Diferença significativa foi

observada entre os grupos quando ajustado por tipo de OI, o grupo não-tratado foi composto em sua maioria por pacientes com OI tipo I. Isso pode ser explicado pelo fato de que o tratamento com BPs é mais frequentemente administrado para pacientes com formas moderada a grave de OI do que para pacientes com formas leves de OI no intuito de diminuir dor músculo-esquelética, incrementar DMO e reduzir a incidência de fraturas (Glorieux, 2008).

Em nossa amostra de adultos com OI, o tratamento com BP oral ou IV a longo prazo não alterou a maioria dos valores de BMO analisados, exceto pela osteocalcina que apresentou níveis mais altos para os pacientes não tratados com BPs. Além disso, embora nós não tenhamos encontrado diferença significativa comparando os grupos BP-tratado e não-tratado, ambos os grupos apresentaram níveis abaixo do valor de referência normal para 25 (OH) vitamina D. Garnero et al. (2009) também encontraram valores mais elevados de osteocalcina em pacientes com OI não tratados com BPs comparando com controles saudáveis. Um estudo histológico em crianças com OI demonstrou um aumento do número de osteoblastos e do *turnover* ósseo em geral (Rauch et al., 2004). Secundário ao tratamento com BP há um declínio em torno de 70% na formação óssea a partir do início do tratamento, o que pode ser relacionado com o valor mais elevado de osteocalcina observado nos pacientes não tratados (Rauch et al., 2006).

Um estudo prospectivo não randomizado avaliou dados de 10 pacientes com OI tratados com zolendronato e não relataram mudanças nos valores séricos de cálcio, fosfato, fosfatase alcalina óssea ou CTX e ainda um decréscimo nas concentrações de vitamina D após 36 meses de tratamento (Pavón de Paz et al., 2010). Em um estudo observacional, incluindo adultos com OI tipo I tratados com risedronato por 24 meses, observou-se uma diminuição no P1NP (*serum procollagen type I aminopropeptide*) em 37%, mas não nas concentrações de fosfatase alcalina óssea. O estudo também relatou uma deficiência de 25

(OH) vitamina D entre os pacientes e nenhum efeito do tratamento com BP oral em relação a taxa de fratura foi observado (Bradbury et al., 2012). Braga et al. (2004) também observaram níveis de marcadores ósseos de 50 a 200% superiores em adultos com OI não tratados em comparação com controles incluindo fosfatase alcalina óssea e total, osteocalcina, NTX e free-deoxipiridinolina-livre urinária (ufDPD).

Seguido o tratamento com BP foi observado um aumento na DMO da coluna lombar, mas não do quadril total ou do colo femural. Além disso, na nossa amostra, tratamento a longo prazo com BP oral ou IV não foi associado com um decréscimo na taxa de fraturas, durante um período de 5 ou 10 anos de observação. Diversos estudos têm relatado aumento da DMO associada ao tratamento com oral ou IV BPs em adultos com OI (Adami et al., 2003; Chevrel et al., 2006; Pavón de Paz et al., 2010; Shapiro et al., 2010; Bradbury et al., 2012; O'Sullivan et al., 2014). De acordo com nossos achados, melhoria da DMO da coluna lombar é o ganho mais descrito na literatura associado a terapia com BPs entre adultos com OI(Adami et al., 2003; Chevrel et al., 2006; Pavón de Paz et al., 2010;. Shapiro et al., 2010; Bradbury et al., 2012; O'Sullivan et al., 2014), no entanto o efeito em relação ao colo femural e quadril total é relatado como inalterado ou com discreta melhora (Adami et al., 2003; Chevrel et al., 2006; Bradbury et al., 2012; Pavón de Paz et al., 2010; Shapiro et al., 2010).

Embora ainda inexplicado, em adultos com OI a densidade óssea parece atingir um platô com 2 a 3 anos de tratamento com BP (Martin & Shapiro, 2007). Um estudo histomorfométrico demonstrou aumento significativo da DMO associada à terapia com BPs, com maior ganho alcançado nos primeiros 2 a 4 anos de tratamento (Rauch et al., 2006). A gravidade da doença também influencia os resultados relacionados a eficácia do tratamento com BP. Um estudo retrospectivo não-randomizado incluindo 90 adultos com OI tratados com BPs oral ou IV, mostrou um aumento significativo da DMO da coluna lombar após o tratamento com BP IV em indivíduos com OI tipos I ou III/IV, no entanto com BP oral houve

aumento da DMO da coluna lombar apenas em indivíduos com OI leve. A incidência de fratura foi marginalmente reduzida com tratamento com BP IV em pacientes com OI tipo III/IV (Shapiro et al., 2010) e não houve efeito para pacientes com OI tipo I tanto com BP oral quanto com BP IV.

A incidência de fratura é um achado importante a ser mensurado, pois reflete a qualidade óssea diferente da densitometria óssea que revela a quantidade óssea. Em adultos com OI, a relação entre a incidência de fraturas e o tratamento com BPs não tem demonstrado consistência. Alguns estudos previamente reportaram inalterada taxa de fratura (Bradbury et al., 2012), uma tendência à diminuição da incidência de fraturas em favor do tratamento (Adami et al., 2003), efeito na redução da incidência de fraturas seguido de tratamento com BPs IV (Pavón de Paz et al., 2010; Shapiro et al., 2010) e alguns estudos não puderam demonstrar um efeito devido a falta de poder estatístico (Chevrel et al., 2006; O'Sullivan et al., 2014).

Estudos recentes da Cochrane avaliaram os resultados do tratamento com BPs de meta-análises de ensaios clínicos randomizados e quasi-randomizado controlados. Foi observado um aumento DMO em crianças e adultos com OI seguindo tratamento com BPs oral ou IV, mas o efeito sobre a incidência de fraturas foi inconclusivo (Phillipi et al., 2008; Dwan et al., 2014). Conjuntamente, duas recente meta-análises concluiram que o efeito dos BPs na diminuição da incidência de fraturas em adultos com OI é "inconclusivo" ou "parecia equivalente ao placebo" (Hald et al., 2015; Shi et al., 2015).

Os benefícios potenciais do tratamento com BP devem ser cuidadosamente investigados em adultos com OI considerando todos os eventos adversos relatados relacionados a esta terapia, tais como dor gastrointestinal, dor muscular e óssea, hipocalcemia assintomática transitória, não-consolidação de fraturas, atraso na consolidação

de locais de osteotomia e fraturas atípicas por fragilidade (Basel & Steiner, 2009; Abrahamsen, 2010; Uihlein & Leder, 2012; Hegazi et al., 2015).

O presente estudo tem várias limitações. Em primeiro lugar, este é um estudo observacional não-randomizado, porém, englobou vários anos de acompanhamento clínico dos pacientes. Alguns dados de biomarcadores metabólicos e ósseos não estavam disponíveis, como por exemplo exames incluindo CTX. Os exames laboratoriais foram executados em diferentes locais de acordo com a facilidade e procedência do paciente, no entanto, a grande maioria do exames de densitometria óssea foram realizados na nossa intituição. Devido ao pequeno tamanho da amostra, homens e mulheres não foram analisados separadamente. A incidência de fraturas foi baseada em auto-relato o que permite perda de dados, a documentação de fraturas em pacientes adultos com OI é difícil devido inadequada confirmação sem raios-X e esquecimento de datas e locais de fratura por parte dos pacientes. Além disso, este estudo não aborda resultados secundários ao tratamento, tais como o alívio do desconforto músculo-esquelético ou melhoria na funcionalidade ou na qualidade das atividades diárias.

CONCLUSÕES

O tratamento a longo prazo com BPs para adultos com OI não foi associado à redução sustentada da incidência de fratura e não se refletiu de forma significativa nos níveis de biomarcadores metabólicos e ósseos. Conforme relatado em diversos estudos, observou-se uma melhora na DMO da coluna lombar associada à terapia com BP. Nossos achados estão em conformidade com a literatura corrente que indica que o tratamento a longo prazo com BPs não pode ser associado com uma redução prolongada na incidência de fraturas em adultos com OI.

Dados abordando a eficácia dos BPs orais e intravenosos em pacientes adultos com OI são conflitantes, uma avaliação crítica da terapia com BP em curso é urgentemente necessária abordando diversos desfechos como incidência de fraturas, biomarcadores metabólicos e ósseos, efeitos adversos e segurança do tratamento a longo prazo.

CONFLITO DE INTERESSES

Os autores declaram não ter conflito de interesses.

CONTRIBUIÇÕES DOS AUTORES

Todos os autores participaram do desenho do estudo, interpretação dos dados e redação do manuscrito. Todos os autores aprovaram a versão final do manuscrito.

AGRADECIMENTOS

Os autores agradecem aos pacientes e suas famílias pela participação neste estudo. Evelise Brizola foi apoiada pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Proc. Número 3770/14-1.

REFERÊNCIAS

Idem as referências na versão em inglês.

Tabela 1. Comparação de biomarcadores metabólicos e ósseos: A) após 5 anos de observação entre os grupos não-tratado e BP-tratado; B) durante o tratamento com BP e após 5 anos de interrupção no grupo BP-tratado

Variável		N	Média	Erro padrão	P
Cálcio	Grupo não-tratado	11	9.41	0.07	0.625*
	Grupo BP-tratado durante tratamento	27	9.47	0.08	
	Grupo BP-tratado após interrupção do tratamento	25	9.46	0.08	0.897+
25 (OH) Vitamina D	Grupo não-tratado	12	33.4	2.40	0.774*
	Grupo BP-tratado durante tratamento	28	34.5	1.99	
	Grupo BP-tratado após interrupção do tratamento	24	40.4	3.19	0.070+
Fosfatase Alcalina Total	Grupo não-tratado	12	83.2	7.22	0.617*
	Grupo BP-tratado durante tratamento	28	94.4	13.9	
	Grupo BP-tratado após interrupção do tratamento	26	85.2	7.57	0.300+
Fósforo	Grupo não-tratado	12	3.69	0.16	0.253*
	Grupo BP-tratado durante tratamento	25	3.49	0.09	
	Grupo BP-tratado após interrupção do tratamento	22	3.47	0.09	0.785+
Osteocalcina	Grupo não-tratado	12	31.6	3.44	**<0.001***
	Grupo BP-tratado durante tratamento	26	13.7	1.52	
	Grupo BP-tratado após interrupção do tratamento	24	15.2	1.61	0.510+
CTX	Grupo não-tratado	11	229.3	21.7	0.371*
	Grupo BP-tratado durante tratamento	15	165.5	55.6	
	Grupo BP-tratado após interrupção do tratamento	19	211.5	59.4	0.558+

* P= comparação entre o grupo não tratado e o grupo BP-tratado durante período de tratamento

+ P= comparação durante o tratamento e após interrupção para o grupo BP-tratado

Tabela 2. Taxa de Fratura no grupo BP-tratado comparando intervalos de 5 ou 10 anos antes a após o início do tratamento com BP

Tempo de tratamento		**Taxa de Fratura**		
		Média	**Std. Error**	**P**
Pré-tratamento	**10 anos**	1.23	0.262	0.109^{+}
	5 anos	0.86	0.181	0.380*****
Pós-tratamento	**5 anos**	1.06	0.156	0.380*
	10 anos	1.71	0.220	0.109^{+}

*P= comparação 5 anos pré e pós tratamento

$^{+}$P= comparação 10 anos pré e pós tratamento

Tabela 3. Comparação da DMO pré e pós-tratamento para o grupo tratado com BP e entre os grupos BP-tratado e não-tratado

Variável		BP tratado				BP-tratado x Não-tratado				
		N	Média	Std. error	P		N	Média	Std. error	P
Quadril	pré-tratamento	15	0.720	0.06	0.461	**não-tratado**	11	0.740	0.05	0.864
	pós-tratamento	17	0.760	0.07		**BP-tratado**	17	0.760	0.07	
Coluna	pré-tratamento	25	0.730	0.03	**0.002**	**não-tratado**	12	0.750	0.05	0.528
	pós-tratamento	26	0.780	0.03		**BP-tratado**	26	0.780	0.03	
FN	pré-tratamento	16	0.700	0.04	0.877	**não-tratado**	10	0.640	0.06	0.537
	pós-tratamento	18	0.690	0.05		**BP-tratado**	18	0.690	0.05	

FN: colo femural

8. CONSIDERAÇÕES FINAIS

A Osteogênese Imperfeita é uma doença rara com expressividade variável. A clássica classificação da doença em quatro fenótipos distintos foi expandida com a inclusão da OI tipo V na tentativa de acomodar as diversas novas descobertas tanto na área clínica quanto molecular.

As características clínicas e radiológicas no momento do diagnóstico da doença podem auxiliar o profissional no reconhecimento dos casos. A presença de escleras azuladas, dentinogênese imperfeita, ossos wormianos e fraturas de ossos longos são características clássicas que compõem o quadro clínico da OI, porém podem ser observadas em outras doenças. O diagnóstico diferencial em relação a casos de maus- tratos infantis também deve ser cuidadosamente investigado e o padrão e a história da fratura são informações relevantes na distinção entre estes casos.

OI tipo V é um dos novos tipos de OI que não apresenta sobreposição fenotípica aos quatro fenótipos prévios descritos por Sillence (Sillence et al., 1979). Na nossa amostra assim como descrito na literatura, os casos suspeitos de OI tipo V representaram 4% da população com OI em tratamento no CROI-HCPA. A mutação c.-14C>T no gene *IFITM5* foi identificada em todos os casos positivos para a OI tipo V. Características clínicas e radiológicas distintas podem ser observadas nestes pacientes, como por exemplo, ausência de dentinogênese imperfeita, formação de calo hiperplásico, calcificação das membranas interósseas entre os ossos dos antebraços e das pernas, deslocamento da cabeça radial e alteração no formato do tórax e posicionamento das costelas.

Após vinte anos de tratamento com bifosfonados questões relacionadas à eficácia e segurança da droga estão em discussão e investigação. No nosso estudo não encontramos

uma relação estatisticamente significante entre o tratamento com bifosfonados, oral ou intravenoso, e redução na incidência de fraturas em pacientes adultos com OI. Biomarcadores metabólicos e ósseos não demonstraram significante diferença entre pacientes tratados e não tratados, exceto para concentrações de osteocalcina que é um marcador da formação óssea. O tratamento com BPs a longo prazo levou ao aumento significativo da DMO na coluna lombar, porém não não houve diferença para os outros locais avaliados.

As novas descobertas nas áreas clínica, molecular e terapêutica parecem estar mudando o perfil de tratamento da doença. A gravidade da doença tem sido relacionada com a resposta ao tratamento, bem como com o genótipo. O estudo clínico e genético nessa população traz um maior entendimento sobre a doença e auxilia no adequado aconselhamento genético das famílias. Correlações fenótipo-genótipo embora complexas são importantes, uma vez que mesmo dentre uma família que apresenta a mesma mutação, as características clínicas não seguem um padrão de apresentação único.

Informações sobre o tratamento medicamentoso no curto, médio e longo prazos fortalecem opções terapêuticas propiciando melhores estratégias de prevenção e de tratamento e ainda evitam administração desnecessária de fármacos com potenciais e conhecidos efeitos adversos. Estudos adicionais com enfoque clínico, molecular e terapêutico na população pediátrica e adulta com OI são necessários.

9. PUBLICAÇÕES ADICIONAIS

9.1. Artigo de Revisão

O artigo intitulado "*Pathophysiology and Therapeutic Options in Osteogenesis Imperfecta: an Update*" foi aceito para publicação no periódico "*Research and Reports in Endocrine Disorders*".

Pathophysiology and Therapeutic Options in Osteogenesis Imperfecta: an Update

Brizola E[1], Félix TM[2], Shapiro JR[1]

[1] Bone and Osteogenesis Imperfecta Program, Kennedy Krieger Institute, Johns Hopkins School of Medicine, Baltimore, MD, USA

[2] Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil

ABSTRACT

Osteogenesis imperfecta is a rare, inherited systemic disorder of bone and connective tissue which in almost 90% of cases is due to mutations affecting the normal synthesis of type I collagen. In 1979, Sillence et al categorized four OI phenotypes which where inherited as autosomal dominant and which were shown due to defective type I collagen synthesis. Individuals with OI present both genetic and phenotypic variability. Major characteristics of OI are bone fragility, blue sclerae, dentinogenesis imperfecta, short stature, scoliosis and joint hyperextensibility. Both autosomal dominant and recessive inheritance are now recognized.

Advances in molecular diagnosis have led to a major expansion in our understanding of the genetic basis for different OI phenotypes. To date, sequence variants in 16 genes are described as causative of OI. These genes regulate the synthesis of type I collagen pro-alpha polypeptide chains, proteins involved in type I collagen processing in the endoplasmic reticulum and proteins involved in osteoblast function. These new genetic associations have also led to uncertainty with regard to the current classification of OI phenotypes.

Bisphosphonates have been widely used to improve bone mass and decrease fractures in both children and adults with OI. While effective in many but not all children when administered for 2-4 years, bisphosphonates have not proven effective in adults with OI. Studies are limited for treatment of adults with teriparatide and denosumab. Advances have been reported in the surgical management of OI. Although the role of physical therapy in the management of children and adults was previously described, this important treatment modality is significantly underutilized.

Key words: osteogenesis imperfecta, sequence variants, collagen, bisphosphonates, bone, treatment

INTRODUCTION

Osteogenesis imperfecta (OI) is a rare clinically and genetically heterogeneous systemic disorder of bone and connective tissue characterized by bone fragility and physical findings related to the underlying connective tissue disorder. The incidence of OI is around 6-7/100.000 births and approximately 500,000 persons worldwide have OI.[1] In United States there are between 25,000 to 50,000 affected individuals.[2]

Individuals with OI have a lifelong risk of fracture which may occur at times spontaneously or following minimal trauma. Other systemic manifestations are blue sclerae, dentinogenesis imperfecta (DI), early onset hearing loss, short stature, kyphoscoliosis, and joint hyperextensibility. Less frequent are basilar invagination, cardiac valvular and vascular disease and ocular complications including glaucoma and decreased corneal thickness.[1-5]

There has been remarkable progress in understanding the molecular basis for this disease. Over the last decade, advances in molecular biology have greatly expanded the association of previously unrecognized gene sequence variants with phenotypes now categorized as OI. As a consequence, the spectrum of OI phenotypes is now considerably enhanced compared to the 4 phenotypes first described by Sillence.[6] That classification employed clinical severity, radiological features and inheritance, dividing OI in four types: mild (OI type I), lethal (OI type II), severe progressive (OI type III) and moderately deforming (OI type IV) (Sillence, 1979). These four types were inherited in an autosomal dominant manner and each was subsequently shown to involve mutations affecting type I collagen pro-alpha chains.

The Online Mendelian Inheritance in Man database (OMIM) currently lists OI types I to XVI based on recently defined sequence variants including several proteins not in the classic collagen pathway. Approximately 90% of OI cases involve autosomal dominant inheritance with mutations in the *COL1A1* or *COL1A2* genes.[5] To date, the Human Collagen

Mutation Database (http://www.le.ac.uk/genetics/collagen/) lists the following genes associated with a phenotype within the broad clinical spectrum considered as OI: *BMP1, COL1A1, COL1A2, CREB3L1, CRTAP, FKBP10, IFITM5, P3H1, P4HB, PLOD2, PLS3, PPIB, SEC24D, SERPINF1, SERPINH1, SP7, TMEM38B,WNT1* and *SPARC*.

OI CLASSIFICATION

A classification of OI can be based on a definitive genotype or on a phenotype that is highly variable and more difficult to categorize in many individuals even within the same family. Furthermore, expression of one gene locus can translate into different skeletal phenotypes. Access to genetic testing is not available for many individuals. Therefore, many individuals with OI do not know what clinical type they represent or the genetic basis for their phenotype. Recognizing the emerging confusion due to the expansion of genetic data in 2010, the International Nomenclature Group for Constitutional Disorders of the Skeleton (INCDS) elected "to retain the Sillence classification with the inclusion of type V OI as the prototypic and universally accepted way to classify the degree of severity in OI; and to free the Sillence classification from any direct molecular reference".[7] The new causatives OI genes are been arranged according to clinical severity into the five OI types (**table 1**).[5,7] In 2010, Van Djik et al. proposed a revised classification of OI with the exclusion of type VII and VIII OI since these types were added based on genotype but with clinical and radiological characteristics indistinguishable from Sillence types II and IV OI.[1]

It is important to emphasize that the diagnosis of OI still requires historical, clinical and radiological evidence of the disorder. Fracture history *per se* in a child does not establish the diagnosis of OI and several other skeletal disorders including secondary causes of osteoporosis can mimic OI clinically. These include hypophosphatasia, idiopathic juvenile osteoporosis, celiac disease, Ehlers-Danlos syndrome, vitamin D deficiency and hypophosphatemic disorders. In addition, child physical abuse is relevant considering this is more frequent than OI and usually presents a history of multiple fractures but in a pattern different from OI.[8]

The recommendation of this review, from a clinical standpoint, is that a clinical classification such as the one suggested by INCDS seems to be the best option. Probably, clinical features will continue being the basis for OI diagnosis and newer genetic findings will be adapted into recognizable phenotypes.

PHENOTYPES AND GENOTYPES OF OI

The discovery of new genotypes related to OI phenotypes has been significantly expanded with the advent of SNP array technology and the increased application of whole exome sequencing.[9] The genes described as OI-causative regulate the synthesis of type I collagen alpha polypeptide chains, several proteins involved in type I collagen processing or proteins involved in osteoblast development and function.[8] Nonetheless, the correlation between genotype and phenotype in OI is extremely complex due high variability in the expressivity of the disorder (**table 2**).

The majority of OI cases are related to autosomal dominant inherence caused by heterozygosity for mutations in the *COL1A1* or *COL1A2* genes corresponding to OI types I, II, III and IV. Individuals with OI type I may experience few fractures associated or not to mild deformities (**figure 1A**). OI type II is characterized by severe bone fragility and neonatal mortality. OI type III is a severe form leading to multiple fractures even *in utero* and bone deformities (**figures 1B and 2**). OI type IV presents moderate bone involvement with high clinical variability.[5,7] *De novo* mutations are responsible for approximately 60% of mild (OI-I) or moderately deforming OI (OI-IV), almost 100% of severely deforming OI (OI-III) and practically all perinatal lethal cases (OI-II).[10] Occasionally types III and IV OI are the result of autosomal recessive inheritance.[1,5]

OI type V is an autosomal dominant disorder with a distinctive phenotype representing 4-5% of OI cases.[5] OI type V is the result of heterozygous mutations (c.-14C>T) in the 5'UT region of the *IFITM5* gene which encodes interferon induced transmembrane protein 5.[5,11,12] It is characterized by variable degree of severity and specific features such as hyperplastic callus, dislocation of the radial head and progressive calcification of the interosseous membranes of forearms and legs.[12] However, a child with clinical features of type III/IV OI was identified with a *de novo IFITM5* mutation (c.-14C>T) but without the classic features described for OI-V.[13]

OI types VI through XVI are recessively inherited. Collectively, these account for approximately 3-5% of individuals with OI and express moderately severe to lethal phenotypes which in some clinically resemble OI types II and III.[8]

OI type VI is associated with homozygous or compound heterozygous mutations in *SERPINF1*. This gene encodes Pigment Epithelium Derived Factor (PEDF) that is a multifunctional secreted glycoprotein of the serpin family with potent antiangiogenic activity.[14,15] It functions in bone homeostasis and osteoid mineralization. Affected individuals have a moderate to severe OI, presenting decreased bone mineral density (BMD) with no fractures at birth and without blue sclerae or DI. Bone biopsy shows a characteristic "fish-scale" pattern caused by disorganization of the bone matrix, with large amount of unmineralized osteoid tissue simulating osteomalacia.[15-17]

Individuals with OI types VII, VIII and IX have severe to lethal phenotypes caused respectively by mutations in the *CRTAP*, *LEPRE1* and *PPIB* genes. Approximately 1.5% of West Africans and 0.4% of African Americans carry a founder mutation in *LEPRE1*gene and three lethal cases of OI VIII were reported in individuals of these ethnicities.[18,19] In addition to features common to severe OI phenotypes, mutations in *CRTAP* and *LEPRE1*

genes (OI-VII, OI-VIII) are associated with formation of bulbous epiphysis with popcorn calcifications at the distal femurs.[19,20]

Type X OI is the result of mutations involving the *SERPINH1* gene which regulates folding and trafficking of the collagen triple helix in the endoplasmic reticulum.[5,21] This mutation was reported in a child from Saudi Arabia with triangular facies, blue sclerae, micrognatia, severe bone deformities, and bilateral renal calculi leading to impaired renal function.[21]

Progressively joint contracture is characteristic of OI type XI secondary to mutation in *FKBP10* gene which is associated with Bruck Syndrome (**figure 3**).[22,23] Bruck Syndrome main features are bone fragility with congenital contractures of large joints.[24] *PLOD2* mutations are also associated with Bruck syndrome and it expression in a boy from nonconsaguineus Turkish parents leaded to congenital join contractures with pterygium and severe OI-like osteopenia including multiple fractures.[25] Interestingly, two brothers from a Spanish family also with *PLOD2* mutations had phenotypes of different severity, one had a mild form with few fractures and white sclerae and the second had a more severe form with multiple fractures and blue sclerae; however, neither had congenital joint contractures.[24] Bruck Syndrome is classified as types 1 and 2 according the gene mutated *FKBP10* and *PLOD2* respectively; however, the phenotypes are indistinguishable.[24]

SP7 gene is associated with OI type XII. This moderate form of OI was described in one child from an Egyptian family displaying decreased BMD, recurrent fractures, mild bone deformities, white sclerae, delayed teeth eruption and absence of DI.[26]

Mutations in *BMP1* gene are categorized as OI type XIII. It was identified in a few individuals from Egyptian and Turkish families with severe but variable phenotypes. The Egyptians individuals had generalized decreased BMD, light blue sclerae, delay of motor development, severe growth deficiency, bone deformities, platispondyly, kyphoscoliosis,

umbilical hernia and several spontaneous fractures.[27] Nonetheless, the Turkish individuals had increased BMD, white sclerae, normal motor development; also multiples fractures after minimal trauma and not all of them had deformities in lower limbs.[28]

A homozygous mutation in the *TMEM38B* gene is associated with OI type XIV which appears to be an ancient mutation in Israeli Bedouin and Saudi families from the Arabian Peninsula.[29,30] Affected individuals had bone involvement of variable severity with decreased BMD, multiple fractures ranging from prenatal onset up to 6 years of age, white or blue sclerae, mild to moderate short stature, and normal teeth and hearing.[29,30]

A heterozygous missense mutation in *WNT1* gene was identified in a family with early-onset osteoporosis by dominant inheritance; illustrating the markedly variable expression seen with certain genes, a homozygous nonsense *WNT1* mutation was also identified in two siblings in a separate family with a recessive and moderately severe form of OI (OI-XV).[31,32] These individuals also had brain malformation and severe intellectual disability not associated with major OI phenotypes.[31]

OI type XVI is associated with mutations in *CREB3L1* gene. It was reported in a Turkish family with consanguinity. One affected was a fetus from a pregnancy medically interrupted at 19 weeks where post-mortem exam showed thin ribs, and long bone fractures. The second child showed intrauterine fractures, pulmonary infections and cardiac insufficiency leading to death in the first year of life.[33]

Mutations in *P4HB* and *SEC24D* genes are causative of Cole-Carpenter syndrome 1 and 2 (CCS-1, CCS-2), respectively.[34,35] CCS-1 is a OI-like disorder that includes severe bone fragility, fractures, craniosynostosis, ocular proptosis, hydrocephalus, and dysmorphic facial features.[34] Individuals initially described with CCS-2 were from a German family and displayed severe pre- and postnatal bone fragility with multiple fractures diminished ossification of the skull, craniofacial dysmorphism and short stature.[35] A study with the

zebrafish model showed that Sec24D plays an important role in the extracellular matrix secretion in cartilage. With reference to facial dysmorphism Sec24D deficiency is shown to affect craniofacial morphogenesis in late stages of chondroblast development.[36]

In 2013, X-linked osteoporosis with early onset fractures occurring in childhood was described in five families associated with a mutation in *PLS3* which encodes the actin-binding protein, plastin 3. The rare heterozygous sequence variant (rs140121121) was associated with decreased BMD and an increased risk of fracture among women in the general population.[37]

SPARC is the gene most recently associated with an OI phenotype. Two distinctive homozygous missense mutations in *SPARC* were reported from two unrelated girls with severe bone fragility. Besides severe bone fragility, one had severe early-onset scoliosis requiring spinal fusion at age 6 years. The second girl was born prematurely of a consanguineous couple, with hypotonia and gross motor developmental delay. Histomorphometric study showed matrix hypermineralization.[38]

CURRENT PHARMACOLOGICAL MANAGEMENT OPTIONS

There is no "optimal treatment" for OI if the primary object of treatment is to limit or prevent fractures in children or adults. Relief of musculoskeletal pain, improvement of compressed vertebral bodies and mobility can also be sought as secondary outcomes. Critical assessment of treatment outcomes is limited by the small numbers of participants in clinical trials and the short duration of many trials which is frequently limited to one or two years of observation.

Bisphosphonates

For over two decades, bisphosphonates (BPs) have been considered the first-line drug for treating children and adults with moderate or severe forms of OI. Individuals with type I OI may be treated with BPs depending on the frequency of fractures. As discussed below, the use of BPs in OI has recently come under critical review. Third generation BPs are nitrogen containing analogues of pyrophosphate which inhibit osteoclast bone resorption by interfering with the mevalonic acid pathway and protein prenelation.[38] Bone formation subsequently is increased as osteoblastic bone formation completes mineralization at sites of active bone formation (transients). BPs have strong affinity for bone mineral, particularly at sites of increased bone turnover.[39] In OI, the response to BP therapy differs between children and adults. BP treatment is generally efficient during childhood due to high bone turnover; however, after puberty there is a decrease in bone turnover which limits the effectiveness of BP in decreasing fracture rate.[2,40]

Currently individual centers in the US and Europe may use different BPs. Nonetheless, alendronate and risedronate by the oral route and pamidronate and zoledronate by intravenous (IV) route are the most frequently used. It is important to note that there are significant differences with regard to schedules, doses and duration of treatment among various centers and published studies (**table 3**).[41]

Oral BPs are advantageous by not requiring IV access; however, the bioavailability is low and compliance may be poor. Several studies have reported the treatment effects of oral BPs in children and adults but to date there is no conclusive evidence its effect on sustained reduction of fracture rate. Two years of alendronate treatment in children with OI was associated with improved lumbar spine areal BMD (LS-aBMD) and decreased bone turnover; however there was no significant difference in regard to the height of the vertebral bodies, cortical thickness, mobility, bone pain and fracture incidence.[42]

Equally beneficial effects following 2 years of BP therapy including increase in BMD, decrease in bone turnover and a trend to decreased fracture incidence was observed comparing alendronate to pamidronate in a sample of 18 children with OI.[43] In a randomized, double blind and placebo-controlled trial, a daily dose of risedronate was administered for one year to children with OI.[44] Eighty-one per cent had a mild form of OI. After 12 months, risedronate treatment increased LS-aBMD and reduced the risk of recurrent fractures by 42%. However, during an extension open-label phase, 2-3 years after the placebo-controlled phase, there was no difference in lumbar spine and total body aBMD between the groups. In addition, 53% of patients in the treated group versus 65% of patients receiving placebo reported vertebral or non-vertebral fractures.[44]

Palomo et al. reviewed the results of pamidronate treatment in 37 children with OI who started BP therapy before 5 years of age, and who had a subsequent follow-up period of at least 10 years.[45] At baseline, 35% of vertebrae were affected by compression fractures, whereas only 6% appeared compressed at the final evaluation, indicating vertebral reshaping during growth. Although long-term pamidronate therapy was associated with higher Z-scores for LS-aBMD and vertebral reshaping, the long-bone fracture rate remained high and the majority of patients developed scoliosis.

Bradbury et al. treated 37 adults with type I OI with risedronate (35 mg/weekly) for 24 months following which there was an increase on lumbar spine BMD and a decrease in bone biomarkers; however, post-treatment there was no difference on bone pain or fracture incidence.[46] In addition, Shapiro et al. reported a marginal decreased fracture incidence associated with IV BP therapy in adult patients with moderate to severe OI but not in individuals with mild OI, and no significant change was observed with oral BP in any type of OI.[47]

Pamidronate is the most used BP for children with OI, however more recently zoledronate also has been adopted as a treatment option for this population. Zoledronic acid given intravenously is 200 fold more potent than pamidronate and longer-lasting, possibly up to 2 years in children, in suppressing bone turnover. To date, information is limited as regards efficacy and safety in OI patients. However, recent publications indicate the relative short-term safety of zolendronate administered at 6 month intervals in children with OI.[48,49] The advantage of this drug compared to pamidronate is the schedule of infusion, zoledronate a shorter infusion requires short time and is less frequent. Zoledronate is given for 30 minutes every 6 months and pamidronate is infused for 4 hours every 3 to 4 months.[49,50] There is no data indicating that zoledronate is more effective in decreasing fracture rate compared to pamidronate. Noting that, zoledronate was designed to be administered yearly in adults with osteoporosis, the current practice of zoledronate therapy in children with OI remains to be assessed.

Positive effects have been described following BP therapy such as increase of BMD, reduce bone turnover, reduction of fractures, increase in vertebral body height, relief of musculoskeletal pain and, improvement in mobility.[47,50-53] Nonetheless, for adults with OI the effect on prevention of fracture still needs further investigation.[53,54]

Recent Cochrane reports assessed the results of BP treatment from meta-analysis of randomized and quasi-randomized controlled clinical trials.[55,56] Including 8 and 14 trials (403 and 819 participants) respectively, both reports stated an increase of BMD for children and adults with OI following either oral or IV BP treatment, but the effect on decreasing fracture rate was inconclusive. Also, the Dwan review found no significant evidence of positive effect of BP on improvement of clinical status such as reduction of pain and functional mobility.[56]

Rijks et al. reviewed 10 randomized controlled trials (519 participants) with regard to effectiveness and safety of BP in children with OI.[57] LS-aBMD increased and bone resorption biomarkers decreased following either oral or IV BP. Significant decrease in fracture incidence was reported in 70% of the studies following BPs therapies. However, the authors highlighted a common problem in interpreting these treatment reports: a) fracture incidence was reported in several ways, b) differentiation between vertebral fractures and fractures of extremities required definition and c) confounding factors need to be considered eg., age, pubertal status and physical activity.

In 2015, Hald and colleagues conducted a meta-analysis specifically to assess the effects of BP therapy on the prevention of fractures.[58] Six randomized, placebo controlled trials (424 subjects) were included. The results of BP therapy on fracture prevention were considered "inconclusive", the proportion of participants who experienced a fracture was not significantly reduced.[58] The meta-analysis by Shi et al. reported the efficacy of BP treatment on BMD and fracture rate in patients with OI.[59] In children, BPs were efficacious in reducing fractures (RR = 0.80; 95% CI: 0.66-0.97); whereas in adults, BP seemed equivalent to placebo (RR = 0.82; 95% CI: 0.42-1.59). There was also no significant difference in fracture reduction comparing oral and IV BP.

BPs are stored in the skeleton for years, although more rapid turnover in children may shorten residence in bone compared to adults.[60] Although data regarding the safety of BP is reported, issues such as bone turnover after several years of BP treatment go unreported. Delayed eruption of primary teeth following BP therapy is reported in children with OI, Kamoun-Goldrat et al. described a mean delay of 1.67 year in tooth eruption associated to BP treatment.[61] Atypical femur fracture following prolonged BP therapy previously described in adults with osteoporosis now is reported in a small number of children and adults with OI.[62-64] It raises the questions about oversuppression of bone

remodeling and its contribution to microdamage accumulation and increased bone fragility.[60,65] Hegazi and colleagues reported the occurrence of unusual femoral stress fractures in six children with OI on long-term pamidronate therapy.[66] These fractures occurred around intramedullary rods in the subtrochanteric or diaphyseal regions of the femur and were caused by no or minimal trauma, similar to the atypical femoral fractures previously described on adults with osteoporosis on long-term BP therapy and with suppression of bone turnover.

Delayed healing of osteotomies sites secondary to BP therapy was previously reported in children and adolescents with OI.[67] However, the same group recently published new data based on a protocol focused on patients treated with zoledronate, interval of 4 months without BP infusion after osteotomy and a different surgical technique using a manual osteotomy rather than a high power saw. [68] It was observed that with the newer approach, delayed healing of the osteotomies over 12 months occurred in 42% of the osteotomies performed versus 72% with the previous approach ($p = 0.001$).

Other effects also associated with BP therapy in OI patients are: gastrointestinal pain associated with oral BPs, flu-like symptoms after the first BP infusion, transient asymptomatic hypocalcemia and fracture non-union.[38,42,69,70]

Teriparatide

Teriparatide (human recombinant parathyroid hormone) is a PTH analog which increases bone mass by increasing osteoblast bone formation. Highly effective in the treatment of age related osteoporosis, teriparatide decreases the risk of fracture by increasing trabecular number and trabecular thickness.[71,72] A randomized, double-blind placebo-controlled trial assessed the effect of teriparatide treatment on 79 adults with OI.[72] Positive effects on bone markers were observed with a significant increase in hip and spine aBMD,

vertebral volumetric BMD, and estimated vertebral strength. This response was more pronounced in type I OI than in individuals with OI types IV and III. There was no significant difference on self-reported fractures when compared treated and control groups.[72]

Thirteen postmenopausal women with type I OI previously treated with BPs, received daily injections of teriparatide for 18 months.[73] Lumbar spine BMD increased significantly up to 3.5% but no difference was observed for total hip BMD. As in the Orwoll et al study, teriparatide significantly increased markers of bone formation and bone resorption suggesting a normal osteoblastic response to therapy. Fracture response was not reported.[73] Teriparatide is reported to promote healing of an atypical femur fracture in an adult patient with OI.[74] This may be related to the ability of this drug on stimulate chondrocyte proliferation and differentiation and cartilage production at the site of fracture.

Denosumab (Anti-RANK-ligand antibody)

The RANK, RANKL complex regulates bone remodeling cycles by regulating osteoblast/osteoclast coupling and osteoclast differentiation. RANK (receptor activator of NF-kB) is present on the osteoclast precursor, and RANK ligand (RANKL) produced by the osteoblast is part of the tumor necrosis factor (TNF) super family and along with the soluble decoy receptor osteoprotegerin (OPG) are essential regulators of osteoclast development and function.[75] Denosumab is a human monoclonal antibody to RANKL; studies involving age-related osteoporosis have showed the efficacy of denosumab in reducing signaling via RANK leading clinically to prevention of bone loss.[75,76] Due to poor response to BP treatment in OI-VI, Semler et al. treated four children with denosumab who had shown continuously elevated urinary bone resorption markers during a previous treatment with BP.[76,77] Treatment was well-tolerated and bone resorption markers decreased to the normal

range. However, this report did not address change in BMD or the impact on fracture rate in these children.[76] Subsequently, these authors described long-term denosumab effects over 8 to 12 cycles of treatment on these same patients.[78] There was a BMD increase, normalization of vertebral shape and decrease in fracture rate. Denosumab treatment also improved BMD and longitudinal bone growth in two children with *COL1A1/A2* mutations previously treated with BPs.[79]

NEW THERAPEUTIC APPROACHES

Anti sclerostin antibody

Sclerostin is a negative regulator of bone formation released from osteocytes which modulates osteoblast activity acting through Wnt/β-catenin pathway. Preclinical studies have demonstrated that treatment with antisclerostin monoclonal antibody improves bone mass and bone strength, and enhances repair of fractures in animal models.[80,81] Interestingly, at the tissue level different mechanisms are involved when comparing osteoanabolic therapy (teriparatide) and sclerostin antibody (Scl-Ab). While osteoanabolic therapy increases bone remodeling through increase on both bone formation and resorption; Scl-Ab increases bone formation while decrease bone resorption.[82]

In humans, antisclerostin antibody (romozumab) was administrated to postmenopausal women and health men (aged 45 to 59 years) and the results showed an increase of bone formation markers and a decrease on bone resorption markers. In addition, there was a significant increase in lumbar spine and hip aBMD.[83] In patients with OI, Palomo et al. did not find a relationship between circulating serum levels of sclerostin and LS-aBMD.[84]

Sinder et al. treated rapidly growing 3 week old Brtl/+ mice model of OI for 5weeks with Scl-Ab. Scl-Ab had anabolic effects in Brtl/+ and led to new cortical bone formation and increased cortical bone mass. This anabolic action resulted in improved mechanical strength to wild type levels without altering the underlying brittle nature of the material.[85]

However, Roschger et al. reported somewhat different results using male Col1a1 (Jrt)/+mice.[86] Once-weekly intravenous Scl-Ab injections for growing and adult mice had no effect on weight or femur length. No significant treatment-associated differences occurred in alkaline phosphatase activity, procollagen type I N-propeptide or C-telopeptide. Micro-CT analyses at the femur showed that Scl-Ab treatment was associated with higher trabecular bone volume and higher cortical thickness in wild type mice at both ages and in growing OI mice, but not in adult OI mice.[86]

Cathepsin K antibody

Cathepsin K is highly expressed in osteoclasts and is an essential enzyme involved in the degradation of type I collagen in the organic bone matrix.[39] In an animal model, the cathepsin K monoclonal antibody (Odanacatib) effectively suppressed bone resorption.[87] A phase 3 randomized, placebo-controlled trial assessed the effect of Odanacatib on fracture risk over 5 years of treatment in 16713 women with osteoporosis aged 65 years or older.[45,88] There was an increase in lumbar spine and total hip BMD over the five years and a significant reduction in the risk of fractures of hip (47% reduction, $p<0.001$), spine (23% reduction, $p<0.001$) and non-vertebral (23% risk reduction, $p<0.001$).[39,88] Applicability to the collagen defect in OI remains to be determined.

Transforming growth factor-β (TGF-β)

TGF-β is produced by osteoblasts and acts to coordinate bone remodeling by coupling osteoblasts and osteoclasts in the process of bone remodeling. TGF-β is secreted predominantly in an inactive latent form and is deposited into the bone matrix.[89] Several studies have reported in the effect of TGF-β overexpression in bone cells and bone. In 1996, Erlebacher et al. used transgenic mice to evaluate the role of TGF-β 2 in bone development and observed that increased expression of TGF-β 2 in osteoblasts resulted in an osteoporosis-like phenotype.[90]

In 2000, Gebken et al. studied the cell surface expression and functional properties of TGF-β receptors I, II and III on osteoblasts from a group of OI patients compared to healthy controls.[91] The number of TGF-β receptors s were significantly higher on OI osteoblasts than on age-matched control osteoblasts in spite of similar steady state levels for TGF-β receptor II mRNA in OI and control cells. Furthermore, receptor affinity was not significantly diminished. Thus, osteoblasts from OI patients were shown have an elevated number of cell surface receptors for TGF-β, without any evidence for a transcriptional regulation of TGF-β receptor II.[91] Excessive TGF-β signaling has been identified in Marfan syndrome involving abnormalities in both lung and aorta.[92]

Grafe et al have reported that excessive TGF-β signaling is a mechanism of OI in both recessive (Crtap-/-) and dominant (Col1a2tm1.1Mcbr) OI mouse models.[89] Higher expression of TGF-β target genes, a higher ratio of phosphorylated Smad2 to total Smad2 protein and higher *in vivo* Smad2 reporter activity were observed in these models. The binding of type I collagen of Crtap (-/-) mice showed reduced binding to decorin, a known regulator of matrix TGF-β activity. Treatment of Crtap (-/-) with the anti-TGF-β neutralizing

antibody 1D11 corrected the bone phenotype and improved the lung abnormalities in both recessive and dominant forms of OI.[89]

Prenatal and postnatal transplantation of mesenchymal stem cells

Severe to lethal forms of OI may be diagnosed *in utero* by ultrasonography starting at the 16th week. Signs include diffuse osteopenia, poor ossification of the skull, increased nuchal transluceny, fractures, delay of growth, micromelia, ventriculomegaly and bowing of long bones.[93] In the *oim/oim* mouse, allogenic transplanted wild type donor mesenchymal stem cells (MSCs) homed to bone improving collagen content and mineralization.[94] In humans, improvement of linear growth and reduction of fracture rate followed prenatal and postnatal cell transplantation in OI.[95,96] Additionally, prenatal transplantation of allogeneic MSC in three OI pregnancies indicated that has appeared to be safe.[95] A clinical trial in human pregnancy is currently in progress.[96]

MULTIDISCIPLINAR MANAGEMENT

Orthopedic Treatment

Orthopedic management and/or surgical treatment may be necessary in cases of severe bone deformity impairing function, with recurrent fractures, nonunion of fracture, severe scoliosis and basilar invagination where neurosurgical intervention may be required. Long periods of immobilization secondary to recurrent fractures or surgery may lead to reduction of bone mass and loss of muscle strength, thereby increasing the risk of further fractures.[97] Straightening, realignment and fixation of long bones by intramedullary rodding

is a classic technique for limb stabilization in OI patients. The purpose of rodding is to provide fracture protection and to improve alignment of long bone thus improving function in growing children.[98] The main complication following intramedullary rod fixation is proximal migration of the rod and breakage or disassembly of the rod creating an unprotected segment in the bone.[97-99]

The need for rodding is not limited to severe types of OI. Lower limb deformities were treated with bone splint technique (osteotomies and internal plating combined with cortical strut allografts) in 9 children with type I OI (aged 5-12 years).[97] Bone healing occurred within 12-16 weeks after surgery in all patients and significant improve on mobility was observed in 8 patients. None participant experienced further fractures, deformity, or nonunion.

Nonunion of fractures occurs in approximately 20% of OI patients (**figure 4**). Surgical repair can be difficult due poor bone quality.[100] Recently, stabilization of long-bone nonunion fractures was performed through compressed sandwich allograft cortical struts method in 12 OI patients (aged 11 to 78 years) at 13 sites of nonunion fractures.[101] All nonunions healed with allograft incorporation to the bone diaphysis and all patients' recovered the pre-fracture level of function, reinforcing this approach as durable and safe.[101]

Rehabilitation

An increased incidence of long bone fractures is the defining feature in individuals with OI. The majority of these fractures occur in early childhood directly affecting the child's motor development.[101-103] The clinical type and severity of OI have a major bearing on functional status including and motor development, range of joint motion, and muscle

strength.[102] Children with moderate to severe forms of OI have greater functional limitation which negatively affects the development of motor function and the level of ambulation when compared to children with mild forms of OI. In addition, gait acquisition is directly affected by joint range of motion and muscle strength.[103] Young adults with type I OI demonstrate full independence, while limitations in self-care, mobility and domestic life activities were observed in some individuals with OI types IV and V.[104]

To date, there are no physiotherapeutic treatment protocols available for children and adults with OI. A recent study investigated a rehabilitation approach combining resistance training, body weight supported treadmill training, neurodevelopmental treatment associated to side-alternating whole body vibration in 53 individuals with OI (ages 2.5 to 24.8 years) for 6 months within a period of 12 months of treatment.[105] There was improvement of mobility between 0 and 6 months of treatment, also an increase on lean mass and aBMD were observed. However, 46 patients had received BP treatment for years, and the program involved different approaches to treatment.

Further investigation is required, including targeted therapy, the acquisition of specific clinical data, and the evaluation of physiotherapy over short- and long-term courses of intervention. The overall approach to effective physiotherapy for the OI patient will improve when experience is enlarged through the publication of more case reports and cohort studies aimed at outcome assessment.

CONCLUSION

Research in clinical and genetic fields have expanded speedily. Multiple new genes are now recognized as OI-causative, reinforcing the need for extensive clinical investigation focused on improving pharmacological and non-pharmacogical therapy linked to these new genetic discoveries. After twenty years of BP therapy in children and adults with OI,

questions have arisen about the long-term and efficacy of this mode of treatment. In addition, new therapeutic approaches and surgical techniques have been investigated in OI. Efforts are required to improve techniques for gene replacement therapy which to date, has shown only limited success.[106] Nevertheless, still there is no cure for OI and the treatment remains based on drug therapy, orthopedic care, rehabilitation and nutrition.

Figure 1. A and B. A: Tibia/fibula of a patient with OI type I showing relative preservation of architecture, both the left tibia and fibula are fractured. **B:** Femur of a patient with type III OI showing undertubulation of the femur.

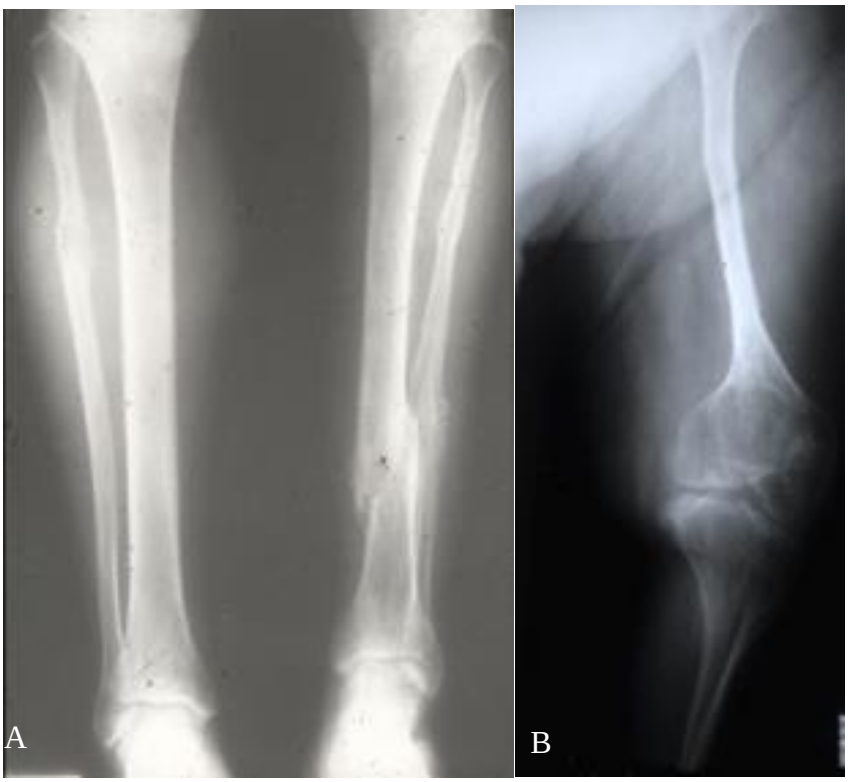

Figure 2. OI type III, 2-month-old baby girl. OI diagnosis in utero with multiple fractures of both long and short bones. On X-ray multiple skeletal deformities and fractures in both the upper and lower extremities.

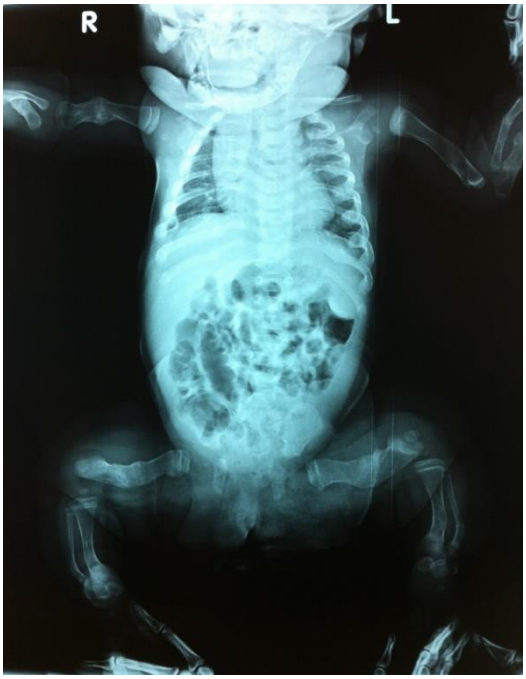

Figure 3. Male, 15-year-old, OI type XI (Bruck syndrome- *FKBP10* mutation). Spine surgery for correction of kyphosis and scoliosis.

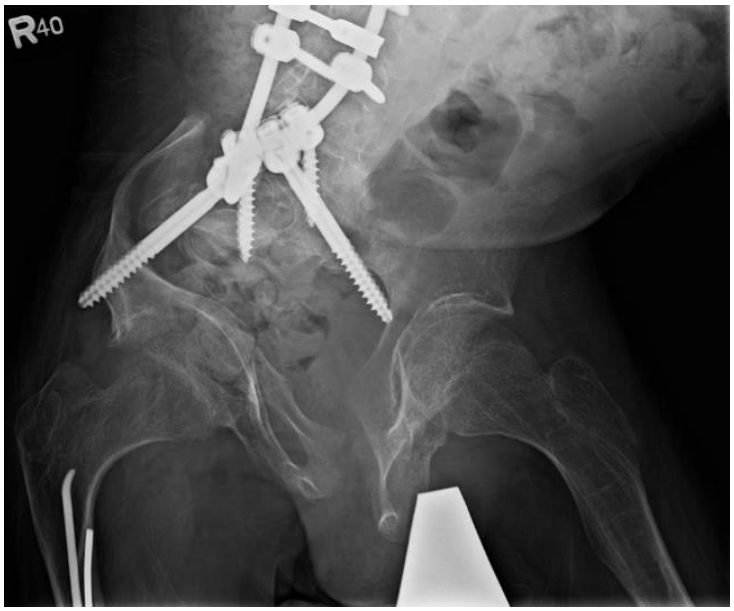

Figure 4. Male, 18 years old, OI type III. **A,B:** Nounion at the site of osteotomies in the left humerus around an intramedullary rod. Patient previously treated with pamidronate.

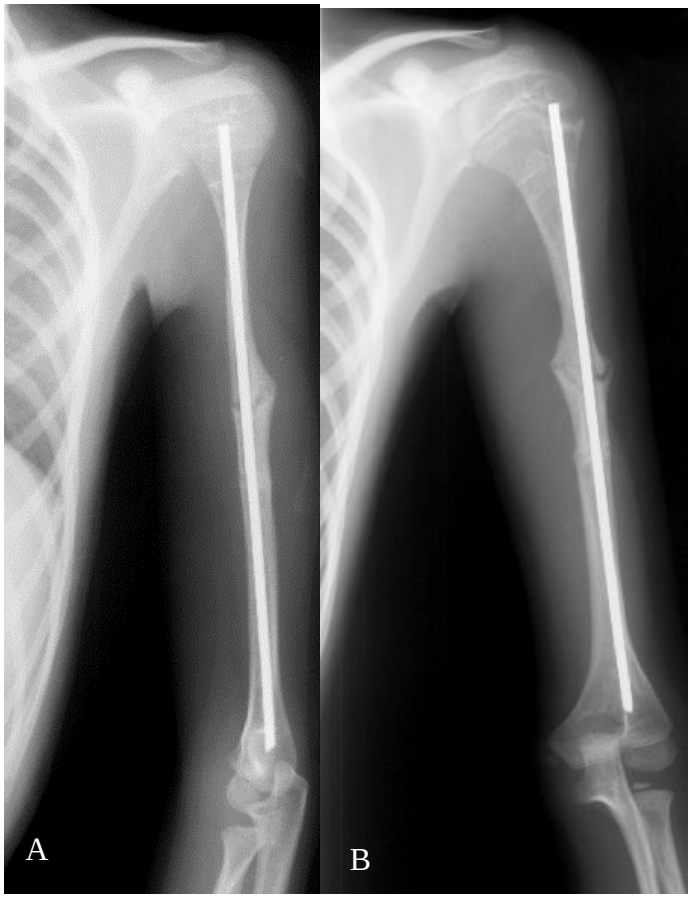

Table 1. OI nomenclature according INCDS

OI Classification	Clinical feature	Gene	Inherance
1	Non-deforming OI with blue sclerae	COL1A1	AD
		COL1A2	AD
2	Perinatally lethal OI	COL1A1	AD
		COL1A2	AD
		CRTAP	AR
		LEPRE1	AR
		PPIB	AR
3	Progressively deforming	COL1A1	AD
		COL1A2	AD
		BMP1	AR
		CRTAP	AR
		FKBP10	AR
		LEPRE1	AR
		PLOD2	AR
		PPIB	AR
		SERPINF1	AR
		SERPINH1	AR
		TMEM38B	AR
		WNT1	AR
		CREB3L1	AR
4	Common variable OI with normal sclerae	COL1A1	AD
		COL1A2	AD
		WNT1	AD
		CRTAP	AR
		PPIB	AR
		SP7	AR
		PLS3	XL
5	OI with calcification in interosseous membranes	IFITM5	AD

AD: autosomal dominant; AR: autosomal recessive
Adapted from Warman et al, 2011[7]

Table 2. Expanded classification of OI according molecular basis

OI Type	OMIM	Gene	Locus	Protein	Defective mechanism	Phenotype	Inherance
I	166200	COL1A1 COL1A2	17q21.33 7q21.3	Collagen alpha-1(I) chain Collagen alpha- 2(I) chain	Collagen quantity	mild	AD
II	166220	COL1A1 COL1A2	17q21.33 7q21.3	Collagen alpha-1(I) chain Collagen alpha- 2(I) chain	Collagen structure	lethal	AD
III	259420	COL1A1 COL1A2	17q21.33 7q21.3	Collagen alpha-1(I) chain Collagen alpha- 2(I) chain	Collagen structure	Progressive deformity	AD; AR
IV	166220	COL1A1 COL1A2	17q21.33 7q21.3	Collagen alpha-1(I) chain Collagen alpha- 2(I) chain	Collagen structure	moderate	AD; AR
V	610967	IFITM5	11p15.5	Interferon-induced transmembrane protein 5	Matrix mineralization/ ossification	Moderate, hypertrophic callus and ossification of the interosseous membrane	AD
VI	613982	SERPINF1	17p13.3	Pigment epithelium derived factor (PEDF)	Matrix mineralization/ ossification	Moderate to severe	AR
VII	610682	CRTAP	3p22.3	Cartilage-associated protein (CRTAP)	Collagen modification	Severe to lethal	AR
VIII	610915	LEPRE1/P3H1	1p34.2	Prolyl 3-hydroxilase 1(P3H1)	Collagen modification	Severe to lethal	AR
IX	259440	PPIB	15q22.31	Peptidyl-prolyl cis-trans isomerase B/ Cyclophilin B	Collagen modification	Severe to lethal	AR
X	613848	SERPINH1	11q13.5	Serpin H1/ Heat shock protein 47 (HSP47)	Collagen chaperoning	Severe	AR
XI	610968	FKBP10	17q21.2	Peptidyl-prolyl cis-trans isomerase FKBP10	Telopeptide hydroxylation	Progressive deformity with contractures (Bruck syndrome)	AR
XII	613849	SP7	12q13.13	Transcription factor 7/Osterix	Osteoblast development	Moderate	AR
XIII	614856	BMP1	8p21.3	Bone morphogenic protein1(BMP1)	Collagen processing	Severe	AR
XIV	615066	TMEM38B	9q31.2	Trimeric intracellular cation channel B (TRIC-B)	Osteoblast development	Variable severity	AR
XV	615220	WNT1	12q13.12	Wingless-type MMTV integration site family, member 1 (WNT1)/Proto-oncogene Wnt-1	Osteoblast development	Variable severity	AR
XVI	616229	CREB3L1	11p11.2	cAMP responsive element binding protein 3-like 1/ Old astrocyte specifically-induced substance (OASIS)	Osteoblast development	Severe	AR
Unclassified	609220	PLOD2	3q24	Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2	Collagen cross-linking	Severe with congenital joint contractures (Bruck Syndrome)	AR
	176790	P4HB	17q25.3	Protein disulfide-isomerase (PDI)	Collagen chaperoning	Severe (Cole-Carpenter syndrome-1)	AR
	607186	SEC24D	4q26	Protein transport protein Sec24D	Collagen modification	Severe (Cole-Carpenter syndrome- 2)	AR
	182120	SPARC	5q33.1	Osteoconectin	Collagen chaperoning	Severe	AR
	300131	PLS3	Xq23	Plastin-3	"bone development" 'assembly and disassembly of the actin cytoskeleton'	Mild	X-linked

OMIM: Online Mendelian Inheritance in Man database; AD: autosomal dominant; AR: autosomal recessive; Adapted from Van Dijk & Sillence, 2014[5]

Table 3. Bisphosphonate treatment protocols for pediatric OI

Author	Year	Drug	Dose	Route	Age
Sakkers et al[107]	2004	Olpadronate	10 mg/m^2 daily	Oral	3 to 18 yr
Antoniazzi et al[108]	2006	Neridronate	2 mg/kg for 2 d, every 3 months	IV	neonatal
Rauch et al[109]	2009	Risedronate	15 mg weekly (< 40kg); 30 mg weekly(> 40kg)	Oral	6 to 17 yr
Bishop et al[44]	2013	Risedronate	2.5mg daily (10–30 kg); 5mg daily (> 30kg)	Oral	4 to 15 yr
Cho et al[110]	2005	Alendronate	10mg/d (> 35 kg), 10 mg every other day (20 to 35 g), 10 mg every 3 days (< 20 kg)	Oral	6.3 to 15 yr
Ward et al[42]	2011	Alendronate	5 mg daily (< 40kg); 10 mg daily (> 40kg)	Oral	4 to 19 yr
DiMeglio et al[43]	2006	Alendronate, Pamidronate	Alendronate, 1 mg/kg/d (max 20 mg/d); Pamidronate, 1 mg/kg/d for each 3d, every 4 months	Oral; IV	> 3 yr
Glorieux et al[111]	1998	Pamidronate	1 mg/kg/d for each 3d, every 4 months	IV	> 3yr
Plotkin et al[69]	2000	Pamidronate	0.5–1.0 mg/kg/d for each 3 d, every 2 to 4 months	IV	≤ 2 yr
Letocha et al[112]	2005	Pamidronate	10 mg/m2/d for each 3 d, every 3 months	IV	4 to16 yr
Kusumi et al[113]	2014	Pamidronate	0.5 mg/kg/d each 3 d, every 2 months (< 2 yr) 0.75 mg/kg/d each 3d, every 3 months (2-3 yr) 1 mg/kg/d each 3d, every 3 months (> 3 yr)	IV	< 24 months
Panigrahi et al[50]	2010	Zoledronate	2 mg (age < 6 months) to 4 mg (age > 6 months) over 30 min to 1h; every 3 to 4 months	IV	2.5 weeks to 7yr
Vuorimies et al[48]	2011	Zoledronate	0.05 mg/kg up to 4.0 mg/d over 45 min every 6 months	IV	1.5 to 16.8 yr
Otaify et al[49]	2015	Zoledronate	0.1 mg/kg, every 6 months	IV	0.2 to 16 yr

d: day; mo: months; yr: years of age; IV: intravenous; min: minutes; h: hour;
Adapted from Bachrach & Ward, 2009[41]

REFERENCES

1. Van Dijk FS, Pals G, Van Rijn RR, Nikkels PG, Cobben JM. Classification of Osteogenesis Imperfecta revisited. *Eur J Med Genet*. 2010;53(1):1-5.
2. Martin E, Shapiro JR. Osteogenesis imperfecta: epidemiology and pathophysiology. *CurrOsteoporos Rep*. 2007;5(3):91-7.
3. Basel D, Steiner RD. Osteogenesis imperfecta: recent findings shed new light on this once well-understood condition. *Genet Med*. 2009;11(6):375-85.
4. Ben Amor IM, Glorieux FH, Rauch F. Genotype-phenotype correlations in autosomal dominant osteogenesis imperfecta. *J Osteoporos*. 2011;2011:540178.

5. Van Dijk FS, Sillence DO. Osteogenesis imperfecta: clinical diagnosis, nomenclature and severity assessment. *Am J Med Genet A*. 2014;164A(6):1470-81.
6. Sillence DO, Senn A, Danks DM. Genetic heterogeneity in osteogenesis imperfecta. *J Med Genet*. 1979;16:101–116.
7. Warman ML, Cormier-Daire V, Hall C, et al. Nosology and classification of genetic skeletal disorders: 2010 revision. *Am J Med Genet Part A*. 2011;155A:943–968.
8. Harrington J, Sochett E, Howard A. Update on the evaluation and treatment of osteogenesis imperfecta. *PediatrClin North Am*. 2014;61(6):1243-57.
9. Maasalu K, Nikopensius T, Kõks S, et al. Whole-exome sequencing identifies de novo mutation in the COL1A1 gene to underlie the severe osteogenesis imperfecta. *Hum Genomics*. 2015;9:6.
10. Steiner RD, Adsit J, Basel D. COL1A1/2-Related Osteogenesis Imperfecta. GeneReviews® [serial on the Internet]. 2005 Jan [updated 2013 Feb 14]. Accessed March 24, 2015.
11. Semler O, Garbes L, Keupp K, et al. A Mutation in the 5'-UTR of IFITM5 Creates an In-Frame Start Codon and Causes Autosomal-Dominant Osteogenesis Imperfecta Type V with Hyperplastic Callus. *Am J Hum Genet*. 2012;91(2):349–357.
12. Cho TJ, Lee KE, Lee SK, et al. A single recurrent mutation in the 5'-UTR of IFITM5 causes osteogenesis imperfecta type V. *Am J Hum Genet*. 2012;91(2):343–348.
13. Grover M, Campeau PM, Lietman CD, et al. Osteogenesis imperfecta without features of type V caused by a mutation in the IFITM5 gene. J Bone Miner Res. 2013;28(11):2333-7.
14. Becker J, Semler O, Gilissen C, et al. Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet*. 2011; 88(3):362-71.
15. Cho SY, Ki CS, Sohn YB, Kim SJ, Maeng SH, Jin DK. Osteogenesis imperfecta Type VI with severe bony deformities caused by novel compound heterozygous mutations in SERPINF1.*J Korean Med Sci.* 2013;28(7):1107-10.
16. Homan EP, Rauch F, Grafe I, et al. Mutations in SERPINF1 cause osteogenesis imperfecta type VI. *J Bone Miner Res*. 2011;26(12):2798-803.
17. Fratzl-Zelman N, Schmidt I, Roschger P, et al. Unique micro- and nano-scale mineralization pattern of human osteogenesis imperfecta type VI bone. *Bone*. 2015;73:233-41.

18. Cabral WA, Barnes AM, Adeyemo A, et al. A founder mutation in LEPRE1 carried by 1.5% of West Africans and 0.4% of African Americans causes lethal recessive osteogenesis imperfecta. Genet Med. 2012;14(5):543-51.
19. Cabral WA, Chang W, Barnes AM, et al. Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. *Nat Genet.* 2007;39(3):359-65.
20. Baldridge D, Schwarze U, Morello R, et al. CRTAP and LEPRE1 mutations in recessive osteogenesis imperfecta. *Hum Mutat.* 2008;29(12):1435-42.
21. Christiansen HE, Schwarze U, Pyott SM, et al. Homozygosity for a missense mutation in SERPINH1, which encodes the collagen chaperone protein HSP47, results in severe recessive osteogenesis imperfecta. *Am J Hum Genet*. 2010;12;86(3):389-98.
22. Alanay Y, Avaygan H, Camacho N, et al. Mutations in the gene encoding the RER protein FKBP65 cause autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet*. 2010;86:551-9.
23. Kelley BP, Malfait F, Bonafe L, et al. Mutations in FKBP10 cause recessive osteogenesis imperfecta and Bruck syndrome. *J Bone Miner Res*. 2011;26:666-72.
24. Puig-Hervás MT, Temtamy S, Aglan M, et al. Mutations in PLOD2 cause autosomal-recessive connective tissue disorders within the Bruck syndrome--osteogenesis imperfecta phenotypic spectrum. *Hum Mutat.* 2012;33(10):1444-9.
25. Ha-Vinh R, Alanay Y, Bank RA, et al. Phenotypic and molecular characterization of Bruck syndrome (osteogenesis imperfecta with contractures of the large joints) caused by a recessive mutation in PLOD2. *Am J Med Genet A*. 2004;131(2):115-20.
26. Lapunzina P, Aglan M, Temtamy S, et al. Identification of a frameshift mutation in Osterix in a patient with recessive osteogenesis imperfecta. *Am J Hum Genet*. 2010; 9;87(1):110-4.
27. Martínez-Glez V, Valencia M, Caparrós-Martín JA, et al. Identification of a mutation causing deficient BMP1/mTLD proteolytic activity in autosomal recessive osteogenesis imperfecta. *Hum Mutat*. 2012;33(2):343-50.
28. Asharani PV, Keupp K, Semler O, et al. Attenuated BMP1 function compromises osteogenesis, leading to bone fragility in humans and zebrafish. *J Hum Genet*. 2012;90(4):661-74.
29. Shaheen R, Alazami AM, Alshammari MJ, et al. Study of autosomal recessive osteogenesis imperfecta in Arabia reveals a novel locus defined by TMEM38B mutation.*J Med Genet*. 2012;49(10):630-5.

30. Volodarsky M, Markus B, Cohen I, et al. A deletion mutation in TMEM38B associated with autosomal recessive osteogenesis imperfecta. *Hum Mutat*. 2013;34(4):582-6.
31. Laine CM, Joeng KS, Campeau PM, et al. WNT1 mutations in early-onset osteoporosis and osteogenesis imperfecta. *N Engl J Med*. 2013;368(19):1809-16.
32. Keupp K, Beleggia F, Kayserili H, et al. Mutations in WNT1 cause different forms of bone fragility. Am J Hum Genet. 2013;92(4):565-74.
33. Symoens S, Malfait F, D'hondt S, et al. Deficiency for the ER-stress transducer OASIS causes severe recessive osteogenesis imperfecta in humans. Orphanet J Rare Dis. 2013;8:154.
34. Rauch F, Fahiminiya S, Majewski J, et al. Cole-Carpenter syndrome is caused by a heterozygous missense mutation in P4HB. *Am J Hum Genet*. 2015;96(3):425-31.
35. Garbes L, Kim K, Rieß A, et al. Mutations in SEC24D, encoding a component of the COPII machinery, cause a syndromic form of osteogenesis imperfecta. *Am J Hum Genet.* 2015;96(3):432-9.
36. Sarmah S, Barrallo-Gimeno A, Melville DB, Topczewski J, Solnica-Krezel L, Knapik EW. Sec24D-Dependent Transport of Extracellular Matrix Proteins Is Required for Zebrafish Skeletal Morphogenesis. *PLoS One*. 2010;5(4): e10367.
37. Van Dijk FS, Zillikens MC, Micha D, et al. PLS3 mutations in X-linked osteoporosis with fractures. *N Engl J Med*. 2013;369(16):1529-36.
38. Mendoza-Londono R, Fahiminiya S, Majewski J; et al. Recessive Osteogenesis Imperfecta Caused by Missense Mutations in SPARC. *Am J Hum Genet*. 2015;96(6):979-85.
39. Russell RG. Pharmacological diversity among drugs that inhibit bone resorption. CurrOpinPharmacol. 2015;22:115-130
40. Hoyer-Kuhn H, Netzer C, Semler O. Osteogenesis imperfecta: pathophysiology and treatment. *Wien Med Wochenschr*. 2015;165(13-14):278-284.
41. Bachrach LK, Ward LM. Clinical review 1: Bisphosphonate use in childhood osteoporosis. *JClinEndocrinolMetab*. 2009;94(2):400-9.
42. Ward LM, Rauch F, Whyte MP, et al. Alendronate for the treatment of pediatric osteogenesis imperfecta: a randomized placebo-controlled study. *J ClinEndocrinolMetab*. 2011;96(2):355-64.
43. DiMeglio LA, Peacock M. Two-year clinical trial of oral alendronate versus intravenous pamidronate in children with osteogenesis imperfecta. *J Bone Miner Res.* 2006;21(1):132-40.

44. Bishop N, Adami S, Ahmed SF, et al. Risedronate in children with osteogenesis imperfecta: a randomised, double-blind, placebo-controlled trial. *Lancet.* 2013;382(9902):1424-32.
45. Palomo T, Fassier F, Ouellet J, et al. Intravenous Bisphosphonate Therapy of Young Children with Osteogenesis Imperfecta: Skeletal Findings During Follow Up Throughout the Growing Years. J Bone Miner Res. 2015 Jun 8. doi: 10.1002/jbmr.2567. Epub 2015 jun 30.
46. Bradbury LA, Barlow S, Geoghegan F, et al. Risedronate in adults with osteogenesis imperfecta type I: increased bone mineral density and decreased bone turnover, but high fracture rate persists. *Osteoporos Int.* 2012;23(1):285-94.
47. Shapiro JR, Thompson CB, Wu Y, Nunes M, Gillen C. Bone mineral density and fracture rate in response to intravenous and oral bisphosphonates in adult osteogenesis imperfecta. *Calcif Tissue Int*. 2010;87(2):120-9.
48. Vuorimies I, Toiviainen-Salo S, Hero M, Mäkitie O. Zoledronic acid treatment in children with osteogenesis imperfecta. Horm Res Paediatr. 2011;75(5):346-53.
49. Otaify GA, Aglan MS, Ibrahim MM, Elnashar M, El Banna RA, Temtamy SA. Zoledronic acid in children with osteogenesis imperfecta and Bruck syndrome: a 2-year prospective observational study. Osteoporos Int. 2015. Epub 2015 Jul 3.
50. Panigrahi I, Das RR, Sharda S, Marwaha RK, Khandelwal N. Response to zolendronic acid in children with type III osteogenesis imperfecta. *J Bone Miner Metab*. 2010;28(4):451-5.
51. Land C, Rauch F, Munns CF, Sahebjam S, Glorieux FH. Vertebral morphometry in children and adolescents with osteogenesis imperfecta: effect of intravenous pamidronate treatment. *Bone.* 2006;39(4):901-6.
52. Shapiro JR, Sponseller PD. Osteogenesis imperfecta: questions and answers. *CurrOpinPediatr*. 2009;21(6):709–716.
53. Lindahl K, Langdahl B, Ljunggren Ö, Kindmark A. Treatment of osteogenesis imperfecta in adults. *Eur J Endocrinol.* 2014;171(2):R79-90.
54. Brizola E, Shapiro JR. Bisphosphonate Treatment of Children and Adults with Osteogenesis Imperfecta: Unanswered Questions. *Calcif Tissue Int*. 2015;97(2):101-3.
55. Phillipi CA, Remmington T, Steiner RD. Bisphosphonate therapy for osteogenesis imperfecta. *Cochrane Database Syst Rev*. 2008;(4):CD005088.

56. Dwan K, Phillipi CA, Steiner RD, Basel D. Bisphosphonate therapy for osteogenesis imperfecta.*Cochrane Database Syst Rev*. 2014;7:CD005088.
57. Rijks EB, Bongers BC, Vlemmix MJ, et al. Efficacy and Safety of Bisphosphonate Therapy in Children with Osteogenesis Imperfecta: A Systematic Review. *Horm Res Paediatr*. 2015;84(1):26-42.
58. Hald JD, Evangelou E, Langdahl BL, Ralston SH. Bisphosphonates for the prevention of fractures in osteogenesis imperfecta: meta-analysis of placebo-controlled trials.*J Bone Miner Res*. 2015;30(5):929-33.
59. Shi CG, Zhang Y, Yuan W. Efficacy of Bisphosphonates on Bone Mineral Density and Fracture Rate in Patients With Osteogenesis Imperfecta: A Systematic Review and Meta-analysis. *Am J Ther.* 2015. Epub 2015 Apr 3.
60. Papapoulos SE, Cremers SC. Prolonged bisphosphonate release after treatment in children. *N Engl J Med.* 2007;356: 1075–1076.
61. Kamoun-Goldrat AS, Le Merrer MF. Osteogenesis imperfecta and dentinogenesis imperfecta: diagnostic frontiers and importance in dentofacial orthopedics. *Orthod Fr*. 2007;78(2):89-99.
62. Schilcher J, Michaelsson K, Aspenberg P. Bisphosphonate use and atypical fractures of the femoral shaft. *N Engl J Med*. 2011;364: 1728–1737.
63. Carpintero P, Del Fresno JA, Ruiz-Sanz J, Jaenal P. Atypical fracture in a child with osteogenesis imperfecta. *Joint Bone Spine*. 2015;82(4):287-8.
64. Meier RP, IngLorenzini K, Uebelhart B, Stern R, Peter RE, Rizzoli R. Atypical femoral fracture following bisphosphonate treatment in a woman with osteogenesis imperfecta--a case report. *ActaOrthop*. 2012;83(5):548-50.
65. Li J, Mashiba T, Burr DB. Bisphosphonate treatment suppresses not only stochastic remodeling but also the targeted repair of microdamage. *Calcif Tissue Int.* 2001;69:281–286.
66. Hegazy A, Kenawey M, Sochett E, Tile L, Cheung AM, Howard AW. Unusual Femur Stress Fractures in Children with Osteogenesis Imperfecta and Intramedullary Rods on Long-term Intravenous Pamidronate Therapy. *J PediatrOrthop*. 2015. Epub 2015 Jun 8.
67. Munns CF, Rauch F, Zeitlin L, Fassier F, Glorieux FH. Delayed osteotomy but not fracture healing in pediatric osteogenesis imperfecta patients receiving pamidronate. *J Bone Miner Res*. 2004;19(11):1779-86.

68. Anam EA, Rauch F, Glorieux FH, Fassier F, Hamdy R. Osteotomy Healing in Children With Osteogenesis Imperfecta Receiving Bisphosphonate Treatment. *J Bone Miner Res*. 2015;30(8):1362-8.
69. Plotkin H, Rauch F, Bishop NJ, et al. Pamidronate treatment of severe osteogenesis imperfecta in children under 3 years of age. *J ClinEndocrinolMetab*. 2000;85(5):1846-50.
70. Falk MJ, Heeger S, Lynch KA, et al. Intravenous bisphosphonate therapy in children with osteogenesis imperfecta. *Pediatrics.* 2003;111(3):573-8.
71. Uihlein AV, Leder BZ. Anabolic therapies for osteoporosis. *EndocrinolMetabClin North Am*. 2012;41(3):507–525.
72. Orwoll ES, Shapiro J, Veith S, et al. Evaluation of teriparatide treatment in adults with osteogenesis imperfecta. *J Clin Invest*. 2014;124(2):491-8.
73. Gatti D, Rossini M, Viapiana O, et al. Teriparatide treatment in adult patients with osteogenesis imperfecta type I. *Calcif Tissue Int*. 2013;93(5):448-52.
74. Holm J, Eiken P, Hyldstrup L, Jensen JE. Atypical femoral fracture in an osteogenesis imperfecta patient successfully treated with teriparatide. *EndocrPract*. 2014;20(10):e187-90.
75. George S, Brenner A, Sarantopoulos J, Bukowski RM. RANK ligand: effects of inhibition. *CurrOncol Rep*. 2010;12(2):80-6.
76. Semler O, Netzer C, Hoyer-Kuhn H, Becker J, Eysel P, Schoenau E. First use of the RANKL antibody denosumab in osteogenesis imperfecta type VI. *J Musculoskelet Neuronal Interact*. 2012;12(3):183-8.
77. Land C, Rauch F, Travers R, Glorieux FH. Osteogenesis imperfecta type VI in childhood and adolescence: effects of cyclical intravenous pamidronate treatment. *Bone.* 2007;40:638-44.
78. Hoyer-Kuhn H, Netzer C, Koerber F, Schoenau E, Semler O. Two years' experience with denosumab for children with osteogenesis imperfecta type VI. *Orphanet J Rare Dis*. 2014;9:145.
79. Hoyer-Kuhn H, Semler O, Schoenau E. Effect of denosumab on the growing skeleton in osteogenesis imperfecta. *J ClinEndocrinolMetab*. 2014;99(11):3954-5.
80. Li X, Warmington KS, Niu QT, et al. Inhibition of sclerostin by monoclonal antibody increases bone formation, bone mass, and bone strength in aged male rats. *J Bone Miner Res.* 2010;25:2647–2656.

81. Ominsky MS, Li C, Li X, et al. Inhibition of sclerostin by monoclonal antibody enhances bone healing and improves bone density and strength of nonfractured bones. *J Bone Miner Res*. 2011;26:1012–1021.
82. Ominsky MS, Niu QT, Li C, Li X, Ke HZ. Tissue-level mechanisms responsible for the increase in bone formation and bone volume by sclerostin antibody. *J Bone Miner Res*. 2014;29(6):1424-30.
83. Padhi D, Jang G, Stouch B, Fang L, Posvar E. Single-dose, placebo-controlled, randomized study of AMG 785, a sclerostin monoclonal antibody. *J Bone Miner Res.* 2011;26:19-26.
84. Palomo T, Glorieux FH, Rauch F. Circulating sclerostin in children and young adults with heritable bone disorders. J ClinEndocrinolMetab. 2014;99(5):E920-5.
85. Sinder BP, Salemi JD, Ominsky MS, Caird MS, Marini JC, Kozloff KM. Rapidly growing Brtl/+ mouse model of osteogenesis imperfecta improves bone mass and strength with sclerostin antibody treatment. *Bone.* 2015;71:115-23.
86. Roschger A, Roschger P, Keplingter P, et al. Effect of sclerostin antibody treatment in a mouse model of severe osteogenesis imperfecta. *Bone.* 2014;66:182-8.
87. Stroup GB, Lark MW, Veber DF, et al. Potent and selective inhibition of human cathepsin K leads to inhibition of bone resorption in vivo in a nonhuman primate. *J Bone Miner Res*. 2001;16:1739-1746.
88. Bone HG, Dempster DW, Eisman JA, et al. Odanacatib for the treatment of postmenopausal osteoporosis: development history and design and participant characteristics of LOFT, the Long-Term Odanacatib Fracture Trial. *Osteoporos Int*. 2015;26(2):699-712.
89. Grafe I, Yang T, Alexander S, et al. Excessive transforming growth factor-β signaling is a common mechanism in osteogenesis imperfecta. *Nat Med*. 2014;20(6):670-5.
90. Erlebacher A, Derynck R. Increased expression of TGF-beta 2 in osteoblasts results in an osteoporosis-like phenotype. *J Cell Biol.* 1996;132(1-2):195-210.
91. Gebken J, Brenner R, Feydt A, et al. Increased cell surface expression of receptors for transforming growth factor-beta on osteoblasts from patients with Osteogenesis imperfecta. *Pathobiology*. 2000;68(3):106-12.
92. Wheeler JB, Ikonomidis JS, Jones JA. Connective tissue disorders and cardiovascular complications: the indomitable role of transforming growth factor-beta signaling. AdvExp Med Biol. 2014;802:107-27.
93. Krakow D. Skeletal Dysplasias. *ClinPerinatol*. 2015;42(2):301-319.

94. Pereira RF, O'Hara MD, Laptev AV et al. Marrow stromal cells as a source of progenitor cells for non hematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl AcadSci U S A.* 1998;95(3):1142–1147.
95. Götherström C, Westgren M, Shaw SW, et al. Pre- and postnatal transplantation of fetal mesenchymal stem cells in osteogenesis imperfecta: a two-center experience. *Stem Cells Transl Med*. 2014;3(2):255-64.
96. Westgren M, Götherström C. Stem cell transplantation before birth - a realistic option for treatment of osteogenesis imperfecta? *PrenatDiagn*. 2015. Epub 2015 May 11.
97. Lin D, Zhai W, Lian K, Ding Z. Results of a bone splint technique for the treatment of lower limb deformities in children with type I osteogenesis imperfecta. *Indian J Orthop*. 2013;47(4):377-81.
98. Lee K, Park MS, Yoo WJ, Chung CY, Choi IH, Cho TJ. Proximal migration of femoral telescopic rod in children with osteogenesis imperfecta. *J Pediatr Orthop*. 2015;35(2):178-84.
99. Nicolaou N, Bowe JD, Wilkinson JM, Fernandes JA, Bell MJ. Use of the Sheffield telescopic intramedullary rod system for the management of osteogenesis imperfecta: clinical outcomes at an average follow-up of nineteen years. *J Bone Joint Surg Am*. 2011;93:1994–2000.
100. Agarwal V, Joseph B. Non-union in osteogenesis imperfecta. *J PediatrOrthop B.* 2005;14(6):451-5.
101. Puvanesarajah V, Shapiro JR, Sponseller PD. Sandwich allografts for long-bone nonunions in patients with osteogenesis imperfecta: a retrospective study. *J Bone Joint Surg Am*. 2015;97(4):318-25.
102. Engelbert RHH, Pruijs HEH, Beemer FA, Helders PJM. Osteogenesis imperfecta in childhood: Treatment strategies. *Arch Phys Med Rehabil.* 1998;79(12):1590–1594.
103. Brizola E, Staub AL, Félix TM. Muscle strength, joint range of motion, and gait in children and adolescents with osteogenesis imperfecta. *PediatrPhysTher*. 2014;26(2):245-52.
104. Montpetit K, Dahan-Oliel N, Ruck-Gibis J, Fassier F, Rauch F, Glorieux F. Activities and participation in young adults with osteogenesis imperfecta. *J PediatrRehabil Med*. 2011;4(1):13-22.
105. Hoyer-Kuhn H, Semler O, Stark C, Struebing N, Goebel O, Schoenau E. A specialized rehabilitation approach improves mobility in children with osteogenesis imperfecta. *J Musculoskelet Neuronal Interact.* 2014;14(4):445-53.

106. Niyibizi C, Li F. Potential implications of cell therapy for osteogenesis imperfecta. *Int J Clin Rheumtol*. 2009;4(1):57-66.
107. Sakkers R, Kok D, Engelbert R, et al. Skeletal effects and functional outcome with olpandronate in children with osteogenesis imperfecta: a 2-year randomized placebo-controlled study. *Lancet*. 2004;363:1427–1431.
108. Antoniazzi F, Zamboni G, Lauriola S, Donadi L, Adami S, Tatò L. Early bisphosphonate treatment in infants with severe osteogenesis imperfecta. J Pediatr. 2006;149(2):174-9.
109. Rauch F, Munns CF, Land C, Cheung M, Glorieux FH. Risedronate in the treatment of mild pediatric osteogenesis imperfecta: a randomized placebo-controlled study. *J Bone Miner Res*. 2009;24(7):1282-9.
110. Cho TJ, Choi IH, Chung CY, Yoo WJ, Park MS, Park YK. Efficacy of oral alendronate in children with osteogenesis imperfecta. *J PediatrOrthop*. 2005;25(5):607-12.
111. Glorieux FH, Bishop N, Plotkin H, Chabot G, Lanoue G, Travers RT. Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. *N Engl J Med*. 1998;339:947–952.
112. Letocha AD, Cintas HL, Troendle JF, et al. Controlled trial of pamidronate in children with types II and IV osteogenesis imperfecta confirms vertebral gains but not short-term functional improvement. *J Bone Miner Res*. 2005;20:977–986.
113. Kusumi K, Ayoob R, Bowden SA, Ingraham S, Mahan JD. Beneficial effects of intravenous pamidronate treatment in children with osteogenesis imperfecta under 24 months of age. *J Bone Miner Metab.* 2014. Epub 2014 Oct 16.

9.2. Editorial

O editorial sobre o estado atual do tratamento com bifosfonados em OI "*Bisphosphonate Treatment of Children and Adults with Osteogenesis Imperfecta: Unanswered Questions*" foi publicado no periódico "*Calcified Tissue International*".

Bisphosphonate Treatment of Children and Adults with Osteogenesis Imperfecta: Unanswered Questions

Brizola E [1,2], Shapiro JR [1]

[1] Bone Disorders Program, Kennedy Krieger Institute, Johns Hopkins University School of Medicine, 707 N. Broadway, Baltimore, MD 21205, USA

[2] Postgraduate Program in Child and Adolescent Health, Faculty of Medicine, Federal University of Rio Grande do Sul, Rua Ramiro Barcelos, 2400, Porto Alegre, RS 90035-003, Brazil

In 1987, Devogelaer et al. [1] first reported the treatment of a 12 year old girl with Osteogenesis Imperfecta (OI) for one year with a newly available bisphosphonate (BP), 3-amino-1-hydroxypropylidene-1,1-bisphosphonate (APD); treatment with APD orally was well-tolerated and, the radiological and clinical improvement was striking. Glorieux et al. [2] reported positive responses to the intravenous administration of pamidronate in 30 children ages 3–16 years old with severe OI who had received between 4 to 12 cycles of

treatment. The mean incidence of radiologically confirmed fractures decreased by 1.7 per year ($p<0.001$) and treatment with pamidronate did not alter rate of fracture healing, growth rate or appearance of the growth plates. In 2003, Shapiro et al. [3] reported on the histologic response of bone to treatment with IV pamidronate (30 mg every 3 months) in 5 adults with OI type I. Treatment led to a significant increase in bone trabecular volume ($p=0.01$), cortical thickness ($p=0.01$) and bone formation rate ($p=0.01$).

There followed many reports from different countries documenting the results of treatment with different BPs, administered orally or intravenously, in children and adults. Reported effects on fracture rate in children were variable but not initially defined for adults. While cautioning that BP treatment be reserved for more severe OI types, it was clear that BP treatment had become the "standard of care" for both children and adults including the very young [4]. Indeed, BP treatment has been associated with multiple positive effects such as an increase bone mineral density and in vertebral height, relief of musculoskeletal pain and fatigue, improvement in muscle strength and mobility and a positive impact on activities of daily living [5].

However, in 2009, Marini [6] urged "caution" as regards BP use in children, specifically with regard to: a) a decline in bone quality with high cumulative doses of BP and, b) the insufficient data at that time supporting decreases in fracture rates. It is clear that BPs lessen fracture rates in many children, but whether BP are uniformly effective and how long treatment should be continued are subjects for discussion.

Recently there have been two Cochrane reports [7,8], and well as 2 recent meta-analyses by Hald et al. [9] and Shi et al. [10] reporting the effects of BP on the fracture incidence in both children and adults with OI. The 2014 Cochrane report [8] surveyed 14 trials (819 patients) focusing on randomized and quasi-randomized controlled trials

comparing BP to placebo, no treatment, or comparative interventions in all types of OI. The authors concluded that it was unclear whether oral or intravenous BP treatment consistently decreases fractures, though multiple studies had reported that independently. The review by Hald et al. [9] was restricted to placebo-controlled randomized clinical trials (n = 6). As with the Cochrane study the conclusions were that the available data did not indicate that BP treatment decreased the incidence of fracture in individuals with OI [8,9]. By contrast, a meta-analysis by Shi et al. [10] concluded BP treatment did decrease fracture rate in children but not in adults. In addition, Dwan et al. [8] did not confirm that treatment decreased musculoskeletal pain or improved mobility.

Both this author's experience and that of Marini indicate that, although an increase in bone mineral density has been widely reported in several studies with BP treatment, it is neither a measure of bone strength nor a predictor of fracture risk: in this context *bone quality* is the important unmeasured variable with regard to fracture risk [6]. In the adult OI residronate study [11] there were modest but significant increases in BMD at LS, and decreased bone turnover but there was no significant difference to fracture incidence. What data may explain these inconsistencies with regard to BP effect on bone quality and fracture risk?

Rauch et al. [12] analyzed bone histomorphometry in 45 children and adolescents with OI treated with pamidronate for 2.4 ± 0.6 years (range 1-4 years). During pamidronate treatment, more samples contained calcified cartilage or abnormally large osteoclasts when compared to non-treatment control. An unexpected finding was that antiresorptive treatment with pamidronate led to a larger relative decrease in bone formation parameters than in bone resorption measures. However, areal and volumetric bone density by dual energy X-ray absorptiometry (DXA) increased. Weber et al. [13] conducted background electron imaging

and nanoindentation on iliac crest bone samples from OI patients who had received 2.5 ± 0.5 years of pamidronate treatment and in controls. It is recognized that in the basal state, bone matrix in OI is hypermineralized. The matrix may be abnormally dense and the bone is stiff at the material level. It appeared that basic bone material properties of the samples were not additionally affected by pamidronate treatment. A conclusion was that long-term treatment might not be associated with an increase in fracture rate. However, this misses potential the long-term effects of BP in the setting of hypermineralization and increased bone stiffness on the resistance of bone to fracture.

A second question addresses fractures rates in children and adults treated with BP and how long treatment should be continued to maximize fracture protection. A recommendation stated at various scientific meetings and published by Bachrach and Ward [14] is that BP treatment should be continued, perhaps a low dose, until growth is completed, in order to avoid fractures in areas unprotected by BP as might occur in the distal femur with growth. However, as Rauch et al. [15] observed in bone biopsies, the gains that can be achieved with pamidronate appear to be largely realized in the first 2-4 years of treatment. Can continued treatment adversely affect fracture risk?

BP localizes to the growth plate and affects chondrocyte maturation and trabecular bone development. In growing wild type mice, treatment with alendronate, pamidronate and zolendronate led to a decrease in the number of chondrocytes in the hypertrophic chondrocyte layer. This was not associated with altered chondrocyte apoptosis or altered vascular invasion at the growth plate [16]. However, this may differ in OI. Evans et al. [17] observed in the *oim* mouse models that pamidronate increased growth plate area secondary to reduced chondrocyte turnover. Furthermore, unlike in the wild type mice, osteoclast numbers were decreased impairing vascular invasion at the growth plate and permitting the accumulation of calcified cartilage in primary trabeculae. Rauch et al. [15] had observed in

patients, an increase in trabecular number but no increase in trabecular mineralization following pamidronate. How does this relate to fracture susceptibility and the question of proposed duration of treatment?

BP treatment is associated with the appearance of metaphyseal “zebra lines” above the growth plate. These sclerotic bands reflect a delay in chondrosseous maturation and decreased osteoclastic activity occurring in response to drug. When growth plate activity is temporarily interrupted by BP, osteoblasts deposit bone matrix on the metaphyseal site of the growth plate. Harcke et al. [18] have proposed that these thin bands of mineralized tissue at the interface between growth plate and metaphysis create a transition area of high and low density, which can produce a stress riser effect and facilitate fracture. Sixty- three per cent of the fractures observed in the children with cerebral palsy (CP) treated with BP were metaphyseal fractures either above, through or below the “zebra lines” [18]. Currently, there is no data indicating that continuing BP until growth ceases will limit fracture at this site. However, there are recent reports of mid-femoral fractures in both young and old individuals with OI, previously treated with BP for 3-5 or more years. Hegazy et al. [19] identified five patients in a group of 72 OI patients who had subtrochanteric fracture and one had mid-diaphyseal femur stress fractures with minimal or no trauma. None were located at stress riser areas (such as tip of the implant) or at metaphysis (site of typical OI-related fractures), or at growth lines related to pamidronate at the metaphysis.

The response to BP treatment is frequently reported as 1 or 2 years post treatment. Fracture rate over several years during and after treatment is not reported. Thus, the question of defining the optimal duration of BP treatment has not been addressed. Nevertheless, in the face of these uncertainties, parents of OI children and many OI adults and their physicians seek prolonged BP treatment. Room exists for expanded and focused clinical research on these topics.

To summarize: The central feature of treatment in OI is fracture prevention. Bisphosphonates are widely prescribed and administered for years to children with OI and treatment is offered to adults. Yet there is an obvious lack of enough properly controlled data to warrant the recommendation that treatment should be continued in the absence of a sustained decrease in fracture rate in children or adults, and an absence of data to counter the concern that continued long duration treatment may incur adverse effect on bone.

Conflict of Interest

The authors have no conflict of interest.

References

1. Devogelaer JP, Malghem J, Maldague B, Nagant de Deuxehaisnes C (1987) Radiological manifestations of bisphosphonate treatment with APD in a child suffering from osteogenesis imperfecta. Skeletal Radiol 16:360-363

2. Glorieux FH, Bishop NJ, Plotkin H, Chabot G, Lanoue G, Travers R (1998) Cyclic administration of pamidronate in children with severe osteogenesis imperfecta. N Engl J Med 339(14):947-52

3. Shapiro JR, McCarthy EF, Rossiter K, Ernest K, Gelman R, Fedarko N, Santiago HT, Bober M (2003) The effect of intravenous pamidronate on bone mineral density, bone histomorphometry, and parameters of bone turnover in adults with type IA osteogenesis imperfecta. Calcif Tissue Int 72(2):103-12

4. Glorieux FH (2000) Bisphosphonate therapy for severe osteogenesis imperfecta. J Pediatr Endocrinol Metab 13(2):989-92

5. Shapiro JR, Sponsellor PD (2009) Osteogenesis imperfecta: questions and answers. Curr Opin Pediatr 21(6):709-16. doi: 10.1097/MOP.0b013e328332c68f

6. Marini JC (2009) Bone: Use of bisphosphonates in children-proceed with caution. Nat Rev Endocrinol 5(5):241-3. doi: 10.1038/nrendo.2009.58

7. Phillipi CA, Remmington T, Steiner RD (2008) Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev 4:CD005088. doi: 10.1002/14651858.CD005088.pub2

8. Dwan K, Phillipi CA, Steiner RD, Basel D (2014) Bisphosphonate therapy for osteogenesis imperfecta. Cochrane Database Syst Rev 7:CD005088. doi: 10.1002/14651858.CD005088.pub3

9. Hald JD, Evangelou E, Langdahl BL, Ralston SH (2015) Bisphosphonates for the Prevention of Fractures in Osteogenesis Imperfecta: Meta-Analysis of Placebo-Controlled Trials. J Bone Miner Res 30(5):929-33. doi: 10.1002/jbmr.2410

10. Shi CG, Zhang Y, Yuan W (2015) Efficacy of Bisphosphonates on Bone Mineral Density and Fracture Rate in Patients With Osteogenesis Imperfecta: A Systematic Review and Meta-analysis. Am J Ther 0:1-11

11. Bradbury LA, Barlow S, Geoghegan F, Hannon RA, Stuckey SL, Wass JA, Russell RG, Brown MA, Duncan EL (2012) Risedronate in adults with osteogenesis imperfecta type I: increased bone mineral density and decreased bone turnover, but high fracture rate persists. Osteoporos Int 23(1):285-94

12. Rauch F, Travers R, Plotkin H, Glorieux FH (2002) The effects of intravenous pamidronate on the bone tissue of children and adolescents with osteogenesis imperfecta. J Clin Invest 110(9):1293-9

13. Weber M, Roschger P, Fratzl-Zelman N, Schöberl T, Rauch F, Glorieux FH, Fratzl P, Klaushofer K (2006) Pamidronate does not adversely affect bone intrinsic material properties in children with osteogenesis imperfecta. Bone 39(3):616-22

14. Bachrach LK, Ward LM (2009) Clinical review: Bisphosphonate use in childhood osteoporosis. J Clin Endocrinol Metab 94(2):400-9. doi: 10.1210/jc.2008-1531

15. Rauch F, Travers R, Glorieux FH (2006) Pamidronate in children with osteogenesis imperfecta: histomorphometric effects of long-term therapy. J Clin Endocrinol Metab 91(2):511-6

16. Zhu ED, Louis L, Brooks DJ, Bouxsein ML, Demay MB (2014) Effect of bisphosphonates on the rapidly growing male murine skeleton. Endocrinology 155(4):1188-96. doi: 10.1210/en.2013-1993

17. Evans KD, Sheppard LE, Rao SH, Martin RB, Oberbauer AM (2009) Pamidronate alters the growth plate in the oim mouse model for osteogenesis imperfecta. Int J Biomed Sci 5(4):345-52

18. Harcke TH, Stevenson KL, Kecskemethy HH, Bachrach SJ, Grisso LE(2012) Fracture after bisphosphonate treatment in children with cerebral palsy: the role of stress risers. Pediatr Radiol 42:76–81. doi: 10.1007/s00247-011-2198-9

19. Hegazy A, Howard A, Sochett E, Tile L, Cheung AM (2012) Femur Stress Fractures in Children with Osteogenesis Imperfecta and Intramedullary Rods on Long-term Intravenous Pamidronate Therapy. J Bone Miner Res 27(1). Acessed April 12, 2015. http://www.asbmr.org/Meetings/AnnualMeeting/Abstracts12.aspx.

10. ANEXOS

Termo de Consentimento Livre e Esclarecido

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

I. Justificativa e os objetivos da pesquisa:

Osteogênese Imperfeita se caracteriza pela diminuição da densidade óssea devido a defeitos no colágeno. Alterações genéticas em diferentes genes causam osteogênese imperfeita.

Estamos convidando seu (Sua) filho(a) para participar do projeto: **Pesquisa de mutações nos genes *COL1A1, COL1A2, CRTAP, PPIB, LEPRE1 E IFITM5* em Osteogênese Imperfeita formas moderada a grave**

II. Procedimentos que serão utilizados:

Serão coletados do (a) seu (sua) filho(a), 5 ml de sangue. As amostras serão estudadas para análise genética relacionada osteogênese imperfeita, no Hospital de Clínicas de Porto Alegre. As amostras serão armazenadas no Laboratório de Medicina Genômica do HCPA e somente serão utilizadas para este projeto.

III. Riscos ou desconfortos potenciais:

No momento da coleta de sangue poderá haver alguma dor em decorrência da punção da pele. Complicações de coleta de sangue rotineira são raras e geralmente são de pequeno porte, como aparecimento de mancha roxa e pequeno desconforto que desaparece em poucos dias.

IV. Sua participação no estudo é voluntária não havendo custos ou despesas por parte do participante no estudo.

V. Benefícios esperados:

Este estudo poderá beneficiar a sua família, pois há um componente genético na osteogênese imperfeita. Isso poderá auxiliar no aconselhamento genético de sua família e em famílias de alto risco de recorrência para osteogênese imperfeita que poderão a ser identificada com este estudo.

VI. Procedimentos alternativos:

Eu entendo que eu tive o direito de recusar a participar deste projeto e que minha recusa não afetará de nenhuma maneira o cuidado de meu filho(a) ou de minha família no Hospital participante.

VII. Formas de acompanhamento e assistência:

O atendimento clínico e as informações sobre o aconselhamento genético da família serão realizadas por médico geneticista.

Pelo presente Consentimento Informado, declaro que fui esclarecido, de forma clara e detalhada, livre de qualquer forma de constrangimento e coerção, dos objetivos, da justificativa, dos procedimentos que serei submetido, dos riscos, desconfortos e benefícios do presente Projeto de Pesquisa, assim como dos procedimentos alternativos aos quais poderia ser submetido, todos acima listados.

Fui, igualmente, informado:

- da garantia de receber resposta a qualquer pergunta ou esclarecimento a qualquer pergunta ou esclarecimento a qualquer dúvida a cerca dos procedimentos, riscos, benefícios e outros assuntos relacionados com a pesquisa;

- da liberdade de retirar meu consentimento, a qualquer momento, e deixar de participar do estudo, sem que isto traga prejuízo à continuação do meu cuidado e tratamento;

- da segurança de que não serei identificado e que se manterá o caráter confidencial das informações relacionadas com minha privacidade;

O pesquisador responsável por este projeto de pesquisa é Têmis Maria Félix, médica do Serviço de Genética Médica do Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos 2350, Porot Alegre, RS , Fone: 51 33598011. Este documento foi revisado e aprovado pelo Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre e qualquer dúvida pode ser esclarecida no fone 51 33598304.

AUTORIZAÇÃO PARA ARMAZENAMENTO DA AMOSTRA

Eu concordo que o DNA seja armazenado ☐

Eu não concordo que o DNA seja armazenado ☐

Nome do paciente: _____

Nome do responsável: _____

Parentesco: _____ Data : _____

_____

Assinatura do responsável

RESPONSABILIDADE DO PESQUISADOR

Eu expliquei a _____ o objetivo do estudo, os procedimentos requeridos e os possíveis riscos e vantagens em participar desse estudo, usando o melhor do meu conhecimento. Eu me comprometo a fornecer uma cópia desse formulário de consentimento ao participante ou responsável.

Nome do pesquisador ou associado: _____

Data: _____

_____

Assinatura do pesquisador ou associado