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Histomorphometric reference data of transiliac bone biopsy in children from 8 to 17 years old

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Abstract

Background: *Histomorphometric analysis of bone samples is a key tool for studying bone metabolism; however, only a few pediatric reference data exist. The aim of the present study is to report more reference data and to investigate if histomorphometric differences exist between age and gender.* **Methods:** *We obtained 19 transiliac bone samples previously marked with tetracycline, from children between 8 and 17 years (13 were male), with normal blood test results and urine biochemical bone markers. We evaluated bone histomorphometric parameters using a digitalizing table with osteomeasure to obtain normative data of means and standard deviations, as well as median and range. Due to the small sample, a Monte Carlo simulation was applied. Structural, static, dynamic, and resorptic histomorphometric parameters were evaluated by age and gender following the American Society for Bone and Mineral Research recommendations.* **Results:** *Bone volume (in the older children) and mineral apposition rate (in the younger children), the eroded surface (in boys), and the new bone wall thickness (in girls) were significantly increased. On the trabecular area of mineralization front, the modeling and the remodeling bone formation were similar (16 and 18%). The rest of the histomorphometric bone parameters by age and gender showed no significant difference.* **Conclusion:** *In healthy children, these bone histomorphometric findings, with these techniques and for this ages could be used as reference values.*

Key words: *Normal bone metabolism. Children bone histomorphometry. Bone modeling and remodeling. Mineralization front. Bone histomorphometric reference.*

Datos histomorfométricos de referencia de la biopsia ósea en niños de 8 a 17 años

Resumen

Introducción: *El análisis histomorfométrico del tejido óseo para el estudio de las enfermedades metabólicas óseas, cuando se correlacionan los hallazgos clínicos, sigue siendo la herramienta con mayor sensibilidad y especificidad para la mayoría de los diagnósticos. En los niños existen pocos reportes histomorfométricos del tejido óseo metabólico normal, por lo que nuestro propósito es reportar más datos de referencia e investigar si hay diferencias histomorfométricas entre edades y sexos.* **Métodos:** *Estudio realizado en 19 niños de 8 a 17 años (13 masculinos) sin anomalías clínicas ni bioquímicas evidentes. Se tomaron muestras de tejido óseo transilíaco marcadas con tetraciclina. Se obtuvieron medias, desviaciones y rangos histomorfométricos totales, y correlación por edad y sexo, siguiendo las recomendaciones para la histomorfometría*

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de la American Society for Bone and Mineral Research. Se realizó una simulación Montecarlo. **Resultados:** *El volumen óseo (en niños mayores), la velocidad de agregación del mineral (en niños menores), la erosión trabecular periférica (en niños) y el grosor de la pared ósea nueva (en niñas) exhibieron aumentos significativos. En el área trabecular del frente de mineralización, el modelado y el remodelado de la formación ósea fueron similares (16 y 18%). El resto de los parámetros histomorfométricos óseos no mostraron diferencias significativas.* **Conclusiones:** *Estos hallazgos histomorfométricos del tejido óseo de niños normales con estas técnicas y para estas edades pueden ser utilizados como valores de referencia.*

Palabras clave: *Hueso metabólico óseo. Histomorfometría ósea. Modelación y remodelación óseas. Frente mineralizado. Datos histomorfométricos óseos de referencia.*

Background

Despite the invasive character of the method, metabolic bone biopsy for the study of bone metabolic diseases in children is gaining fans. Several studies in children, without tetracycline or calcein labeling, showed histomorphometric static normal or abnormal values, but data in children with dynamic parameters are scarce[1-3]. This may be because histomorphometry requires an intense labor and needs special equipment and expertise. Other possible reason includes overestimation of the utility of laboratory bone diagnostics and poor information about what the bone histomorphometry does[4-6]. Our aim was to validate in a normal children group, bone normality by clinical, biochemical, and bone histomorphometric parameters. In the cortical and trabecular zones, the bone structure (size, form, and number), static (fixed bone formation parameters) with remodeling (with resorption) and modeling (without resorption), the dynamic (movably) bone formation, and the resorptic (bone degradation) parameters were analyzed[7,8]. Because in the growing children, the remodeling and modeling bone formations (RBF MBFs) have been rarely reported, it is important to qualified data obtained from a control group to compare with a given patient. In the present histomorphometric study, we are searching for reference results to be used as control in our laboratory and by others in the field. Since it is well known that the sensibility and specify of the clinical findings are not always exactly[9,10] (e.g., osteoporosis vs. rickets), we need the bone biopsy as help.

Methods

This study was performed according to the recommendations of the World Medical Association of Helsinki declaration and approved by the Hospital Internal Review Board and ethics committee[11]. In all cases, parental written consent and assent of the children were obtained.

In a prospective protocol, the study comprised 20 Mexican children, age 8–17 years; 13 boys and 7 girls with bone transiliac biopsies obtained during surgery to correct congenital pale fissure. At the beginning, 20 subjects were studied, but one girl was excluded from the histomorphometry analysis because her bone biopsy was not representative (crushed biopsy core). All the children had normal renal function before surgery and no evidence of any clinic metabolic bone disease. Patient nutrition was evaluated through Z-score from body mass index considering the following rage: normal ≥ 1 to 1; low ≤ - 1; overweight ≥ + 1 to < 2, and obese ≥ + 2[12]. None of the children were immobilized before surgery or received medications known to affect bone metabolism. Blood and 24 h urine were collected. In blood, Ca, P, Mg, creatinine, alkaline total phosphatase and its bone enzyme[13,14], parathyroid hormone (PTH), calcidiol ($25(OH)D_3$), and calcitriol ($1,25(OH)_2D_3$) were measured by radio immunoanalysis and in urine, volume, creatinine, Ca, phosphorous, cross-linked N-telopeptide of type collagen evaluated with ELISA[13], and a urine culture was performed. To compare normal biochemical metabolic bone parameters, biochemical controls from 170 children were used[15].

Under general anesthesia and after dual labeling with tetracycline (1 g/day taken orally during 2 days separated by a 10 day free interval), the biopsies were collected 48 or 72 h after the dual labeling. Transiliac bone biopsies were obtained with a Bordier trephine (7-8 mm core diameter) from 2 cm below and behind the anterior superior iliac spine in 20 children. No side effects were noticed.

Biopsy specimens were fixed in 70% alcohol and kept at room temperature. They were dehydrated in increasing concentrations of ethanol, cleared with xylene, and embedded undecalcified in methyl methacrylate. Sections (4-5 µ - thick) were cut with a Polycut M microtome (Reichert-Jung, Heidelberg, Germany). The sections were desplastified with ethylene glycol monoethyl acetate and rehydrated for optimal staining. They

were stained with Masson Goldner trichrome, toluidine blue, and the third mounted unstained for fluorescence microscopy[3,15].

Histomorphometry

The histomorphometric analysis was studied using a digitizing table with osteomeasure software (Osteometrics, Atlanta, GA) nomenclature and abbreviations followed the recommendations of the Committee from the American Society for Bone and Mineral Research[16]. According to this definition, "bone" is bone matrix (mineralized or not), "tissue" refers to bone and associated soft tissue as bone marrow, muscles, etcetera. Histomorphormetric measure is performed in two-dimensional sections. Nevertheless, to stress the three-dimensional nature of the bone, the nomenclature committee favored a three-dimensional nomenclature. To study the bone histomorphometric parameters, they are analyzed as structural, static with MBF and RBF, dynamic bone formation, and bone resorption parameters[3,16,17].

Structural parameters

These parameters are defined as cortical width (Ct. Wi μm), which is the combined thickness, in mm, of both cortices; cortical bone area (Ct.B.Ar in mm^2); cortical porosity (Ct.Po%), the percentage of intracortical holes in the total cortical area; trabecular thickness (Tb. Th μm), which is the mean distance across individual trabecule; trabecular number (Tb.N/mm), which is the number of trabecule that a line through a trabecular compartment would hit per millimeter of its length; osteocytes number in cortical tissue area per mm^2 (Ct. Ot/mm^2); and bone volume/tissue volume (BV/TV%), which is the percentage of the total marrow area occupied by trabecular mineralized and unmineralized bone (bone area/tissue area) × 100[3,16,17].

Static formation parameters

Static formation parameters comprise the quantity of osteoid matrix, the osteoblast cells covering the bone surface and the MBF and RBF. They are evaluated as osteoid thickness (O.Th μm), which is the distance between the surface of the osteoid seam and mineralized bone (with a width of > 1.5 μm); osteoid surface/bone surface (OS/BS%), which is the percentage of bone surface covered by osteoid; osteoid volume/bone volume (OV/BV%), which is the percentage of bone volume that consists of osteoid; osteoblast surface/bone surface (Ob.S/BS%), which is the percentage of bone surface covered by osteoblast; and wall thickness (W.Th μm from average of the new bone formed per activation event, on bone surface), which is the mean thickness of bone tissue that has been deposited at a bone-forming site[3,16,17].

MBF and RBF static parameters

We studied three static parameters evaluating the trabecular bone formation: RBF preceded by resorption (RBF), MBF with no prior resorption (MBR), and the quiescent surface (QS) (no RBF neither MBF). This assessment was based on the bone mineralization front (MF) identified with the toluidine blue stain, which reflected the architecture of the cement lines underlying the active bone-forming surfaces[15,18-20]. The RBF was identified with toluidine blue as having cement lines scalloped in appearance due to prior osteoclast resorption. The MBF was identified as having underlying smooth cement lines, as if they were generated by direct bone formation on a quiescent bone surface. RBF and MBF were separately quantified as a percentage of total MF on the trabecular surface. The percentage of QS, no RBF, and no MBF area was observed negative with toluidine blue stain, calculated with 100-(MBF+RBF+ES/BS)[17,18].

Dynamic formation parameters

Dynamic bone formation parameters yield information about *in vivo* bone cell function and can only be measured when patients have received two courses of tetracycline label before biopsy. We worked with two basic parameters: the mineralizing surface activity, by tetracycline (MS/BS% is calculated as the length of double tetracycline label plus one half of the single label length perimeter×100) and the mineral appositional rate (MAR), rated in μm/day that the new bone has added by the osteoblast to trabecular surface, calculated as the distance between tetracycline labels divided by the labeling interval in days. The adjusted apposition rate (Aj. AR μm/d) is given by MAR × (MS/OS)/100. The mineralization lag time (MLT/day) is the time interval between the deposition and mineralization of the bone matrix, and is given by O.Th/AJ.AR. Osteoid maturation time (Omt/day) is the time interval between osteoid deposition to be prepared for its mineralization and is given by O.Th/MAR (in human is shorter than MLT). The BFR/BS $\mu m^3 \times \mu m^2$/year is the bone formation rate/bone surface and is given by MAR × (MS/BS) × 3.65 = Activity of bone turnover on a given bone surface. These last four

parameters are derived mathematically from the two primary dynamic measures[1,3,20].

Bone resorption parameters

Eroded surface/bone surface (ES/BS%) is a static parameter represented by the percentage of eroded spicule surfaces covered with or without osteoclast. Osteoclast surface/bone surface (Oc.S/BS%) is the percentage of bone surface covered by osteoclast. Peritrabecular fibrosis (Pm.Tb.Fb), perimeter trabecular fibrosis, is the histological finding that usually accompanies the high resorption state, and was evaluated as present or absent[1,2,10,21], and was expressed as percentage. We compared the histomorphometric parameters between ages and sexes and analyzed the differences.

Statistical analysis

The 19 participants were separated into two age groups: 8–11 years (n = 10) and 12–17 years (n = 9); of this group, 13 were male and 6 were female. The statistical analysis was done with the SPSS program version 23, adapted to windows with interphase graphic.

The histomorphometric parameters (structural, static, dynamic, and resorptic) were described with a median and central dispersion tendency. Because not all histomorphometric parameters were normally distributed, median and ranges are also given.

We used the Mann–Whitney non-parametric test to compare between sexes and ages. Since our sample of normal bone metabolism in children was limited, but assuming that we had a normal distribution, we decided to use a Monte Carlo simulation obtained with 1000 hypothetic children, to verify that the results are similar that those obtained with the histomorphometric results. For more precise results, we used percentiles to calculate RBF and MBF in these pediatric populations; the median and range of each parameter in all the studied population are also given[22].

Results

The Z-score was normal in the 100% of children (Z-score = 0.84). On table 1, we compare blood and urine biochemical parameters of the 19 children, with their control values[14,23]. We observed that in blood: serum Ca, phosphorus, Mg, creatinine, total alkaline phosphates', and its osseous enzyme are between normal limits. The calciotropic parameters: vitamin D, calcitriol, and PTH hormones also are normal. The findings in the 24 h. urine: volume, creatinine, P, Ca, and the cross-linked N-telopeptide of type 1 collagen (as marks of bone resorption) are within normal levels too.

Table 1. Biochemical hospital data controls compared with our children studies

Blood	Controls (N = 170)[21]	Studied children (N = 19)
Ca (mg/dl)	9.53 ± 0.68	8.72 ± 1.26
Mg (mg/dl)	2.14 ± 0.31	2.29 ± 0.53
P (mg/dl)	4.11 ± 0.96	5.04 ± 0.88
Creatinine (mg/dl)	0.67 ± 0.30	0.57 ± 0.31
ALP (total) (U/L)	< 400[23]	264.9 ± 184.4
ALP (bone enzyme) (%)	< 20%[13]	77.88 ± 13.96%
$25(OH)D_3$ (ng/ml)	9.0 ± 37.6	20.67 ± 7.51
$1,25(OH)_2D_3$ (pg/ml)	25.1 ± 66.1	50.97 ± 20.08
PTH (pg/ml)	33.94 ± 12.7	27.57 ± 13.76
24 h urine		
Volume ml	307.59-1,058.33	800-1,400
Creatinine/24 h	> 1 mg/24 h	673.27 ± 655.3
P mg/24 h	400 a 1300	651.4 ± 255.8
CaU/mc	≤ 4 mg/kg/24 h	1.73 ± 1.12
Cross-linked N-telop nMBCE/liter	< 20	15.6

Values are expressed as mean ± SD.
N: cases number, PTH: parathyroid hormone, CaU/mc: urinary calcium/body mass, nMBCE/liter: Nanomoles of bone collagen equivalents/liter, Mg: magnesium, P: phosphorus, Ca: calcium, ALP: alkaline phosphatase, SD: standard deviation

Table 2 summarizes the mean and the SD, as well the median and ranges of each bone histomorphometric parameters (structural, static bone formation, dynamic bone formation, and bone resorption) from the biopsies of all children.

Seven of the structural bone parameters were analyzed. The results of the histomorphometric structural parameters represent the individual normative values for bone volume and sides. When the BV/TV% and the MAR were compared by ages (Table 3), the oldest group significantly increases (21.20 ± 1.57 vs. 23.73 ± 3.26) as well as the MAR (0.68 ± 0.34 vs. 0.88 ± 0.29 in both with p = 0.043). The rest of the histomorphometry structural parameters are similar.

Table 2. Bone histomorphometric parameters from transiliac bone biopsies in 19 healthy children of 8–17 years

Parameters	Parameters abbreviation and units	Values and mean ± SD	Median and range
Structural parameters of bone	Ct.Wi μm	650.13 ± 243.8	594.86 (366.8-1317.5)
	Ct.B.Ar mm^2	2.19 ± 0.62	2.18 (1.43-3.7)
	Ct.Ot/mm^2	142.94 ± 42.65	136.11 (59.5-181.0)
	Ct.Po%	6.2 ± 2.67	6.23 (2.11-12.15)
	BV/TV%	22.40 ± 3.17	22.73 (16.1-28.0)
	Tb.Th μm	77.2 ± 17.4	74.19 (56.9-129.4)
	Tb.N/mm	3.0 ± 0.65	2.88 (1.95-4.4)
Static parameters of bone formation	O.Th μm	7.40 ± 1.56	7.84 (4.7-9.8)
	OS/BS%	23.69 ± 13.01	23.21 (6.2-48.7)
	Ob.S/BS%	9.48 ± 3.0	9.28 (5.0-14.9)
	OV/BV%	2.99 ± 2.01	2.22 (1.2-8.3)
	W. Th μm	30.49 ± 2.86	31.6 (25.45-35.26)
	MBF%	15.92 ± 3.2	15.36 (11.18-23)
	RBF%	18.09 ± 4.0	18.22 (11.2-24.6)
	QS%	65.06	65.66 (56.3-74.1)
Dynamic parameters of bone formation	MS/BS%	9.57 ± 4.80	7.39 (3.9-19.0)
	MAR μm/d	0.79 ± 0.32	0.75 (0.32-1.6)
	Aj.AR μm/d	0.24 ± 0.2	0.21 (0.03-0.65)
	Omt μm/d	9.14 ± 3.0	10.97 (2.9-14.8)
	MLT μm/d	18.38 ± 6.3	18.32 (7.0-39.3)
	BFR/BS $\mu m^3/\mu m^2/y$	25.80 ± 13.7	26.85 (6.6-51.8)
Static parameters of bone resorption	ES/BS%	16.9 ± 5.9	15.34 (9.6-31.2)
	Oc.S/BS%	0.92 ± 0.3	0.93 (0.61-1.5)
	Fb.Pm.Tb%	Absent	Absent

Mean ± SD in the central row and the median and range in the right row.
Ct.Wi μm: thickness of the both cortices, Ct.BAr mm^2: cortical bone area, Ct.Ot/mm^2: osteocytes in cortical tissue, CtPo%:cortical porosity, BV/TV%: bone volume/tissue volume, Tb.Th μm: trabecular thickness, Tb.N/mm: trabecular number/mm, O.Th μm: osteoid thickness, OS/BS%: osteoid surface/bone surface, Ob.S/BS%: osteoblast surface/bone surface, OV/BV%: osteoid volume/bone volume, W.Th μm: wall thickness of the new bone, MBF%: modeling bone formation, RBF%: remodeling bone formation, QS%: quiescent bone surface, MS/BS%: mineralizing bone surface, MARμm/d: mineral appositional rate, Aj.ARμm/d: adjusted apposition rate, Omt μm/d: osteoid maturation time, MLT μm/d: mineralization lag time, BFR/BS $\mu m^3/\mu m^2$/year: activity of bone turnover on a given bone surface, ES/BS%: eroded surface/bone surface, OcS/BS%: osteoclast surface/bone surface, Fb.Pm.Tb: % peritrabecular fibrosis, SD: standard deviation

From the bone static formation parameters, eight were histomorphometric reviewed. The wall thickness (W.Th mm), when compared by gender, was significantly higher in girls (girls 41.3 *vs.* boys: 37.6 $p = 0.02$) (Table 4). With the toluidine blue stain, we can observe and identify the bone MF[15]. The MBF looks as smooth cement lines, which represent 16.0% of the MF. The RBF as scalloped cement lines, which exhibits 18.0% of the MF (Table 2, Figs. 1-3). Even when the RBF parameter is slightly higher that the MBF, there is not statistical difference between them. The QS (without RBF or MBF) represents 65.0% of the MF (Fig. 1). The rest of the static histomorphometry parameters were similar (Table 2, Figs. 1-3).

Dynamic bone formation parameters from all the children can be observed in table 2. We evaluate six dynamic parameters. The mineral apposition rate (MAR) was significantly increased in the youngest group compared the oldest (0.88 ± 2.9 vs. 0.68 ± 34 $p = 0.043$) (Table 3). The rest of the dynamic histomorphometric parameters were similar.

We analyzed only three histomorphometric parameters to evaluate the bone metabolic resorption. The eroded surface/bone surface (ES/BS%) exhibits a significant difference when compared between genders (ES/BS%; boys 19.19 ± 5.76 vs. girls 12.16 ± 2.53; $p = 0.005$) (Table 4). Other bone histomorphometric gender parameter comparisons yielded no significant differences. The osteoclast percentage on the trabecular surface (Oc.S/BS%) and the percentage of peritrabecular fibrosis were similar. On table 5, the Monte Carlo simulation with 1000 iterations and handled with percentiles from 5% to 95%, the histomorphometry parameters between 50% and 90% verified a tendency of normal distribution, which helped to identified histopathological abnormalities in the children.

Discussion

Since reference data for bone metabolic histomorphometric children data are poor, we tried to fill this gap, testing histomorphometric results obtained from

Table 3. Bone histomorphometric results from the 19 children compared between ages

Parameters	Parameters abbreviation and units	Group 1 from 8 to 11 years (N = 10) Mean ± SD	Group 2 from 12 to 17 years (N = 9) Mean ± SD	P
Structural parameters	Ct.Pf.Wi µm	650.89 ± 198.94	649.29 ± 298.79	0.549
	Ct.B.Ar mm^2	2.38 ± 0.60	1.99 ± 0.61	0.156
	Ct.Ot/mm^2	136.66 ± 11.66	149.92 ± 61.93	0.549
	Ct.Po%	5.81 ± 1.57	6.63 ± 3.58	0.518
	BV/TV%	21.20 ± 2.71	23.73 ± 3.26	0.043
	Tb. Th µm	75.9 ± 11.6	78.7 ± 23	0.730
	Tb. N mm	2.84 ± 0.54	3.16 ± 0.73	0.293
Static parameters of bone formation	OS/BS%	20.59 ± 11.97	27.13 ± 13.95	0.315
	Ob.S/BS%	8.49 ± 2.68	10.58 ± 3.12	0.133
	O.Th µm	6.76 ± 1.50	8.11 ± 1.37	0.095
	OV/BV%	2.37 ± 0.90	3.68 ± 2.67	0.661
	W.Th µm	0.31 ± 0.02	0.30 ± 0.02	0.22
	MBF%	15.40 ± 3.15	16.49 ± 3.43	0.549
	RBF%	18.37 ± 3.67	17.77 ± 4.37	0.842
	QS%	65.20 ± 4.98	64.91 ± 6.35	1.0
Dynamic parameters of bone formation	MS/BS%	9.11 ± 4.32	10.08 ± 5.51	0.842
	MAR µm/d	0.88 ± 0.29	0.68 ± 0.34	0.043
	AjAr µm/d	0.25 ± 0.15	0.22 ± 0.25	0.10
	Omt µm/d	8.41 ± 3.17	9.96 ± 2.90	0.211
	MLT µm/d	17.22 ± 4.82	19.67 ± 7.68	0.780
	BFR/BS $µm^3/µm^2/y$	28.47 ± 13.06	22.83 ± 14.69	0.497
Static parameters of bone resorption	ES/BS%	17.11 ± 5.57	16.81 ± 6.64	0.720
	Oc.S/BS%	0.99 ± 0.17	0.83 ± 0.49	0.497
	Fb.Pm.Tb%	Absent	Absent	

Bone histomorphometric results in Group 1 (8–11 years) compared with Group 2 (12–17 years).
Ct.Wi µm: thickness of the both cortices, Ct.BAr in mm^2: cortical bone area, Ct.Ot/mm^2: osteocytes in cortical porosity, Ct.Po%: cortical porosity, BV/TV%: bone volume/tissue volume, Tb.Th µm: trabecular thickness, Tb.N/mm: trabecular number/mm, O.Th µm: osteoid thickness, OS/BS%: osteoid surface/bone surface, Ob.S/BS%: osteoblast surface/bone surface, OV/BV%: osteoid volume/bone volume, W.Th µm: wall thickness of the new bone, MBF%: modeling bone formation, RBF%: remodeling bone formation, QS%: quiescent bone surface, MS/BS%: mineralizing bone surface, MARµm/d: mineral appositional rate, Aj.ARµm/d: adjusted apposition rate, Omt µm/d: osteoid maturation time, MLT µm/d: mineralization lag time, BFR/BS $µm^3/µm^2$/year: activity of bone turnover on a given bone surface, ES/BS%: eroded surface/bone surface, Oc.S/BS%: osteoclast surface/bone surface, Fb.Pm.Tb: % peritrabecular fibrosis

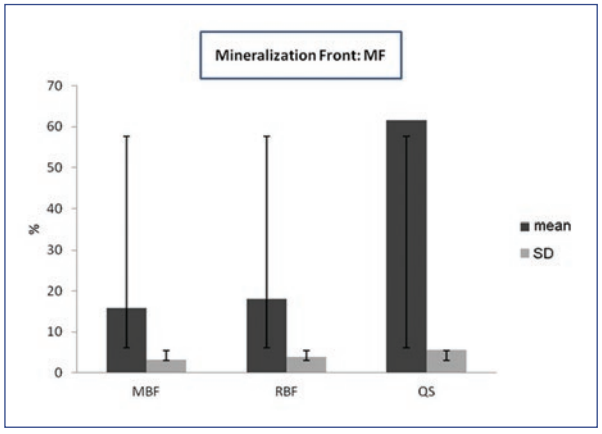

Figure 1. This figure represents the percentage of bone formation analyzed on the mineralization front (stained with toluidine blue) and analyses through the remodeling, the modeling, and the quince zones. The percentage of the remodeling and the modeling bone formation is similar, and the quince zone (free of stain) is the larger. MF: mineralization front, MBF: modeling-based bone formation, RBF: remodeling-based bone formation, QS: quiescent surface.

19 children, apparently with no evidence of metabolic bone disease. On table 1, we observe that their serum electrolytes, bone alkaline phosphates, and calciotropic hormones were normal, and in urine, the electrolytes and the cross-linked N-telopeptide of type 1 collagen were within normal limits, so the children clinically and biochemically were apparently healthy.

The structural histomorphometric parameters observed in table 2 are similar to other reference material reports, using a similar histomorphometric methodology. We only found two parameter results that differed from thus observed by Glorieux et al[·]. In our study, the bone trabecular thickness appears thinner (Tb.Th µm = 77.2±17.4 vs. 139 ± 28) and the number of bone trabecular increased (Tb.N mm = 3.0 ± 0.65 vs. 1.72 ± 0.23). The rest of the histomorphometric parameters were similar, though we think the low thickness of the bone trabecular is compensated by the increase in its number, or might have a geographic or ethnic cause.

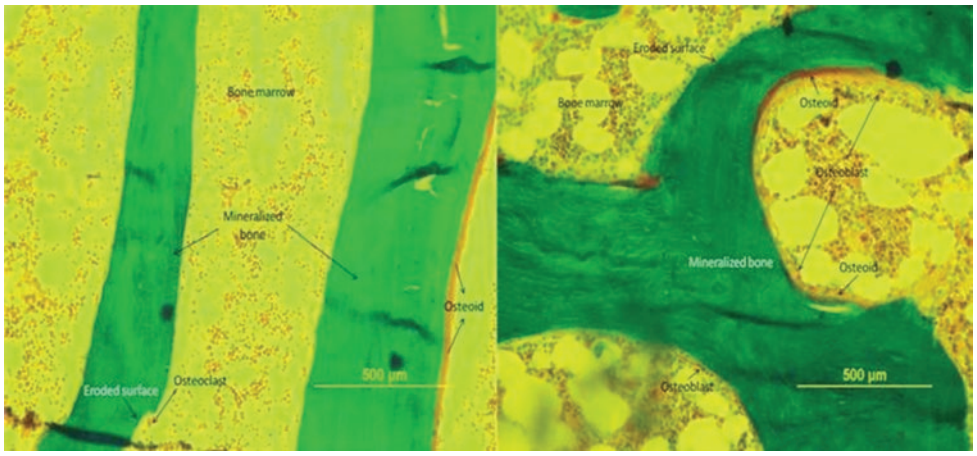

Figure 2. Microphotographs from trabecular bone stains with "Goldner trichrome on children of 11 and 15 years old." The BV/TV% (21.7 vs. 23.73) is slightly thinner on the younger children (left) when is compared with the older (right). The number of osteoblast and the osteoid volume are normal. Undecalcified bone, 10 × 12.5 mm.

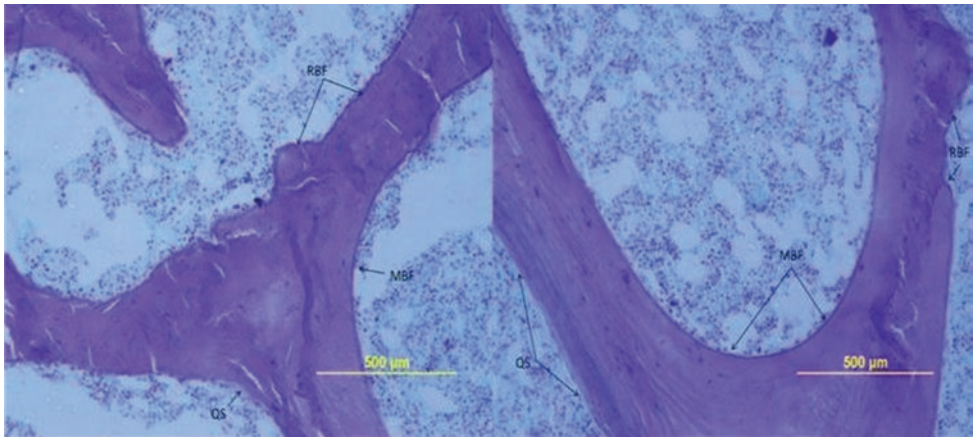

Figure 3. Microphotographs from normal mineralization front, from an 11 and 15 years to old boys stained with toluidine blue and observed as blue-stained lines, underlying the active bone-formation surfaces. The remodeling formation (RBF) surfaces are associated with scalloped cement lines reflecting previous resorption of the remodeling cycle. The modeling bone formation (MBF) is associated with straight cement lines consistent to have been originated on quiescent surface. The quiescent surface (QS) does not exhibit stained surface, which means there is no mineralizing formation activity. Undecalcified bone and photomicroscograph 10.0 × 12.5 mm. Toluidine blue of undecalcified bone.

The measure of the cortical volume, its porosity, and the number of its osteocytes are rarely mentioned, but these histomorphometric parameters are an important data to be correlated with fractures, phosphaturias, and bone volume. Observing the structural histomorphometric parameters, classified by ages (Table 3), we found that the children in the oldest group exhibited a significant greater bone volume than that observed in the youngest group (BV/TV% = 21.20 ± 2.71 vs. 23.73 ± 3.26; p = 0.043); this finding of bone volume increase is an issue normally seen in the oldest group[3,20]. The W.Th measure by gender was significantly thicker in the girls (Table 4) and was similar compared by ages (Table 3). In the static formation parameters, comparing the amount of osteoid on the bone surface and the number of osteoblast, the results are similar too.

To evaluate the MF and its cement lines apparently for the 1st time, we used the toluidine blue stain[15]. With

Table 4. Bone histomorphometric results from the 19 children compared between sexes

Parameters	Bone parameters abbreviation units	Boys (N = 13) Mean ± SD	Girls (N = 6) Mean ± SD	P
Structural parameters	Ct.Pf.Wi µm	698.46 ± 255.14	545.42 ± 196.49	0.152
	Ct.B.Ar mm^2	2.32 ± 0.60	1.92 ± 0.62	0.179
	Ct0t/mm^2	151.98 ± 45.11	123.34 ± 31.46	0.282
	Ct.Po%	6.68 ± 2.85	5.16 ± 2.06	0.261
	BV/TV%	21.85 ± 2.71	23.58 ± 4.03	0.210
	Tb. Th µm	77.2 ± 18.84	77.2 ± 15.51	0.898
	Tb. N mm	2.91 ± 0.49	3.17 ± 0.9	0.424
Static parameters of bone formation	OS/BS%	25.07 ± 12.26	20.69 ± 15.28	0.416
	Ob.S/BS%	10.15 ± 2.96	8.04 ± 2.82	0.244
	O.Th µm	7.28 ± 1.77	7.67 ± 1.05	0.831
	OV/BV%	3.16 ± 2.21	2.63 ± 1.61	0.639
	W.Th µm	37.6 ± 3.18	41.3 ± 2.11	0.022
	MBF%	15.39 ± 3.66	17.05 ± 1.87	0.127
	RBF%	17.90 ± 3.77	18.49 ± 4.55	0.701
	QS%	65.79 ± 5.53	63.48 ± 5.62	0.368
Dynamic parameters of bone formation	MS/BS%	10.19 ± 4.66	8.23 ± 5.27	0.323
	MAR µm/d	0.84 ± 0.36	0.67 ± 0.20	0.323
	MLT µm/d	16.92 ± 4.37	21.54 ± 8.86	0.323
	Omt µm/d	8.56 ± 3.22	10.42 ± 2.45	0.282
	BFR/BS $\mu m^3/\mu m^2/y$	27.64 ± 11.98	21.81 ± 17.59	0.282
Static parameters of bone resorption	ES/BS%	19.19 ± 5.76	12.16 ± 2.53	0.005
	Oc.S/BS%	0.93 ± 0.35	0.89 ± 0.41	0.966
	Fb.Pm.Tb	Absent	Absent	

Bone histomorphometric abbreviation parameters and units in the left row. Boys histomorphometric values in the central row and girls in the right row. Ct.Wi µm: thickness of the both cortices, CtBAr in mm^2: cortical bone area, Ct.Ot/mm^2: osteocytes in cortical, Ct.Po%: porosity, BV/TV%: bone volume/tissue volume, Tb. Th µm: trabecular thickness, Tb.N/mm: trabecular number/mm, O.Th µm: osteoid thickness, OS/BS%: osteoid surface/bone surface, Ob.S/BS%: osteoblast surface/bone surface, OV/BV%: osteoid volume/bone volume, W.Th µm: wall thickness of the new bone, M/BF%: modeling bone formation, R/BF%: remodeling bone formation, QS/BF%: quiescent bone surface, MS/BS%: mineralizing bone surface, MARµm/d: mineral appositional rate, Omt µm/d: osteoid maturation time, MLT µm/d: mineralization lag time, BFR/BS $\mu m^3/\mu m^2$/year: activity of bone turnover on a given bone surface, ES/BS%: eroded surface/bone surface, Oc.S/BS%: osteoclast surface/bone surface, Fb. Pm.Tb: % peritrabecular fibrosis

this method, if the lines are scalloped, they reflect an initial resorption phase of the remodeling cycle (RBF), whereas if they are straight, they represent MBF, which is associated with a previously QS17,[18] (Figs. 2 and 3). Using these techniques, the proportion of active MBF was 16% and the RBF was 18%, statistically not different (Fig. 1). These findings suggested that the normal bone formation developed on the trabecular area by two different mechanisms: remodeling and modeling, and their activity is similar. Recently, some authors have started to identify the MF with tetracycline to measure the MBF and RBF[15,17,18]. The results with the toluidine blue stain are similar to Villanueva's15 and easier to interpret. This means that we have to emphasize this finding to evaluate the bone formation activity in each bone histomorphometric study and try to investigate the biochemical markers to correlate them, but up to date, there is no specific biochemical marker for the MBF. Dynamic bone formation parameters can be observed in table 2. They yield information on *in vivo* bone cell function only when the children or the patient have received dual labeling with tetracycline before biopsy20. We evaluate six dynamic parameters, two of them are basic. One is the surface extent of mineralization activity per bone surface identified with the tetracycline MS/BS% (9.57 ± 4.80) and the second is the MAR or the distance between two tetracycline labels divided by the length of the labeling interval (0.79 ± 0.32). When we compared between ages in table 3, the MAR is significantly higher in the youngest group (0.88 ± 0.29 vs. 0.68± 0.34 µm/d; $p = 0.043$); this is explained by a more active osteoid formation by the osteoblast in the youngest group[16,17,19]. The MAR could be different too, with the dosage of the MF marker[3]. The other four histomorphometric parameters are the adjusted opposition rate (Aj.AR), the MLT, osteoid maturation time (Omt), and the bone formation rate per bone surface (BFR/BS)[1,10]. Comparing between ages, there were no differences.

We analyzed only three parameters to evaluate the bone metabolic resorption. The first one consists in counting the percent of eroded trabecular perimeter or eroded surface/bone surface (ES/BS). A significant

Table 5. Percentile to show the distribution of histomorphometric parameters using Monte Carlo simulations with 1000 iterations

Abbreviations	5%	10%	50%	90%	95%
BV/TV	19.65	20.54	23.05	26.04	26.84
Tb.Th	37.65	52.22	83.9	105.43	110.3
Tb.N	1.88	2.11	2.98	3.78	3.99
O.Th	5.21	5.66	7.41	9.17	9.63
OS/BS	13.57	16.39	26.28	35.69	37.78
OV/BV	1.5	1.76	2.59	3.48	3.78
Ob.S/BS	5.85	6.63	9.93	13.18	14.17
MS/BS	6.4	7.86	12.96	18.36	19.34
MAR	0.35	0.47	0.93	1.38	1.5
Omt	3.08	4.41	9.18	13.92	15.28
Mlt	13.37	14.59	18.77	22.8	23.45
BFR/BS	25.48	28	37.31	46.53	49.79
ES/BS	0.33	1.29	4.72	7.99	8.92
Oc.S/BS	0.52	0.63	0.99	1.34	1.44
MFB	11.64	13.06	15.36	16.62	17.02
RFB	11.02	14.11	18.22	20.55	23.27
QS	58.41	60.94	65.87	71.54	74.11

BV/TV%: bone volume/tissue volume, Tb.Th μm: trabecular thickness, Tb.N/mm: trabecular number/mm, O.Th μm: osteoid thickness, OS/BS%: osteoid surface/bone surface, Ob.S/BS%: osteoblast surface/bone surface, OV/BV%: osteoid volume/bone volume, MS/BS%: mineralizing bone surface, MARμm/d: mineral appositional rate, Omt μm/d: osteoid maturation time, MLT μm/d: mineralization lag time, BFR/BS $\mu m^3/\mu m^2$/year: activity of bone turnover on a given bone surface, ES/BS%: eroded surface/bone surface, Oc.S/BS%: osteoclast surface/bone surface, MBF%: modeling bone formation, RBF%: remodeling bone formation, QS%: quiescent bone surface

difference in histomorphometric parameters between genders was found when we compared eroded surface/bone surface from the boys group versus girls (boys 19.19 ± 5.76 vs. girls12.16 ± 2.53, p = 0.005) (Table 4). This measure is rather subjective for interpretation3, but in the present study, erosion surface observed by golden stained was similar to that quantified with toluidine blue stain (ES/ES% = 17 vs. RBF% = 18). The rest of the gender histomorphometric parameters (Oc.S/BS and the Fb.Pm.Tb)[21,24,25] yielded not significant differences. The individual parameter forms are reported in table 2, and these values represent our histomorphometric bone reference data.

Our study has several limitations. The children number was small. The biomarker profiles are consistence with the histomorphometry findings, but their clinical studies were limited, for instance, the pubertal stages were not evaluated. The effect of toluidine blue at the cortical area was not described and could be an interesting area for future investigation. Finally, characterizing the MF on MBF across human ages and across species could be interesting[3,9].

In summary, the present report exhibited the bone histomorphometry reference data for children between 8 and 17 years. There were few differences related to their ages and gender. The assessment of MBF and RBF with toluidine blue stain is reported, and they had similar extension, suggesting equal activity in the normal children (MBF% = 16 and the RBF% = 18) (Table 2). Additional studies are needed to elucidate the cellular and molecular mechanisms regulating the activity of bone formation. Children with fractures not explained with non-invasive examination should have a bone biopsy that provides data that cannot be obtained in any other way. We believe that all these bone histomorphometric parameters for children of this age and with these techniques can be used as bone histomorphometric reference data.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

Conflict of interest

The authors declare no conflicts of interest.

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References

1. Merz WA, Schenk RK. Quantitative structural analysis of human cancellous bone. Acta Anat (Basel). 1970;75:54-66.
2. Frost HM. Tetracycline-based histological analysis of bone remodeling. Calcif Tissue Res. 1969;3:211-37.
3. Glorieux FH, Travers R, Taylor A, et al. Normative data for iliac bone histomorphometry in growing children. Bone. 2000;26:103-9.
4. Salusky IB, Coburn JW, Brill J, et al. Bone disease in pediatric patients undergoing dialysis with CAPD or CCPD. Kidney Int. 1988;33:975-82.
5. Sanchez CP, Salusky IB, Kuizon BD, et al. Bone disease in children and adolescents undergoing successful renal transplantation. Kidney Int. 998; 53:1358-64.
6. Schnitzler CM, Daniels ED, Mesquita JM, et al. Bone disease in african children with slipped capital femoral epiphysis: histomorphometry of iliac crest biopsies. Bone. 1998;22:259-65.
7. Erben RG. Trabecular and endocortical bone surfaces in the rat: modeling or remodeling? Anat Rec. 1996;246:39-46.
8. Yao W, Jee WS, Zhou H, et al. Anabolic effect of prostaglandin E2 on cortical bone of aged male rats comes mainly from modeling-dependent bone gain. Bone. 1999;25:697-702.
9. Qi Q, Monier-Faugere MC, Geng Z, Malluche HH. Predictive value of serum parathyroid hormone levels for bone turnover in patients on chronic maintenance dialysis. Am J Kidney Dis. 1995;26:622-31.
10. Llach F, Felsenfeld AJ, Coleman MD, et al. The natural course of dialysis osteomalacia. Kidney Int Suppl. 1986;18:S74-9.
11. The World Medical Association. The World Medical Association's Declaration of Helsinki; 2012. Available from: https://www.wma.net/es/policies-post/declaracion-de-helsinki-de-la-amm-principios-eticos-para-las-investigaciones-medicas-en-seres-humanos/.
12. Dibley MJ, Staehling N, Nieburg P, Trowbridge FL. Interpretation of Z-score anthropometric indicators derived from the international growth reference. Am J Clin Nutr. 1987;46:749-62.
13. McComb RB, Bowers GN Jr. Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. Clin Chem. 1972;18:97-104.
14. Calvo MS, Eyre DR, Gundberg CM. Molecular basis and clinical application of biological markers of bone turnover. Endocr Rev. 1996;17:333-68.
15. Villanueva AR, Kujawa M, Mathews CH, Parfitt AM. Identification of the mineralization front: comparison of a modified toluidine blue stain with tetracycline fluorescence. Metab Bone Dis Relat Res. 1983;5:41-5.
16. Dempster DW, Compston JE, Drezner MK, et al. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR histomorphometry nomenclature committee. J Bone Miner Res. 2013;28:2-17.
17. Velásquez-Forero FH, Esparza-García M, Canseco-Jiménez JF, Villanueva-Moreno NL, Rincón-Rodríguez H, Aguado-Reyes VH. Histomorfometria de la modelación y remodelación ósea con azul de toluidina en el tejido trabecular pediátrico. Patol Rev Latinoam. 2017;55:7-17.
18. Ominsky MS, Niu QT, Li C, Li X, Ke ZH. Tissue-level mechanisms responsible for the increases in bone formation and bone volume by sclerostin antibody. J Bone Miner Res. 2014;29:1424-30.
19. Miller SC, Marks SC. Local stimulation of new bone formation by prostaglandin E1: quantitative histomorphometry and comparison of delivery by minipumps and controlled-release pellets. Bone. 1993;14:143-51.
20. Rauch F. Watching bone cells at work: what we can see from bone biopsies. Pediatr Nephrol. 2006;21:457-62.
21. Sherrard DJ, Hercz G, Pei Y, et al. The spectrum of bone disease in end-stage renal failure – An evolving disorder. Kidney Int. 1993;43:436-42.
22. Lindgren BW. Statistical Theory. New York: Macmillan; 1968.
23. Velásquez-Forero FH, Esparza M, Salas A, Medeiros M, Toussaint G, Llach F. Risk factors evaluation for urolithiasis among children. Bol Med Hosp Infant Mex. 2016;73:228-36.
24. Rehman MT, Hoyland JA, Denton J, Freemont AJ. Age related histomorphometric changes in bone in normal British men and women. J Clin Pathol. 1994;47:529-34.
25. Bakaloglu SA, Wesseling-Perry K, Pereira RC, et al. Value of the bone classification system in pediatric renal osteodystrophy. Clin J Am Soc Nephrol. 2010;5:1860-6.