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Lamellation in fibrous dysplasia: a clinicopathologic study

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Summary. Background. Fibrous dysplasia (FD) is a maturation defect characterized by immature woven bones and stroma. However, especially in craniofacial bones, lamellation can be seen and this is associated with the maturation. Aim: To show maturation in FD and discuss the factors that may affect the maturation. Materials and Methods. Ninety-five FD cases were divided into three subgroups according to the lamellation percentage as Groups 1, 2 and 3 (low, moderate and high lamellation, respectively). Each group was compared in terms of the peritrabecular clefting (PTC), stromal cellularity and the age. The lesions under pressure and the ones that are not were compared in terms of lamellation percentage. Results. A significant statistical difference was found between Groups 1 and 3 in terms of PTC, stromal cellularity, histologic pattern suggesting maturation ($p<0.001$, $p<0.001$, $p=0.002$, respectively). Conclusion. The findings suggested a strong relation between lamellation and maturation. Lamellation was more prominent in the bones under pressure than the others. Considering lamellation as a finding of maturation, it is possible to establish a relation between maturation and pressure. Therefore, future studies should focus on the question if the pressure could be a factor for maturation and it could be used for treating FD.

Key words: Fibrous dysplasia, Lamellation, Pressure, Retraction, Weight-bearing exercise

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Introduction

Fibrous dysplasia (FD) is a benign, medullary fibro-osseous lesion that may present in the monostotic or polyostotic form (WHO, 2013). The monostotic form comprises approximately 70-75% of all cases and usually occurs in craniofacial bones, ribs, femurs, tibia and humerus (Riddle and Bui, 2013). The diagnosis of the disease depends on the combination of clinical, radiological and histological features, which reflect the etiopathogenic aspects of the lesions. The somatic mutation on GNAS gene (20q13) that encodes the alpha subunit of G-protein receptors (Gs-α mutation) increases intracellular cAMP leading to abnormal proliferation and incomplete differentiation of osteoblastic cells and stromal cells in bone marrow (Riddle and Bui, 2013). Thus, a prominent fibroblastic stroma and irregular, curvilinear bone trabeculae are replaced by normal marrow space. These newly formed bone trabeculae are composed of abnormal, spindle-shaped osteoblastic cells surrounded by irregularly arranged bundles of collagen, a structure called 'woven bone' (Marotti et al., 2013). In the early phase of the lesion, the stromal fibroblastic element is proliferative and hypercellular, and bone trabeculae are woven and rimmed by osteoblasts. In the later stages of the disease, woven bone is replaced by lamellar bone trabeculae (Eversole et al., 2008). It is believed that this phenomenon occurs particularly in craniofacial lesions as an indicator of the maturation process (El-Mofty, 2014). In this study, we aimed to show certain histological findings of maturation including lamellation, peritrabecular clefting (PTC) and stromal cellularity in craniofacial as well was non-

craniofacial FD lesions. We also discuss possible factors that may affect maturation, such as gender, age and anatomic localization.

Materials and methods

Study design

This study was designed as retrospective analytic observation based on clinical and morphological findings. Ninety-five patients diagnosed as FD between 2000 and 2015 were included in the study. Demographic data on the patients including age, gender and localization of lesions were obtained from the patient files. Radiologic images were analyzed again to make a correlation between the radiologic and histopathologic diagnosis. All lesions showed predominantly ground glass opacity pattern with expansion of related bone consistent with fibrous dysplasia by radiology. Paraffin blocks were obtained from the pathology archives for histologic examination.

Histologic examination

Five micron-thickness sections were obtained from each paraffin block and stained with H&E and Masson-trichrome stain. All the slides were reviewed by two pathologists under a light microscope and polarized light for lamellation (%), PTC (%), stromal cellularity and stromal histologic pattern as follows:

Lamellation was defined as the presence of parallel striation within the bone trabeculae (Fig. 1). A semi-quantitative method was used to evaluate bone trabeculae under polarizing light according to birefringent patterns. At least 100 bone trabeculae were assessed on each slide. Those that showed parallel striation were counted and a value of percentage was obtained for each case. Striation observed within an entire trabecula was counted as '1' while incomplete striation was considered 'half' and scored as 0.5. For example, if a case contained 40 trabeculae with complete lamellation and 60 trabeculae with incomplete lamellation, it was scored as 70% (40+60/2=70).

PTC was defined as the space between the bone trabecula and the stroma. PTC was calculated using a method similar to that of lamellation as described above. At least 100 bone trabeculae were assessed and those that had a surrounding space were counted as PTC. For example, if a case contained a total of 35 bone trabeculae with a space between the bone and the stroma, it was scored as 35%.

To assess the stromal cellularity, image analysis software (ImageJ 1.46 National Institutes of Health, US) was used. Microscopic images from five randomly selected high-power fields were captured and measured as a pixel value. Only the stromal areas were measured. Bone trabeculae and vascular spaces were excluded. Then, spindle shaped stromal cells in each field were counted. Endothelial cells and inflammatory cells within the stroma were excluded. The stromal cellularity was calculated by dividing the number of the stromal cells by the area. For example, an area measured as "1302152 pixel" and having 72 stromal cells, gained the score of "$5.53x10^{-5}$" (72/1302152). The mean value of stromal cellularity for each case was performed by dividing the total stromal score of five areas by five. "10^{-5}" was ruled out to simplify the results.

Stromal histologic pattern is defined as 'fascicular' when the stroma is hypocellular and the stromal cells are loosely arranged and as 'storiform' when the stroma resembles dermatofibroma. A meningioma-like appearance was defined as 'whorl'.

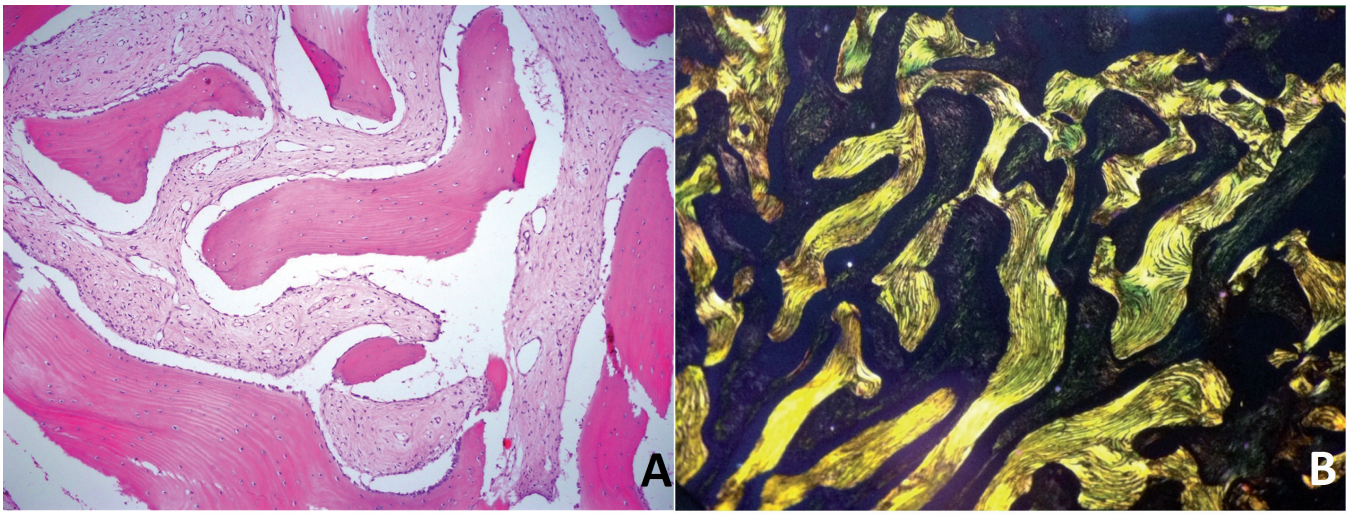

Fig. 1. Histologic section showing parallel striations (lamellation) within the bone trabeculae **(A)** and they are demonstrated under polarized light **(B)** (H&E) x 200.

Categorization and comparison of the cases

In terms of anatomic localization, the cases were investigated mainly in two categories; craniofacial and non-craniofacial. Each category was divided into two subcategories according to the exposure to pressure. For craniofacial cases, the bones which constitute the sinus wall (ethmoid, frontal, sphenoid and maxilla) and those surrounded by a group of masticator muscles (maxilla and mandibula) were defined as being exposed to pressure while temporal, parietal, and occipital bones were considered the bones that were not exposed to pressure. For non-craniofacial cases, the bones carrying the body weight such as vertebra, pelvis and lower extremities were defined as being exposed to pressure whereas the bones that do not carry as much body weight as other bones such as costae and upper extremities were defined as not being exposed to pressure. Craniofacial and non-craniofacial cases were assessed separately for reasons, which will be given in the discussion section.

According to their lamellation percentages, each category (craniofacial and non-craniofacial) was divided into three groups as Group 1, Group 2 and Group 3. The cases representing lower lamellation with a percentage of 0 to 33 were placed in Group 1; those that had a lamellation percentage of 34% to 66% were included in Group 2 (representing moderate lamellation) and those that had 67% to 100% lamellation indicated higher lamellation and were placed in Group 3. These groups were then compared in terms of the mean percentage of PTC, stromal cellularity and age of the patients. In addition, the cases that are exposed to pressure and the cases that are not exposed to pressure, gender and histologic pattern were compared to each other in terms of the mean percentage of lamellation.

Statistical analysis

The software of IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis. The difference between independent variables was assessed by student t-test, Kruskal-wallis test and MannWhitney U test. "P" value under 0.05 was considered statistically significant.

Results

The total number of cases was 95, of which 55 (57.8%) were craniofacial and 40 (42.2%) were non-craniofacial. Of the craniofacial cases, 31 (56.3%) were located on the bones exposed to pressure [maxilla (15), mandible (7), nasal (3), sphenoid (3), ethmoid (1), orbita (1), and zygoma (1)]; 24 (43.7%) were located on the bones that are not exposed to pressure [frontal (16), parietal (4), occipital (2) and temporal (2)]. Of the non-craniofacial cases, 25 (62.5%) were located on the bones exposed to pressure [tibia (12), femur (10), fibula (1), vertebra (1) and pelvis (1)] while 15 (37.5%) were located on the bones that are not exposed to pressure such as costa (10), ulna (4) and humerus (1). The mean age of the patients was 28.5 (min: 4, max: 64) (Table 1). Seventy-one (75%) patients were male and 24 (25%) were female. The clinical and demographic features of the patients are summarized in table 1.

In craniofacial lesions, the mean percentage of lamellation was 40.4%. Of the 55 craniofacial lesions, 28 (51%) were in Group 1 (lower lamellation group), 10 (18%) were in Group 2 (moderate lamellation group) and 17 (31%) were in Group 3 (higher lamellation group). (Table 1). The mean percentage of PTC was 18% in Group 1, 46% in Group 2 and 77% in Group 3. There was a significant statistical difference between Group 1 and 2 (18% and 46%, respectively, $p=0.02$); Group 2 and 3 (46% and 77%, respectively, $p=0.01$); Group 1 and 3 (18% and 77%, respectively, $p<0.0001$) in terms of mean percentage of PTC. The mean stromal cellularity was 7.2 in Group 1, 4.3 in Group 2 and 2.6 in Group 3 (Table 1). There was a significant statistical difference between lamellation groups in terms of mean stromal cellularity (Group 1 and 2, $p<0.001$; Group 2 and 3, $p=0.010$ and Group 1 and 3, $p<0.001$) (Table 2).

In non-craniofacial lesions, the mean percentage of lamellation was 21.6%. Of the 40 non-craniofacial lesions, 32 (80%) were in Group 1, 4 (10%) were in Group 2 and 4 (10%) were in Group 3 (Table 1). The mean percentage of PTC was 15% in Group 1, 55% in Group 2 and 91% in Group 3. There was a significant

Table 1. The clinical and demographic features of the cases.

	Craniofacial	Non-craniofacial
Patient Age (year)	29,1±10,9	27,9±8,1
Range (year)	4-64	12-59
Gender		
Male	37 (67,3%)	34 (85,0%)
Female	18 (32,7%)	6 (15,0%)
Exposure to pressure		
Yes	32 (58,2%)	15 (37,5%)
No	23 (41,8%)	25 (62,5%)
Histological pattern		
Fascicular	25 (45,5%)	13 (32,5%)
Storiform	29 (52,7%)	25 (62,5%)
Whorl	1 (1,8%)	2 (5,0%)
Lamellation (%)	40.4 (min: 0-max: 95)	21,6 (min: 0-max: 95)
Group 1(0-33)	28 (51%)	32 (80%)
Group 2 (34-66)	10 (18%)	4 (10%)
Group 3(67-100)	17 (31%)	4 (10%)
Retraction (PTC) (%)	41,5 (min: 0-max: 100)	26,8 (min: 0-max: 95)
Stromal cellularity	5,25 (min: 1,76-max: 10,65)	5,87 (min: 2,57-max: 11,08)
Total (n=95)	**n=55 (57.8%)**	**n=40 (42.2%)**

The lesions exposed to pressure: Maxilla, madibula, sphenoid and frontal bones (craniofacial) Lower extremity bones, pelvic bones and vertebrae (noncraniofacial). The lesions not exposed to pressure: Parietal, temporal and occipital (craniofacial), Upper extremity bones and costae (noncraniofacial) PTC: Peritrabecular clefting.

statistical difference between Group 1 and 2 (15% and 55%, respectively, p=0.04; Group 1 and 3 (15% and 91%, respectively, p<0.0001) in terms of mean percentage of PTC (Table 2). The mean stromal cellularity was 6.59 in Group 1, 3.36 in Group 2 and 2.67 in Group 3 (Table 1). There was significant statistical difference between Group 1 and 2 (6.59 and 3.36, respectively, p<0.001); group 1 and 3 (6.59 and 2.67, respectively, p<0.001). There was positive correlation between lamellation and PTC (Fig. 2) and inverse correlation between lamellation and stromal cellularity (Fig. 3).

Another significant statistical difference was found between histological patterns (fascicular and storiform) of craniofacial cases in terms of lamellation percentages (55.4% and 28.7%, respectively, p= 0.002) (Table 3). This finding may suggest that a high percentage of lamellation may be related with the fascicular pattern. Another remarkable finding of this study was a relationship between pressure and lamellation. Even if there was no significant statistical difference, the lesions under pressure showed higher percentage of lamellation. (44.2% and 35.2%, respectively, p=0.323 for craniofacial bones; 25.2% and 15.3%, respectively, p=0.2288 for non-craniofacial bones) (Table 2). There was no significant statistical difference between Group 1, 2 and 3 in terms of age (Table 2) and there was no statistically significant relation between gender and lamellation (Table 3).

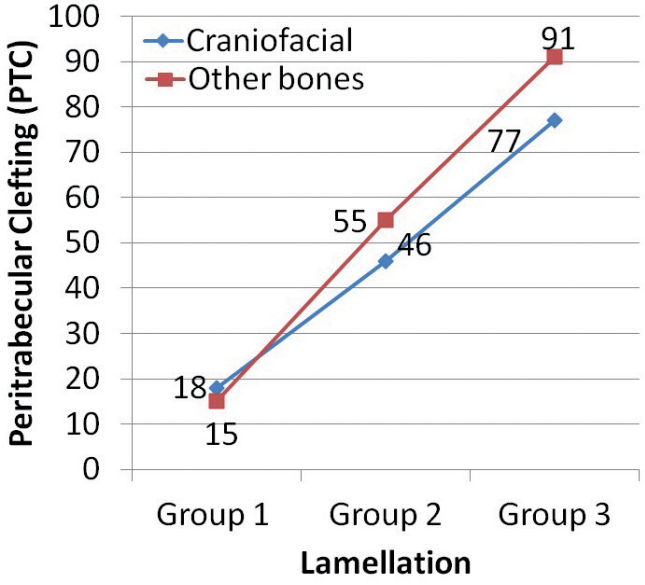

Fig. 2. Positive correlation between lamellation and peritrabecular clefting (PTC).

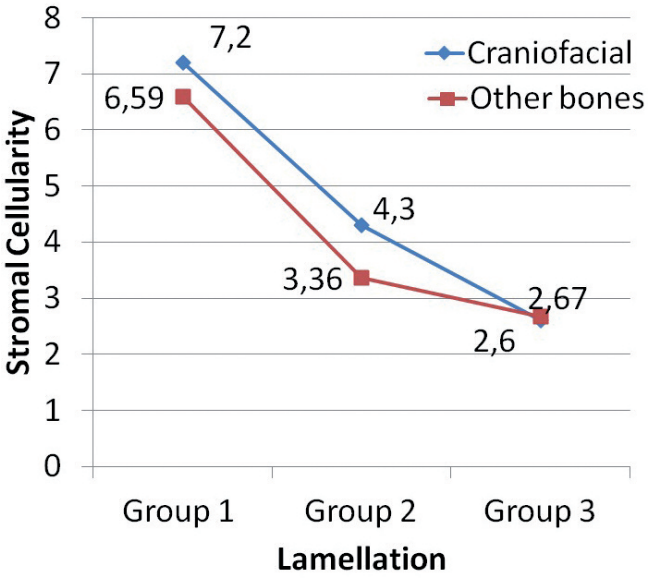

Fig. 3. Inverse correlation between lamellation and stromal cellularity.

Table 2. Relation between lamellation and age, peritrabecular clefting, stromal cellularity.

Characteristics	Overall	Lamellation (%)						p value					
		Group 1 (0-33)		Group 2 (34-66)		Group 3 (67-100)		Group 1 vs 2		Group 2 vs 3		Group 1 vs 3	
		CF	NCF	CF	NCF	CF	NCF	CF	NCF	CF	NCF	CF	NCF
Total no of Patient	95												
Mean Lamellation Percentage	CF: 40.4% (n: 55) NCF:21.6% (n:40)	28	32	10	4	17	4						
Mean Patient Age	28.5 (min:4 max:64)	30.18	27.3	28.3	29.25	27.82	31.25	0.61†	0.62†	0.56†	0.75†	0.5†	0.5†
Mean Peritrabecular Clefting (%)	35.3 (min:0 max:100)	18%	15%	46%	55%	77%	91%	0.02†	0.04†	0.01†	0.054†	<0.001†	<0.001†
Mean Stromal Cellularity	5.51 (min:1.76 max:11.08)	7.2	6.59	4.3	3.36	2.6	2.67	<0.001†	<0.001†	0.01	0,488†	<0.001†	<0.001†

CF, Craniofacial; NCF, Non-craniofacial; †, Kruskal Wallis Test. Difference was considered significant when the p value was less than 0.05.

Table 3. Relation between lamellation and gender, localization, histologic pattern.

Characteristics		Lamellation (%)																		
		Gender						Localization						Histologic Pattern						
	Overall	Craniofacial			Non-craniofacial			Craniofacial			Non-craniofacial			Craniofacial			Non-craniofacial		
		M	F	p value	M	F	p value	EP	NEP	p value	EP	NEP	p value	F	S	p value	F	S	p value
Total no of Patient	95																			
Gender	M: 71 F: 24	38.6%	44%	0.584§	20.7%	26.6%	0.694§													
Localization	EP: 47 NEP: 48							44.2%	35.2%	0.323#	25.2%	15.3%	0.228#							
Histologic Pattern	F: 38 S: 54 *W: 3													55.4%	28.7%	0.002†	37	15	0.054†	

M, Male; F, Female; EP, Exposed to pressure; NEP, Not exposed to pressure; F, Fascicular; S, Storiform; W, Whorl *Since the number of the patients is very low, this parameter is not available for statistical comparison. Difference was considered significant when the p value was less than 0.05. §, Mann Whitney U test; #, Student t test; †, Kruskal Wallis Test.

Discussion

Fibrous dysplasia is a maturation defect which is due to the somatic mutation on the 20th chromosome that causes a defective synthesis of the α-subunit of G-protein receptors (GNAS-1 mutation). This results in an overproduction of intracellular cAMP as well as hyper-proliferation and incomplete differentiation of marrow stromal cells to abnormal osteoblasts. cAMP also activates Fos, which plays a role in inhibiting stimulator cytokines that promote bone resorption by osteoclasts (Riddle and Bui, 2013). All these factors lead to an uncontrolled bone formation. Therefore, immature bone lamella is expected to be seen during the histologic examination of FD.

Recent publications suggest that craniofacial FD, unlike that of long bones, may undergo a process of maturation leading to lamellar bone formation (El-Mofty, 2014). Many researchers now consider bone lamellation as well as PTC as an indicator of maturation (Eversole et al., 2008; George et al., 2013; Kulkarni et al., 2014; Macdonald-Jankowski et al., 2009). In our study, we found a mean percentage of lamellation of 40.4% for craniofacial bones and 21.6% for non-craniofacial bones supporting the hypothesis that lamellation is predominantly seen in craniofacial FD cases.

PTC, also called retraction artifact, is a histologically well-known phenomenon; however, there is very little attention paid to this issue in routine practice. Pathologists usually consider it an artificially produced tissue alteration. Some authors support the argument that that PTC depends on tissue fixation, decalcification, preparation or sectioning (George et al., 2013), while others claim an association with an abnormality in the expression of basement membrane proteins, collagenases, or other enzymes (Tomas et al., 2011; Prado Ribeiro et al., 2012). Prado Ribeiro et al. even suggest that PTC can be a diagnostic criterion allowing the differentiation of FD from ossifying fibroma (Prado Ribeiro et al., 2012). Whether it is regarded as an artificial artifact produced by tissue processing or a specific feature of FD, researchers conclude that PTC in FD is related to bone maturation (Prado Ribeiro et al., 2012; George et al., 2013). In a study conducted by Kulkarni et al, stromal cellularity was found to be low in all FD cases, which all had a fascicular growth pattern. Furthermore, all the cases showed retraction spaces and parallel lamellation (Kulkarni et al., 2014). These findings indicate that low stromal cellularity as well as fascicular growth pattern is related to bone lamellation and retraction artifact. In our study, we came across similar findings. The stromal cellularity was remarkably low for the cases with higher percentage of lamellation and also, a higher percentage of lamellation was related to fascicular growth and retraction (peritrabecular clefting). All of these findings suggest that parallel lamellation of bone trabeculae is strongly associated with PTC, low stromal cellularity and fascicular growth pattern, all of which indicate maturation.

The most interesting aspect of this study is the relation between the exposure to pressure and the percentage of lamellation which has not been investigated previously. We noticed that the lesions in the bones under pressure or carrying body burden tend to have higher percentage of lamellation. Interestingly, this relation was revealed when we seperated the cases as craniofacial and non-craniofacial. This may result from the following reasons: 1) There is a difference between the craniofacial and long bones concerning their physiologic development pattern with the former being formed by intramembranous ossification and the latter being constructed by endochondral ossification. 2) The craniofacial bones, in contrast to other bones, originate from the neural crest. 3) The blood supply of the jaw

bones is more prominent than the other bones; therefore, ischemic necrosis is not expected to be seen in these bones. 4) Dental and surrounding periodontal tissues affect bone maturation and normalization.

In the craniofacial group, the jaw bones such as maxilla and mandibula are surrounded by highly dynamic masticator muscle groups. Therefore, they are under constant pressure during mastication and speaking activities. In addition, the bones which are located around the sinuses are also affected by the changes in the sinusoidal spaces. For instance, in case of an infection, intrasinusoidal pressure increases and this may lead to an exposure to internal pressure for the bones around the sinuses. However, the cranial bones covering the brain do not suffer from such exposure as much as the facial and jaw bones. Similarly, other bones such as vertebrae, femur, tibia, fibula and the pelvic bones carry the body burden and therefore, they are exposed to more pressure than the upper extremity bones or costae.

The impact of pressure on lamellation in both craniofacial and non-craniofacial groups was found to be similar in our study. The lesions located in the bones exposed to constant pressure and body burden had a histologically higher percentage of lamellation than those that are exposed to less pressure (Table 3). Considering lamellation as an indicator of maturation, this finding may suggest that pressure may lead to maturation in FD. From this point of view, it can be asked whether pressure can be used to treat FD. Actually, there are a number of studies supporting this hypothesis some of which concluded that the disease becomes quiescent after bone maturation (MacDonald-Jankowski, 2004; Alvares et al., 2009; Macdonald-Jankowski and Li, 2009). Alvares et al. suggested that FD might be triggered by the retardation of bone lamellation resulting in the overgrowth of immature woven bone. After puberty, this overgrowth may stop with the reduced pubertal growth hormones (Alvares et al., 2009). Another report suggests that most FD cases "burn out" in early adulthood by skeletal maturation (Waldron, 1993). Another reason supporting this hypothesis is that we found the same relation between lamellation and pressure in both craniofacial and non-craniofacial bones, which have a different growth pathway. In addition, there are some publications indicating the efficacy of weight-bearing physical activities on bone lamellation and maturation (Chad et al., 1999; Wallace et al. 2015).

There are certain limitations to the present study. We conducted this study with a restricted number of cases, especially when dividing them into subgroups. Another limitation of this study is being a retrospective analysis rather than being experimental.

In conclusion, the findings of this study revealed a positive correlation between the percentage of retraction spaces, stromal histologic pattern and lamellation, which can be considered an indication of maturation in FD. On the other hand, we found an inverse correlation between lamellation and stromal cellularity indicating that lesions with hypocellular stroma tend to be more mature. In addition, there was a striking association between lamellation and the localization of lesions, albeit not statistically significant. Interestingly, the lesions located in the bones under pressure and body burden demonstrated a higher lamellation percentage than others. This can be interpreted as the lesions under pressure being more likely to be mature. In the light of these findings, we put forward the hypothesis that weight-bearing physical activities can be helpful to treat FD. However, further studies including more cases and experimental trials need to be undertaken to corroborate this hypothesis.

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