Yuri Silvestre Barbosa

Avaliação da Cortical e do Trabeculado Ósseo Mandibular de Pacientes com Polipose Adenomatosa Familial

Brasília
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Trabalho de Conclusão de Curso apresentado ao Departamento de Odontologia da Faculdade de Ciências da Saúde da Universidade de Brasília, como requisito parcial para a conclusão do curso de Graduação em Odontologia.

Orientadora: Profª. Drª. Eliete Neves da Silva Guerra

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2020

Às pessoas com doenças raras.

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A Deus, autor da vida e de todas as muitas graças em minha vida;

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EPÍGRAFE

“Não é na resignação, mas na rebeldia em face das injustiças que nos afirmaremos.”

Paulo Freire

RESUMO

SILVESTRE-BARBOSA, Y. Avaliação da cortical e do trabeculado ósseo mandibular de pacientes com Polipose Adenomatosa Familial. 2020. Trabalho de Conclusão de Curso (Graduação em Odontologia) – Departamento de Odontologia da Faculdade de Ciências da Saúde da Universidade de Brasília.

A Polipose Adenomatosa Familial (FAP) é uma condição rara predisponente ao câncer colorretal e que pode apresentar manifestações dento-ósseas anteriores às alterações intestinais. Assim, objetivo do estudo foi avaliar o perfil de alterações ósseas de pacientes com essa condição utilizando radiografias panorâmicas da face. Os pacientes com FAP (n=15) tiveram suas radiografias analisadas e comparadas a um grupo controle pareado em idade e gênero (n=45). Os índices radiomorfométricos para análise da cortical óssea, como o Índice Mandibular Cortical e Espessura Cortical Mandibular, e a análise da dimensão fractal para o trabeculado foram explorados. A análise estatística foi feita por meio do “teste t” e “qui-quadrado”, sendo o nível de significância estabelecido em 5%. Os resultados da análise da dimensão fractal foram estatisticamente menores nos pacientes com FAP ($p<0,05$). Entretanto os índices que avaliaram a cortical óssea não apresentaram diferença estatisticamente significativa entre os grupos. Pode-se concluir que a análise da dimensão fractal diferencia os pacientes com FAP dos indivíduos com padrão de normalidade óssea enquanto o Índice Mandibular Cortical e a Espessura Cortical Mandibular não são capazes de identificar alterações nos pacientes com FAP.

ABSTRACT

SILVESTRE-BARBOSA, Y. Evaluation of mandibular cortical and trabecular bone of patients with Familial Adenomatous Polyposis. 2020. Undergraduate Course Final Monograph (Undergraduate Course in Dentistry) – Department of Dentistry, School of Health Sciences, University of Brasília.

Familial Adenomatous Polyposis is a rare condition predictor of colorectal cancer. The disease presents dento-osseous manifestations before typical intestinal alterations. Thus, this study aims to evaluate mandibular osseous changes of patients with this condition in panoramic radiographs exams. Fifteen radiographs of patients with FAP were analyzed and compared to gender and age paired control group. Radiomorphometric indices for analysis of cortical bone, such as Mandibular Cortical Index and Mandibular Cortical Width, and fractal dimension for trabecular bone were explored. T test and Chi-square were used for statistical analysis and the significant level was standardized in 5%. Results of fractal dimension analysis were significantly lower in FAP patients ($p<0.05$) and cortical indices did not show any difference statistically significant between groups. So, we concluded that fractal dimension analysis may differentiate FAP patients from non-FAP individuals while Mandibular Cortical Index and Mandibular Cortical Width are not able to identify alterations in FAP.

SUMMARY

SCIENTIFIC ARTICLE

This undergraduate final paper is based on the scientific article:
SILVESTRE-BARBOSA, Y.; PACHÊCO-PEREIRA, C.; ALMEIDA, F.T.; LEITE, A.F.; GUERRA, E.N.S. Evaluation of mandibular cortical and trabecular bone of patients with Familial Adenomatous Polyposis.
Presented following Orphanet Journal of Rare Diseases' guidelines.

TITLE PAGE

Avaliação da cortical e do trabeculado ósseo mandibular de pacientes com Polipose Adenomatosa Familial

Evaluation of mandibular cortical and trabecular bone of patients with Familial Adenomatous Polyposis

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RESUMO

Avaliação da cortical e do trabeculado ósseo mandibular de pacientes com Polipose Adenomatosa Familial

A Polipose Adenomatosa Familial (FAP) é uma condição rara predisponente ao câncer colorretal e que pode apresentar manifestações dento-ósseas anteriores às alterações intestinais. Assim, objetivo do estudo foi avaliar o perfil de alterações ósseas de pacientes com essa condição utilizando radiografias panorâmicas da face. Os pacientes com FAP (n=15) tiveram suas radiografias analisadas e comparadas a um grupo controle pareado em idade e gênero (n=45). Os índices radiomorfométricos para análise da cortical óssea, como o Índice Mandibular Cortical e Espessura Cortical Mandibular, e a análise da dimensão fractal para o trabeculado foram explorados. A análise estatística foi feita por meio do “teste t” e “qui-quadrado”, sendo o nível de significância estabelecido em 5%. Os resultados da análise da dimensão fractal foram estatisticamente menores nos pacientes com FAP ($p<0,05$). Entretanto os índices que avaliaram a cortical óssea não apresentaram diferença estatisticamente significativa entre os grupos. Pode-se concluir que a análise da dimensão fractal diferencia os pacientes com FAP dos indivíduos com padrão de normalidade óssea enquanto o Índice Mandibular Cortical e a Espessura Cortical Mandibular não são capazes de identificar alterações nos pacientes com FAP.

Palavras-chave

Radiologia Oral, Polipose Adenomatosa Familial; Alterações Dento-Ósseas.

Clinical Relevance

FAP is a rare hereditary condition, so its prevalence is low. However, it is a condition predictor of severe complications, such as colorectal cancer. It is necessary to promote health for affected individuals and stablish more efficient protocols to help early diagnosis and decrease costs for the Health System.

ABSTRACT

Evaluation of mandibular cortical and trabecular bone of patients with Familial Adenomatous Polyposis

Background and Aim: Familial Adenomatous Polyposis (FAP) is a rare condition predictor of colorectal cancer. The disease presents dento-osseous manifestations before typical intestinal alterations. Thus, this study aims to evaluate mandibular osseous changes of patients with this condition in panoramic radiographs exams. **Methods:** Fifteen radiographs of patients with FAP were analyzed and compared to gender and age paired control group. These radiomorphometric indices were explored: Mandibular Cortical Index (MCI) and Mandibular Cortical Width (MCW), besides fractal dimension (FD) for trabecular bone. T test and Chi-square were used for statistical analysis and significant level was standardized in 5%. **Results:** Fractal dimension analysis were significantly lower in FAP patients ($p<0.05$) and cortical indices did not show any difference statistically significative between groups. **Conclusion:** Fractal dimension analysis may differentiate FAP patients from individuals with a considered normal bone while Mandibular Cortical Index and Mandibular Cortical Width are not able to identify alterations in FAP.

Keywords

Oral Radiology; Familial Adenomatous Polyposis; Dento-Osseous Alterations.

INTRODUCTION

Colorectal cancer (CRC) is the third most common type of malignant tumor, being responsible for almost 9% of all death by cancer in the world[1]. In Brazil, more than 36,000 new cases were estimated in 2018, considered the third most common cancer in men and the second in women[2].

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disease characterized by the development of multiple colorectal adenomas[3–6]. It is caused by mutation of the *APC* gene (Adenomatous Polyposis Coli), a tumor suppressor gene[7]. FAP is a rare condition and its global prevalence is estimated in 1 case for 5,000-10,000 live births while Brazilian prevalence is around 1 for 30,000 live births[5,8]. FAP also affects both genders equally and 1% of CRC cases are caused by this condition[5,8]. These cases usually occur in the third or fourth decade of life in 95% of non-treated patients[4,9,10].

APC protein controls β-catenin, an oncoprotein in the Wnt pathway. When *APC* is mutated, it leads to the accumulation of β-catenin, which upregulates the transcription of genes involved in cell cycle progression[11,12]. As a result, it unsettles the Wnt pathway, which plays a central role in many processes during embryologic development and adult homeostasis[13]. This way, not just colorectal alterations affect FAP patients, but a series of extracolonic manifestations may be present, such as desmoid tumors, multiple osteomas, odontomas, epidermoid cysts, congenital hypertrophy of the retinal pigment epithelium, vascular abnormalities in oral mucosa and malignant tumors of several organs[6,12,14].

As many of these extracolonic phenotypes are in the head and neck region, Almeida et al. (2016), in a systematic review and meta-analysis, described the occurrence of the dento-

osseous lesions in FAP patients. By analyzing 16 studies with a total of 1635 FAP patients, they concluded that the frequencies of osseous and dental manifestations were 65.3% and 30.5%, respectively.

Many of these manifestations can be explored by dental panoramic radiographs (DPR), which are the most used imaging modality for diagnosis in dentistry. Besides identifying classic dental disorders, conventional and advanced imaging modalities, as cone beam computed tomography (CBCT), may opportunistically identify systemic disorders that appear in the maxillomandibular complex[15]. Accordingly, the use of DPR for the screening of patients with systemic disorders or stablishing new protocols in treatment has been reported widely in several conditions, like osteoporosis, obesity, hyperparathyroidism, celiac disease, multiple myeloma, thalassemia, Paget disease and also FAP[16–23].

Given that, previous authors have applied radiomorphometric indices and analysis of fractal dimension (FD) for assessment of cortical and trabecular bone. Two of these indices are Mandibular Cortical Index (MCI) and Mandibular Cortical Width (MCW), that evaluate the intern cortical margin qualitatively and measure the thickness of the cortex. FD, in turn, is an economical and easily available method for analyzing bone texture by a mathematical morphology image process system[20]. These methods can measure patterns of osseous alterations in imaging exams and scale them for analysis and comparison with healthy patients[24–30].

Although a series of studies that evaluate osseous alteration in DPR exist, they mostly observe differences resultant from systemic conditions that cause low bone mineral density (BMD). Until now, there was no investigation regarding the trabecular and cortical bone pattern of affected FAP patients. Therefore, this study aims to evaluate the quality of mandibular

cortical and trabecular bones through radiomorphometric indices and FD in DPRs of patients diagnosed with FAP in comparison with matched healthy patients.

As specific objectives, this project aims to answer the following research questions:

- Are the methods for assessment of cortical and trabecular bone (MCI, MCW and FD) reliable and reproducible?
- Is there any difference in the assessment of cortical and trabecular bone between individuals with FAP and healthy controls?
- Are there any alterations in cortical bone of FAP patients that may be assessed by radiomorphometric indices?
- Do trabecular alterations of FAP patients show different fractal dimension means compared to healthy control?

METHODS

Approval from the Ethics Committee of the Health Sciences Faculty, University of Brasília, Brazil approved for the protocol and informed consent process of this study under number 493.502. A case-control study was planned to analyze the mandibular bone pattern of FAP patients.

Fap Patients and Control Group

An available pool of FAP patients' DPRs from the Brazilian University Hospital (HUB) was explored. Due to the nature of this rare disease and the cross-sectional study design no sample size was calculated. We included the patients whose confirmation of FAP was made by colonoscopy, the reference standard diagnosis. Excluded from the sample were the patients those DPRs were not derived from the conventional imaging modalities such as reconstructions from CBCT scans and inadequate radiographs with a suboptimal quality or ghost images superimposing essential anatomic landmarks/incidental findings in the region of interest (ROI).

A convenience sample of all patients having the diagnostic of the FAP treated by the department of Dentistry was evaluated. The newest recruited patients received a paper-pamphlet containing all the information regarding the study and the informed consent to be signed. They had an opportunity to clarify their concerns verbally with a representative of the research project in the location. All invited patients had access to the emails and phone numbers of the research project team for further contact if more clarification was needed.

Group 1 was composed of DPRs of FAP patients recruited from the HUB. Group 2, a matched control group, was composed by DPRs of healthy (non-FAP) Brazilian individuals produced by the same hospital or clinic, machines and

technicians. The patients from Groups 1 and 2 were matched by gender and year of birth.

Dental Panoramic Radiographs Exam

The selected radiographs were taken with the same machine (Rotograph Plus, Villa Sistemi Medicalli, Buccinasco, Milan, Italy) and the patients were positioned for acquisition following a standardized protocol, the vertical orientation line was aligned with the patient mid sagittal plane and the horizontal one based on the Frankfort plane parallelism to the floor. All identifiers were coded by one member and transferred to an Excel spreadsheet for statistical analyses.

For calibration purposes, the measurements were done independently by two experienced evaluators, an oral and maxillofacial radiologist (Evaluator 1) and an experienced undergraduate student in dentistry (Evaluator 2), repeated with a wash out period of 7 days. A computer-generated randomized list of 10 panoramic radiographs was used for this purpose. The same evaluators made all FD calculations and the mandibular cortical measurements. These observers were blinded to the diagnosis of FAP, risk or normality status of patients included in the sample.

The Fractal Dimension Analysis

For the fractal dimension (FD) analysis, four mandibular ROI were selected. These four well known trabecular regions were selected based on previously published papers[29,31] and were standardized as a square of 100 x100 pixels at following:

- ROI 1- right mandibular angle;
- ROI 2- 2mm anterior to right mental foramen;
- ROI 3- 2mm anterior to left mental foramen;
- ROI 4- left mandibular angle.

The trabecular bone structure was analyzed using the same imaging processor, the ImageJ Figi 1.52a for Mac OS (public domain software available at http://rsbweb.nih.gov/ij).

All digital DPRs were stored with a matrix of 7008 x 2975 pixels. The digital images were processed following the methods of a published study from the same research group[29]. First, the ROI was selected, cropped and duplicated. Then, the duplicated image was blurred with a gaussian filter (sigma, 35) to remove large-scale variations in brightness on the image. The blurred image was subtracted from the original ROI image and a gray value of 128 was added at each pixel location. The resultant image was made binary and, within this process, the regions that represent trabecular bone were set to black and marrow spaces were set to white. The image was eroded and dilated to reduce the noise. After dilation, the image was skeletonized, and the FD analysis pursuit. The FD was calculated by the box-counting method, the widths of these square boxes were 2, 3, 4, 6, 8, 12, 16, 32 and 64 pixels. Figure 1 presents the FD analysis sequence adopted by this study.

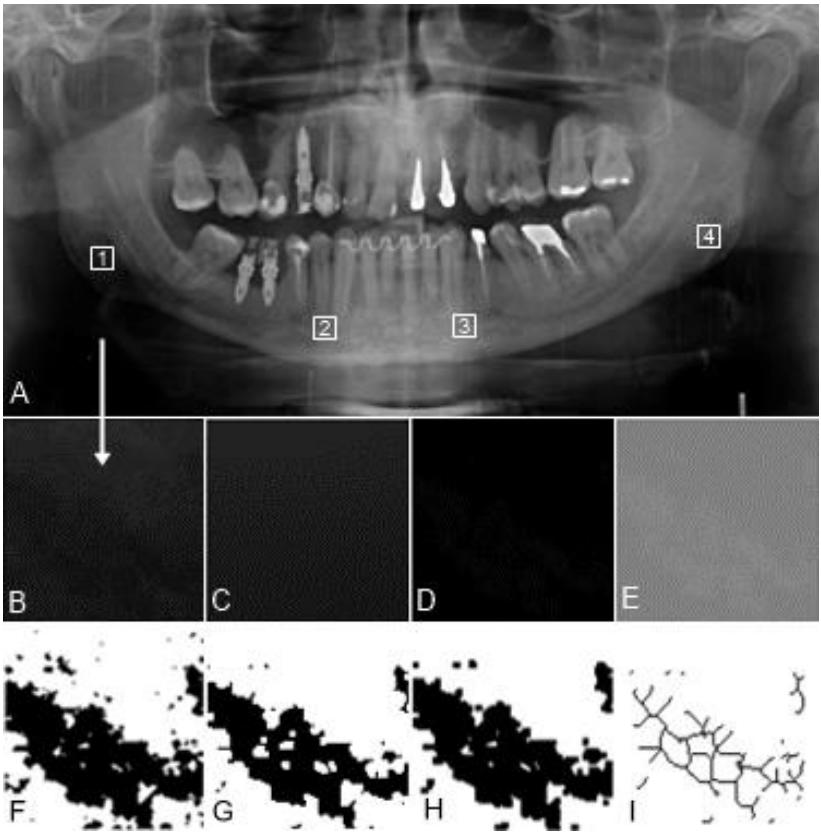


Figure 1. The FD sequences. The stages of image processing for the calculation of Fractal Dimension (FD). A, selection of regions of interest (ROI); B, an enlarged image of ROI 1; C, a Gaussian blurred image; D, a subtraction image; E, an added gray value of 128 image; F, a binary image; G, an eroded image; H, a dilated image; I, a skeletonized image. Adapted from Sindeaux (2014).

The Mandibular Cortical Index Measurements

The qualitative measurements of the right and left cortical width were made according to the classification of Klemetti et al. (1994)[27]. MCI is the appearance of the inferior mandibular cortical thickness, which is as follows:

- C1: The endosteal margin of the cortex is even and sharp on both sides;
- C2: The endosteal margin shows semilunar defects (lacunar resorption) or it seems to form endosteal cortical residues (one to three layers) on one or both sides;
- C3: The cortical layer forms heavy endosteal residues and it is porous.

Cortical bone is assessed by MCI mainly in the mental foramen area, just like presented by Figure 2.

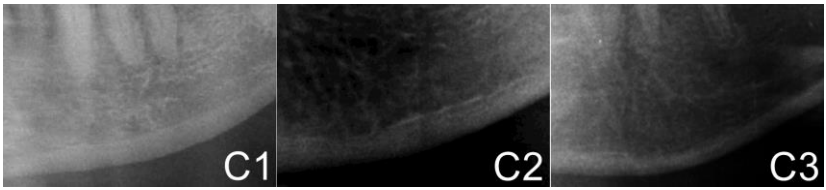


Figure 2. The Mandibular Cortical Index. Cortical bone is classified according to its endosteal margin

The Mandibular Cortical Width Measurements

The quantitative measurements of the cortical width followed Taguchi et al. (1995) validated protocol[30]. The main measurement was defined based on the mandibular cortical width of the right (r) and left sides (l) of the mandible's body. The center of the foramen was located, a line extending inferiorly and reaching in 90 degrees the lower mandible border was the point of reference. Support lines are drawn to define the slope and position of the mandibular axis. All the indices are measured along this extension line presented in Figure 3 and the same software, Image J, was used for this purpose.

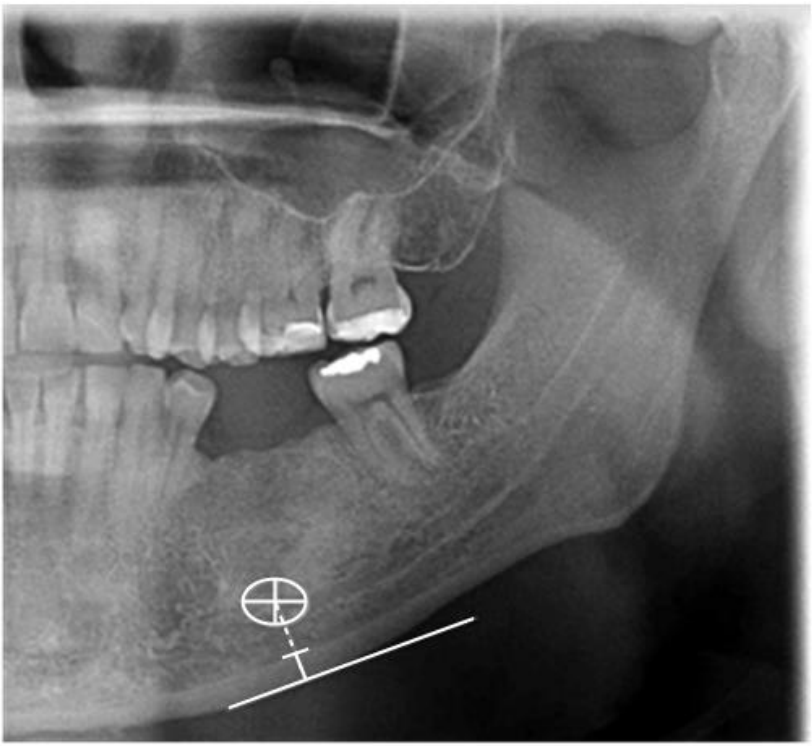


Figure 3. Panoramic radiograph with tracing for Mandibular Cortical Width (MCW) measurement.

Statistical Analysis

A reliability test was applied at two different times, within 1-week wash out interval during calibration and following the final sample measurements. The Intraclass Correlation Coefficient (ICC) was used and interpreted by Portney and Watkins guidelines: 0-0.2 indicating poor agreement: 0.3-0.4 = fair agreement; 0.5-0.6 = moderate agreement; 0.7-0.8 = strong agreement; and more than 0.8 indicated almost perfect agreement[32].

Extreme values of the observed variables that were not normally distributed or homoscedastic were checked with Cook's Distance and excluded from the sample. Age, fractal dimension

measurements and mandibular cortical width were compared between FAP and control groups by parametric student T test. These variables were also compared between men and women with FAP and control group verified by T test. The Chi-square statistic was applied to verify the distribution of the MCI based on the observed counts and the expected counts if there were no relationship at all in the population. A p-value <0.05 was considered statistically significant.

Statistical Package for Social Sciences (IBM, SPSS Statistics 24 software) was used for statistical analysis and the data were analyzed by an independent statistical service.

RESULTS

The Calibration Shows Strong Reliability

The intra-reliability coefficient was as follows: Evaluator 1, ICC=0.958 (CI 0.846-0.989) and Evaluator 2, ICC= 0.78 (CI 0.917-0.995). The inter-reliability coefficient for the two evaluators was considered an almost perfect agreement, ICC= 0.992 (CI= 0.976-0.998). Cronbach's alpha at 0.991 when intra and inter-reliability were considered. These observers were blinded to the diagnosis of FAP, risk or normality status of patients included in the sample. For the final sample measurements, the Evaluator 2 consistency (intraclass correlation reliability) were as follows: MCI = 0.967 (CI 0.945-0.980), MCW right side = 0.995 (0.991-0.997) and MCW left side= 0.983 (CI 0.972-0.990). For the FD analysis for each ROI, there was no variation between the first and second values of FD-ROI 1 to 4, resulting in an ICC=100%.

Table 1 shows the demographics of both groups. The FAP group was composed of 33.3% of females (N=5) and 10 males, the mean age=41.20 years (SD 15.20). The matched control group had 15 females and 30 males, with a mean age=41.20 years (SD 14.85).

Considering the gender of the patients, no significant differences were found for all cortical and trabecular radiographic measurements between males and females in Group 1 (Table 2 and Graph 1). In Group 2, significant differences between males and females were only found for MCW values (Table 3 and Graph 2).

Fractal Dimension measurement values were significantly lower for the FAP group.

This lower mean corroborates with previous studies outcomes that evaluate other conditions, such as osteoporosis and hyperparathyroidism and cemento-osseous dysplasia[20,31,33]. Table 4 and Graph 3 present the mean FD for each ROI and the comparison between FAP patients and controls. FD values were significantly lower for the FAP group when compared with the control group, and more expressive for the FD-ROI 2 (anterior to

right foramen) ($p<0.001$); except for the FD-ROI 1 in which the difference was not statistically significant ($p=0.91$).

Table 1. Demographic details.

	Group 1 (n = 15)		Group 2 (n = 45)		't'/x^2 value	P value
	Mean / n	**± SD / %**	**Mean / n**	**± SD / %**		
Age (Years)	41.20	15.209	41.20	14.859	0.000	1.000; NS
Sex (F/ M)	5 / 10	33.33% / 66.77%	15 / 30	33.33% / 66.77%	0.000	1.000; NS

Student t test/ Chi-Square Test; NS: $p > 0.05$ - Not significant; SD: Standard Deviation; F: Females; M: Males.

Table 2. Comparing mean Mandibular Cortical Width and Fractal Dimension between Males and Females in Group 1.

	Males (n = 5)		Females (n = 10)		't' value	P value
	Mean	**± SD**	**Mean**	**± SD**		
MCW (R)	3.540	0.587	3.329	0.478	0.749	0.467; NS
MCW (L)	3.404	0.546	3.371	0.759	0.086	0.933; NS
FD-ROI 1	1.209	0.095	1.184	0.053	0.675	0.511; NS
FD-ROI 2	1.131	0.094	1.178	0.065	1.138	0.276; NS
FD-ROI 3	1.214	0.094	1.169	0.106	0.801	0.438; NS
FD-ROI 4	1.079	0.086	1.166	0.080	1.944	0.074; NS

Student t test; NS: $p > 0.05$ - Not significant; SD: Standard Deviation; MCW: Mandibular Cortical Width; (R): Right Side; (L) Left Side; FD-ROI: Region of Interest.

Table 3. Comparing mean Mandibular Cortical Width and Fractal Dimension between Males and Females in Group 2.

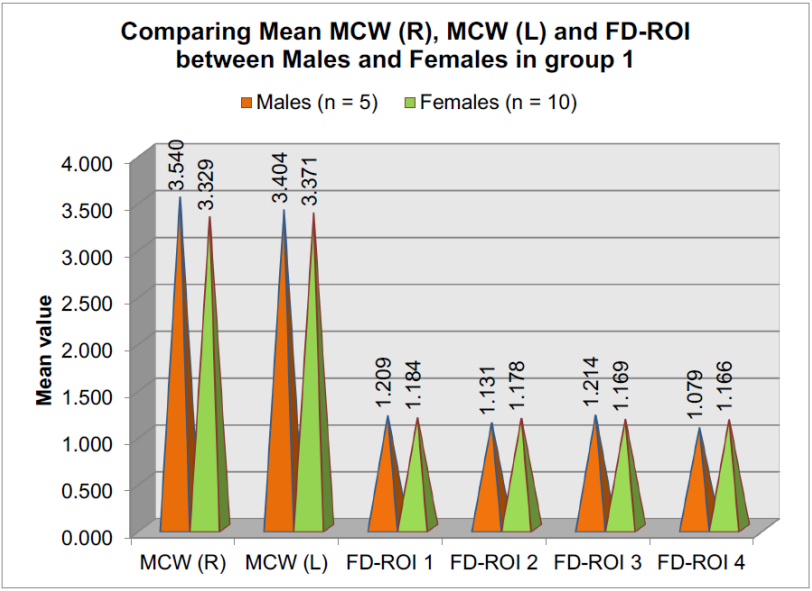
	Males (n = 15)		Females (n = 30)		't' value	P value
	Mean	**± SD**	**Mean**	**± SD**		
MCW (R)	4.085	0.559	3.415	0.712	3.181	0.003*
MCW (L)	4.001	0.446	3.577	0.608	2.395	0.021*
FD-ROI 1	1.219	0.051	1.239	0.093	0.785	0.437; NS
FD-ROI 2	1.257	0.051	1.251	0.074	0.260	0.796; NS
FD-ROI 3	1.261	0.052	1.249	0.085	0.477	0.636; NS
FD-ROI 4	1.239	0.092	1.189	0.119	1.437	0.158; NS

Student t test; NS: p > 0.05 - Not significant; SD: Standard Deviation; MCW: Mandibular Cortical Width; (R): Right Side; (L) Left Side; FD-ROI: Region of Interest.

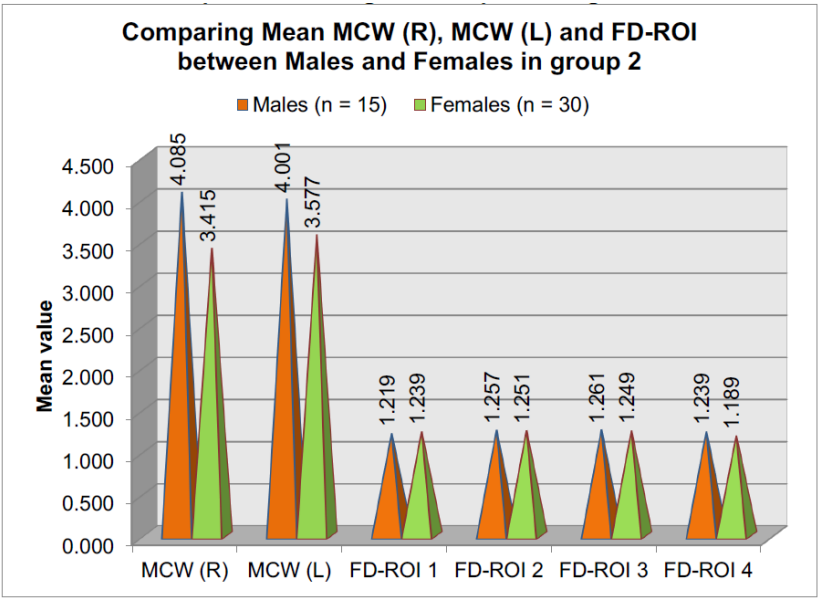
Table 4. Mean of Fractal Dimension in two groups.

	Group 1 (n = 15)		Group 2 (n = 45)		't' value	P value
	Mean	**± SD**	**Mean**	**± SD**		
FD-ROI 1	1.193	0.067	1.233	0.082	1.716	0.091; NS
FD-ROI 2	1.162	0.076	1.253	0.067	4.403	<0.001**
FD-ROI 3	1.184	0.101	1.253	0.075	2.824	0.006*
FD-ROI 4	1.137	0.089	1.206	0.112	2.151	0.036*

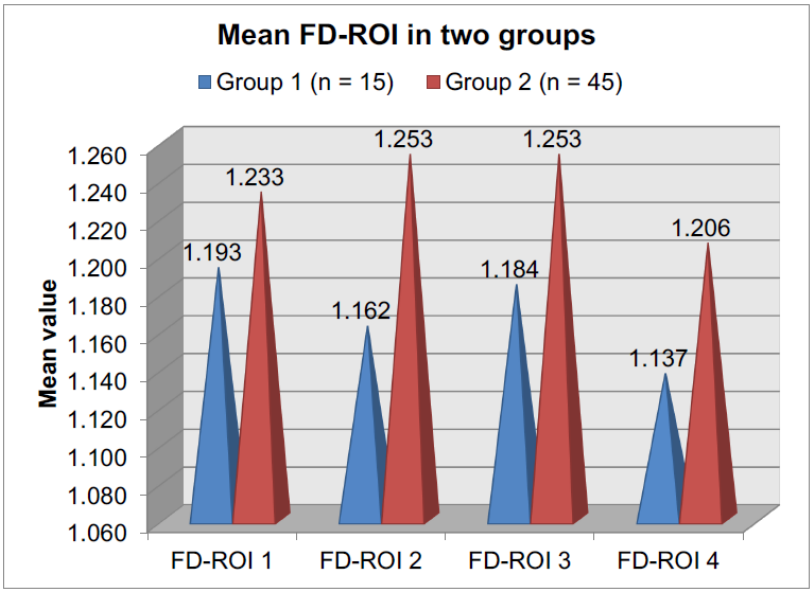
Student t test; NS: p > 0.05 - Not significant; SD: Standard Deviation; MCW: Mandibular Cortical Width; FD-ROI: Region of Interest.



Graph 1. Comparing Mean Mandibular Cortical Width and Fractal Dimension between Males and Females in Group 1. MCW: Mandibular Cortical Width (R): Right Side; (L) Left Side; FD-ROI: Region of Interest.



Graph 2. Comparing Mean Mandibular Cortical Width and Fractal Dimension between Males and Females in Group 2. MCW: Mandibular Cortical Width (R): Right Side; (L) Left Side; FD-ROI: Region of Interest.



Graph 3. Mean Fractal Dimension in two groups. FD-ROI: Region of Interest.

Mandibular Cortical Index measurements and Mandibular Cortical Width values were similar in both groups, unlike outcomes of studies that evaluate conditions that cause low BMD[26,28,34,35]. Table 5 and Graph 4 presents the MCI distribution in two groups, Group 1 showed 12 FAP cortical qualitatively classified as even and sharp on both sides - C1, and 20% of the sample presenting semilunar defects - C2. The control groups had 75.6% as C1, 20% as C2 and two as having cortical layer forming heavy endosteal residues and being porous - C3. The MCI was not significant when both groups were compared, p=0.706 and x^2= 0.696.

Mandibular Cortical Width tends to be lower in FAP patients, but there is no statistical relevance. For Group 1, the $MCW(R)_{mean}$ = 3.399 (SD 0.506) and $MCW(L)_{mean}$= 3.382 (SD 0.678). Group 2 showed $MCW(R)_{mean}$ = 3.399 (SD 0.506) and $MCW(L)_{mean}$= 3.382 (SD 0.678). The values show no statistical significance for both sides when the groups were compared, p=0.247 and t=1.170 and for MCR(R) t=1.848 and p=0.070 (see Table 6 and Graph 5).

Table 5. Distribution of Mandibular Cortical Index in two groups.

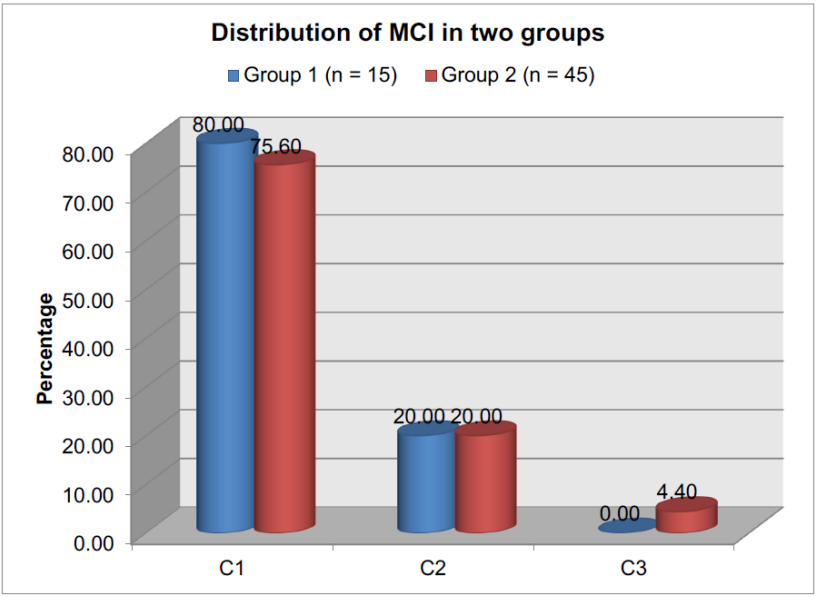
MCI	Group 1 (n = 15)		Group 2 (n = 45)		x^2 value	P value
	N	%	N	%		
C1	12	80.0	34	75.6		
C2	3	20.0	9	20.0		
C3	-	-	2	4.4		0.706;
Total	15	100.0	45	100.0	0.696	NS

Chi-Square Test; NS: p > 0.05 - Not significant; MCI: Mandibular Cortical Index

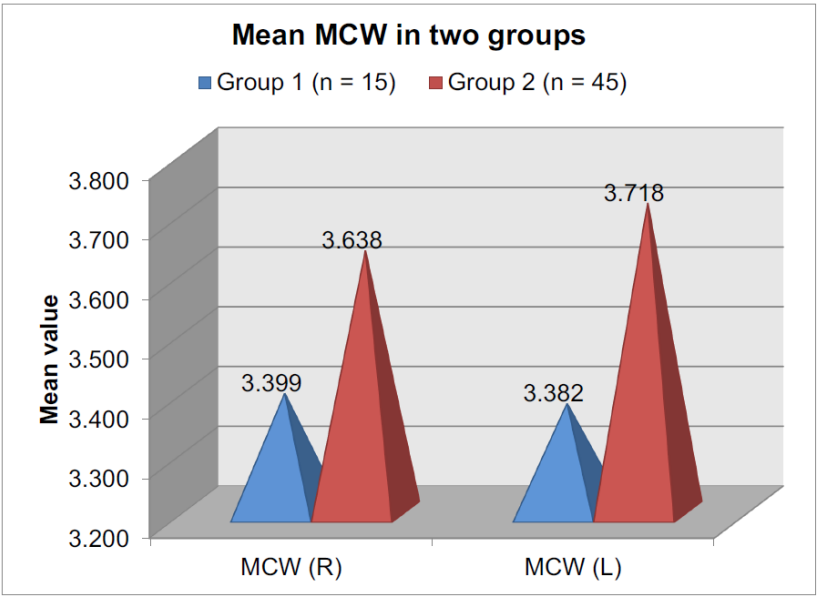
Table 6. Mean Mandibular Cortical Width in two groups.

	Group 1 (n = 15)		Group 2 (n = 45)		't' value	P value
	Mean	**± SD**	**Mean**	**± SD**		
MCW (R)	3.399	0.506	3.638	0.732	1.170	0.247; NS
MCW (L)	3.382	0.678	3.718	0.589	1.846	0.070; NS

Student t test; NS: p > 0.05 - Not significant; SD: Standard Deviation; MCW: Mandibular Cortical Width; (R): Right Side; (L): Left Side.



Graph 4: Distribution of Mandibular Cortical Index in two groups.



Graph 5. Mean Mandibular Cortical Width in two groups. MCW: Mandibular Cortical Width (R): Right Side; (L) Left Side;

DISCUSSION

Despite FAP is a rare condition, it is necessary to deeply investigate genetic and phenotypical aspects to better understand it and give patients with the disease an early diagnosis, effective treatment and greater quality of life. Besides, research on it may lead to the improvement of protocols in attendance, optimizing the care by the health service. This way, our study assessed FAP patients' alterations in the maxillomandibular region to contribute to individuals lives and all health system as well.

From the best of our knowledge, this is the first study to evaluate osseous patterns in FAP patients through radiomorphometric indices and FD analysis. In this study, MCI and MCW proved highly reliable and reproducible by analyzing numbers of intra and inter-reliability for two evaluators in the calibration phase and final measurements. Our methodology and further analysis confirm previous studies results which conclude that MCI and MCW are viable for DPR assessment[17]. All evaluations were performed with a wash-out period to decrease memory bias. In FD analysis, the perfect agreement may be explained by the standardization of the ROI in both measurements and for the error-free computerized system.

There was no statistical significance in comparison of MCW and FD on genders in Group 1. On the other hand, it is observed a mean difference in comparison of MCW in Group 2 with lower values in women. Previous studies reported that the cortical thickness of postmenopausal patients exhibits some alterations, tending to be thinner/eroded. This condition may be explained by lower BMD and conditions such as osteoporosis[26,28,33–35]. Some hypotheses are considered for the same fact that is not present in the group of FAP-patients. One of them is about the smaller sample size in Group 1(n=15), which limited the statistical analysis; another fact that may influence it is the BMD increase in patients with *APC* mutations reported by literature[36,37].

FD is a mathematical expression of fractal analysis (FA) that, in turn, is a method for the description of complex shapes and structure patterns, where geometry is difficult to be

applied[38,39]. This way, FD becomes an excellent method for the assessment of trabecular bone, using digital DPR as a tool[40]. In a systematic review of literature, Kato et al. (2019) observe that there are many available protocols for image processing and FD calculation and they raised the attention for the higher demand to establish a reference standard protocol for FD analysis in the maxillomandibular complex. Thus, the protocol used in this study is the same adopted by Sindeaux et al. (2014) and described by White and Rudolf (1999) because of familiarity with the technique[29,41].

The FD values of this study shown FD mean values of FAP patients generally smaller and statistically significant compared to healthy controls. BMD increase in FAP patients with *APC* mutation can change trabecular bone structure and was explained by Miclea et al. (2010). They evaluated bone mineral density in FAP patients and described that the increase BMD is caused by activation of the Wnt signaling cascade, promoting osteoblast differentiation, which leads to bone mass acquisition[37]. Hence bone quality is altered, and trabecular bone microarchitecture becomes less complex, resulting in lower FD values, as observed in our analysis.

In Group 1, three of four assessed ROI showed lower means with a significant difference. Despite there was no statistical relevance in FD-ROI 1 result, it is noted a tendency to lower values in this same group as well. A more precise way to determine the area for assessing FD-ROI 2 and FD-ROI 3 by locating the mental foramen may be the cause for the highly significant outcomes. Although the method for determining FD-ROI 1 and FD-ROI 4 are well described[31], there is still a greater subjectivity and variation comparing to the other ROI. It makes a perfect precision in analysis between individuals challenging.

Although there were studies with similar findings[20,31,33], literature reports opposite outcomes[29,42]. However, all these studies are heterogeneous methodology and differ on investigated condition, adopting different protocols for FD and choosing sundry ROI. In this study, ROI were selected based on previous articles[29,31] and avoiding areas with the interference of typical FAP lesions, such as osteomas, odontomas and supernumerary or impacted teeth.

In the mandibular cortical assessment, MCI and MCW methods are useful, mainly executed together, since one evaluates qualitative aspects and the other, quantitative. In this study, it was observed that dento-osseous manifestations in FAP affect trabeculae much more than cortical bone and there was no significant difference between FAP-patients and control group in measurements made by both radiomorphometric indices. As cited, relationship between low BMD and inferior cortical of the jaw defects is well established[26,28,33–35]. However, there is not any evidence of which cortical alterations could be present in conditions that increase bone density.

One of the limitations of our study was the impossibility to calculate the sample size because FAP is a rare condition. However, to enable a better and robust statistical analysis, each FAP-patient was paired with three healthy patients, matched in gender and age. Nevertheless, outcomes must be interpreted with caution. Further studies that assess BMD in FAP-patients by the reference standard exam (DXA) and correlate it with radiomorphometric indices via the mandibular cortical bone are recommended.

CONCLUSION

The FD values were statistically significant while FAP patients from healthy ones were compared by analyzing the trabecular bone structure. Despite MCI and MCW were not able to identify alterations on FAP-patients, they are a viable way to assess this imaging exam due to the high reliability and reproducibility. This study highlights DPR as a powerful and cost-effective screening tool for FAP.

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Appendix

JOURNAL GUIDELINES

Style

Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/.

Format. Manuscripts should use 1.5 line spacing.

Spelling. The journal uses US spelling and authors should therefore follow the latest edition of the Merriam-Webster's Collegiate Dictionary.

Units. All measurements must be given in SI units as outlined in the latest edition of Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Royal Society of Medicine Press, London).

Abbreviations should be used sparingly and only where they ease the reader's task by reducing repetition of long technical terms. Initially use the word in full, followed by the abbreviation in parentheses. Thereafter use the abbreviation.

Trade names should not be used. Drugs should be referred to by their generic names, rather than brand names.

Parts of the Manuscript

The manuscript should be submitted in separate files: title page; main text file; figures.

Title page

The title page should contain:
(i) A short informative title that contains the major key words. The title should not contain abbreviations (see Wiley's best practice SEO tips). Titles must not be longer than 120 characters (including spaces);
(ii) The full names of the authors;
(iii) The author's institutional affiliations at which the work was carried out;
(iv) The full postal and email address, plus telephone number, of the author to whom correspondence about the manuscript should be sent;
(v) Disclosure statement; and
(vi) Acknowledgements.

The present address of any author, if different from that where the work was carried out, should be supplied in a footnote.

Disclosure statement

The source of financial grants and other funding should be acknowledged, including a frank declaration of the authors' industrial links and affiliations. In the case of clinical trials or any article describing use of a commercial device, therapeutic substance or food must state whether there are any potential conflicts of interest for each of the authors: failure to make such a statement may jeopardise the article being sent out for peer-review.

For details on what to include in this section, see the ‘Conflict of Interest’ section in the Editorial Policies and Ethical Considerations section below. Authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Acknowledgments

The contribution of colleagues or institutions should also be acknowledged. Thanks to anonymous reviewers are not allowed.

Main text

As papers are double-blind peer reviewed the main text file should not include any information that might identify the authors.

The main text of the manuscript should be presented in the following order: (i) title, abstract and key words, (ii) text, (iii) references, (iv) tables (each table complete with title and footnotes), (vii) figure legends. Figures and supporting information should be submitted as separate files. Footnotes to the text are not allowed and any such material should be incorporated into the text as parenthetical matter.

Abstract and keywords

All articles, with the exception of Editorials, must contain a structured abstract that states in 250 words or less the purpose, basic procedures, main findings and principal conclusions of the study. Divide the abstract with the headings: Background and Aim, Methods, Results, Conclusions. The abstracts of reviews need not be structured. The abstract should not contain abbreviations or references.

Three to five keywords should be supplied below the abstract and should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser (http://www.nlm.nih.gov/mesh/meshhome.html).

Text

Authors should use subheadings to divide the sections of their manuscript: Introduction, Methods, Results, Discussion.

References

The Vancouver system of referencing should be used. In the text, references should be cited using superscript Arabic numerals in the order in which they appear. If cited only in tables or figure legends, number them according to the first identification of the table or figure in the text.
In the reference list, the references should be numbered and listed in order of appearance in the text.
Cite the names of all authors when there are six or less; when seven or more list the first three followed by et al.
Names of journals should be abbreviated in the style used in MEDLINE.
Only literature that is published or in press (with the name of the publication known) should be cited in the reference list. Abstracts and letters to the editor may be cited, but they must be less than 3 years old and identified as such. Reference to unpublished data, submitted manuscruots and personal communications should appear in the text only as in the following example: (Chercheur X, unpublished data). If the owner of the unpublished data or personal communication is not an author of the manuscript under review, a signed statement is required verifying the accuracy of the attributed information and agreement to its publication.

List all authors up to six, using six and "et al." when the number is greater than six.
References should be listed in the following form:

Tables

Tables should be self-contained and complement, but not duplicate, information contained in the text. Number tables consecutively in the text in Arabic numerals. Type tables on a separate page with the legend above. Legends should be concise but comprehensive – the table, legend and footnotes must be understandable without reference to the text. Vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶ should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figure legends

Type figure legends on a separate page. Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement Indicate the stains used in histopathology. Identify statistical measures of variation, such as standard deviation and standard error of the mean.

Figures

All illustrations (line drawings and photographs) are classified as figures. Figures should be numbered using Arabic numerals, and cited in consecutive order in the text. Each figure should be

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