**CAMILA PACHECO DE OLIVEIRA PEREIRA**

**FAMILIAL ADENOMATOUS POLYPOSIS: NEW INSIGHTS INTO THE CRANIOFACIAL RADIOGRAPH FEATURES**

Brasília

2020

**UNIVERSIDADE DE BRASÍLIA**

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Tese apresentada como requisito parcial para obtenção do título de Doutor em Ciências da Saúde, do Programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília.

Orientadora: Prof. Dr. Eliete Neves da Silva Guerra

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Brasília

2020

*"...Carpe Diem..."*

*Horace, Odes (23BC)*

# AGRADECIMENTOS INSTITUCIONAIS

# AGRADECIMENTOS

Agradeço a Deus por me permitir a trilhar minha caminhada,

Aos meu pais por criarem um ambiente propenso ao aprendizado,

Aos educadores que acreditam que cada pessoa tem uma forma de aprender e que abriram as portas para que eu pudesse participar de experiências acadêmicas incríveis;

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Dedico esse trabalho aos indivíduos e famílias que foram vítimas do COVID-19, que a comunidade científica continue ativa e empenhada nos projetos de pesquisa para desvendar esse enigma. Que os corações das famílias vítimas desse vírus, incluindo minha família, sejam acalentados.

Enfim, que esse título não seja em vão,

Que eu possa transmitir meu conhecimento com simplicidade e humildade,

Que os projetos que desenvolvemos abram as portas para essa instituição, esse laboratório e esses pesquisadores.

E que os pacientes FAP e famílias em risco possam ser beneficiados pelos conhecimentos adquiridos.

Meu sincero muito obrigada,

Camila

# ABSTRACT

Familial Adenomatous Polyposis (FAP) is an autosomal dominant disorder caused by mutations in the Adenomatous Polyposis Coli gene (*APC*). Worldwide, colorectal cancer (CRC) is within the third most frequent malignant neoplasm. CRC ranked as the third modality associated-death with females and males. The FAP patients, in addition to present extraintestinal manifestations, also show dento-osseous alterations. These alterations are mostly associated with odontomas, osteomas, supernumerary teeth, and idiopathic osteosclerosis. These last could precede the clinical evidence of intestinal polyps. A systemic review of the literature demonstrated the importance of the systemic disease investigation through mandibular trabecular bone alterations using conventional panoramic radiographs – which are routinely prescribed by dentists in general practice. Based on the articles included in this systematic review, regions of interest were mapped and used as reference-points to investigate radiomorphometric indexes. Besides, trabecular and cortical bone alterations were possibly associated with systemic conditions. A second project demonstrated that the mandibular trabecular bone pattern in FAP patients when compared to healthy individuals, showed texture discrepancies and narrow bone alterations via the fractal dimension analysis. In an attempt to radiographically assess FAP children and adults in different locations, we developed a multicentric study in partnership with the Children's Mercy Hospital in Kansas City, United States. Pediatric FAP demonstrated osseous alterations that were similar to the adults affected by the same disease. Compared to the healthy controls, the FAP patients, presented alterations in the trabecular bone texture of the mandible. These studies aim to recommend the annual dental follow-up on the FAP patients and families at risk using the panoramic radiograph. In addition to emphasizing the importance of a dentist collaborating in the FAP multispecialty team. Thus, our objective is to alert and create critical thinking, based on scientific evidence, in the dental health teams about the importance of the opportunistic surveillance and screening of systemic diseases and FAP extraintestinal manifestations on the routinely taken dental radiographs.


**Keywords:** Familial adenomatous polyposis. FAP. Colorectal cancer. Fractal dimension analysis. Osseous alterations.

**RESUMO**

A Polipose Adenomatosa Familial (FAP) é uma doença com padrão de herança autossômico dominante predisponente ao câncer colorretal. No Brasil, o câncer colorretal está entre as quatro neoplasias malignas mais frequentes e é o terceiro em mortalidade em ambos os sexos. Os pacientes com FAP, além de apresentarem manifestações intestinais, apresentam alterações dento-ósseas. Dentre essas, são relatados presença de osteomas, odontomas, dentes supranumerários, escleroses ósseas no complexo maxilomandibular que podem se manifestar precocemente - antes do aparecimento dos pólipos intestinais. Uma revisão sistemática da literatura demonstrou a importância da investigação de doenças sistêmicas por meio de alterações ósseas presentes em radiografias panorâmicas - rotineiramente requisitadas por cirurgiões-dentistas. Baseando em artigos incluídos na revisão, regiões de interesse foram mapeadas como pontos de referência para uma futura área de análise de índices radiomorfométricos. Alterações ósseas foram detectadas quando condições sistêmicas acometiam os pacientes. Um segundo projeto demonstrou que o trabeculado ósseo mandibular de pacientes FAP, quando comparados com controles pareados, apresentou alterações micro estruturais no osso trabecular mandibular quando submetidos a análise de dimensão fractal. Numa tentativa de englobar pacientes pediátricos e adultos em países diferentes, um estudo multicêntrico foi elaborado em parceria com a Universidade de Brasília e o *Mercy's Children Hospital* nos Estados Unidos. Pacientes pediátricos FAP mostraram alterações ósseas similares aos adultos. Quando esses pacientes foram comparados aos controles, os pacientes FAP apresentaram alterações no padrão trabeculado ósseo, além de alterações dentais. Esse último estudo têm como objetivo recomendar o acompanhamento odontológico periódico através de radiografia panorâmica convencional anual em pacientes FAP e nas famílias em risco. Além de enfatizar a necessidade de participação do dentista nas equipes médicas multiprofissionais que acompanham essas famílias. Assim, esse trabalho alerta e conscientiza de forma crítica, baseada em evidências, nas equipes de saúde bucal sobre a importância de investigar doenças sistêmicas, alterações ósseas e FAP nos exames radiográficos rotineiros.


**Palavras-chave :** Polipose adenomatosa familial. FAP. Câncer colorretal. Dimensão fractal. Alterações ósseas.

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# LIST OF ABREVIATIONS AND ACRONYMS

| | |
|---|---|
| AAOMS | American Association of Oral and Maxillofacial Surgery |
| AAOMR | American Academy of Oral and Maxillofacial Radiology |
| ADA | American Dental Association |
| Apc | Adenomatous polyposis coli (animal gene) |
| *APC* | Adenomatous polyposis coli (human gene) |
| APC | Adenomatous polyposis coli (protein) |
| AXIN2 | Axis Inhibition Protein 2 |
| BMD | Bone Mineral Density |
| BP | Bisphosphonate |
| CBCT | Cone-Beam Computed Tomography |
| CCD | Charge-coupled Device |
| CMKC | Children's Mercy's Kansas City |
| CMOS | Complementary Metal Oxide Semiconductor |
| CRC | Colorectal Cancer |
| CT | Computed Tomography |
| DXA – DEXA | Dual Energy X-ray Absorptiometry |
| DP3 | Dual Photon Densitometer (Gd153 source) |
| DPR | Dental Panoramic Radiograph |
| F | Female |
| Ft | Levine's test |
| FAP | Familial Adenomatous Polyposis |
| FD | Fractal Dimension Analysis |
| H | High Risk of Bias |
| HU | Hounsfield Units |
| HUB | Hospital Universitário de Brasília |
| ICC | Intraclass Correlation Coefficient |
| IOPA | Intraoral Periapical Radiograph |

| | |
|---|---|
| IRB | Institutional Research Board |
| L | Low Risk of Bias |
| M | Male |
| MF | Mental Foramen |
| Mo | Moderate Risk of Bias |
| N | No |
| ND | Not Declared |
| OI | Osteogenesis Imperfecta |
| OMR | Oral and Maxillofacial Radiology |
| OR | Odds Ratio |
| p | Statistical p-value |
| PTH | Primary Hyperparathyroidism |
| RoB | Risk of Bias |
| ROI | Region of Interest |
| SD | Standard Deviation |
| SOS | Speed of Sound |
| T2DM | Type 2 Diabetes Mellitus |
| T | t-test |
| TBA | Trabecular Bone Area |
| U | Unclear |
| UnB | University of Brasilia |
| US | United States of America |
| WHO | World Health Organization |
| Y | Yes |

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# 1 BACKGROUND

Familial Adenomatous Polyposis (FAP) [Online Mendelian Inheritance in Man (OMIM) # 175100] is an autosomal dominant syndrome caused by mutations in the Adenomatous Polyposis Coli gene (*APC*), a tumor suppressor gene on chromosome 5q21 (Groden et al., 1991; Kinzler et al., 1991; Goss; Groden, 2000; Galiatsatos; Foulkes, 2006; Half et al., 2009; Fitzmaurice et al., 2019). *APC* is a large gene encoding a protein involved in numerous cellular processes; the gene mutation results in a premature codon-interruption resulting in the protein malfunction (Goss; Groden, 2000; Lesko et al., 2014). Up to now, 1000 *APC* germline mutations have been reported, according to the Human Gene Mutation Database. The FAP dominant inheritance disease predisposes to Colorectal Cancer (CRC) (Miclea et al., 2010; Aihara et al., 2014).

From an epidemiological perspective, FAP is responsible for 1% of all cases of CRC (Bisgaard et al., 1994; Half et al., 2009). Almost all FAP patients will have adenocarcinomas, bringing the mortality rate of these patients to around 100%. CRC is the third cause of cancer-associated death worldwide and responsible for almost 9% of all deaths (Lynch; de la Chapelle, 2003; de la Chapelle, 2004; Half et al., 2009; Jasperson et al., 2010; Fitzmaurice et al., 2019). As per the Cancer Statistics 2020, CRC is the second most common neoplastic malignancy when men and women are combined and the third cause of cancer death (Siegel et al., 2019; Siegel et al., 2020). In cases caused by *APC* mutation, the prevalence is 1 case for 6,800-29,000 people. Besides, the FAP could be caused by two gene variations, the MYH gene mutations result in MAP, and the Attenuated FAP (AFAP) there is a resultant of the mutation in the *APC* gene (Torrezan et al.,2013).

In Brazil, CRC has ranked among the 4th most common malignant neoplasia, and it is the third in the mortality rank among males and females. Its prevalence ranks below breast cancer in females, leading the ranking; in males, it ranks below prostate cancer (INCA, 2020). A study has explored the CRC mortality index in the Brazilian population preview an increase in the mortality rate through the next years due to the aging population (Souza et al., 2014; INCA, 2020). From 2014 onwards, they expected a robust increase of death related to CRC in the underdeveloped Brazilian regions; these have reduced public health agencies designated to screen, diagnose, and monitor the patients (Souza et al., 2014). For

2020, the Health Agency of Brazil estimated 25,520 CRC new cases in males, and 20,470 in females, with a calculated risk, averaged at around 19 cases for every 100 individuals (INCA, 2020).

Individuals with FAP are susceptible to a myriad of extracolonic features encompassing both malignant and benign neoplastic and non-neoplastic features (Bülow et al., 1996). Clinically, nearly 100% of adult FAP patients develop multiple colorectal adenomas, in most cases, by the fourth decade of life. In case these polyps are not diagnosed and/or missed by a prophylactic intervention, the patient experiences deterioration in the quality of life and a decrease in the survival rate (Half et al., 2009; Almeida, 2010; Miclea et al., 2010). Besides, estimates that 11 to 25% of the FAP cases will occur for the first time due to *de novo* mutations without a family history (Bisgaard et al., 1994).

Besides the typical colorectal alterations, FAP patients present several well-known extraintestinal manifestations (Half et al., 2009; Miclea et al., 2010). Individuals with FAP are susceptible to a myriad of extracolonic features encompassing both malignant and benign neoplastic and non-neoplastic features. These manifestations include gastric polyps, desmoid tumors, and congenital hypertrophy of the retinal pigment epithelium, epidermoid cysts, osteomas, and alterations in the maxillomandibular complex (Gardner; Richards, 1953; Gardner, 1962; Galiatsatos; Foulkes, 2006; Almeida et al., 2010; Almeida et al., 2012; Septer et al., 2018). And, the dento-osseous alterations were reported firstly by Gardner, that is why the "Gardner Syndrome" is known since 1995 by the dental and medical community.

Amongst the radiographic findings, the most commonly reported in the dento-osseous anomalies of FAP patients are osteomas. These are followed by odontomas, unerupted and supernumerary teeth, and a dense bone island in the maxilla and mandible that could be appreciated early in life (Gardner, 1962, Wijn et al., 2007; Septer et al., 2018; Almeida et al., 2020). These alterations can predate and can be detected years before the intestinal polyps are detected. (Wjin et al., 2007). However, oral alterations' detection is challenging due to the absence of screening practices and dental community awareness.

Several clinical reports presented dento-osseous anomalies such as osteomas, dense bone islands, osteomas, odontomas, and supernumerary teeth associated with adults

with a FAP diagnosis (Payne et al., 2002; Butler et al., 2005; de Oliveira Ribas et al., 2009; Almeida et al., 2012). A systematic review described the extraintestinal alterations' frequency, including 1,635 affected individuals from 16 included articles. From their meta-analysis, the observed prevalence was 65.3% for osseous alterations and 35.5% for dental alterations, showing a higher prevalence of osseous alterations than the dental ones (Almeida et al., 2016).

Recently, two studies expanded the spectrum of the *APC* gene. Almeida et al. (2020) followed-up a cohort of 11 FAP individuals using panoramic radiographs and CBCT in addition to genetic testing. The study demonstrated how essential radiographic imaging is as a screening tool for early diagnosis of FAP.  Septer et al. (2018) discussed the genotype of pediatric FAP related to phenotype. They showed an association between a genetic variant at the upstream of codon 1309 and dental radiographic anomalies. Both studies expanded the expression mutation of the APC gene, presenting dento-osseous abnormalities in young patients. The increased detection of osseous alterations during adolescence highlighted the utility of a periodic dental follow-up in FAP children and families at high risk.

The frequency of focal areas of sclerotic bone in FAP patients is meaningful, and its basis should be emphasized (Almeida et al., 2020). In FAP patients, animal studies investigated the structural alterations in the bone. As shown by Holmen et al. (2005), *APC* mutated mice developed a bone in which the vast majority of the marrow component is absent. This study linked the *APC* mutation to a dramatically increased bone deposition associated with bone architecture disturbances (Holmen et al., 2005). Interestingly, studies demonstrated that FAP patients with heterozygous *APC* mutations might present increased bone mineral density (BMD) (Miclea et al., 2010; Chew et al., 2012). Miclea et al. (2010) described the *APC* suppressor effects in the cancellous bone. FAP patients display increased mean BMD, maybe because of *APC* gene regulates bone density. The increased BMD is possible due to the activation of the β-catenin that regulates the pathophysiology of bone formation and disorders (Chew et al., 2012). The increased accumulation of bone matrix is a consequence of the Wnt's activation. It is the gatekeeper of the β-catenin pathway that promotes osteoblast differentiation, leading to bone mass acquisition. The typical architecture bone structure is outweighed; the trabeculae structure becomes less complex, disrupting trabecular bone texture.

Despite the high-frequency of osseous alterations in FAP patients, no publications evaluate their cortical bone and the trabecular pattern of the mandibular bone. The textural marrow bone disruption is translated radiographically to a radiolucent area. This incidental radiographic finding is often appreciated in dental imaging. A previous clinical study conducted at the University of Brasília regarding FAP families evaluated dental and osseous manifestations of FAP. They found 90% of bone alterations in the affected patients (Almeida et al., 2016). The results of this study raised questions regarding the cortical bone and the trabecular bone pattern in the FAP patient's mandible and the maxilla.

Screening decreases mortality rates when disorders are diagnosed in their initial stages (Greenberg; Glick, 2012). Screening of some systemic conditions that affect bone density via routinely obtained dental imaging is a potentially beneficial practice. Studies from the United States (US) have shown that, on average, 68% of the population visit their dentist annually, yet around 15% of those patients have not seen their physician in the last year (Glick; Greenberg, 2005; CDC, 2011). Since the estimated number of dental radiographs produced per year surpasses millions (White; Pharoah, 2014), the dental radiographs should be used opportunistically as a screening adjuvant to systemic disorders.

The dental panoramic radiograph (DPR) is one of the most common extraoral dental imaging modalities. Besides, the DPR is considered an accurate tool to opportunistically identify systemic disorders (Pacheco-Pereira et al., 2019). Although several studies evaluate osseous alterations detected via DPR, they mostly observed differences in bone mineral density in a systemic condition such as osteoporosis (Leite et al., 2010).

The oral and maxillofacial complex contains multiple structures affected secondarily by some systemic diseases. A recent systematic review reported that analysis of mandibular cortical width on Cone-Beam Computed Tomography (CBCT) images has screening capability to suggest differences in bone mineral density (BMD) in patients with osteoporosis (Guerra et al., 2017). Cortical bone changes have also been reported in other systemic diseases such as Paget's disease, renal osteodystrophy, and Rickets (Annanuntana et al., 2011).

Unlike cortical bone, trabecular bone has an internal structure of interconnected rod and plate-like structures with great surface area (Parkinson; Fazzalari, 2012). This structure promotes a functional balance between maximum strength and minimal mass. Anatomical

landmarks with greater trabecular bone volume show significant bone metabolism and are most susceptible to osteoporotic activity (Johnell; Kanis, 2004).

The radiomorphometric indexes and the fractal dimension of the mandibular trabecular bone were performed in conventional panoramic radiographs and CBCT, mostly for diagnosising BMD (Sindeaux et al., 2014; Calciolari et al., 2015; Mostafa et al., 2016). Although cortical bone mineral density indices have been validated and demonstrate to have good diagnostic performance (Guerra et al., 2017), bone microstructure investigation through its trabecular architecture for screening systemic disorders has received limited attention. These methods could be an option to assist in the early identification and screening of FAP patients utilizing bone mineral density evaluation.

Lastly, the dento-osseous anomalies in FAP children and adults are under-reported. The published literature to date is limited by the rarity and the usually delayed FAP diagnosis. Therefore, given the preponderance of case reports in pediatric patients (Nissen; Wynn, 2014) and the limited sampling in the existent studies, there is a gap in our understanding of the age-dependent dental manifestations FAP risk. A recent follow-up study in Brazilian FAP patients (Almeida et al., 2020) showed an osseous lesion more frequent than dental lesions. An increase in the size and number of lesions found in adolescents was detected, while the adult lesions stagnated for some time (Almeida et al., 2020). We believe that a multicenter study encompassing diverse ages and ethnicities could provide a broader perspective regarding the dento-osseous anomalies in the maxillomandibular complex of children and adults with FAP.

# 2    OBJECTIVES

The objectives were constructed according to the structured research question followed the "PICO principle" (O'Connor et al., 2011). ***Population***: FAP patients and matched controls; ***Index test/Exposure***: panoramic radiograph of the maxillomandibular complex; ***Comparison:*** healthy patients; ***Outcome:*** alterations of the trabecular bone structure, and radiographic findings in the maxillomandibular complex.

*Question:* ***Do FAP adults and children differ from non-FAP while evaluating radiographic trabecular structure in the maxillomandibular complex?***

In light of the evidence previously presented, the objectives of this thesis are:

1) To perform a systematic literature review exploring the systemic diseases that affect the mandibular trabecular bone pattern.

2) To evaluate the cortical bone of the mandible using radiomorphometric indexes applied to panoramic radiographs of FAP patients and controls.

3) To assess the mandible trabecular bone of FAP patients using fractal dimension analysis applied on panoramic radiographs.

4) To compare the specific objectives #2 and #3 of FAP patients with healthy individuals without family history or risk of FAP, these matched by sex and age.

5) To compare phenotypes of the Brazilian and United States samples of patients through a multicentric project involving FAP children and adult patients and paired controls.

## 3    HYPOTHESES

1)    The published literature indicates that the panoramic radiograph is a useful adjunct tool for screening the cortical and trabecular bone in patients affected with systemic diseases.

2)    FAP individuals, when compared to healthy controls, will demonstrate an altered cortical bone (quantitatively and qualitatively) and a disrupted microarchitecture of the trabecular bone in the mandible.

3)    A multicentric approach, even if composed of samples from a different continent/country, will show consistent dental and osseous alterations in FAP patients. These findings are related directly or indirectly to FAP adults and children.

# 4    ARTICLE 1

**Title: Dental imaging of trabecular bone structure for systemic disorders screening: a systematic review**

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**Conflict of Interests**

**Author Contributions**

## Abstract

The purpose of this systematic review is to evaluate the potential use of dental imaging assessment of trabecular bone structure in the maxillomandibular complex as an adjuvant screening tool to identify systemic disorders. Five electronic databases and grey literature were searched. Studies were included if they investigated subjects with altered trabecular bone determined by dental radiographs. The QUADAS-2 assessed the risk of bias (RoB) among the studies, while the GRADE determined the strength of evidence. A total of 14 studies that included 1,466 individuals were considered eligible for the qualitative analysis. All studies presented an overall low RoB and low concern regarding applicability. Systemic disorders such as osteoporosis, osteogenesis imperfecta, diabetes and primary hyperparathyroidism were analyzed amongst the included studies with their respective control groups. Osteoporosis was the condition presenting the most significant results; 72% of the studies detected changes in the maxillomandibular trabecular bone structure. Studies exploring diabetic edentulous patients found a less-dense trabecular bone pattern ($p < 0.05$). In summary, periapical and panoramic radiographs, Computed Tomography, and Cone-Beam Computed Tomography imaging could be considered useful for assessing the mandibular trabecular bone structure of affected by osteoporotic and diabetes patients.

**Introduction**

Disease screening has been found to decrease mortality rates when disorders are diagnosed in the initial stages (Greenberg; Glick, 2012). Opportunistic screening of some systemic disorders that affect bone density via routinely obtained dental imaging is potentially a beneficial practice. Studies from the United States (US) have shown that on average 68% of the population visit their dentist annually, yet around 15% of those patients have not seen their physician in the last year (Glick; Greenberg, 2005; CDC, 2011). The estimated number of dental radiographs produced per year surpasses millions (White; Pharoah, 2014), and these could be screened for trabecular bone structure alterations.

The oral and maxillofacial complex contains multiple structures that can be secondarily affected by some systemic diseases. A recent systematic review reported that analysis of mandibular cortical width on Cone-beam computed tomography (CBCT) images has screening capability to suggest differences in bone mineral density (BMD) in patients with osteoporosis (Guerra et al., 2017). Cortical bone changes have also been reported in other systemic diseases such as Paget's disease, renal osteodystrophy and Rickets (Unnanuntan et al., 2011).

Unlike cortical bone, trabecular bone has an internal structure of interconnected rod and plate-like structures with great surface area (Parkinson; Fazzalari, 2012). This promotes a functional balance between maximum strength and minimal mass. Areas with greater trabecular bone volume have greater active bone metabolism and therefore are most effected by osteoporotic activity (Johnell; Kanis, 2004).

Although cortical bone mineral density indices have been extensively explored by the literature and demonstrated to have valid diagnostic performance (Guerra et al., 2017), the investigation of the bone microstructure through its trabecular architecture for screening systemic disorders has received limited attention. Therefore, the purpose of this systematic review is to evaluate the potential use of dental imaging assessment of trabecular bone structure in the maxillomandibular complex as a screening tool to identify systemic disorders that affect bone density.

**Methods**

**Protocol and Registration**

This systematic review adhered to the Preferred Reporting Items for Systematic reviews and Meta-analyses - PRISMA (Moher et al., 2009; McInnes et al., 2019). The protocol was peer reviewed and registered at International Prospective Register of Systematic Reviews (Centre for reviews and dissemination, University of York, York, United Kingdom) under protocol CRD 42017079783 (Pacheco-Pereira et al., 2018).

**Identification of relevant studies**

The following research question was considered: Which systemic disorders that affect trabecular structures in the maxilla or/and mandible complex can be determined/assessed by dental radiographic imaging?

**Eligibility criteria**

This systematic review exclusively ***included*** articles investigating subjects with altered trabecular bone structure in the maxillofacial complex determined by intraoral and extraoral radiograph imaging modalities. No language, cortical unspeakable release participant's age or original study design restrictions were set. Any study type that included relevant cross-sectional data was included. Studies were ***excluded*** if they met the following criteria: 1) animals or ex-vivo studies; 2) studies on patients with no systemic disorders; 3) studies in which the sample included craniofacial anomalies; 4) studies that assessed bone density exclusively from cortical bone and did not assess trabecular structure; 5) case reports, and 6) reviews, letters, conference abstracts, personal opinions. In phase 2, we augmented one additional exclusion criterium: 7) studies in which trabecular bone patterns were evaluated by visual assessment; these were excluded due to the subjectivity of this methodology. A study was excluded if there was insufficient information on the reference standard of the systemic condition composing the final diagnosis or if only the risk of the condition was investigated. In cases where the article did not provide enough information, attempts were made to contact the corresponding author to retrieve the unpublished information.

**Information Sources and Search**

Appropriate truncation and word combinations were selected and adapted for specific databases used with the aid and expertise of a health sciences librarian (L.C). Detailed individual search strategies were tailored for the following electronic databases: EMBASE, Latin American and Caribbean health sciences (LILACS), MEDLINE (and adapted for all EBM Reviews including Cochrane library), PubMed and Web of Science. Google was elected as the search engine to partially explore grey literature. Searches were inclusive to January 08, 2018 (Appendix1). To reach a comprehensive identification of relevant studies, the reference lists of the selected articles were manually screened by the first author to identify any additional references overlooked during the electronic search. Articles obtained from consultations with experts in the field were also considered.

In this phase, the references and duplicate titles were managed with appropriate software (RefWorks – COS, ProQuest communications).

**Study Selection**

Studies were selected via a two-phase process after reviewers were appropriately calibrated. In Phase 1, two reviewers (C.P-P and F.T.A) independently screened titles and abstracts of all the gathered references. All articles that did not meet the eligibility criteria were rejected. In Phase 2, the same reviewers applied the criteria to the full-text articles. Any disagreements between these two reviewers (C.P-P and F.T.A) were reconciled by agreeing on a final and mutual decision through a discussion with two additional reviewers (E.G and A.L).

The phase-2 selection process was carried out to support a web application tool specific for the systematic review screening process – the Rayyan Application (Qatar Computing Research Institute, Doha, Qatar) (Ouzzani et al., 2016). All reviewers and collaborators were blinded to each other, but they had access to the total number of screened articles.

**Data collection process**

One author (C.P-P) extracted the key features associated with the research question from the included articles. An extraction template was developed based on Cochrane Handbook recommendations (O'Connor et al., 2011; Schünemann et al., 2011). A second collaborator (S.C) reviewed all the retrieved information. Any disagreement was solved by a discussion with two or three collaborators. An expert in the field (A.L) was involved when disagreements were not settled between the first and second reviewers at this level.

**Definitions for data extraction**

The authors extracted pertinent characteristics of the included articles following the "PICO principle" (O'Connor et al., 2011). This principle was applied to provide a structured approach in identifying the relevant data from each study:

*Population*: the main characteristics of the sample that have systemic disorder. Specific characteristics of the reference standard method used to establish the diagnosis from the affected patients were collected; *Index test/*Exposure: dental imaging in the maxillomandibular complex to detect a target condition and trabecular structure; *Comparison*: healthy patients or patients with different levels of the same disorder was considered. *Outcome*: alterations of the trabecular bone structure in the maxillomandibular complex as a result of systemic diseases; as a co-variable, the imaging modality used.

**Risk of Bias and applicability**

Risk of bias (RoB) and applicability assessment was used to evaluate the methodology of the included studies. QUADAS-2 specifically evaluated the methodological quality of diagnostic studies in distinct domains: patient selection, index test, reference standard, flown, and timing.  (Whiting et al., 2011) A collaborator (S.C) and one author (C.P-P) assessed the selected articles for methodological quality. If these authors disagreed on any aspect of the quality assessment, a second author (F.A) offered their assessment to resolve the disagreement.

**Summary measures and approach to synthesis**

The primary outcome of interest in this study was trabecular bone structure in patients diagnosed with a systemic condition/alteration. The following imaging parameters were used for evaluating bone structure on radiographic imaging: fractal dimension analysis, pixel intensity, and grayscale analysis.

The imaging modality was considered as co-variable. Imaging modalities such as computer tomography (CT), computerized microtomography (micro-CT), CBCT, periapical and panoramic radiographs were considered. A quantitative analysis of these studies was performed.

**Level of evidence and Additional analysis**

The GRADE system (McMaster University, 2015), an approach recommended by Cochrane guidelines (Schünemann et al., 2011), was used to rate the evidence level across the studies. The quality of evidence was assessed based on the study design, RoB, inconsistency, indirectness, imprecision, and publication bias at the outcome level. Then, based on the assessment, the quality of evidence was characterized as high, moderate, low, or very low (Balshem et al., 2011).

Due to substantial methodological and clinical heterogeneity among studies involving distinct images modalities and systemic conditions a valid mathematical combination of data though a meta-analysis was prevented.

**Results**

**Study Selection**

The search results and the flow of the selection approach are shown in Figure 1. The initial phase summed up to a total of 52 articles. In Phase 2, the authors executed the full-text manuscript analysis. A total of 14 studies were found to be appropriate for qualitative synthesis. The flow diagram (Figure 1) presents the excluded articles from the selection process and lists the reasons for exclusion (Appendix 2).

**Studies characteristics**

The included studies examined systemic disorders summing to a total of 1,466 investigated individuals, 854 were an experimental group, and 612 were controls.

Groups of participants diagnosed with osteoporosis were compared against patients considered as having normal BMD in 10 studies (Lee; White, 2005; Tosoni et al., 2006; Khojastehpour et al., 2013; Oliveira et al., 2013; Roberts et al., 2013; Yamashita-Mikama et al., 2013; Chai et al., 2014; Kathirvelu; Anburajan,2014; Sindeaux et al., 2014; Mostafa et al., 2016). Radiographic modalities such as conventional film and digital panoramic, intra-oral film radiographs, CT and micro-CT scans were explored and compared to the BMD score. The reference standard for these studies, the BMD measurements by dual-energy x-ray absorptiometry (DXA) had the combined values of the total hip, femoral neck, posteroanterior lumbar spine or full-body. Except for one study that measured the calcaneal bone density by ultrasound, a validated and reliable tool generally used for osteoporosis screening (Yamashita-Mikami et al., 2013).

In two studies, Diabetes Mellitus Type 1 and Type 2 (Nemtoi et al., 2013; Kayipmaz et al., 2017) patients were compared to control non-diabetic subjects using trabecular bone qualitative and quantitative assessment via CBCT imaging.

Primary Hyperparathyroidism (HPT) (Padbury et al., 2006) patients diagnosed by the endocrinology department had their panoramic radiographs compared to a match case-control group. The control patients were specifically selected according to the absence of signs of altered HTP skeletal metabolism and abnormality.

None of the diabetes, HPT and Osteogenesis Imperfecta studies had the patient assess by the DXA. All original studies included in this systematic review had strict eligibility criteria regarding exclusion of patients undergoing hormone therapy and/or bone-related disorders that could be considered as a confounding factor regarding the bone density investigation. The standardized anatomic location, named as region of interest (ROI), and its trabecular structure analysis were determined by each included study. Key features from included articles such as the investigated systemic disorder, sample size, reference standard, the radiographic method used to obtain patient's imaging and the methodology applied to explore alterations in the trabecular pattern is shown on the summary of findings in Table 1.

**Risk of bias and applicability within studies**

We judged that all studies presented an overall low RoB and low concern regarding applicability. However, three studies (Lee; White, 2005; Mostafa et al., 2016; Kayipmaz et al., 2017) were evaluated as unclear in one RoB domain (index test) and one study (Kayipmaz et al., 2017) presented applicability concerns in the patient selection domain. No study was judged as having high RoB as per Figure 2a, b. Appendix 3 details QUADAS-2 criteria applied to each included study.

**Results of individual studies**

Four systemic disorders with their respective control groups were analyzed amongst all the included studies. These conditions will be individually discussed:

*Osteoporosis*

Osteoporosis was the most explored systemic condition with ten included studies. In these studies, the DXA was applied as the reference standard and the measurements classified by the World Health Organization criteria (WHO, 2004).

Lee and White (2005) investigated osteoporotic females and males using periapical imaging. They identified lower density in osteoporotic patients when compared to normal or osteopenic adults with 82% sensitivity and 86% specificity with 79% agreement between evaluators.

For panoramic radiographs, Oliveira et al. (2013) found a strong positive rank coefficient ($r$) correlation ($r= 0.90$) between fractal dimension analysis of the mandibular body and osteoporosis in postmenopausal women ($p< 0.05$). Kathirvelu and Anburajan (2014) showed 84% inter-reliability between radiologists while measuring radiographs. They segmented the average values of pixels as a threshold. Presenting 92% sensitivity and 78% specificity and strong correlation ($r= 0.76$), the authors compared bony structural deterioration/fragility (bilateral mental foramen surrounding area) between normal and low bone density women groups ($p< 0.01$). Khojastehpour et al. (2013) found a statistical difference in trabecular bone pattern posterior to mental foramen when they compared osteoporotic and healthy patients ($p< 0.05$). In contrast, Sindeaux et al. (2014) applied fractal

dimension analysis on mandibular sites and found no significance (p= 0.33) in trabecular bone around (p= 0.62), anterior (p= 0.27) and inferior (p= 0.89) to the mental foramen between normal and osteoporotic subjects' groups. Roberts et al. (2013) judged trabecular pattern analysis not sufficiently effective as an osteoporosis biomarker given the higher false-positive value (FP= 41%) when compared to DXA scores. The diagnostic performance of mandibular CT images reported by Chai et al. (2014) found to have diagnostic value in detecting edentulous patients with low BMD. Using Hounsfield units (HU), they established cut-off values to correlate with the spine T-score values. The optimal range (judged capable of detecting the osteoporotic status) found ranged from 530 to 730 HU in mandibular sites, these measurements were correlated with DXA values.

Using micro-CT images, Yamashita-Mikami et al. (2013) explored alveolar microstructural changes of the trabecular before and after menopause. They had the patients' BMD status checked via calcaneus ultrasound, a tool used for osteoporosis screening, on the same day a dental implant surgery was performed. These patients had also a CT scan performed for implant placement planning. During surgery, samples of the premolar and molar alveolar sites requiring implants were collected and micro-CT scans of these cancellous bone (consisting of trabecular) specimens were performed. The correlation of these groups by their alveolar bone samples analysis and the calcaneus BMD values were explored. The correlation was strong $r > 0.7$ and p= 0.01 between pre- and post-menopause/bone turnover women.

In a prospective study using CBCT imaging, Mostafa et al. (2016) presented fractal dimension analysis comparing post-menopausal osteoporotic and health control patients. They did not show significant difference in trabecular assessment when both groups were compared (p= 0.05 and $t = 1.99$).

### *Osteogenesis Imperfecta*

Apolinario et al. (2016) used the endocrinologists' diagnosis of Osteogenesis Imperfecta as the reference standard for the condition. Children's trabecular pattern was assessed via panoramic radiographs, and the fractal dimension analysis of the trabecular bone did not demonstrate significant changes in the Osteogenesis Imperfecta patients (p= 0.05). It is known by the literature that this systemic disease alters bone density; however, the authors did not assess the patients' bone density through the reference standard.

*Diabetes Mellitus*

Kayipmaz et al. (2017) reported that CBCT imaging was a useful adjunct tool when assessing diabetic patients. The trabecular fractal dimension analysis demonstrated no statistically significant difference in the posterior mandible trabecular bone of health controls and Type 2 diabetes mellitus patients (p= 0.712). In contrast, Nemtoi et al. (2013) found a significant difference in the trabecular bone when comparing well-controlled vs. moderate vs. poorly controlled diabetes mellitus patients using the same imaging modality and assessing the posterior mandible bone on edentulous areas. Their study showed the presence of higher glycosylated hemoglobin values on patients with less bone mineral density (p<0.05).

*Primary Hyperparathyroidism*

Padbury et al. (2006) investigated HPT effects on oral bone structures via panoramic images. Their elected ROI was the interdental area, in the middle third of the first mandible molar's root. They found a trend, not statistically significant p=0.07, towards quantitative measurements (computer-assisted) of low interdental alveolar bone density in the HPT patients' group. Calibrated evaluators also qualitatively analyzed the radiographic trabecular pattern by scoring its density from 1-3 (visual impressions). Osseous calcifications such as increased incidence of mandibular tori on HPT patients' early adulthood were constantly noted on these affected patients.

**Synthesis of Results**

Regarding osteoporosis studies (n=10), seven of them found evidence that alveolar cancellous bone and its trabecular alter its pattern when a systemic disorder is present (Lee; White, 2005; Tosoni et al., 2006; Khojastehpour et al., 2013; Oliveira et al., 2013; Roberts et al., 2013; Chai et al., 2014; Kathirvelu; Anburajan, 2014). Three studies demonstrated that isolated fractal dimension or pixel intensity analysis from the trabecular bone could not distinguish between diseased and health control groups (Yamashita-Mikama et al., 2013; Sindeaux et al., 2014; Mostafa et al., 2016).

Regarding Diabetes (n=2), just one study found evidence that the trabecular bone analysis provides useful information on trabecular bone's quality (Nemtoi et al., 2013). Primary HPT seems to protect, by a qualitative categorization, the trabecular bone increasing its density (Padbury et al., 2006) and the Osteogenesis Imperfecta study did not demonstrated changes on the trabecular bone pattern (Apolinario et al., 2016).

In general, retrospective and prospective studies that investigated the trabecular bone pattern via dental radiographs found a positive correlation with the reference standard for a variety of systemic diseases. Although there were differences in study design, these studies found common ground in concluding that the trabecular bone density appears to be a useful screening index for systemic disorders which alter bone density (Lee; White, 2005; Tosoni et al., 2006; Khojastehpour et al., 2013; Nemtoi et al., 2013; Oliveira et al., 2013; Roberts et al., 2013; Yamashita-Mikama et al., 2013; Chai et al., 2014; Kathirvelu; Anburajan, 2014).

The studies that presented trabecular bone alterations selected a variety of anatomical ROI located in the mandible. These anatomical areas elected by the studies showed a significant difference when healthy and diseased patients were compared. Figure 3 maps the effective ROIs for trabecular bone pattern screening.

**Risk of bias across studies**

When exploring RoB across the included studies, the concern identified was related applying of the index test without knowing the results of the reference standard (blindness). In most of these studies, this information was not clear and with regards to retrospective studies, it is challenging to be blinded to these conditions due to their inclusion criteria being based on the health record information. All of the studies had a convenience sample based on their investigated condition. The potential bias is related to the fact that the knowledge of the reference standard may influence the interpretation of the index test results (Whiting et al., 2011). Most studies' applicability did not report the interval between the reference and index tests leading to a potential bias. Delays and different intervals could be problematic in assessing patients with chronic conditions. Figure 2b reviews a graph on the authors judgements on each domain presented as percentages across included studies.

**Additional Analysis**

Overall, the quality of the evidence from the outcomes evaluated by the GRADE system was determined to be low (see Appendix 4). It suggested limited confidence in the estimated effect of the assessed primary outcome and co-variable. Heterogeneity of the study's methodology and design (cross-sectional) to measure the trabecular density (considering distinct imaging modalities and systemic conditions) and its pattern was the main factor responsible for the limited level of evidence and the unlikelihood of a meta-analysis.

**Discussion**

*Summary of evidence*

Dental images are potentially valuable diagnostic sources for the screening of systemic disorders as these are routinely administered and relatively low-dose exposures to patients with anatomical and pathological data.

A rationale for screening oral radiographs for health conditions is their frequency. Dental imaging is common and frequent, and intraoral radiographs are the most common dental radiographic performed with numbers approaching 400 million worldwide (UNSCEAR, 2010). Although out of the scope of the routine dental practice, the screening of CT and micro-CT imaging of the mandible were demonstrated as effective screening tools (Yamashita-Mikama et al., 2013; Chai et al., 2014). The possibility of an early referral to the physician by screening the bone changes reflect the importance and the opportunistic use of dental screenings (Gausden et al., 2017).

A study that investigated the usefulness of the cortical bone density to screening for systemic conditions opened the door for the dental professional to detect these alterations and support a referral (Guerra et al., 2017). Their assessment required a panoramic or a CBCT imaging modalities are not are routinely prescribed on a recall appointment. However, two of the included studies in the current systematic review utilized intraoral radiographs. These radiograph modalities are commonly and annually obtained on dental follow-ups; therefore, a more accessible screening opportunity (Lee; White, 2005; Padbury et al., 2006).

The current systematic review demonstrated that the complex trabecular microarchitecture of the mandible could be affected by systemic diseases which alter the bone structure and turn over (Lee; White, 2005; Khojastehpour et al., 2013; Nemtoi et al., 2013; Chai et al.; 2014; Kathirvelu; Anburajan, 2014). Previous *in vitro* research supports a combination of trabecular bone analysis and its geometry for the prediction of the bone mechanical competency as accurately as DXA in osteoporotic patients (Pulkkinen et al., 2008).

A previous study addressed the opportunistic use of CTs to infer bone quality (Gausden et al., 2017). The HU measurements were inconsistent due to calibration and heterogeneity of parameters. However, the potential of CT to assess osteoporotic patients was clearly demonstrated by 93% sensitivity and 97% specificity when correlated to DXA. Considering a different scope, Taniguchi et al. (2016) compared CTs from patients under medication-related osteonecrosis of the jaw at various stages of the condition. The cancellous bone pattern changes on affected patients was significantly higher when comparing affected and unaffected areas (p= 0.01 and p= 0.47). These reported by HU of each investigated area. It could be argued that these changes are drug-induced; however, this is a trabecular bone structure that should be accessed on patients requiring dental implants and/or intraoral surgeries (Nemtoi et al., 2013). The prevalence of cancer survivors requiring treatment increased these past decades significantly, and the dental professional examination should serve as a screening tool for the disease recurrence and bisphosphonates side effects in the jaws (American Dental Association Council on Scientific Affairs, 2006).

Several methods could do texture analysis of the trabecular bone could; however, majority of our included studies elected the fractal dimension analysis to evaluate the trabecular structure. This analysis is based on fractal mathematics for describing complex shapes and patterns of the bone (Mostafa et al., 2016). A prior study mentioned a positive association between the combination of cortical bone and trabecular bone density parameters and the clinical findings (Roberts et al., 2013). In our systemic review, all of the studies using fractal dimension analysis of the trabecula were negatively correlated to the reference measurement for mineral bone density. This could be due to the size and shape of the anatomical site elected for investigation. The imaging modality and the size of the ROI explored by the included studies varied considerably (Figure 3 maps the ROI explored by the studies that found evidence of trabecular bone changes).

Our study results indicate that the trabecular bone pattern could be considered a potential screening tool for systemic disorders involving changes in bone structure during dental examination. The majority of the included studies assessed osteoporosis, this fact is consistent with its domination in the published literature (Cummings; Eastell, 2018). We demonstrated that diseases that highly affect the world's population, such as osteoporosis and diabetes, may be opportunistically screened in routine dental imaging (Lalla et al., 2011; Office of the Surgeon General (US), 2004). The mandibular cancellous bone, cortical and trabecular bone begins to deteriorate at the early stages of the systemic disorders. These conditions could not be neglected in the screening of dental radiographs such as Panoramic and 3D scans.

### *Limitations*

There are some limitations to this systematic review to be considered. The drawbacks of the cross-sectional design and computer-based studies using pixels intensity and greyscale have to be considered. CBCT machines present a shift on their greyscale over the course of the day. Subsequent HU's accuracy and consistency could be affected if a careful geometric calibration is missed (Pauwels et al., 2015). The studies investigating Osteogenesis Imperfecta and HPT did not have the patient's BMD assessed by the reference standard; this could affect assumptions regarding alterations in the bone pattern.

### Future directions

This paper highlighted dentist's potential role of in screening various bone diseases based on the analyses of the trabecular structure in dental imaging modalities. As radiographic imaging is routinely used in dental treatment, they can become powerful adjuvant tools for detecting systemic bone diseases. However, the challenge is standardizing 2D and 3D imaging analyses and developing a method that has acceptable reliability.

Recent studies have assessed trabecular changes through bone structure visual estimation (Neves et al., 2012; Venela et al., 2012), which supports this paper's clinically applicable findings. This subjective evaluation should not be neglected as the dental professional could apply it on a regular daily basis. Under this perspective, a significant number of Sickle Cell Disease patients (n=71) presented increased trabecular bone marrow spacing when compared to the controls (Neves et al., 2012). Interestingly, Venela et al., (2012) investigated intraoral images of patients with Chronic Renal Failure, their findings revealed the "granular chalky and white" trabecular shown by the dental imaging fields of view. These last studies and the qualitative assessment of the trabecular bone pattern have identified an opportunity for further research and practice translation. However, the poor reproducibility of these subjective visual assessments could compromise the method application.

Future research efforts should focus on studies investigating the most effective imaging modality and specific tools to analyze the trabecular pattern and bone density on a bigger scope. This review did not elucidate these points due to the heterogeneity of studies, systemic conditions and diverse image modalities. In addition, the development of automated approaches that could be part of the routine practice will optimize processes that detect threshold changes warranting the referral of these patients. The automated analysis of image texture in projection images of trabecular bone structure could be useful and further explored. This systematic review is the start point for studies in this specific scope. Furthermore, it contributes to the general health of the population that frequently visit their dentists. The concept of clinical utility, radiographic screenings, or diagnostic tests could be applied in the dental examination based on maintaining public health, restoring function, and preventing premature death (Bossuyt et al., 2012).

**Conclusion**

This systematic review demonstrated the potential of the trabecular bone assessment through routine dental imaging, it can serve as a screening tool for the detection of osteoporosis involving bone density changes.

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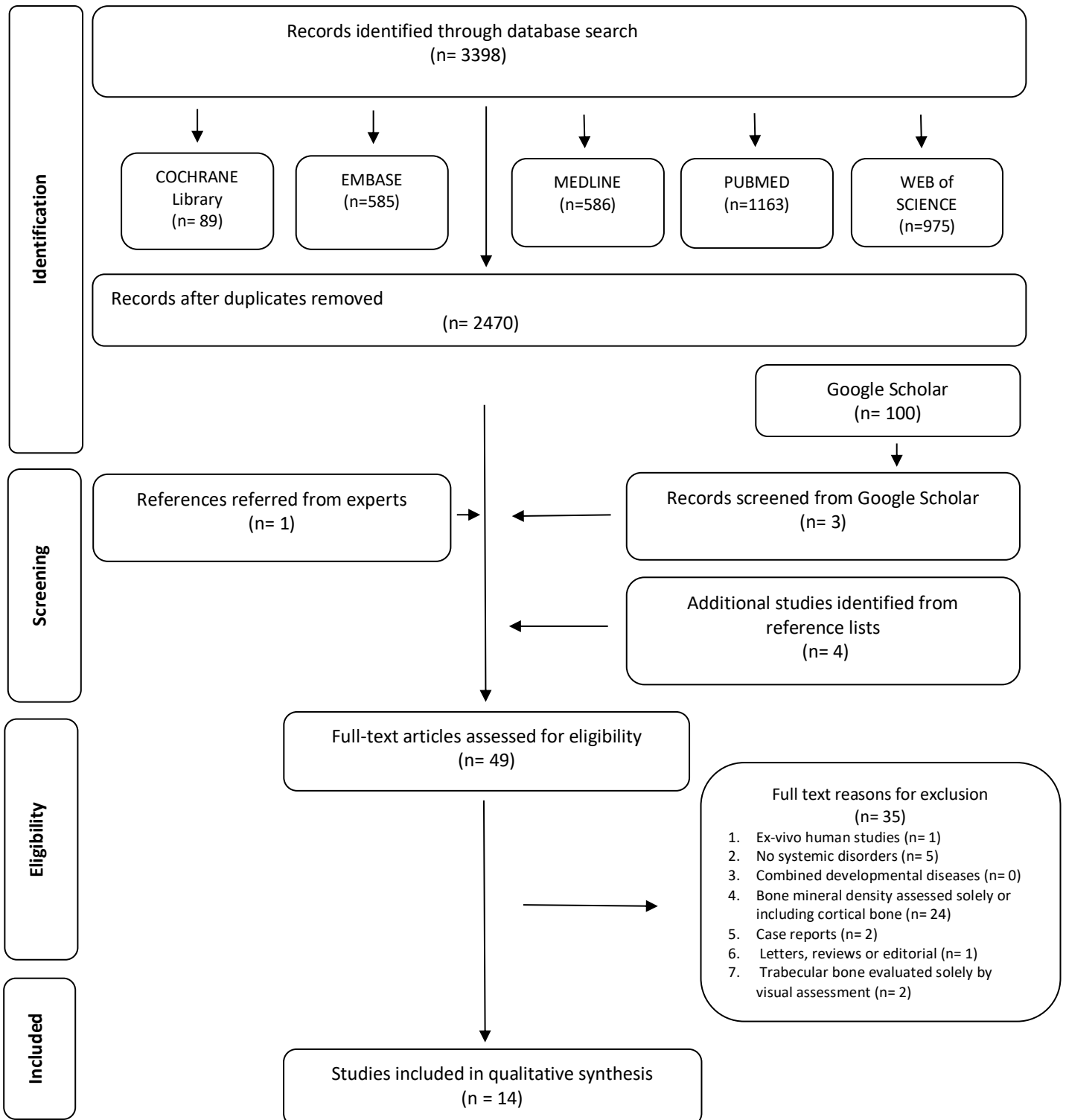
**Figure 1** - Flow Diagram of Literature Search and Selection Criteria

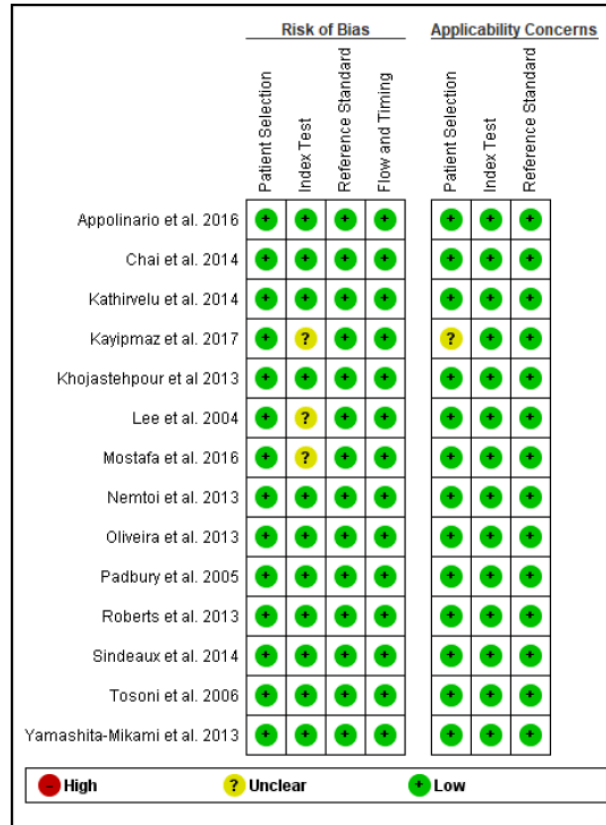**Figure 2a** - Risk of bias and applicability concerns summary



**Figure 2b** - Risk of bias and applicability concerns graph
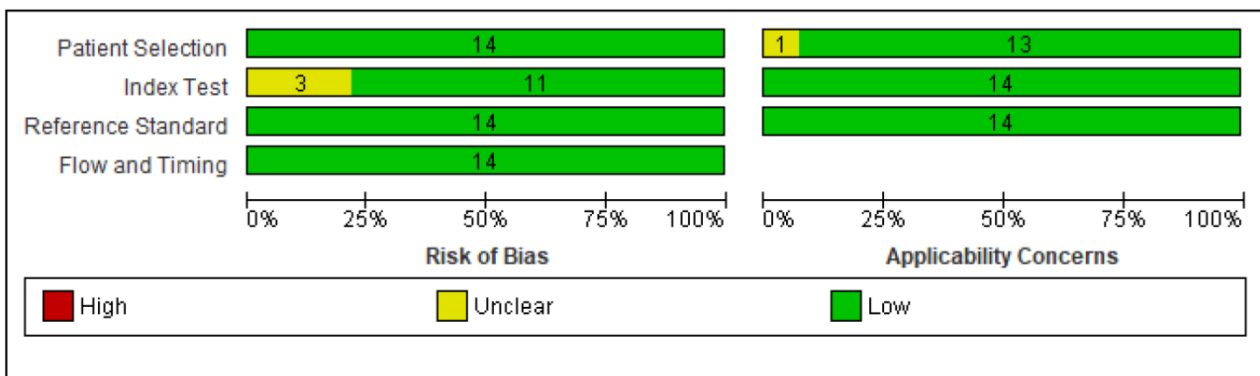
**Figure 3** - Region of Interest location in the radiographic imaging
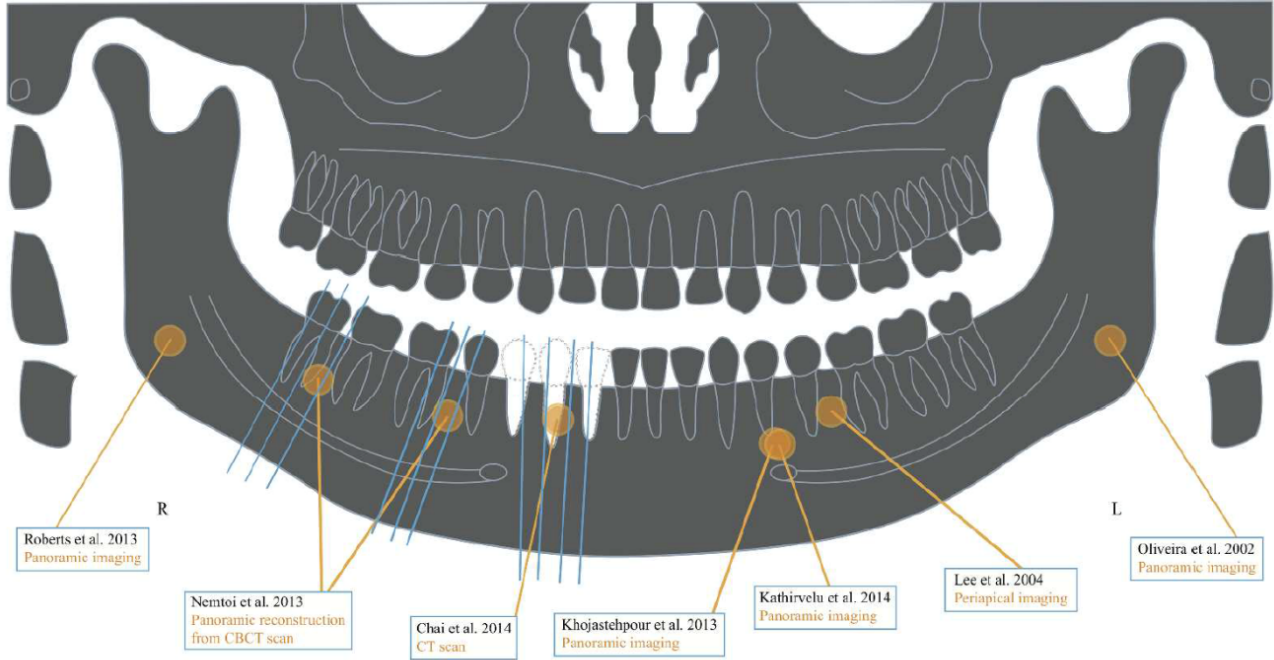
**Table 1** - Summary of descriptive characteristics of included articles (n=14)

| Authors Year Country | Study Design & Radiograph Sample Size | Condition Sample size (N) & Reference Standard | Comparison modality & Sample size | Dental imaging | Trabecular Measurement & Methodology | Region of interest (ROI) Trabecular bone | Results related to trabecular bone density |
|---|---|---|---|---|---|---|---|
| *Apolinario et al.* 2016 Brazil | Retrospective Cohort N=197 sites (F & M) | OI N=62 Pediatric Endocrinology Center diagnosis | OI distinct types Types I (N=58) Types III (N=83) Types IV (N=56) | DPR Rotograph Plus (Villa Sistemi Medicali) | FD analysis ImageJ v. 1.45s software (National Institutes of Health, Bethesda, MD | ROI (1): a square of 25 x 25 pixels in the geometric center of the mandibular ramus ROI (2): a square of 25 x 25 pixels in the geometric center of the mandibular angle | FD measurements of the trabecular bone were not statistically different among OI types (p= 0.05). FD measurements did not present association with qualitative indices and had weak correlation with cortical bone measurements. |
| *Chai et al.* 2014 Japan | Prospective Cohort N= 122 sites F=40 M=28 | Osteoporosis N=116 BMD by DEXA* (Explorer, Hologic) | Normal BMD Type 1 (N=100) Type II (N=21) Type III (N=1) | CT Spiral HiSpeed/ Fxi (General Electric) | Bone density in HU of each side of the mandible. HU bone density software (Simplant 3D Implant Planning System, Materialise Dental) | Markers were placed in the canine region, right and left. A midline marker served as orientation and positioning landmark. | CT bone density in HU of mandibular sites have moderate but significant correlation with BMD T-score of full-body. The optimal cut-off value ~530, ~600 and ~640 (hip, spine and total body T-score) sensitivity of 80%. The HU value representing trabecular density was effective on detecting "non-osteonormal" condition. |
| *Kathirvelu et al.* 2014 Indian | Prospective Cohort F=64 | Osteoporosis Low BMD N=36 BMD by DPX Prodigy DXA Scanner (GE Lunar Corporation) | Normal BMD N=28 | DPR Kodak 8000C | Pixel analysis formula TBA=White pixels/ white + black pixels Image analysis via MATLAB 7 (Windows 7 Core i3 processor with 2 GB of RAM). | ROI: 128 X 128 pixels manually placed and cropped in the region around the mental foramen on both sides of the DPR. | The results showed a significant correlation (r= 0.401; p< 0.01) between TBA and BMD. |
| *Kayipmaz et al.* 2017 Turkey | Retrospective Case-control N=46 M=29 F= 17 | T2DM N=23 | Non-diabetic N=23 | CBCT Kodak 9500 Cone Beam 3D System (Carestream Health) | FD analysis Image J software (version 1.3; National Institutes of Health, Bethesda, MD, USA). | ROI: superior to the mandibular canal in the basal bone on each 200-μm-thick section image. A 64 x 64-pixel. | The FD analysis failed to establish a statistically significant difference between T2DM patients and the controls f= 0.712; p=0.655 (0; 0.03); t=0.450. |
| *Khojastehpour et al.* 2013 Iran | Cross-sectional F=104 | Osteoporosis N=59 DXA by BMD* | Normal BMD N=45 | DPR Promax panoramic X-ray (Planmeca,) | Measurements using DfW software. Digora PCT Soredex (DfW software) | ROI: 4×4 mm dimension near the distal edge of the right mental foramen in the DPR was selected and density was calculated. | Trabecular density of the ROI differs significantly between the normal and the osteoporotic group (p=0.033) and the osteoporotic women in the femoral region (FBMD T-Score ≤ -2.5) vs. normal significance (p=0.028). |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Lee; White** 2004 Korea | Cross-sectional Prospective N=66 F= 37 M=29 | Osteoporosis N=4 DXA* | Normal BMD N=7 Osteopenia N=17 | IOPA Kodak using Ektaspeed Plus film | Gray levels/bone pixel intensities Scion image-procession program (Scion Corporation, Frederick, Md) | ROI: the interdental bone between the premolars or first and second molars from the alveolar crest to the level of the apices, excluding the crestal bone and lamina dura. | Clinical information (age) combined with morphologic analysis of IOPA can assist on the screening of low femoral/lumbar BMD. Maxila interdental k= 0.24; (CI = 0, 0.76) & Alveolar k=0.82 (0.66, 0.96) Mandible interdental k=0.79 and 0.46 & Alveolar k=0.73 (0.48, 0.97). |
| **Mostafa et al.** 2016 Egypt | Prospective F=50 | Osteoporosis N=25 Lumbar spine BMD measured by DXA | Normal BMD N=25 | CBCT Planmeca ProMax® 3D Classic | FD analysis Image J software (Image J; US National Institutes of Health, Bethesda, MD) | Circular ROI with 20 pixels diameter below the roots of the premolar and the mental foramen and above the inferior border of the mandible. | FD values not significant difference between groups. The control group showed lower values than the osteoporotic group. Indices mean=1.173 ±0.048 vs 1.199 ± 0.0425 respectively. Negative significant correlation between FD and lumbar spine BMD measured by DXA. p=0.05; r=0.359. |
| **Nemtoi et al.** 2013 Romania | Retrospective N=50 M=28 F=22 | Diabetes Mellitus N=23 Blood sampling-glycated haemoglobin (HbA1c) levels | Non-diabetic N=27 | Panoramic reconstructed from CBCT Planmeca ProMax® 3D Classic | Bone density classified according to the software measurement as D1, D1, D3 and D4. Romexis software (Plammeca, Helsilnk, Finland) | ROI: between mandibular canal to alveolar ridge. Bilateral analysis of the premolar and molar trabecular bone in reconstructed panoramic CBCT image. | Presence of significant correlation between bone quality and values of HbA1c. For High levels of HbA1c, low levels of bone density were found (p=0.00). |
| **Oliveira et al.** 2013 Brazil | Retrospective F=73 | Osteporosis N=35 Bone densitometry | Normal BMD N=38 | DPR Veraview epocs 2D | FD analysis ImageJ 1.44s (National Institutes of Health, Bethesda, MD, USA). | A 230 x130 pixels keeping the same image resolution. Mandible: ROI (1) body: below the apex of the canine, right anteriorly to the mental foramen. ROI (2) angle: below the mandibular canal, posteriorly to the molar. | FD and pixel intensity analysis statistically significant (p<0.05) The mandibular trabecular bone was effective in detecting osteoporotic changes in postmenopausal Brazilian women. |
| **Padbury et al.** 2006 United States | Prospective N=59 F=42 M=17 | PTH N=39 Elevated serum calcium confirmed by concomitant elevation of PTH | Thyroid control patients N=20 | IOPA ND | FD analysis ImageJ v1.34 (National Institutes of Health, Bethesda, MD, USA) | The interdental alveolar bone density - average density of two 1 mm². ROI (1): center of the trabecular crestal bone area (2mm) ROI (2): below the bone crest and at the interradicular area at the middle third of the root length. | The pattern of trabecular bone with no significant differences between groups (P =0.076). There was a trend toward a correlation between the quantitative measurements of alveolar density and the qualitative categorization of trabecular pattern. |
| **Roberts et al.** 2013 United Kingdom | Prospective Cohort F=663 | Osteoporosis BMD by DXA* N=140 | Normal BMD N=423 | DPR ND | Applied classical Haralick bone texture features based on gray-level co-occurrence matrices. | Below the canal location and the small border, superior basal bone above the mandible cortex. | A smaller but significant improvement was obtained when diagnosing osteoporosis at any of the three ROI sites by combining cortical width and similar texture features of the superior basal bone above the cortex. |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Sindeaux et al.*<br><br>2014<br>Brazil | Retrospective Cohort<br>N=133<br>F=84<br>M=49 | Osteoporosis N=87<br>BMD by DXA* | Normal BMD N=46 | DPR Rotograph Plus (Villa Medical System) | FD analysis<br><br>ImageJ 1.45s (National Institutes of Health, Bethesda, MD, USA). | ROI (1): a square of 100 x 100 pixels in the trabecular bone, 2mm anterior to the mental foramen<br>ROI (2): 50 x 50 pixels in the trabecular bone, 2mm anterior to the MF, inside ROI 1<br>ROI (3): 50 x 50 pixels in the trabecular bone, 2mm inferior to the MF. | No significant differences were found between FD on the trabecular bone in men and women with normal BMD and osteoporosis in the ROI 1, 2 and 3 (p=0.621; p=0.276; p=0.896) |
| *Tosoni et al.*<br><br>2006<br>United States | Prospective Cohort<br>F=38 | Osteoporosis N=16<br>DXA by lunar DPX-IQ bone densitometry unit (GE Medical Systems, Madison, WI) | Normal BMD N=1<br>Osteoporotic N=15 | DPR Planmeca Proline PM 2002 CC Panoramic) | FD analysis Image J software (Image J version 1.3v; NIH, Bethesda, MD)<br><br>Pixel intensity analysis Values measured by MetaMorph 4.5r6 software (Universal Imaging Corp., Downingtown, PA) | ROI (1): mandible angle<br>ROI (2): mid-posterior mandible body<br>ROI (3): canine/premolar region, a square in the trabecular bone anterior to the MF and superior to the inner cortical boundary of the inferior cortex. | FD analyses and Pixel intensity analysis failed to differentiate the 3 groups.<br><br>Canine/premolar ROI (3) analysis detected osteoporotic changes when using robust image analysis paradigm. |
| *Yamashita-Mikami et al.*<br><br>2013<br>Japan | Prospective Case-control<br>F=18 | Osteoporosis N=18<br><br>BMD by calcaneal SOS with an ultrasound device (CM-200; Furuno Electric Co., Hyogo, Japan) | Premenopausal N=5<br><br>Postmenopausal Early stage N=3<br><br>Late stage N=10 | micro-CT midsagittal plane<br><br>CT Xvigor Real (Toshiba Medical Systems Co.) | 3D trabecular analysis software (TRI/3DBON; Ratoc System Engineering Co., Tokyo, Japan). Morphometry and densitometry of the alveolar cancellous bone were performed | ROI in the cancellous bone was used for bone morphometry and BMD measurement.<br>Lower region: cancellous bone. | Women's alveolar cancellous bone already begins to deteriorate early after menopause and that the alveolar cancellous bone microstructure and BMD both respond quickly to changes in systemic bone turnover markers (r=0.7 and p<0.05). |

\* Based the World Health Organization criteria (WHO): patients with a BMD standardized T-score value between 1.0 and 2.5 SD were considered osteopenic and those having T-score more than 2.5 SD at three sites are considered osteoporotic.

AAOMS= American Association of Oral and Maxillofacial Surgery; BMD= Bone Mineral Density; BP= Bisphosphonate patients; CCD= Charge-coupled Device; CBCT= Cone Beam Computed Tomography; CMOS= Complementary metal–oxide–semiconductor; CT= Computed Tomography; IOPA= Intraoral Periapical; DPR= Dental Panoramic Radiograph; DP3= Dual Photon Densitometer (Gd153 source); DEXA= DXA=Dual Energy x-ray absorptiometry; f= Levine's test; F=Female; FD= Fractal Dimension Analysis; HU= Hounsfield unit; IOPA=Intraoral Periapical Radiograph; M= Male; MF= Mental Foramen; ND= Not declared; OI= Osteogenesis imperfecta; OR= Odds ratio; p=statistical P value; PTH=Primary Hyperparathyroidism; ROI= Region of Interest; SD= Standard Deviation; SOS= Speed of Sound; t= t-test; T2DM= Type 2 diabetes mellitus; TBA= Trabecular Bone Area; WHO= World M Health Organization

## **Appendix 1** – Databases and Individualized truncations of words

| Database (up to January 8, 2018) | Key Words |
|---|---|
| Cochrane Library<br>http://onlinelibrary.wiley.com/cochranelibrary/search/ | bone mineral density or BMD or Bone density AND maxilla* or mandible* or maxillomandibul* or alveolar* or "dental arch" or "hard palate" AND sensitiv* or sensitivity and specificity or diagnose or diagnosed or diagnoses or diagnosing or diagnosis or diagnostic or diagnosis or diagnostic* or diagnosis, differential or diagnosis |
| Google Scholar<br>www.scholar.google.ca | Bone mineral density OR BMD OR Bone Width AND maxilla OR mandible Including Patents and Citations<br>Sort by "relevance"- 100 1st hits were screened |
| LILACS<br>lilacs.bvsalud.org | Densidade mineral ossea AND complex maxillomandibular OR mandibular OR maxilla<br>Search in English: Bone mineral density AND maxillomandibular complex |
| MEDLINE<br>http://www.ncbi.nln.nih.gov/pubmed<br><br>&<br><br>EMBASE<br>http//embase.com/search | 1-exp jaw/<br>2-(maxilla* or mandible* or maxillomandibul* or alveolar* or "dental arch" or "hard palate").mp.<br>or/1-2<br>4-exp Bone Density/<br>5-((bone adj2 (density or mass)) or BMD).mp.<br>7- 3 AND 6<br>8-limit to "diagnosis (maximizes sensitivity)"<br>9-Diagnosis/ or diagnostic*/ or diagnosis,differential/<br>10-diagnosis.fs.<br>11-(sensitiv* or diagnose or diagnosed or diagnoses or diagnosing or diagnosis or diagnostic).ti,ab.<br>12-specificity.ti,ab.<br>13-exp "sensitivity and specificity"/<br>14- or/9-13<br>15- 7 AND 14<br>16- 8 AND 15<br>17- Case repost.ti.<br>18- 16 AND 17 |
| PubMed<br>http://www.ncbi.nln.nih.gov/pubmed | (((((bone mineral density OR bone density OR bone mass OR BMD))) AND ((maxilla* OR mandible* OR maxillomandibul* OR alveolar* OR "dental arch" OR "hard palate")))) AND ((((sensitiv*[Title/Abstract] OR sensitivity and specificity[MeSH Terms] OR diagnose[Title/Abstract] OR diagnosed[Title/Abstract] OR diagnoses[Title/Abstract] OR diagnosing[Title/Abstract] OR diagnosis[Title/Abstract] OR diagnostic[Title/Abstract] OR diagnosis[MeSH:noexp] OR diagnostic *[MeSH:noexp] OR diagnosis,differential[MeSH:noexp] OR diagnosis[Subheading:noexp]))) |
| Web of Science<br>http://apps.webofknowledge.com | TOPIC: (bone mineral density OR BMD OR Bone density) AND TOPIC: (maxilla* OR mandible* OR maxillomandibul* OR alveolar* OR "dental arch" OR "hard palate") AND TOPIC: (sensitiv* OR sensitivity and specificity OR diagnose OR diagnosed OR diagnoses OR diagnosing OR diagnosis OR diagnostic OR diagnosis OR diagnostic* OR diagnosis, differential OR diagnosis)<br>Timespan=All years<br>Search language=Auto |

*Refined search not included in the last selection because the result was 0. Selection of the articles was being done manually.

**Appendix 2** - Excluded articles and reasons for exclusion (N=34)

| Author | Reasons for Exclusion* |
|---|:---:|
| Atalaya et al., 2016 | 4 |
| Cakur et al., 2010 | 4 |
| Damilakis; Vlasiadis, 2011 | 4 |
| Drozdzowska et al., 2002 | 4 |
| Gearets et al., 2008 | 2,4 |
| Geibel et al., 2016 | 1 |
| Govindraju; Chandra, 2014 | 4 |
| Horner et al, 1996 | 2 |
| Horner; Devlin, 1992 | 2 |
| Jagelaviciene et al., 2010 | 4 |
| Kavitha et al., 2015 | 4 |
| Kavitha et al., 2016 | 2 |
| Klemetti et al., 1993a | 4 |
| Klemetti et al., 1993b | 4 |
| Klemetti et al., 1994 | 4 |
| Klemetti et al., 1997 | 5 |
| Kribs, 1990 | 5 |
| Muramatsu et al., 2016 | 4 |
| Naitoh et al., 2007 | 4 |
| Naitoh et al., 2014 | 4 |
| Neves et al., 2012 | 7 |
| Passos et al., 2012 | 4 |
| Saviv Pavicin et al., 2014 | 4 |
| Polat et al., 2002 | 6 |
| Shintaku et al., 2013 | 4 |
| Stagraczynski et al., 2016 | 4 |
| Taguchi et al., 1996 | 4 |
| Taguchi et al., 2003 | 4 |
| Taguchi et al., 2006 | 4 |
| Taguchi et al., 2008 | 4 |
| Taniguchi et al., 2016 | 2 |
| Torres et al., 2012 | 4 |
| Torres et al., 2015 | 4 |
| Venela et al., 2012 | 7 |

*1- Ex-vivo human studies; 2- No systemic disorders; 3- Combined developmental diseases; 4- Bone mineral density exclusively assessed from cortical bone; 5- Case reports; 6- Letters, reviews, conference paper or editorial; 7- Trabecular bone evaluated solely by visual assessment.

**Appendix 3** - Evidence level assessment

## Dental imaging for trabecular bone density screening: a systematic review

**Patient or population**: adults, children and adolescents

**Setting**: patients with systemic disorders

**Intervention**: dental imaging

**Comparison**: reference standard for each systemic disorder

| Outcomes | Anticipated absolute effects* (95% CI) | Relative effect (95% CI) | № of participants (studies) | Certainty of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|
| | **Risk with reference standard for each systemic disorder** | | | | |
| Trabecular bone structure alteration | Trabecular bone structure alteration | not estimable | 1516 (observational studies, n=14) | ⊕⊕◯◯ LOW | |

**\*The risk in the intervention group** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI). **CI:** Confidence interval

**GRADE Working Group grades of evidence**
**High certainty:** We are very confident that the true effect lies close to that of the estimate of the effect
**Moderate certainty:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different
**Low certainty:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect
**Very low certainty:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect

## Appendix 4 - Risk of Bias in Individual Studies - QUADAS 2 criteria fulfilled

| | Item | Kayipmaz et al., 2017 | Mostafa et al., 2016 | Apolinario et al., 2016 | Chai et al., 2014 | Sindeaux et al., 2014 | Kathirvelu; Anbu,rajan, 2014 | Khojastehpour et al., 2013 | Nemtoi et al., 2013 | Oliveira et al., 2013 | Roberts et al., 2013 | Yamashita-Mikami et al., 2013 | Tosoni et al., 2006 | Padbury et al.,2006 | Lee and White, 2005 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Domain 1: Patient Selection** | Was a consecutive or random sample of patients enrolled? | N | U | N | U | N | Y | Y | Y | N | U | Y | U | N | N |
| | Was a case-control design avoided? | N | N | Y | Y | N | N | N | N | N | Y | N | Y | N | Y |
| | Did the study avoid inappropriate exclusions? | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | U | Y |
| | **Could the selection of patients have introduced bias?** | L | L | L | L | L | L | L | L | L | L | L | L | L | L |
| | **Concerns regarding applicability:** Is there concern that the included patients do not match the review question? | L | L | L | L | L | L | L | L | L | L | L | L | L | L |
| **Domain 2: Index Test** | Were the index test results interpreted without knowledge of the results of the reference standard? | U | U | U | U | N | U | U | U | N | U | Y | U | Y | U |
| | If a threshold was used, was it pre-specified? | U | U | U | Y | U | Y | Y | U | Y | Y | U | Y | Y | U |
| | **Could the conduct or interpretation of the index test have introduced bias?** | U | U | U | L | L | L | L | L | L | L | L | L | L | U |
| | **Concerns regarding applicability:** Is there concern that the index test, its conduct, or interpretation differ from the review question? | L | L | L | L | L | L | L | L | L | L | L | L | L | L |
| **Domain 3: Reference Standard** | Is the reference standard likely to correctly classify the target condition? | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| | Were the reference standard results interpreted without knowledge of the results of the index test? | U | U | U | U | N | U | U | U | N | Y | Y | Y | N | U |
| | **Could the reference standard, its conduct, or its interpretation have introduced bias?** | L | L | L | L | L | L | L | L | L | L | L | L | L | L |
| | **Concerns regarding applicability:** Is there concern that the target condition as defined by the reference standard does not match the review question? | L | L | L | L | L | L | L | L | L | L | L | L | L | L |
| **Domain 4: Flow and Timing** | Was there an appropriate interval between index test(s) and reference standard? | U | U | U | Y | U | Y | Y | Y | U | Y | U | U | U | U |
| | Did patients receive the same reference standard? | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| | Were all patients included in the analysis? | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| | **Could the patient flow have introduced bias?** | L | L | L | L | L | L | L | L | L | L | L | L | L | L |

# 5 ARTICLE 2

**Title: Trabecular and cortical mandibular bone structure in Familial Adenomatous Polyposis patients**

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*Article presented and formatted as submitted at the Scientific Reports journal, with minor grammar corrections and adaptations.*

## Authors' contributions

C.P-P. was responsible for conceptualization, investigation, formal analysis of the collected data and was a major contributor in writing the original draft. Y.S-B. was responsible for the measurements and the background literature. F.T.A. was responsible for data curation, review and editing. H.G. assisted in the interpretation of the results. A.F.L. and E.N.S.G were responsible for conceptualization, study design and manuscript revision. All authors read and approved the manuscript.

**Additional information**

**Abstract**

To address bone changes in rare disease patients, this study evaluates the mandibular cortical and trabecular bone pattern in patients with Familial Adenomatous Polyposis (FAP) through dental panoramic radiomorphometric indices and fractal dimension (FD). Dental panoramic radiographs (DPRs) were obtained from FAP patients and healthy paired controls. The parameters analyzed were: FA of the trabecular bone in four different regions of interest (ROI), mandibular cortical index (MCI) and mandibular cortical width (MCW). Sixty panoramic radiographs, from FAP patients (n=15) and non-FAP (n=45) were investigated. Fractal values were lower (5%) for the FAP group. Statistical significance difference was shown by the ROI 2 and 3 anteriorly to the mental foramen bilaterally, $p$=0.001 and $p$=0.006. The ROI 1 and 4, at the mandibular angle trabeculae, indicated statistical significance on the right side ($p$=0.036) and no difference on the left side ($p$=0.091). There was no significant difference in MCI and MCW when the groups were compared, MCW $_{(L)}$ $p$=0.247, and MCW $_{(R)}$ $p$=0.070. Fractal values of FAP patients' mandibular trabecular bone were lower than healthy controls. The radiomorphometric indices MCI and MCW were not useful for the analysis of the cortical bone pattern. Therefore, FD is a possible tool for bone structure evaluation in DPRs and supports the appropriate referral of FAP patients.

**Keywords:** Familial Adenomatous Polyposis. Trabecular bone. Mandibular cortical bone. Fractal Dimension. Panoramic radiograph.

**Introduction**

Colorectal cancer (CRC) is the third cause of cancer-associated death worldwide and responsible for almost 9% of all deaths (Fitzmaurice et al., 2019). FAP is an autosomal dominant disorder caused by mutations in the Adenomatous Polyposis Coli gene (*APC*) (Half et al., 2009; Miclea et al., 2010). Its prevalence is estimated in 1 case for 6,800-29,000 people, in whom 1% of CRC cases are caused by *APC* mutation (Fitzmaurice et al., 2019). Clinically, nearly 100% of adult FAP patients develop multiple colorectal adenomas, in most cases, by the fourth decade of life, leading to a deteriorated quality of life and survival rate (Half et al., 2009; Aihara et al., 2014; Almeida et al., 2016). It is estimated that 11 to 25% of the FAP cases will occur for the first time due to *de novo* mutations, without a family history (Ripa et al., 2002).

Besides the typical colorectal alterations, FAP patients present several well-known extraintestinal manifestations (Gardner, 1962; Half et al., 2009; Miclea et al., 2010). Amongst these, the literature describes classic dental and osseous alterations of the jaws (Gardner, 1962; Septer et al., 2018). A systematic review revealed the frequency of focal areas of sclerotic bone/osteosclerosis (65.3%) was meaningful (Almeida et al., 2016). Interestingly, studies have been demonstrated that FAP patients with heterozygous *APC* mutations may present increased bone mineral density (BMD) (Miclea et al., 2010; Chew et al., 2012). The *APC* mutation's role in the alteration of the bone turnover is through β-catenin regulation and activation of the Wnt-protein pathway resulting in increased bone deposition (Holmen et al., 2005).

Maxillomandibular complex bone pattern changes are commonly detected during routine dental examinations. The dental panoramic radiograph (DPR) is one of the most common extraoral dental imaging modalities. Besides, the DPR is considered a very useful tool to opportunistically identify systemic disorders (Pacheco-Pereira et al., 2019). Although several studies are evaluating osseous alterations detected via DPR, these mostly observed differences result of low BMD in a systemic condition such as osteoporosis (Leite et al., 2010). Given that, authors have established radiomorphometric indices and fractal dimension (FD) analysis for assessment of the cortical and trabecular bone patterns using dental imaging (Taguchi et al., 1996; Gomes et al., 2014; Sindeaux et al., 2014; Mostafa et al., 2016). The FD is the most commonly used mathematical tile-counting method to assess

the bone architecture (Geraets; van der Stelt, 2000). Previous studies established the bone texture evaluation of patients with multiple conditions (Leite et al., 2010; Apolinário et al., 2016; Pacheco-Pereira et al., 2019). The method is a promising and cost-effective tool used to predict the complex trabecular bone architecture (Ergün et al., 2009).

The trabecular bone pattern of FAP patients is affected by osseous manifestations. It is accepted that the high incidence of sclerotic osseous lesions in the maxillomandibular complex of FAP patients is related to the aforementioned mechanisms, while *APC* mutation slightly increases BMD (Miclea et al., 2010). Although this skeletal effect may occur in FAP patients, the tumour burden is a crucial factor underlying BMD decline and bone fragility in CRC patients. Therefore, the positive effect of *APC* mutation on bones might be abrogated by elevated intestinal tumor burden. Thus, patients with CRC may even present decreased BMD and elevated fracture risk (Saul et al., 2019). To our knowledge, there is no available data on the pattern of the mandibular bone in FAP patients showing the influence of this condition on the mandibular bone turnover. Therefore, we aim to evaluate the mandibular cortices and trabecular bone pattern in subjects with FAP and matched controls through DPR radiomorphometric indices and FD.

**Material and Methods**

The Ethics Committee of the Health Sciences Faculty at the University of Brasilia approved the study protocol number 493.502. The matched case-control study was carried out following the Declaration of Helsinki and planned to analyze the mandibular bone structure of FAP patients through dental radiographs.

***Study population: Familial adenomatous polyposis and a matched-control group***

An available pool of patients that performed DPRs at the Oral Care Center, University Hospital of Brasilia was assessed. Group 1 was composed of DPRs of FAP patients diagnosed by the standard of care, with no associated metabolic diseases. These patients presented a classic FAP with more than 100 polyps. Five patients of our sample had previously been identified with a heterozygous mutation in the *APC* gene. Their genetic characterization was recently detailed by Almeida et al. (2020).

Group 2 was composed of non-FAP individuals, with no risk of FAP due to familial expression, absence of metabolic bone diseases, neither a declared developmental and genetic disease. Due to the nature of this rare population, convenience sampling was adopted. The groups were matched by nationality, sex and age on a proportion of one FAP to three healthy controls; both groups were composed of dentate individuals. DPRs were excluded from the sample, whether these resulted from 3D techniques reconstruction or presenting inadequate positioning during acquisition that could compromise the evaluation of findings. As well, inadequate radiographs with ghost images superimposition, local bone reactions/dense lamellar bone associated with inflammation that could alter the areas of interest or interfere in the evaluation were also excluded.

Clinical characteristics from 10 FAP patients were described in a previous study (Almeida et al., 2020). The newest recruited FAP patients (n=5) received a paper-pamphlet containing all the information regarding the research and the informed consent to be signed. They had access to the research project team for further contact if more clarification was needed.  The selected DPRs were taken in one of these three machines, the analogic Rotograph Plus (Villa Sistemi Medicalli, Buccinasco, Milan, Italy) and the digital systems from Kodak 8000C (Digital Panoramic, Trophy, France) and Orthophos CD (TS, Dentsply Sirona, Germany). The patients were positioned for acquisition following a standardized protocol in which the vertical orientation line was aligned with the patient mid-sagittal plane and the horizontal one based on the Frankfort plane parallel to the floor; the technicians were calibrated by the same institution and Oral and maxillofacial radiologist (OMR).

For training purposes, an OMR (Evaluator 1) and a 5th-year dental student experienced in FA (Evaluator 2) did the measurements independently using the same computer (Lenovo 15.6" Intel Core i5-7200U 8GB RAM 1TB HDD Windows 10 LED HD resolution 1366 x 768) at two different times, within a 1-week washout interval following a computer-generated randomized list of 10 DPRs. Both observers were blinded to the diagnosis of FAP, risk, or normality status of patients. One evaluator made the final FD and the mandibular cortical measurements in all 60 DPRs.

*The fractal dimension analysis*

Trabecular bone areas designated as Regions of Interest (ROI) were chosen for the FD analysis, as per Figure 1. These were standardized as a square of 100 x 100 pixels and were selected based on previously validated methods as follows. ROI 1: the area above the mandibular angle, below the right mandibular canal, and posterior to the molar region, to avoid interference of the masticatory stress in the trabecular bone; (Oliveira et al., 2013; Roberts et al., 2013; Apolinário et al., 2016). ROI 2: in the trabecular bone, 2 mm anterior to right mental foramen; (Taguchi et al., 1995; Sindeaux et al., 2014). ROI 3: in the trabecular bone, 2 mm anterior to left mental foramen; (Kathirvelu; Anburajan; 2014; Sindeaux et al., 2014). ROI 4: the area above the left mandibular angle, below the mandibular canal, and posterior to the molar's region (Oliveira et al., 2013; Apolinário et al., 2016). These areas were cautiously selected to avoid overlapping of anatomical landmarks such as mandibular canal and cortical bone, and usual radiographic findings of FAP patients such as osteomas, idiopathic osteosclerosis, and supernumeraries.

All digital DPRs (n=11) were stored with a matrix of 7008 x 2975 pixels; the analogic ones (n=46) were digitalized using the same scanner (Epson 1680 Pro; Seiko Epson Corporation, Japan), with 8-bit grayscale, 300 dpi resolution and stored using the same parameters. The trabecular bone structure was analyzed using Image J 1.52a (US National Institute of Health, public domain software available at http://rsbweb.nih.gov/ij). The images were processed similarly to a methodology validated by previous studies, especially following the methods of studies from the same research group (Sindeaux et al., 2014; Apolinário et al., 2016) and based on the steps validated by White and Rudolph (1999). Figure 2 details the FD analysis sequence adopted by this study.

*The mandibular cortical index (MCI) assessment*

The qualitative assessment of the right and left mandibular cortical bone were made according to the well-established MCI classification validated by Klemetti et al. (1994). MCI categorizes the appearance of the inferior mandibular cortical thickness as per Figure 3. Classified as C1: the endosteal margin of the cortex is even and sharp on both sides, C2: the margin shows semilunar defects, lacunar resorption appearance and/or it seems to form endosteal cortical residues in one or both sides, C3: the cortical layer forms heavy endosteal

residues. The MCI analysis was performed bilaterally and not allowed to adjust image brightness and contrast to prevent possible interferences.

### *The mandibular cortical width (MCW) measurement*

The quantitative measurements of the MCW followed Taguchi et al. (1996) validated protocols. The ultimate measurement was defined based on the MCW of the mandible body on both sides. 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 were drawn to define the slope and position of the mandibular axis 2D. All indices were measured along this extension line presented in Figure 3.

### *Statistical analysis*

A reliability test was applied at two different times, within a 1-week washout interval during training and following the final sample measurements. The Intraclass Correlation Coefficient (ICC) was interpreted by Portney and Watkins guidelines (2009). Extreme values of the observed variables that were not normally distributed or homoscedastic were checked with Cook's Distance. The outcomes of the ROI were independently analyzed, left and right sides were assessed. Age, FD measurements, MCI, and MCW, were compared between FAP and control groups by parametric Student t-test. The number of teeth was compared between both groups by Mann-Whitney non-parametric test. The Chi-square statistic verified the distribution of the MCI based on the observed and 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 (SPSS Statistics 24 software, IBM, Armonk NY) was used for statistical analysis.

### Results

Of the 60 investigated DPRs, 15 were from FAP patients and 45 matched controls. FAP group was composed of 33.3% of females (n=5) and 77.7% of males (n=10), with a mean age of 37.2 years (SD 15.79). The non-FAP group had 15 females and 30 males, with a mean age of 38.3 years (SD 15.55), $p$=1.000. Regarding the number of teeth, there was

no significant difference between FAP patients (median = 26 teeth, 6-32 teeth) and the control group (median = 28 teeth, 11-32 teeth), $p$ = 0.13 A different dentition status could interfere in the trabecular pattern (Supplementary Table S1).

### Strong agreement and consistency between evaluators

During training, the intra-rater reliability coefficient was as follows: evaluator 1, ICC=0.958 (CI= 0.846-0.989) and evaluator 2, ICC= 0.98 (CI= 0.917-0.995). The tow evaluators's excellent inter-rater reliability is confirmed by an ICC = 0.992 (CI= 0.976-0.998). Also, a Cronbach's alpha at 0.991 was found when the intra and inter-reliability were considered. For the final sample measurements, the evaluator consistency shows an almost perfect agreement. MCI = 0.967 (CI 0.945-0.980), $MCW_{(R)}$ = 0.995 (0.991-0.997) and $MCW_{(L)}$ = 0.983 (CI 0.972-0.990). For the FD analysis, there was no variation between the first and second fractal values, resulting in an ICC=100%.

### Fractal dimension values were lower in patients with FAP

The FD analysis value reflects how much a fractal completes the trabecular bone spaces; it is considered a texture evaluation. Previous authors established that a high FD correlates to a greater bone complexity, and a lower FD is indicative of a simpler structure (Sánchez; Uzcátegui, 2011). The present results indicate that FD measurements may differentiate FAP patients from individuals considered being healthy and having a normal trabecular bone. Table 1 presents the mean FD values for each ROI and the comparison between groups. The mean FD values of FAP were lower than that of the non-FAP in ROI 2 (1.162 SD 0.076, $p<0.005$), ROI 3 (1.184 SD 0.101, $p=0.006$) and ROI 4 (1.137 SD 0.089, $p=0.036$). A more expressive difference was found for ROI 2, and no statistical difference was found in ROI 1 (1.193 SD 0.067, $p=0.91$).

### No difference in the MCI and MCW between groups

The mandibular cortex was associated with an ordinal classification of the morphology of the inferior cortex (Klemetti; Kolmakow, 1997). The MCI is claimed to have a very high diagnostic capability compared to the reference-standard technique for estimating of the BMD, the dual X-ray absorptiometry (DXA).

Table 2 presents the MCI distribution in two groups, Group I showed 12 FAP cortical qualitatively classified as C1, and 20% of the sample presenting semilunar defects - C2. The matched controls had 75.6% of the mandibular cortices as C1, 20% as C2 and 4.4% as being porous - C3. The MCI was not significant when both groups were compared, $p$=0.706 and $x^2$= 0.696. The C1 degree was the most frequent in both groups ($p$=0.706).

The MCW and the morphology of the mandible's inferior cortex are considered the best predictors of BMD in panoramic radiographs (Taguchi et al., 1996). A common ground showed by previous studies is an MCW lower than 4mm in healthy individuals (Demontiero et al., 2012). Besides, the cortical bone dimensional changes were associated with systemic diseases (Apolinário et al., 2016; Iwata et al., 2017; Kurşun-Çakmak; Bayrak, 2018). For FAP, the mean MCW $_{right (R)}$= 3.399(SD 0.506) and MCW $_{left(L)}$= 3.382 (SD 0.678). The non-FAP showed a mean MCW$_{(R)}$= 3.368(SD 0.732) and MCW$_{(L)}$= 3.718 (SD 0.589). These values showed no statistical significance for both sides when the groups were compared, MCW$_{(L)}p$=0.247 and t=1.170 and for MCW$_{(R)}$ t =1.848 and $p$=0.070, see Table 3.

Considering the patients' sex, no significant differences were found for all radiomorphometric indices and fractal values in FAP, p>0.074. In the non-FAP, significant differences between males and females were only found for MCWR (p=0.003) and MCWL (p=0.021) values. When Table 2 is compared to Supplementary Tables S3 and S4, we assume that FD variability is higher in the male group influenced by age. Age in male groups presented significantly higher variance when compared to age in female groups. On the other hand, the mean age between male (42.5 years) and female (38.6 years) were not statistically different, $p$>005. See Supplementary Tables S2, S3, S4, and S5.

**Discussion**

Although the extraintestinal manifestations of FAP, specifically the osseous and dental alterations, were addressed previously (Gardner, 1962; Thakker et al., 1995; Miclea et al., 2010; Almeida et al., 2016; Septer et al., 2018), this study adds unpublished and relevant knowledge while evaluating the mandibular cortical and trabecular radiomorphometric indices of FAP and matched healthy patients in DPRs.

For the fractal analysis, we have selected the ROI 100 x 100-pixel size (4.23mm) based on validated fractal studies (Parkinson; Fazzalari, 2000; Huh et al., 2011). These studies showed that FD of the mandibular trabecular bone has optimal characterization when the tile size ranges from 0.025 to 4.25mm. While the BMD is frequently used in studies investigating bone mass changes, the FD values are related to the bone texture and the complexity of patterns. It is essential to understand that FD is independent of the BMD, not synonymous. The BMD scores represent the disruption of the bone structure and replacement of the cancellous bone by non-collagenous proteins and monitor bone fragility (Demontiero et al., 2012). In FAP, the structural alterations in bone were investigated in animal studies. As shown by Holmen et al. (2005), *APC* mutated mice developed a bone in which the vast majority of the marrow component is absent; they found that the *APC* mutation was linked to a dramatically increased bone deposition with its architecture disturbances(Holmen et al., 2005). The mean fractal values found in our sample confirm the assumption of FAP patients having altered mandibular bone architecture.

The fractal values of FAP patients were generally smaller when compared to controls. Areas anterior to the mental foramen showed significant differences (*p*=0.001 and *p*=0.006) when both groups were compared. In agreement with a previous study using a smaller ROI at the same location, this area demonstrated an altered bone structure when comparing osteoporotic and healthy patients (*p*=0.03) (Khojastehpour et al., 2013). However, fractal values can be contradictory when the same disease is investigated. Another study found no significance on a larger squared ROI (*p*=0.621) on osteoporosis (Sindeaux et al., 2014). This variation could be due to disagreement of the results in their studies could be due to Anatomical variations, discrepancies in the methods, FA techniques and/or the differences in selecting the regions to be measured could be all considered responsible for their results discrepancy. The second area explored by our study, the trabecular bone at the angle of the mandible bilaterally, showed a difference in the left side (*p*=0.036) between the two groups. In spite of the lack of statistical significance on the right side, we still consider the angle of the mandible an adequate area to assess the bone structure; this, in agreement with previous studies (Oliveira et al., 2013; Roberts et al., 2013; Apolinário et al., 2016).

Furthermore, our study carefully eliminated the areas affected by the radiographic findings and normal anatomical variants to prevent misrepresentation of the mandibular trabecular structure represented by the fractal values (Chappard et al., 2005). The ROIs

explored by this study are considered good predictors of bone texture (Taguchi et al., 1995; Leite et al., 2010; Oliveira et al., 2013; Roberts et al., 2013; Kathirvelu; Anburajan, 2014; Sindeaux et al., 2014; Apolinário et al., 2016). Once these areas are demarcated, the methodology followed is computerized and carries an error-free calculation. For this reason, the one calibrated-evaluator measured the entire sample twice without differences, the evaluator consistency tends to be perfect, and the double measurement is questionable.

The *APC* suppressor effects in the cancellous bone were described by Miclea et al. (2010). FAP patients display increased mean BMD, maybe because of *APC* gene regulates bone density. The increased BMD is possible due to the inhibition of the β-catenin that regulates the pathophysiology of bone formation and disorders (Chew et al., 2012). The increased accumulation of bone matrix results from the activation of the Wnt/β-catenin pathway that promotes osteoblast differentiation, leading to bone mass acquisition. Hence, the typical architecture bone structure is altered, and the trabecular structure becomes less complex, resulting in lower FD of trabecular bone projection texture values, as observed in our analysis. The FAP fractal values were approximately 5% lower than the controls. Studies exploring low BMD also show lower fractal values, the bone pattern disruption rationale is not similar, but the bone texture caused by bone cell disbalance will decrease fractal values. Interestingly, CRC and osteoporosis represent two global challenges in public health (Unnanuntana et al., 2011; Fitzmaurice et al., 2019).

The radiomorphometric indices did not show differences between groups. Previous studies investigating systemic diseases found MCW values higher than those considered in the normal range (MCW < 4mm) (Leite et al., 2010). Considering our sample patients in our sample are relatively young (~37 years), a distinct scenario could be seen with aging individuals since the bone turnover tends to disbalance (Demontiero et al., 2012). By experiments in CRC mice showed the integrity of the cortical bone. This could also be due to the bone turnover or a short treatment time causing cancellous bone alterations and not affecting the cortical bone (Hamdani et al., 2008).

Our study also aims to create awareness in both the dental and medical community, so that these results could be translated clinically. Despite previous literature on FAP patients (Gardner, 1962; Septer et al., 2018), the utility of the DPRs may surpass the dental evaluation; the opportunistic evaluation of the already taken DPR is essential for the surveillance of systemic conditions and osseous manifestations (Pacheco-Pereira et al.,

2019; Kato et al., 2020). The clinical importance of this study for FAP patients is the early detection of osseous disorders, as the lower fractal numbers (5% lower in FAP) found in our study showed the expected trabecular texture disruption. These values represent a loss on the bone texture even though radiographically, the findings are resultant from bone accumulation. Once the radiographic inspection of the trabecular bone is performed, a base-pattern analysis should be investigated clinically.  Thus, the radiographic findings should be attentively correlated to the medical history and further investigated if a systemic disorder is suspected. We emphasize the selection criteria for radiographs (American Dental Association Council on Scientific Affairs, 2006), DPRs explicitly not recommended specifically for FAP surveillance, but rather that the dentists evaluate radiographs taken for dental purposes for this condition as well. If the condition is suspected, referral to a physician is advised. Confirmed FAP children and adults should undergo a colonoscopy assessment before genetic testing (Herzig et al., 2017). Preventative measures such as the evaluation of existent DPRs could assist in the early detection of CRC to reduce its incidence by approximately 55% and improve their survival rates (Heiskanen et al., 2000; Fitzmaurice et al., 2019).

Pointing out the limitations of this study, we should consider that few DPRs were conventional film-based and the majority were acquired digitally. The conventional ones were digitalized and imported to Image J using the same spatial parameters and resolution to minimize the difference. For analysis, all radiographs' parameters were standardized. Besides, DPRs represent a 2D image of a 3D trabecular structure. A 3D analysis will undoubtedly give more valuable information; however, cone beam computed tomography is not a method routinely used for a radiographic follow for the FAP patients due to the ionizing radiation dose concerns and radiation safety precautions.

As a future approach, the correlation of FAP patients' fractal analysis with the BMD through DXA could elucidate and reveal the likelihood or risk of bone fracture or possible fracture protection in FAP patients. The standardization of the ROI pixel size in the mandibular body and fractal analysis parameters could ease the comparison of the results between distinct research projects.

**Summary and Conclusion**

Routinely taken panoramic radiographs are essential for monitoring and surveillance of systemic conditions. By creating awareness in the dental community, our study could be clinically translated by implementing the evaluation of the mandible trabeculae's evaluation. Preventive measures reduce colorectal cancer incidence and improve the survival rates of FAP patients.

The analysis of the trabecular bone structures of FAP patients showed that most fractal values of the mandibular trabecular bone were 5% lower than the matched controls. Therefore, the FA could be possibly considered as a tool for evaluating of the bone structure in these patients. The radiomorphometric indices MCI and MCW were equal for the FAP and non-FAP groups.

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**Figure 1 - Schema on the elected regions of interest (ROI).** ROI 1: Right mandibular angle; ROI 2: In the trabecular bone, 2mm anterior to right mental foramen; ROI 3: In the trabecular bone, 2mm anterior to left mental foramen; ROI 4: Left mandibular angle
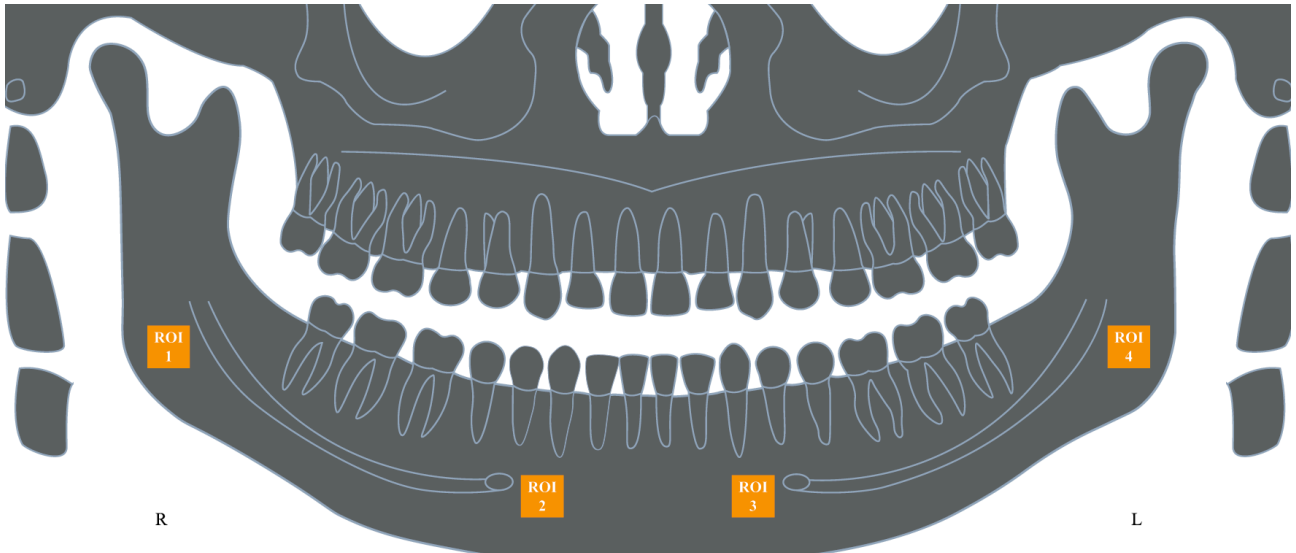
**Figure 2 - Fractal Dimension analysis sequence adopted by this study.**FD adopted methodology. The ROIs were standardized as a square of 100 x 100 pixels, cropped and duplicated (A). The duplicated image was blurred with a gaussian filter (sigma, 35) to remove large-scale variations in brightness on the image (B). The blurred image was subtracted from the original ROI image and a gray value of 128 was added at each pixel location (C). 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 (D). The image was eroded and dilated to reduce the noise (E). After dilation, the image was skeletonized (F), and the FD analysis pursuit (G). The FD was calculated by the box-counting method (H); the widths of these square boxes were 2, 3, 4, 6, 8, 12, 16, 32 and 64 pixels (I)
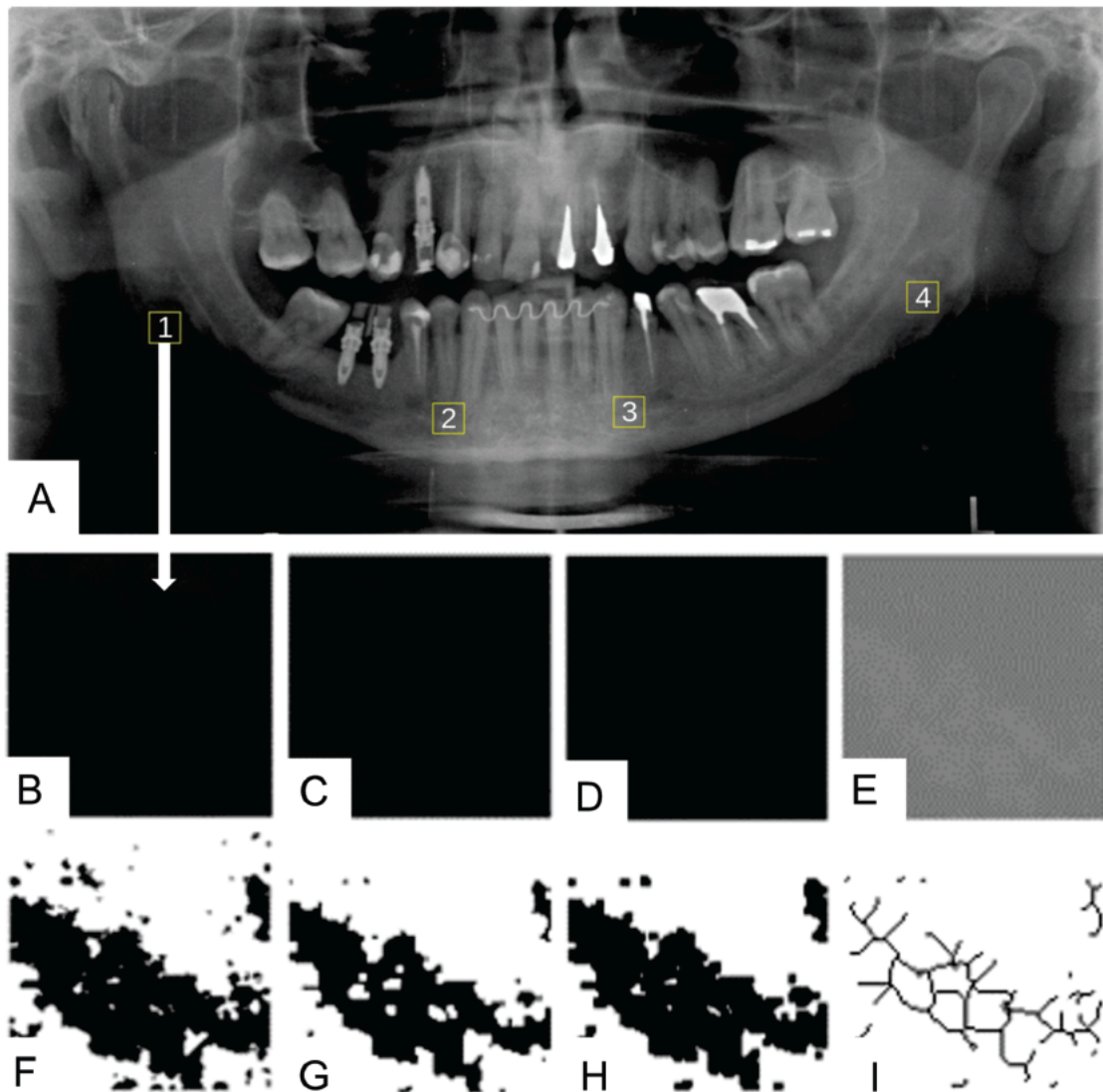
**Figure 3 - Panoramic radiograph representing the MCI assessment and tracing for MCW measurement.** The left cropped DPR shows the inferior cortical border of the mandible that was evaluated qualitatively as C1=considered homogeneous, C2= mild erosions and C3= multiple erosions appearance, clearly porous. The right cropped DPR radiograph showing the mandibular cortical width index. The mental foramen is delimited on the radiograph, and two parallel lines are drawn to demarcate the upper and lower edges of the mandibular cortex. A third line is drawn in the center of the mental foramen and perpendicular to the two cortical lines. The cortical width is determined by measuring the parallel line that surrounds the two structures
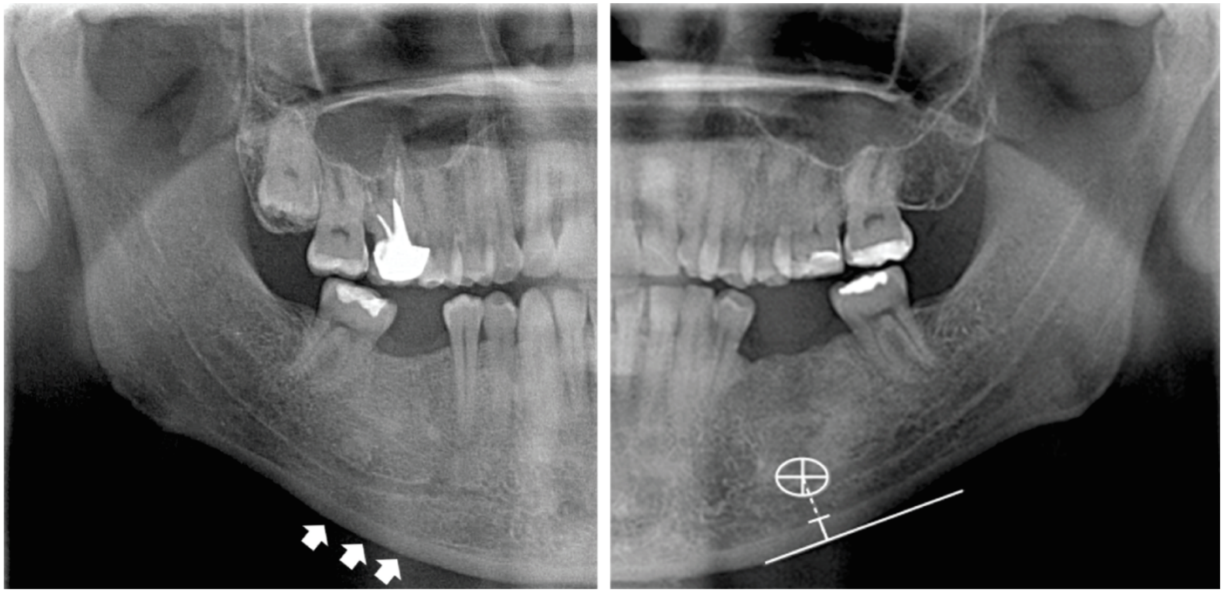
**Table 1-** Mean Fractal Dimension on each trabecular area in the FAP and non-FAP groups

| | FAP (n = 15) | | non-FAP (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 | <0.005* |
| 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* |

NS: *p*>0.05; *p*<0.05: Significant; 't' = Student t test.

FD= Fractal Dimension; NS=Not Significant; ROI= Region of Interest; ROI 1: Right mandibular angle; ROI 2: In the trabecular bone, 2mm anterior to right mental foramen; ROI 3: In the trabecular bone, 2mm anterior to left mental foramen; ROI 4: Left mandibular angle; SD=Standard Deviation.

**Table 2** - Distribution of Mandibular Cortical Index in the two groups.

| MCI | FAP (n = 15) | | non-FAP (n = 45) | | $x^2$ value | $p$ value |
|---|---|---|---|---|---|---|
| | N | % | N | % | | |
| C1 | 12 | 80.0 | 34 | 75.6 | 0.696 | 0.706; NS |
| C2 | 3 | 20.0 | 9 | 20.0 | | |
| C3 | - | - | 2 | 4.4 | | |
| Total | 15 | 100.0 | 45 | 100.0 | | |

Results are given as percentages. $p$-values were determined using Chi-Square Test ($x^2$); NS: $p>0.05$.

C1: The endosteal margin of the cortex is even and sharp on both sides; C2: The margin shows semilunar defects, lacunar resorption appearance and/or it seems to form endosteal cortical residues in one or both sides; C3: The cortical layer forms heavy endosteal residues and it is clearly porous; MCI= Mandibular Cortical Index; N=Number; NS=Not Significant.

**Table 3 -** Mandibular cortical width in the FAP and non-FAP groups

| | FAP (n = 15) | | non-FAP (n = 45) | | 't' value | *p* value |
|---|---|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD | | |
| MCW (R) | 3.399 mm | 0.506 | 3.638 mm | 0.732 | 1.170 | 0.247; NS |
| MCW (L) | 3.382 mm | 0.678 | 3.718 mm | 0.589 | 1.846 | 0.070; NS |

Results are given as mean ± SD. p-values were determined using Student t test; NS: p>0.05.

L=Left Side; MCW=Mandibular Cortical Width; mm= millimeters; NS=Not Significant.; R= Right Side.

**SUPPORTING INFORMATION**

**Table S1**- Teeth Number of Individuals in the FAP and non-FAP groups

|  | Number of teeth | | Median | CI | *p* value |
|---|---|---|---|---|---|
|  | Lowest | Highest |  | 95% |  |
| FAP (n=15) | 6 | 32 | 26 | 18.3 to 30.2 |  |
| Non-FAP (n=45) | 11 | 31 | 28 | 27.0 to 29.3 | 0.13; NS |

P-values were determined using Mann-Witney test; NS: *p* > 0.05.

CI=Confidence interval; NS=Not Significant.

**Table S2 -** Comparing Mean MCW, MCW and FD-ROI between sex in the FAP group

|  | 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 |

"t" = Student t test; NS: *p*> 0.05.

CI=Confidence Interval; FD=Fractal Dimension; L=Left Side; MCI=Mandibular Cortical Index; MCW=Mandibular Cortical Width; NS=Not Significant.; R= Right Side; ROI=Region of Interest; ROI 1= Right mandibular angle; ROI 2= In the trabecular bone, 2mm anterior to right mental foramen; ROI 3= In the trabecular bone, 2mm anterior to left mental foramen; ROI 4= Left mandibular angle; SD=Standard Deviation.

**Table S3 -** Comparing Mean MCW, MCW and FD-ROI between sex in non-FAP group

| | 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 |

"t" = Student t test; NS: *p*> 0.05. *p<0.05; Significant

CI=Confidence Interval; FD=Fractal Dimension; L=Left Side; MCI=Mandibular Cortical Index; MCW=Mandibular Cortical Width; NS=Not Significant.; R= Right Side; ROI=Region of Interest; ROI 1= Right mandibular angle; ROI 2= In the trabecular bone, 2mm anterior to right mental foramen; ROI 3= In the trabecular bone, 2mm anterior to left mental foramen; ROI 4= Left mandibular angle; SD=Standard Deviation.


**Table S4** - Comparing Mean MCW, MCW and FD-ROI between FAP and non-FAP males

| | FAP (n = 5) | | Non-FAP (n = 15) | | 't' value | *p* value |
|---|---|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD | | |
| MCW (R) | 3.540 | 0.587 | 4.085 | 0.559 | 1.864 | 0.079; NS |
| MCW (L) | 3.404 | 0.546 | 4.001 | 0.446 | 2.462 | 0.024* |
| FD-ROI 1 | 1.209 | 0.095 | 1.219 | 0.051 | 0.292 | 0.774; NS |
| FD-ROI 2 | 1.131 | 0.094 | 1.257 | 0.051 | 3.842 | 0.001* |
| FD-ROI 3 | 1.214 | 0.094 | 1.261 | 0.052 | 1.421 | 0.172; NS |
| FD-ROI 4 | 1.079 | 0.086 | 1.239 | 0.092 | 3.449 | 0.003* |

"t" = Student t test; NS: *p*> 0.05. *p<0.05; Significant

CI=Confidence Interval; FD=Fractal Dimension; L=Left Side; MCI=Mandibular Cortical Index; MCW=Mandibular Cortical Width; NS=Not Significant.; R= Right Side; ROI=Region of Interest; ROI 1= Right mandibular angle; ROI 2= In the trabecular bone, 2mm anterior to right mental foramen; ROI 3= In the trabecular bone, 2mm anterior to left mental foramen; ROI 4= Left mandibular angle; SD=Standard Deviation.

**Table S5** - Comparing Mean MCW, MCW and FD-ROI between FAP and non-FAP females

| | FAP (n = 10) | | Non-FAP (n = 30) | | 't' value | *p* value |
|---|---|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD | | |
| MCW (R) | 3.329 | 0.478 | 3.415 | 0.712 | 0.353 | 0.726; NS |
| MCW (L) | 3.371 | 0.759 | 3.577 | 0.608 | 0.872 | 0.389; NS |
| FD-ROI 1 | 1.184 | 0.053 | 1.239 | 0.093 | 1.777 | 0.084; NS |
| FD-ROI 2 | 1.178 | 0.065 | 1.251 | 0.074 | 2.786 | 0.008* |
| FD-ROI 3 | 1.169 | 0.106 | 1.249 | 0.085 | 2.435 | 0.020* |
| FD-ROI 4 | 1.166 | 0.080 | 1.189 | 0.119 | 0.564 | 0.576; NS |

"t" = Student t test; NS: *p*> 0.05. *p<0.05; Significant

CI=Confidence Interval; FD=Fractal Dimension; L=Left Side; MCI=Mandibular Cortical Index; MCW=Mandibular Cortical Width; NS=Not Significant.; R= Right Side; ROI=Region of Interest; ROI 1= Right mandibular angle; ROI 2= In the trabecular bone, 2mm anterior to right mental foramen; ROI 3= In the trabecular bone, 2mm anterior to left mental foramen; ROI 4= Left mandibular angle; SD=Standard Deviation.

# 6    ARTICLE 3

**Title: A Multicentric Approach on Dento-osseous Alterations in Familial Adenomatous Polyposis**

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## Declarations

**Conflict of interest Disclosure:** The authors have no conflicts of interest relevant to disclose.

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**Ethical approval:** Ethical approval all procedures performed in studies involving human participants were in accordance with ethical standards of each institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Research involving human participants and informed consent:** Informed consent was obtained from all individual participants or legal responsible included in the study.

**Abstract**

Familial Adenomatous Polyposis (FAP) is an inherited disorder that predisposes one to the early onset of colorectal cancer. We aimed was to characterize the dento-osseous radiographic findings of patients with FAP from Brazil and the United States. A multicenter study designed to assess FAP children and adults' dental radiographic panoramic (DPR). Both groups were diagnosed with FAP by standard criteria and paired with a healthy control group by sex and age. Blinded Oral and Maxillofacial Radiologists analyzed all DPR's. Of 114 DPRs, from 38 patients with FAP, composed of Group I (n=22), Group II (n=16), and 76 matched controls. Evaluators' reliability reached an excellent agreement, ICC $_{mean}$=0.89. The prevalence of osseous anomalies was higher in FAP adults (75%) than in children (65.4%) and a total prevalence of 73.6% in all FAP populations. The dental anomalies frequency was higher in the FAP children sample with a prevalence of 15.4% than in FAP adults (8.3%). We highlight significant differences between children and adult patients with FAP. The prevalence of osseous abnormalities was 65.4% and 75%, whereas dental anomalies were present in 15.4% and 8.3% of children and adults. The presence of these abnormalities in pediatric dental patients, even if diagnosed with FAP, should be borne in mind as possibly indicating *de novo* or unrecognized disease. Liaison with a multidisciplinary team including gastroenterology and genetics, may be necessary to investigate the related risk further. Iron studies suggest that dental abnormalities in children with FAP are similar to those in adults.

**Introduction**

Familial Adenomatous Polyposis (FAP) is an autosomal inherited disorder characterized by intestinal pre-cancerous polyps which, if untreated, results in colorectal cancer (CRC) by a mean age of 35 years (Global Burden of Disease Cancer Collaboration et al., 2019). Besides, individuals with FAP are susceptible to a myriad of extracolonic features encompassing both malignant and benign neoplastic and non-neoplastic features. The latter include congenital hypertrophy of the retinal pigment epithelium and dento-osseous anomalies (Gardner; Richards, 1953; Galiatsatos; Foulkes, 2006; Almeida et al., 2016). Extraintestinal involvement can predate and can be detected years before the intestinal polyps are identified (Half et al., 2009; Almeida et al., 2020). Most individuals with FAP have a detectable mutation in the Adenomatous Polyposis Coli (*APC)* gene on chromosome 5q21 (Kinzler et al., 1991; Bisgaard et al., 1994; Galiatsatos; Foulkes; 2006; Half et al., 2009).

There is an intriguing overlap between dental abnormalities and the risk of colon cancer, central to which has been the understanding of the function of Axis Inhibition Protein 2 (AXIN2), which has been linked to both familial oligodontia and CRC (Callahan et al., 2009; Hlouskova et al., 2017). The AXIN2 gene product is part of the Wnt wingless pathway, which is central to the *APC* gene product's function in the normal cells. Disrupted Wnt signaling has been related to the failure of tooth development/morphogenesis causing agenesis (Lammi et al., 2004). Conversely, individuals with *APC* mutation are reported to have supernumerary teeth formation resulting from overstimulation of Wnt signaling (Liu et al., 2008; Wang et al., 2009; Miclea et al., 2010). Unrelatedly, the oral microbiome is understood to influence intestinal dysbiosis and consequently colon cancer risk (Flemer et al., 2018; Koliarakis et al., 2019).

FAP included extraintestinal manifestations encompassing dento-osseous manifestations (Gardner; Richards, 1953; Gardner, 1962). More recently, awareness of FAP related cancer risk by the dental community has been emphasized (Septer et al., 2018; Global Burden of Disease Cancer Collaboration et al., 2019). The serendipitous discovery of FAP through incidental radiographic findings either in the context of unrecognized family history or with a *de novo* mutation has staggering, lifesaving potential (Bülow, 2003; Half et al., 2009; Septer et al., 2018).

The published literature to date is limited by the rarity and usually a later diagnosis of FAP. Therefore, given the preponderance of case reports in pediatric patients (Nissen; Wynn, 2014) and limited large studies, there is a gap in our understanding of the age-dependent dental manifestations of FAP risk. A multicenter study encompassing diverse ages and ethnicities could provide a broader perspective regarding the maxillomandibular complex's alterations/anomalies. Our study represents a collaborative effort to address the deficit with two principal aims. First, to characterize the dento-osseous anomalies of FAP patients from a diverse multinational cohort, Brazil and the United States (US), and describe the radiographic findings and affected individuals compared to matched controls.

**Material and Methods**

Independent institutional ethical conduct of research review board approval was obtained through both participating institutions (Ethics Committee of the Health Sciences Faculty, University of Brasília, Brazil – CEP/UnB #493.502, and the Pediatric Research Board at Children's Mercy's Kansas City – CMKC IRB #12020134, Missouri, US).

The study inclusion criteria included a diagnosis of FAP by standard clinical diagnostic criteria. Group I (n=66) was composed of DPRs of 22 CMKC FAP in the pediatric age range and their respective controls (n=44), the FAP diagnostic was confirmed by the APC mutation via genetic test. Group II (n=48) consisted of Dental Panoramic Radiographs (DPR) of 16 Brazilians FAP subjects diagnosed by the colonoscopy and their respective controls (n=32). The diagnostic test found clinically more that 100 polyps and confirmed the FAP diagnostic. Besides, patients in Group I had identification of an *APC* pathogenic variant previously described by Septer et al. (2018), and five FAP of Group II patients had previously been identified with a heterozygous mutation in the *APC* gene, having their genetic characterization presented by Almeida et al. (2020).

Group I was composed by 22 FAP children and Group II by 12 FAP adults and 4 children. were considered pediatric patients/children if younger than 18 years old. The patients from Group I and II were paired by sex and age on a proportion of one FAP to two healthy controls. These control individuals had neither family history indicating a risk of FAP nor contributory medical history, as declared in their electronic health records.

### *The evaluators' training*

Two oral and maxillofacial radiologist (OMR) evaluators were trained using the Dental Panoramic Radiograph Score (Thakker et al., 1995), a standardized weighted score system validated for high-risk FAP patients. This index assesses and scores the diagnostic significance of the FAP dento-osseous manifestations. The radiographic evaluation training took place until the evaluators reached a satisfactory agreement using five DPRs, in two consecutive trials repeated under identical conditions, and after a two-week washout period.

The entire sample's final radiographic interpretation was completed by the two trained OMRs (CP-P and FTA) and crosschecked by a 20-year expert OMR (H.G). To avoid potential biases, the evaluators were blinded to FAP diagnosis and any other information from the patients' records. Each DPR was evaluated, in a standardized radiological finding report, the number and severity of these dental anomalies and osseous manifestations were recorded. Groups I and II were compared with their respective matched controls and between them.

### *Statistical Analysis*

Analysis of the data was conducted using The Statistical Package for the Social Sciences (version 22; SPSS, IBM, Armonk, NY). The Intraclass Correlation Coefficient (ICC) was applied to study intra- and inter-observer reliability for calibration purposes. The ICC value was interpreted as per Portney and Watkins (2009).

Descriptive data were computed. The Fisher exact test was applied to compare the frequency of dental anomalies. The Pearson Chi-Square statistic was used to evaluate Tests of Independence on the cross-tabulation. These bivariate tables present the distributions of two categorical variables simultaneously, with the intersections of the categories of the variables appearing in the cells of the table. The Test of Independence assessed whether an association exists between the two variables by comparing the observed pattern of responses in the cells to the pattern that would be expected if the variables were genuinely independent of each other. The severity of the radiographic finding was recorded and tested using the Chi-square test. *P*-values below 0.05 indicate statistical significance.

### Results

*Study population: Familial adenomatous polyposis and matched controls*

Out of 114 evaluated DPRs, 38 were FAP patients including children (n=24) and adults (n=14) and paired controls (n=76). Group I (n=66) had a mean age of 13.50 years (SD 4.54) and Group II (n=48), a mean age of 35.35 years (SD 16.36). Appendix 1 displays the demographics, mean ages for cases and controls were virtually identical (*p*=0.925 and *p*=0.995).

The intra-observer reliability of 0.89 [CI 95% (0.84, 0.98)] indicated an excellent agreement between evaluators. The inter-evaluator consistency in both rounds showed a strong agreement reflecting the efficacy of the training process. Evaluator 1, ICC=0.96 [CI 95% (0.75, 0.99)] and evaluator 2, ICC=0.85 [CI 95% (0.82, 0.96)]. The OMR expert crosschecked the final radiographic findings of the entire sample, and a consensus was reached in case of discrepancies.

*Radiographic findings of FAP patients and controls in Group I*

Group I showed a higher frequency of idiopathic osteosclerosis in the FAP group (63.3%, *p*<0.001) when compared to their paired controls, followed by odontomas (13.6%, *p*=0.010), long tapered roots (13.6%, *p*=0.038), altered bone trabecular pattern (13.6%, *p*=0.010), and osteomas (9.1%, *p*=0.038). Almost all lesions had significant differences between the FAP patients and controls (*p*<0.05), except tapered long roots and impacted teeth, p=0.60 and p=0.333, respectively.

The severity, expressed by the frequency of each significant finding within the same individual, was explored in Group I, as per Table 1. Osteomas were found in two patients, one 16-year-old and another 17-year-old presenting with nine osteomas (Figure 1). However, this finding was not seen in the majority of the children (90.9%). Odontomas were identified in three children, representing a frequency of 13.6% (*p*=0.010) and three odontomas were found in one 17-year-old patient. The presence of idiopathic osteosclerosis was significantly more common in the mandible of 63.6% of FAP patients in Group I an absent in the controls, p<0.001. Figure 2 illustrates this finding. An altered trabecular bone

pattern was identified in three of the FAP patients, while none were found in the controls ($p$=0.038).

### Group II shows a difference between radiographic findings in FAP patients and controls

In Group II, we found idiopathic osteosclerosis present in 75% of FAP patients and osteomas in 5 of the 16 individuals (31.5%). These findings were statistically significant when compared to the controls, $p$=0.001 for both scenarios. The severity of the statistically significant differences between the findings of FAP and controls was also explored. The higher incidence of idiopathic osteosclerosis (75.2%, $p$<0.001) and osteomas (31.3%, $p$<0.001) was also confirmed in the FAP patients of Group II, see Table 2.

### Comparative radiographic findings analysis of Group I and II

The radiographic findings from FAP patients of Group I (n=22) and Group II (n=16) were compared. There were no significant differences in dental or osseous findings between groups ($p$>0.05). Regarding the osseous lesions, the FAP Group I showed a prevalence of 68.2% and Group II of 81.2%, resulting in an overall prevalence of 74.7% for osseous anomalies and 13.1% dental anomalies in all FAP individuals. Table 3 lists the findings and shows the consistency of the FAP dento-osseous manifestations in the two groups.

### FAP children show similar radiographic findings as FAP adults

To further explore the radiographic manifestations in children (n=26) and adults (n=12), age subgroups were created categorizing affected children (<18-year-old) and adults. We observed no significant differences in radiographic findings between children and adults with FAP (Appendix 2). Notwithstanding the increased number of osteomas in the adult sample, there was no statistical difference between both subgroups ($p$>0.05), confirming the findings' consistency independent of the age. Idiopathic osteosclerosis was the most common finding with 69.2% in FAP children and 66.7% in adults ($p$=1.00).

Upon comparing the radiographic findings in children with FAP (n=26) with controls (Table 4), statistically significant differences in the prevalence of idiopathic osteosclerosis

(*p*=0.001), the number of osteomas (*p*=0.009), odontomas (*p*=0.009) and altered trabecular bone pattern (*p*=0.032) were observed. Condensing osteitis was also statistically significant; however, it is an inflammatory condition (*p*=0.032). Similar observations could be made upon comparing adult FAP patients and corresponding controls (Table 5). Areas showing idiopathic osteosclerosis (*p*=0.001) and osteomas (*p*=0.31) were also seen in adults with FAP.

Idiopathic osteosclerosis, osteomas, and altered trabecular bone patterns were regrouped to assess FAP children and adults' overall osseous manifestations. Table 6 shows a higher prevalence of osseous anomalies in FAP adults (75%) than in FAP children (65.4%) and a total prevalence of 73.6% in all FAP populations. The dental anomalies were higher in the FAP children sample, with 15.4% prevalence than in the FAP adults with 8.3%. The odontomas prevalence was 15.4% in FAP children, and a higher prevalence of supernumeraries (8.3%) was noted in the FAP adult group.

**Discussion**

This study is a multicenter project that aimed to systematically characterize the pattern of osseous and dental anomalies in FAP patients in two different populations, one from the US and the other from Brazil. The results provide insight to the medical and dental community since the oral manifestations could appear years before the colorectal polyps and are reported in more than 58% of affected individuals ( Gardner, 1962; Ida et al., 1981; Aggarwal et al., 2003; Wijn et al., 2007; Lee et al., 2009; Almeida et al., 2012). Our data creates awareness and may help develop better surveillance practices regarding FAP radiographic findings and the dentists' role in screening high-risk FAP families.

The prevalence of the dento-osseous anomalies reported by this multicenter study differs from the ones presented by a systematic review regrouping patient from various studies (Almeida et al., 2016). Regarding osseous lesions, we found that they occur more frequently than dental lesions, which is in line with the frequency of such lesions reported by the systematic review. However, in terms of prevalence, our study shows a 10% higher prevalence of osseous manifestations (73.6%) and a 50% decreased prevalence of dental anomalies (13.7%) than the prevalence reported in the systematic review (65.3% and

30.5%, respectively). However, we should be cautious in comparing their stated prevalence due to the distinct study designs.

Osteoma in the jaw is a classic oral finding of FAP reported in 46-93% of these patients (Gardner; Plenk, 1952; Ida et al., 1981; Aggarwal et al., 2003; Bülow, 2003; Wijn et al., 2007). Interestingly, the lower prevalence of osteomas (18.4%) found in our FAP sample contrasted significantly with previous studies (Takeuchi et al., 1993; Bülow, 2003). Moreover, we found a high prevalence of idiopathic osteosclerosis (68.4%), not a standard classification seen in FAP reports. These findings could be justified because the majority of the data available about FAP comes from medical reports with no involvement of dentists in the diagnosis of maxillomandibular osseous anomalies. This latter could cause a divergence in the diagnosis and the classification of osseous lesions. Previous authors raised the attention of discrepancies and the absence of standardization, leading to misreports of the dental radiographic findings related to the disease (Almeida et al., 2016). Further systematic phenotyping with a unique classification of dental osseous lesions of the jaw is necessary in FAP patients to better understand their frequency. Despite this, it is essential to emphasize the diagnostic method used to evaluate the lesions. At present, there are advanced diagnostic imaging techniques to better assist in the characterization of the lesions in the jaw and to minimize the ionizing radiation concerns.

In our study, the osseous anomalies of FAP patients were calculated by the sum of osteomas, idiopathic osteosclerosis, and altered trabecular pattern. Studies have demonstrated an association between *APC* mutations and bone mass increase in FAP patients due to the *APC* regulatory role in the differentiation of skeletal progenitor cells (Miclea et al., 2010). Group II, which presented a mean age of 35.35 years, had a higher frequency of osseous anomalies than Group I (mean age 13.5 years). The gradual growth of odontogenic lesions during adolescence and its continued development in adulthood has been discussed previously (Almeida et al., 2020). This reinforces the necessity of long-term dental follow-up in conjunction with the medical FAP surveillance protocol.

A previous study showed that *Apc* mutation presents an alteration of the Wnt/β-catenin pathway, which participates in dental development (Liu et al., 2008). Our current understanding of this pathway builds on animal studies (Wang et al., 2009; Wang et al., 2014). For example, the Wnt/β-catenin pathway signals are necessary for multiple tooth morphogenesis stages, and inactivation of *Apc* in murine dental development results in

supernumerary tooth formation (Wang et al., 2009). Such a study suggests that the induction of supernumerary teeth by Wnt/β-catenin signaling involves mechanisms that resemble those used in endogenous murine tooth development. Note that our study is not longitudinal, investigating DPRs at one point in time, which could explain the reduced number of supernumeraries (8.3% in all affected patients).

The low prevalence of 13.6% for dental anomalies presented by our study was an unexpected finding. Hypothetically, this could be due to the mutation location, an understudied scope of the FAP; what is known is that the mutation location alters the phenotype. Besides, Group I was mostly composed of pediatric patients, and Group II adults receive routine dental care. Thus, we cannot rule out the possibility that supernumeraries and odontomas were previously removed.

Tooth agenesis was not observed in our study sample. Previous reports have suggested that tooth agenesis, resulting from *AXIN2* mutations, could be associated with Attenuated FAP, and consequently, an indicator of CRC predisposition (Lammi et al., 2004; Rivera et al., 2014; Beard et al., 2019). Patients from our sample had the diagnosis of classical FAP, and most of them with confirmed *APC* mutation. So far, there is no evidence in the literature associating *APC* mutation and agenesis; our results reinforce that.

Our study's important finding is that the radiographic alterations in the trabecular pattern could be detected since childhood; these alterations were shown on a 6-year-old patient. Also, the prevalence of dento-osseous anomalies in FAP children was significantly higher than in the controls. Therefore, idiopathic osteosclerosis and altered trabecular bone areas may be clinically significant in children at risk of FAP and require attention and follow-up. The radiographic pattern identified in DPRs emphasizes the essential role of dentists in detecting these anomalies. Pediatric surveillance is the key to prevention (Thakker et al., 1995; Aggarwal et al., 2003). A need for prompt care highlights the importance of routine radiography in pediatric patients. It should be emphasized to avoid complications in such patients and their families. Besides, confirmed FAP children and adults and their families should be referred to genetic mapping and testing.

We acknowledge the limitations to our study. It focused on the radiographic characterization of osseous and dental anomalies at a point in time. A multilocation monitoring of these individuals on a longitudinal approach could give better insight into the

natural history of the anomalies associated with FAP. Also, longitudinal studies, including genetic mapping of all FAP patients, opens up the possibility of performing genotype-phenotype correlations for regrouping data with characterized radiographic findings (Half et al., 2009; Talseth-Palmer, 2017). Future studies may be needed and may include larger multicenter consortia or data analytic methodologies.

We recommend that FAP families be referred to dentists as a protocol - the same rationale as the referral of these patients to ophthalmologists to follow up on the retinal pigment epithelium (Chapman et al., 1989). We emphasize that a dental examination followed by a conventional panoramic radiograph at mixed dentition, preferably coupled with a multidisciplinary, coordinated approach, be adopted for FAP children and the families at risk (American Dental Association, 2012). Dentists should perform an annual dental and radiographic surveillance of these children until adulthood, as well as for their families. The coordination of care between dental and genetic or gastroenterology providers is essential to identify and follow patients at risk and their families.

**Conclusion**

In our study, we highlight the presence of FAP dento-osseous lesions since childhood and could be, in the future, considered a predictive factor. With all the limitations of a small FAP sample, the prevalence of osseous abnormalities was 65.4% and 75% in children and adults, respectively, whereas dental anomalies were almost equivalent for both groups (15.4% of children and 8.3% in adults). These abnormalities in dental patients, even if not diagnosed with FAP, should be borne in mind as possibly indicating *de novo* or unrecognized disease. Panoramic dental radiographs in FAP children in mixed dentition (around 7-9 years old) and adults at risk should be adopted as an annual surveillance protocol until the age of 18 and prescribed every two years for the adults.

**Acknowledgements**

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**Figure 1 - FAP radiographic findings described as a "classic".** A panoramic radiograph of a FAP female (17-year-old) presenting five osteomas (white arrows), and some areas of altered trabecular bone pattern in the maxillomandibular complex represented by the white circles.
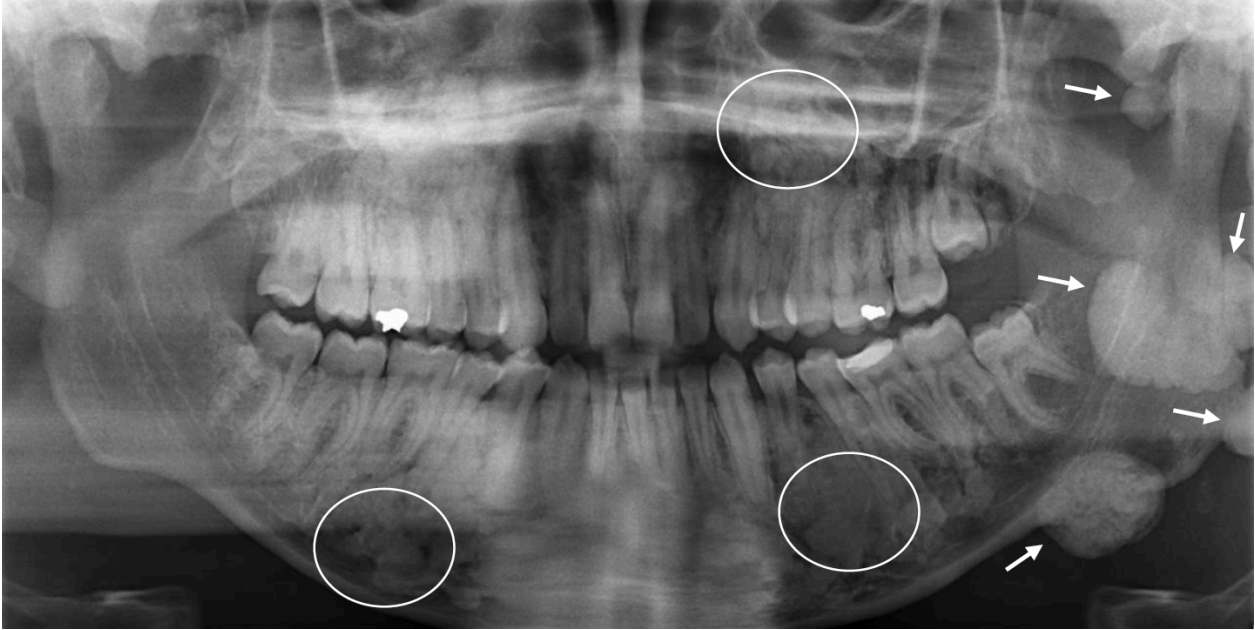
**Figure 2 - FAP pediatric radiographic findings**. A 15-year-old FAP children showing areas of altered trabecular bone and idiopathic osteosclerosis in the mandible represented by the four white arrows.

**Table 1** - Severity of significant findings in Group I

| Osseous anomalies | Severity score | FAP (n=22) | | Controls (n=46) | | p-value |
|---|---|---|---|---|---|---|
| | | N | % | N | % | |
| *Osteoma* | ND | 20 | 90.9 | 46 | 100.0 | 0.116 |
| | 1 | 1 | 4.5 | 0 | 0.0 | |
| | 9 | 1 | 4.5 | 0 | 0.0 | |
| *Odontomas* | ND | 19 | 86.4 | 46 | 100.0 | 0.038* |
| | 1 | 2 | 9.1 | 0 | 0.0 | |
| | 3 | 1 | 4.5 | 0 | 0.0 | |
| *Idiopathic Osteosclerosis* | ND | 8 | 36.4 | 46 | 100.0 | **<0.001**\*\* |
| | 1 | 4 | 18.2 | 0 | 0.0 | |
| | 2 | 1 | 4.5 | 0 | 0.0 | |
| | 3 | 2 | 9.1 | 0 | 0.0 | |
| | 4 | 1 | 4.5 | 0 | 0.0 | |
| | 5 | 2 | 9.1 | 0 | 0.0 | |
| | 6 | 1 | 4.5 | 0 | 0.0 | |
| | 7 | 1 | 4.5 | 0 | 0.0 | |
| | 9 | 1 | 4.5 | 0 | 0.0 | |
| | 10 | 1 | 4.5 | 0 | 0.0 | |
| *Altered trabecular bone pattern* | ND | 19 | 86.4 | 46 | 100.0 | **0.038*** |
| | 1 | 2 | 9.1 | 0 | 0.0 | |
| | 2 | 1 | 4.5 | 0 | 0.0 | |

Chi-Square Test: *p<0.05; Significant; **p<0.001 Highly significant.
Severity score represents the frequency of findings. FAP=Familial adenomatous polyposis; ND=Not detected.

**Table 2** – Severity of significant findings in Group II

| Osseous anomalies | Severity score | FAP (n=16) | | Controls (n=32) | | p-value |
|---|---|---|---|---|---|---|
| | | N | % | N | % | |
| *Osteoma* | ND | 11 | 68.8 | 32 | 100.0 | 0.001* |
| | 1 | 5 | 31.3 | 0 | 0.0 | |
| *Idiopathic Osteosclerosis* | ND | 4 | 25.0 | 31 | 96.9 | <0.001** |
| | 1 | 3 | 18.8 | 1 | 3.1 | |
| | 2 | 4 | 25.0 | 0 | 0.0 | |
| | 3 | 3 | 18.8 | 0 | 0.0 | |
| | 9 | 1 | 6.3 | 0 | 0.0 | |
| | 14 | 1 | 6.3 | 0 | 0.0 | |

Chi-Square Test: *p<0.05; Significant; **p<0.001; Highly significant.
Severity score represents the frequency of findings. FAP=Familial adenomatous polyposis, ND=not detected.

**Table 3** - Dento-osseous findings in FAP patients, Group I and II

| Dento-osseous anomalies | FAP Group I (n=22) | | FAP Group II (n=16) | | p-value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Idiopathic Osteosclerosis | 14 | 63.6 | 12 | 75.0 | 0.457 |
| Osteoma | 2 | 9.1 | 5 | 31.3 | 0.082 |
| Impacted teeth | 3 | 13.6 | 4 | 25.0 | 0.372 |
| Odontomas | 3 | 13.6 | 1 | 6.3 | 0.464 |
| Altered trabecular bone pattern | 3 | 13.6 | 1 | 6.3 | 0.464 |
| Long roots | 3 | 13.6 | 0 | 0.0 | 0.124 |
| Condensing osteitis | 3 | 13.6 | 0 | 0.0 | 0.124 |
| Rarefying osteitis | 2 | 9.1 | 1 | 6.3 | 0.748 |
| Long tapered root | 2 | 9.1 | 0 | 0.0 | 0.215 |
| Retained tooth | 1 | 4.5 | 1 | 6.3 | 0.816 |
| Dentigerous cysts | 0 | 0.0 | 1 | 6.3 | 0.235 |
| Supernumerary | 0 | 0.0 | 1 | 6.3 | 0.235 |
| Hypercementosis | 0 | 0.0 | 1 | 6.3 | 0.235 |
| Congenitally missing teeth | 0 | 0.0 | 0 | 0.0 | - |
| *Osseous Anomalies** | *15* | *68.2* | *13* | *81.2* | *0.657* |

Chi-Square Test: *$p<0.05$; Significant; FAP=Familial adenomatous polyposis.
*Any one of Osteoma, Idiopathic Osteosclerosis or Altered trabecular bone pattern present.

**Table 4** – Comparison of dento-osseous anomalies in FAP children and controls

| Dento-osseous anomalies | FAP children (n=26) | | Controls (n = 54) | | p-value[#] |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Idiopathic Osteosclerosis | 18 | 69.2 | 0 | 0.0 | **0.001*** |
| Osteoma | 4 | 15.4 | 0 | 0.0 | **0.009*** |
| Odontomas | 4 | 15.4 | 0 | 0.0 | **0.009*** |
| Impacted teeth | 4 | 15.4 | 5 | 9.3 | 0.462 |
| Long roots | 3 | 11.5 | 1 | 1.9 | 0.098 |
| Condensing osteitis | 3 | 11.5 | 0 | 0.0 | **0.032*** |
| Altered trabecular bone pattern | 3 | 11.5 | 0 | 0.0 | **0.032*** |
| Long tapered root | 2 | 7.7 | 0 | 0.0 | 0.103 |
| Rarefying osteitis | 2 | 7.7 | 0 | 0.0 | 0.103 |
| Retained tooth | 1 | 3.8 | 3 | 5.6 | 1.000 |
| Dentigerous cysts | 1 | 3.8 | 0 | 0.0 | 0.325 |
| Supernumerary | 0 | 0.0 | 2 | 3.7 | 1.000 |
| Hypercementosis | 0 | 0.0 | 0 | 0.0 | - |
| *Osseous Anomalies*** | *17* | *65.4* | *2* | *3.7* | *0.001** |

#Fisher Exact Test: $p>0.05$; Not Significant and *$p<0.05$; Significant.
**Any one of Osteoma, Idiopathic Osteosclerosis or Altered trabecular bone pattern present.
Group I had 22 FAP children and Group II 4 FAP children.

**Table 5** – Comparison of dento-osseous anomalies in FAP adults and controls

| *Dento-osseous anomalies* | FAP adults (n=12) | | Controls (n=24) | | *p*-value[#] |
|---|---|---|---|---|---|
| | N | % | N | % | |
| *Idiopathic Osteosclerosis* | 8 | 66.7 | 1 | 4.2 | **0.001*** |
| *Osteoma* | 3 | 25.0 | 0 | 0.0 | **0.031*** |
| *Impacted teeth* | 3 | 25.0 | 6 | 25.0 | 1.000 |
| *Rarefying osteitis* | 1 | 8.3 | 4 | 16.7 | 0.646 |
| *Hypercementosis* | 1 | 8.3 | 0 | 0.0 | 0.333 |
| *Supernumerary* | 1 | 8.3 | 0 | 0.0 | 0.333 |
| *Altered trabecular bone pattern* | 1 | 8.3 | 0 | 0.0 | 0.333 |
| *Retained tooth* | 1 | 8.3 | 0 | 0.0 | 0.333 |
| *Condensing osteitis* | 0 | 0.0 | 1 | 4.2 | 1.000 |
| *Odontomas* | 0 | 0.0 | 0 | 0.0 | - |
| *Dentigerous cysts* | 0 | 0.0 | 0 | 0.0 | - |
| *Long tapered root* | 0 | 0.0 | 1 | 4.2 | 1.000 |
| *Long roots* | 0 | 0.0 | 1 | 4.2 | 1.000 |
| *Osseous Anomalies*** | *9* | *75.0* | *1* | *4.2* | *0.001*** |

#Fisher Exact Test: *p*>0.05; Not Significant and *p<0.05; Significant.
**Any one of Osteoma, Idiopathic Osteosclerosis or Altered trabecular bone pattern present.
All 12 FAP adults were from Group I.

**Table 6** - Prevalence of findings in FAP children, FAP adults and All FAP cases

| *Dento-osseous anomalies* | FAP children (n=26) | | FAP adults (n=12) | | All FAP (n=38) | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| *Idiopathic Osteosclerosis* | 18 | 69.2 | 8 | 66.7 | 26 | 68.4 |
| *Osteoma* | 4 | 15.4 | 3 | 25.0 | 7 | 18.4 |
| *Altered trabecular bone pattern* | 3 | 11.5 | 1 | 8.3 | 4 | 10.5 |
| *Osseous anomalies*[#] | **17** | **65.4** | **9** | **75.0** | **28** | **73.6** |
| | | | | | | |
| *Odontomas* | 4 | 15.4 | 0 | 0.0 | 4 | 10.5 |
| *Supernumerary* | 0 | 0.0 | 1 | 8.3 | 1 | 2.6 |
| *Dental anomalies** | **4** | **15.4** | **1** | **8.3** | **5** | **13.1** |

#Any one of Osteoma, Idiopathic Osteosclerosis or Altered trabecular bone pattern present.
*Any one of Odontomas or Supernumeraries present.
Group I had 22 FAP children and Group II 4 FAP children.
All 12 FAP adults were from Group I.

## Appendix 1 - Demographics of the participants

| | | Group I | | | | Group II | | | |
| | | FAP | | Controls | | FAP | | Controls | |
| | | N | % | N | % | N | % | N | % |
|---|---|---|---|---|---|---|---|---|---|
| **Sex** | **Male** | **10** | **45.5** | **21** | **45.6** | **11** | **68.8** | **22** | **68.8** |
| | **Female** | **12** | **54.5** | **25** | **54.3** | **5** | **31.3** | **10** | **31.3** |
| | | *p*=0.726 | | | | *p*=1.000 | | | |
| | | Group I composed by 22 FAP children + matched controls | | | | Group II composed by 12 FAP adults and 4 children + matched controls | | | |
| **Age** | **Mean ± SD** | **13.50 ± 4.54** | | **13.70 ± 4.49** | | **35.35 ± 16.36** | | **35.72 ± 15.59** | |
| | | *p*=0.925 | | | | *p*=0.995 | | | |

FAP=Familial Adenomatous Polyposis patients; NS=Not significant; SD=Standard deviation.
Group I: Brazilian FAP and controls.
Group II: American FAP and controls.

## Appendix 2 - Dento-osseous anomalies in FAP children and FAP adults

| *Dento-osseous anomalies* | FAP children (n=26) | | FAP adults (n=12) | | *p*-value[#] |
| | N | % | N | % | |
|---|---|---|---|---|---|
| *Idiopathic Osteosclerosis* | 18 | 69.2 | 8 | 66.7 | 1.000 |
| *Osteoma* | 4 | 15.4 | 3 | 25.0 | 0.656 |
| *Odontomas* | 4 | 15.4 | 0 | 0.0 | 0.287 |
| *Impacted teeth* | 4 | 15.4 | 3 | 25.0 | 0.656 |
| *Altered trabecular bone pattern* | 3 | 11.5 | 1 | 8.3 | 1.000 |
| *Long roots* | 3 | 11.5 | 0 | 0.0 | 0.538 |
| *Condensing osteitis* | 3 | 11.5 | 0 | 0.0 | 0.538 |
| *Long tapered root* | 2 | 7.7 | 0 | 0.0 | 1.000 |
| *Rarefying osteitis* | 2 | 7.7 | 1 | 8.3 | 1.000 |
| *Retained tooth* | 1 | 3.8 | 1 | 8.3 | 0.538 |
| *Dentigerous cysts* | 1 | 3.8 | 0 | 0.0 | 1.000 |
| *Supernumerary* | 0 | 0.0 | 1 | 8.3 | 0.084 |
| *Hypercementosis* | 0 | 0.0 | 1 | 8.3 | 0.316 |
| *Congenitally missing teeth* | 0 | 0.0 | 0 | 0.0 | - |

#Fisher Exact Test*: p*>0.05; Not Significant and **p*<0.05; Significant
*Any one of Osteoma, Idiopathic Osteosclerosis or Altered trabecular bone pattern present
Group I had 22 FAP children and Group II 4 FAP children
All 12 FAP adults were from Group I

# 7    DISCUSSION

The oral and maxillofacial complex can be secondarily affected by some systemic diseases. Firstly, we built up the background for this thesis by performing a systematic review of the literature. The already published systematic review included studies that examined systemic disorders, summing to 1,466 investigated individuals. The findings demonstrated the potential use of dental imaging assessment of cortical bone and trabecular bone structure in the maxillomandibular complex as an adjuvant screening tool to identify a systemic disorder – osteoporosis and a possibility to identify Diabetes (Pacheco-Pereira et al., 2018).

Although cortical bone mineral density indices have been extensively explored by the literature and demonstrated to have good diagnostic performance (Guerra et al., 2017); the bone microstructure investigation through its trabecular architecture for screening systemic disorders has received limited attention. In general, retrospective and prospective studies investigating the trabecular bone pattern via dental radiographs found a positive correlation with the reference standard for various of systemic diseases (Pacheco-Pereira et al., 2018). Even though the existence of differences in study design, these specific studies found common ground in concluding that the trabecular bone density appears to be a useful screening index for systemic disorders which alter bone density (Nakamoto et al., 2003; Tosoni et al., 2006; de Oliveira et al., 2009; Lee et al., 2009; Khojastehpour et al., 2013; Nemtoi et al., 2013; Roberts et al., 2013; Yamashita-Mikama et al., 2013; Chai et al., 2014; Kathirvelu; Anburajan, 2014). From our systematic review, osteoporosis was the condition presenting the most significant results as 72% of the studies detected changes in the maxillomandibular trabecular bone structure (Delvin; Horner, 2002; Geraets et al., 2007; Lindh et al., 2008; Leite et al., 2010; Ferreira et al., 2011). Besides, few studies exploring diabetic edentulous patients found a less-dense trabecular bone pattern ($p<0.05$) in the affected individuals (Pacheco-Pereira et al., 2018).

Although the trabecular bone pattern of FAP patients is affected by osseous alterations, there is no study evaluating the mandibular bone pattern on this condition. By applying a systematic approach to identify studies that assessed the trabecular bone alterations, we were able to identify various anatomical Regions of Interest (ROI) located in the mandible and map the most consistent areas. At the end of this preliminary phase, we

determined the ROI to be used in our further studies on the FAP patient sample.

Texture analysis of the trabecular bone can be done using several methods (Parkinson; Fazzalari, 2000); however, most of the published studies in this field elected the fractal analysis to explore the trabecular microarchitecture. Since this analysis is based on fractal mathematics for describing complex shapes and patterns of the bone (White; Rudoph, 1999); our second study - Article 2 - proved to be a suitable method to assess the trabecular bone pattern FAP patients using panoramic radiograph. It is known that the *APC* gene mutation interferes in the function of the gatekeeper β-catenin, alters the osteoblast differentiation, and increases bone deposition (Holmen et al., 2005; Miclea et al., 2010). Thus, the fractal values found in the FAP patients were generally 5% lower when compared to the matched controls; it confirms the assumption that the mandibular trabecular bone of FAP individuals is systemically altered. However, the radiomorphometric indices MCI and MCW were equal when the young patients' sample of FAP and health controls were compared. A different scenario could be seen in older individuals' radiographs were assessed; this due to the tumor burden that affects bone remodeling. Since radiomorphometric indices are claimed to have a very high diagnostic capability to detect bone mineral density, it could reveal a fracture protection or even bone fragility in FAP-affected individuals (Taguchi et al., 1999). Thus, a question regarding the bone mineral density of not so young FAP patients should be investigated further.

Since FAP is a rare disease and a few regional papers were published, our research group approached a North American research team to join efforts. A multilocation group brought a broader perspective regarding the alterations in the maxillomandibular complex – Article 3. The dento-osseous alterations of FAP children and adults were investigated through the assessment of their dental panoramic imaging. We characterized the pattern of dental and osseous radiographic findings of FAP and matched control groups; these different populations were from the University of Brasilia, Brazil (n=48) and from the Mercy's Children Hospital in Kansas City, United States of America (n=68). This study confirmed the higher prevalence of osseous anomalies in FAP adults of 75%, then in FAP children of 65.4%. Also, we observed a higher prevalence of dental anomalies in FAP adults (15.4%) and 8.3% in children, this dental finding should be considered with caution because Group I was composed basically by children. The presence of radiographic abnormalities in pediatric dental patients should be born in mind as a possibility indicating de novo or

unrecognized disease. We could also confirm that osseous alterations could be detected since childhood, as some of the trabecular bone alterations found in our sample were shown on a 6-year-old patient.

Our data aims to create awareness and help develop better surveillance practices regarding FAP radiographic findings and the dentists' role in screening high-risk FAP patients. The radiographic pattern identified in DPRs emphasized the essential role of dentists in detecting these anomalies clinically. Iron studies suggest that dental abnormalities in children with FAP are similar to those in adults. Pediatric surveillance may be the key to prevention (Thakker et al., 1995; Aggarwal et al., 2003; Septer et al., 2018; Almeida et al., 2020). Prompt care should be emphasized with the dental community to avoid complications in such patients and their families.

This thesis brings radiographic insights into the craniofacial features in familial adenomatous polyposis. It also highlighted the trabecular bone alterations of FAP patients through the fractal analysis (5% lower in this population) and the necessity of their opportunistic screening using dental radiographs. We emphasize the necessity of a liaison with a multidisciplinary team, including the medical specialists such as pediatricians, gastroenterologists, and proctologists, and the importance of a dentist in the health care team that follows up on FAP patients, families, and the individuals at high risk.

### *Final Considerations*

As aforementioned, our goal was to display dental radiographic imaging as powerful adjuvant tools for detecting systemic bone diseases. Specifically, for FAP patients, we aimed to demonstrate the importance of the dento-osseous alterations in the screening and the diagnostic context of the disease. The early detection of the disease's extraintestinal manifestations may identify affected individuals and promote the referral and screening of entire families at risk; these contribute to the population's general health. Using the concept of clinical utility, radiographic screenings and diagnostic tests should be applied in the dental examination based on maintaining public health, restoring function, and preventing premature death (Bossuyt et al., 2012).

In terms of our study's clinical relevance, we emphasize that the panoramic radiograph should not be limited to the dental evaluation. These three studies showed a

significant association between bone structural alterations and FAP diagnosis. Also, it showed that the proper assessment and detection of bone structural changes are crucial in the early screening and management of FAP patients and families at risk. We believe that any preventative measure could reduce the incidence of colorectal cancer. Early detection of the disease's extraintestinal findings leads to the improvement of early treatment and survival rates. Therefore, new protocols, such as the dentists' active participation, could be introduced in the screening of FAP, resulting in a better clinical procedure. Based on scientific evidence, enhanced guidelines, may improve the quality of life in patient's affected families.

Finally, the dental professional should attentively correlate the radiographic findings to the medical history and investigate if a disorder is suspected. Confirmed FAP children and adults should undergo an endoscopy assessment before genetic testing (Herzig et al., 2017). Preventative measures such as evaluating the assistant DPR's could initiate an early investigation of the disease in families at risk and further referral to the specialists.

### *Future directions*

As a future approach, the correlation of FAP patients' fractal analysis with the BMD through DXA could elucidate and reveal the likelihood or risk of bone fracture or even consider possible fracture protection in FAP patients. Besides, the standardization of the ROI pixel size in the mandibular body in fractal dimension analysis parameters could ease comparing the results between distinct research projects.

Also, longitudinal studies, including genetic mapping of all FAP patients, open up the possibility of performing genotype-phenotype correlations for regrouping data with characterized radiographic findings (Bertario et al., 2003; Bisgaard et al., 2006; Half et al., 2009; Talseth-Palmer, 2017). Future studies may be needed and may include larger multicenter consortia or data analytic methodologies.

From a clinical perspective, dental radiographs may play an important role in screening extra-intestinal manifestations of FAP individuals and families at high-risk, contributing to the disease's early diagnosis. A 3D radiograph longitudinal study, using a FAP pool of available CBCT scans, may also be useful in the analysis of the bone texture

using advanced imaging. In addition to monitoring the long-term development of FAP dento-osseous anomalies.

***Our recommendation***

We recommend that FAP families be referred to dentists as a protocol - the same rationale as the referral of these patients to ophthalmologists to follow up on the retinal pigment epithelium (Chapman et al., 1989). We emphasize that a dental examination followed by a conventional panoramic radiograph at mixed dentition, preferably coupled with a multidisciplinary, coordinated approach, be added to the management protocol for FAP children. Panoramic dental radiographs in FAP children in mixed dentition (~7 to 9 years old) and adults at risk should be adopted as an annual surveillance protocol until the age of 21 and prescribed every two years for the adults.

# 8    CONCLUSIONS

Based on our hypothesis, we can conclude that:

1) The systematic review demonstrated the potential of periapical and panoramic radiographs, Computed Tomography, and CBCT imaging to assess the mandibular trabecular bone structure of patients affected by systemic conditions. The findings also revealed that the frequency of sclerotic bone and or osteosclerosis in focal areas was meaningful for systemic disease screening, such as osteoporosis.

2) Routinely taken panoramic radiographs are essential for opportunistic monitoring and surveillance of systemic conditions. By creating awareness of the dental community's incidental findings, our study could be translated clinically. Any preventive measure could reduce colorectal cancer incidence and improve the survival rates of FAP patients. The analysis of the trabecular bone structures of FAP patients showed that most fractal values of the mandibular trabecular bone were, on average 5% lower than the matched controls. Therefore, the fractal analysis has a potential as a predicting tool for evaluating the bone structure in FAP patients. The radiomorphometric indices, MCI and MCW, were equal for our young sample of FAP patients and matched controls.

3) On a more significant scope, the third study embraced the challenge of a multicentric project. We highlighted the presence of FAP dento-osseous lesions since childhood, and these findings had the potential to be a predictive factor in the future. The FAP prevalence of osseous abnormalities was 65.4% in children and 75% in adults, whereas dental anomalies were not so different between children (15.4%) and adults (8.3%). These abnormalities in dental patients should be borne in mind as possibly indicating de novo or unrecognized disease. Liaison with a multidisciplinary team may be necessary to further investigate the related risk, including gastroenterology and genetics approaches.

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