Correlation Between Single Photon Emission Computed Tomography, AgNOR Count, and Histomorphologic Features in Patients With Active Mandibular Condylar Hyperplasia

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Purpose: To analyze the association between nuclear medicine examination (single photon emission computed tomography [SPECT]), histology, and Argyrophilic Nuclear Organizer Region (AgNOR) count in patients with active condylar hyperplasia who have undergone condylectomy.

Patients and Methods: Eight patients with a diagnosis of active condylar hyperplasia and evidence of facial asymmetry, with progressive deformation in time and on SPECT studies, were evaluated. The relationship between the rate of technetium Tc 99 intake, cartilage layer thickness, and cellular activity measured by recounting nucleolar organizers with AgNOR was evaluated.

Results: The 4 pathologic layers of condylar hyperplasia (fibrous, mesenchymal, and hypertrophic chondrocyte layers and ossification layer) showed great variability and different thicknesses among the cases analyzed. As age increased, the histologic layer thickness decreased ($r = -0.73$, $P = .04$). The age of the patients was inversely related to the number of AgNOR dots ($r = -0.65$, $P = .08$). The thickness of both mesenchymal and hypertrophic chondrocyte layers was related to cartilage island depth ($r = 0.81$, $P = .02$).

Conclusions: Younger patients with condylar hyperplasia had a thicker condylar layer and more cellular activity measured by AgNOR count. The histologic features of this group of patients could not be associated with their SPECT findings.

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Condylar hyperplasia is a pathologic temporomandibular joint condition resulting in a unilateral, progressive, non-neoplastic growth involving both the size and configuration of the neck and the mandibular condyle,[1-3] presenting increased multiplication in the affected mesenchymal layer (ML) of the affected condyle, in terms of hyperactivity of the proliferative layer of the articular cartilage. This condition causes mandibular and facial asymmetry, occlusal alterations, and occasionally, temporomandibular dysfunction.[3-5]

Nitzan et al[6] have classified condylar hyperplasias into 3 types according to their clinical features:

1. Vertical condylar hyperplasia, showing a predominantly vertical growth pattern

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2. Transversal condylar hyperplasia, showing a predominantly horizontal growth pattern
3. Mixed

The etiology of this alteration remains uncertain.[7,8] Articles in the relevant literature describe the following probable etiologic factors: intrinsic factors, such as local vascularization problems and endocrine alterations, and extrinsic factors, such as traumatic injuries and infections.[1,8]

The histology of a normal adult mandibular condyle consists of a surface fibrous layer, a layer of mesenchymal cells, and a fibrocartilage layer, whereas a growing articulation presents 4 layers: a surface fibrous conjunctive tissue, a mesenchymal (proliferative) layer, a hypertrophic chondrocyte layer (HCL), and an ossification layer.[9]

The condylar hyperplasia diagnosis depends on a good anamnesis, a clinical examination, and a standardized imaging study. A nuclear medicine test is a good tool to establish the actual activity status of this condylar hyper growth. Asymmetry progression as recounted by the patient, facial balance alterations, malocclusions, and temporomandibular dysfunction symptomatology are typical among these patients. Prospective imaging studies based on profile and frontal teleradiography, orthopantomography, and CT scan are basic to hyper growth quantification. Nuclear medicine studies, such as scintigraphy, and single photon emission computed tomography (SPECT) studies are intended for diagnostic confirmation of the increased metabolic activity at the condylar head. Through the administration of radio medicine (technetium Tc 99), we obtain 1 or several bidimensional (scintigraphy) or multiplanar (SPECT) images that show the distribution of this radioactive compound in the patient.

The SPECT study consists of 3 phases[7]:

1. Angiographic phase: At this early phase, the medicine is distributed by the venous and arterial blood vessels. The vascularization level is detected by means of quick images (2-5 seconds per image).

2.Tissular perfusion phase: These vessels (veins and arteries) later split up into capillaries and irrigate the soft tissues, thus permitting us to see how a tissue is being irrigated.

3.Osseous phase: This phase measures the degree of the existing metabolism and bone formation. This is what we want to know about this pathology. The value regarded as normal (nonpathologic) is on the average of 50% ± 5%.

Scintigraphy-based evaluation of condylar hyperplasia is performed in 2 ways:

1. By comparatively detecting the uptake rate between the 2 condyles—any difference between both temporomandibular joints above 10% is an abnormality indicator of a total 100% between both condyles.
2. By relating the uptake of both articulations through the fourth lumbar vertebra, which is used as a reference, because it is easy to locate and because of its symmetric uptake.[7]

These nuclear medicine methods are nonspecific because a positive result may be obtained after any kind of rise in bone metabolism, whether infectious, inflammatory, and traumatic or neoplastic, even by normal growth processes. Relating them to the clinical examination is therefore essentially important.

The etiologic treatment for this pathology is to remove the proliferative layer associated with the bone segment with hyper growth.[5]

The histopathologic study will allow confirmation of the diagnostic hypothesis through the morphologic evaluation of the resected condylar segment, which will permit the characterization of the condylar hyperplasia growth into 4 types.[10] This also permits the ruling out of other possible diagnoses, such as osteochondroma or some other neoplasia.[11]

Cell proliferation in the tissues can be estimated through immune-histochemistry tests with such markers as antibody Ki-67 and antibody proliferating cell nuclear antigen (PCNA), as well as the Argyrophilic Nucleolus Organizer Region (AgNOR) recount. The AgNOR technique uses silver salts for detecting those non-histone fibrillar proteins located in the nucleolus organizing regions (NOR). NOR are DNA segments that codify the ribosomal RNA, which is directly associated with protein synthesis and cell proliferation. The set of genes that transcribe ribosomal RNA are located in the secondary constrictions of chromosomes 13, 14, 15, 21, and 22. The nucleolus size may vary depending on the need for the cell to generate ribosomes, consequently increasing the protein production. The number and size of the NORs reflect the activity, proliferation, or transformation of the cells.[12,13]

This study aims to analyze the association between nuclear medicine examination (SPECT), histology, and AgNOR recount in patients with active condylar hyperplasia who have undergone condylectomy.

Patients and Methods

This retrospective study included 8 patients from the Hospital del Salvador and Dr Rodrigo Fariña's private office, with a diagnosis of active condylar hyperplasia. (This study was exempt from requiring approval from the ethical board of the Hospital del

Salvador.) A full clinical record was issued for all of them, confirming progressive deformation in time. Their clinical examination yielded evidence of facial asymmetry, and the imaging study confirmed condylar unit excess.

All the patients presented hyper uptake on their SPECT studies, and their images were analyzed by use of ImageJ software (National Institutes of Health, Bethesda, MD) for the purpose of determining the condyle uptake difference for each patient.

The patients' mean age was 18.1 years (range, 14-25 years), and the male-female ratio was 1:1, with the right and left sides equally affected.

All the patients underwent a low condylectomy performed at the Hospital del Salvador's Department of Maxillofacial Surgery.

The hyperplastic condyle consistently presented higher Tc-99 uptake values from the SPECT studies, with a mean rate of 62.5% (range, 65%-69%) (Fig 1, Table 1).

The removed hyperplastic condyle samples were included in paraffin blocks following cuts 3 μm thick, from which samples were produced for 2 studies:

1. Hematoxylin-eosin staining tinction was used to study histomorphologic features.
2. For the AgNOR-based tinction, a nitrate solution (1 part of a 2-g gel in 100 mL of 1% formic acid with 2 parts of 50% silver nitrate) was prepared. The samples were then washed in distilled water for 30 minutes, dehydrated, mounted, and observed for their analysis by use of 1,000× magnification.

The data obtained were submitted to a SYSTAT statistical analysis (Systat Software, San Jose, CA) to determine whether there was any relationship between the different variables to be studied (patient age, rate of Tc-99 intake, cartilage layer thickness, and recount of nucleolar organizers with AgNOR). Spearman correlation analysis was performed.

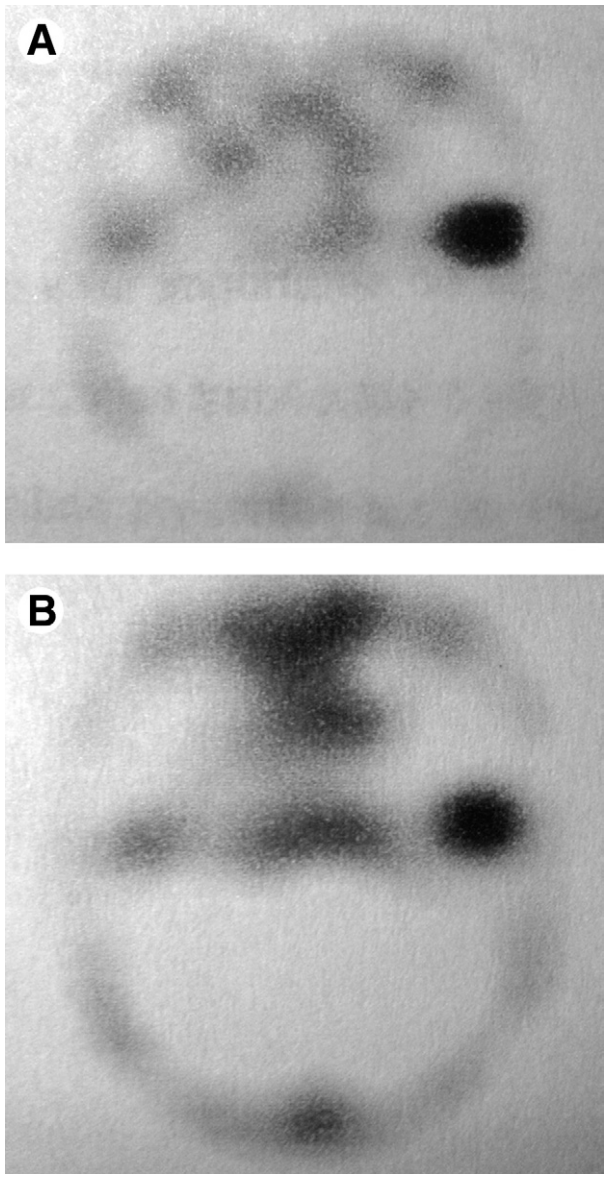

FIGURE 1. *A* and *B*, SPECT studies of 2 patients, with radiomedicine hyper uptake at affected temporomandibular joint.

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Results

HISTOMORPHOLOGIC FEATURES

In the 8 patients studied, hyperplastic condylar samples stained with hematoxylin-eosin were analyzed. The 4 layers that are typical of a pathologic condylar condition showed great variability in all cases. The surface fibrous layer presented a mean thickness of 0.146 mm (minimum, 0 mm; maximum, 0.326 mm). The mean thickness of the ML was 0.123 mm (minimum, 0.059; maximum, 0.239 mm). The mean thickness of the underlying HCL was 0.316 (minimum, 0.206 mm; maximum, 0.477 mm).

The sums of the ML plus the hypertrophic chondrocytes were calculated because these layers show the heaviest cellularity and provide the main source of activity. Their values ranged between 0.265 and 0.624 mm, with a mean of 0.439 mm.

The millimeter depth of the cartilage islands inside the bone trabeculae was estimated. The mean distances for these islands were 1.348 mm (minimum, 0.474 mm; maximum, 1.699 mm) (Fig 2, Table 1).

AgNOR

Regarding nucleolar organizers with affinity for silver, which were visualized as black or dark-brown

Table 1. VARIABLES OBTAINED

	Age (yr)	Gender	Affected Side	SPECT (%)	FL (mm)	ML (mm)	HCL (mm)	ML + HCL (mm)	Island Depth (mm)	AgNOR (n)
Case 1	14	Female	Right	65	n/o	0.134	0.434	0.568	1.538	2.1
Case 2	25	Male	Right	56	0.257	0.059	0.206	0.265	0.474	1.48
Case 3	18	Male	Left	69	0.029	0.092	0.263	0.355	0.938	1.53
Case 4	14	Female	Left	55	n/o	0.132	0.293	0.425	1.066	2.09
Case 5	20	Female	Right	67	0.326	0.059	0.268	0.327	1.174	2.07
Case 6	19	Male	Left	64	0.213	0.119	0.342	0.461	1.471	1.95
Case 7	15	Male	Left	56	0.056	0.147	0.477	0.624	1.699	1.95
Case 8	16	Female	Right	63	0.286	0.239	0.248	0.487	1.139	1.89
Mean	17.625	1:01	1:01	61.875	0.146	0.123	0.316	0.439	1.348	1.88

Abbreviation: FL, fibrous layer.

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dots, a number of 100 cells were counted in each case. The dot average per nucleus was 1.88, with a per-case average range of 1.48 to 2.1.

AgNOR quantification could be carried out especially in the cells that make up the HCL and in calcification inside the bone tissue[12,13] (Fig 3, Table 1).

A Spearman correlation analysis was done to evaluate the association between the variables of age, percentage difference with SPECT, ML and HCL, cartilage island depth, and AgNOR recount (Fig 4). Significant association was observed between the following:

1. Age/(ML + HCL): As the age increases, the histologic layer thickness decreases ($r = -0.73$, $P = .04$).
2. Age/AgNOR: As the patient's age increases, a smaller number of AgNOR dots are counted ($r = -0.65$, $P = .08$).
3. (ML + HCL)/island depth: As the thickness of both the ML and hyperchondrocyte layer increases, cartilage island depth is seen to increase to a statistically significant degree ($r = 0.81$, $P = .02$).

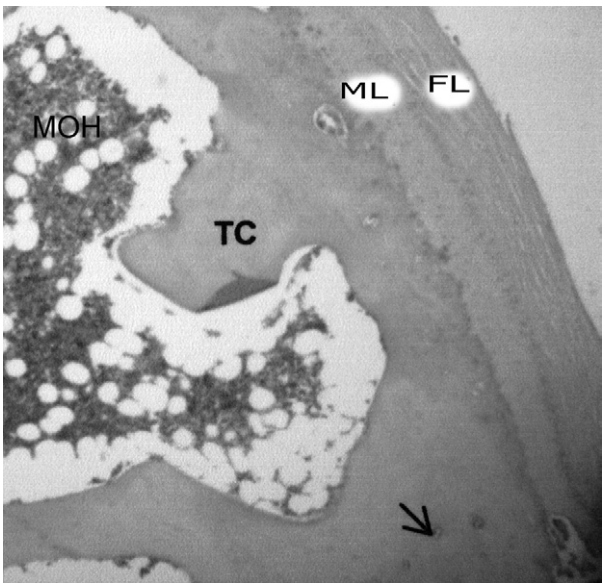

FIGURE 2. Condyle sample showing a wide fibrous surface layer (FL); beneath, a well-defined area of cells that make up the ML; and next, a small hypertrophic and bone tissue layer (TC) with cartilage islands inside of it (*arrow*) (hematoxylin and eosin stain, original magnification ×40). (MOH, hematopoietic bone marrow.)

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Discussion

The results allow us to confirm active condylar growth in the entire specimen being examined through SPECT but with different histologic features.

The relationship found between the thickness of the ML and HCL and the depth of the cartilage islands suggests that patients who are active condylar hyperplasia carriers have greater cell multiplication in the proliferative layer during active distant mineralization.

In the cases analyzed we observed a trend of an inverse relationship between AgNOR count (related with active cellular metabolism) and age. For this reason, we propose that histopathologic features of the cases with condylar hyperplasia should be analyzed considering the age of the patients. Slootweg and Müller[10] share our postulation that histopathologic features of condylar hyperplasia should depend on age.

So far, the best clinical approach to the diagnosis of condylar hyperplasia has possibly been through the clinical history and SPECT-assisted confirmation of greater Tc-99 uptake. Regrettably, the mechanism used to measure this uptake is the uptake rate difference between the affected side and that not affected. In our sample the patients presented affected condyle ranges between 56% and 69%. However, it was not possible to confirm an association between these results with the his-

topathologic characteristics. The great variability of values obtained with SPECT probably did not permit us to relate them to any of the other variables. Moreover, Tc-99 hyper uptake may possibly be a late development from the variables already studied, presenting higher values at moments of greater bone apposition, and not necessarily encompassed by the initial biological changes (like those studied) or by increased protein syntheses. Therefore increased Tc-99 uptake is evidence of a condylar hyperplasia undergoing ossification, rather than a protein synthesis–phased hyperplasia on SPECT.

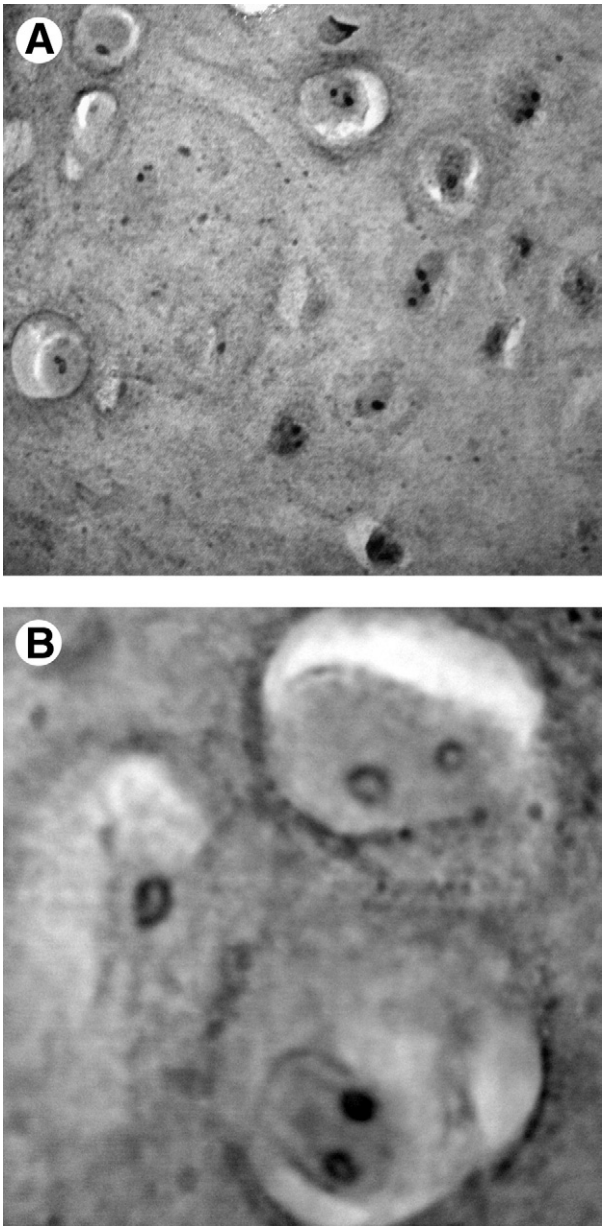

FIGURE 3. *A*, Chondrocytes that are part of hypertrophic cartilage (AgNor stain, original magnification x500). *B*, Presence of 1 to 3 AgNoR-stained nucleolar organizers for each nucleus (AgNor stain, original magnification ×1,000).

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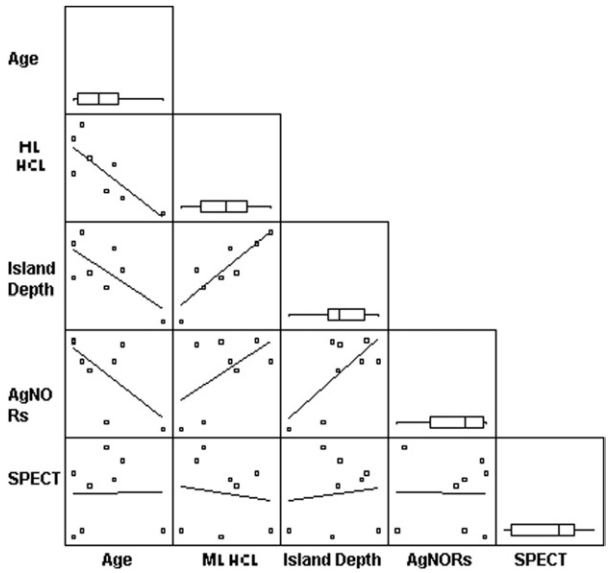

FIGURE 4. Dispersion of variables under study.

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One other factor to consider accounting for the lack of a relationship is that the time between the administration of SPECT and the moment of surgery was variable for the different patients (1-6 months). For this reason, the scintigraphic activity for the examination was not necessarily coincidental with that at surgery and during the histologic examination.

The histomorphologic features in patients with active mandibular condylar hyperplasia show an inverse association between AgNOR count and patient age. Younger patients with condylar hyperplasia had a thicker condylar layer and more cellular activity measured by AgNOR count. Although the best clinical approach to the diagnosis of condylar hyperplasia has been the clinical history with SPECT confirmation of greater Tc-99 uptake, the histologic features observed in our patients have not been linked to the SPECT findings, showing that examination is not sensitive enough to detect the histopathologic variations possibly found in patients with condylar hyperplasia.

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