International Journal of Research (IJR)

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

Ossification of the temporal bone and mandibular condyle in the human fetus: a comparative study

Dr. Wilfredo Molina Wills

DDS, MSc, MShr, PhD

(https://orcid.org/0000-0001-7233-4972)

Researcher at the University Latina of Panamá and Visiting researcher of the department of morphological sciences of the School of Medicine of the University of Los Andes, Venezuela.

Dr. Vanessa Rodriguez DDS, private practice.

Abstract

Histological studies of the temporomandibular joint in human fetuses in the gestational stages of 12 and 14 weeks were performed. The aim of the study was to compare the formation of osteoid tissue between the temporal bone and the mandibular condyle at the indicated stages of development. Methods: Histological sections were sectioned serially in sagittal planes 8 μm wide and stained with hematoxylin-eosin. The total amount of osteoid tissue was calculated for each histological section in the temporal bone and mandibular condyle by ImageJ software. Statistical analysis**:** Student's T was used to compare the results of the ossification areas Results. The percentages of osteoid tissue formation in the temporal bone were higher than in the mandibular condyle and were even more evident at 14 weeks of prenatal development. The differences were extremely significant for both weeks of gestation studied. Conclusion: osteoid tissue was observed occupying larger areas in the zone near the glenoid cavity in the temporal bone at 12 and 14 weeks of gestation than in the mandibular condyle. In the condyle the formation of osteoid tissue was located at the neck level

Key words: Mandibular condyle; Ossification; Temporal bone; Temporomandibular joint.

Introduction

The processes of craniofacial growth and development are complex and begin from the very early stages of gestation. During the eighth week of fertilization, the mandibular trabecular bone grows rapidly to form muscular insertions of the masseter, temporal and pterygoid. From week 14 there is histological evidence of a conical structure as well as trabecular growth patterns in the mandibular body, Suk keun Lee et al (2001)

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

Anatomically the jawbone is connected to the temporal bone through the temporomandibular joint and phylogenetically seems to have evolved towards a complete development involving muscular, alveolar, neural, and articular structures, Goret-Nicaise and Dhem (1984). With respect to joint involvement, joint disc formation has been reported at 12 weeks of prenatal development, Molina W et al (2005) and likewise, opening and closing mouth movements at the same stage of development, Molina W (2017) in week 12 of fertilization, the conical shaped condyle was elongated toward the temporal squama to form the temporomandibular joint, there is a rapid growth process for the mandibular condyle in the early stages of gestation. From week 14 of fertilization, the growth of the condyle became apparent on histological and radiological examination and, the growth of the proximal end of condyle was also approximated to the mandibular primary growth center. Between weeks 13-15 of intrauterine life mandibular trabecular growth occurs from the centers of ossification or primary growth, Suk K et al (2001).

The temporal bone undergoes a mixed ossification process. The ossification of the squamous portion is intramembranous, while the rest of the bone does so by endochondral ossification, Sperber GH, Sperber SM, Guttmann GD (2010). The condyle and glenoid cavity originate from endochondral ossification. The first evidence of TMJ formation during development is the appearance of distinct mesenchymal condensations, the temporal and condylar blastemas, Avery JK (2001).

The objective of this study was to compare the formation of osteoid tissue in the areas of the temporal bone near the glenoid cavity and the articular portion of the condylar head and neck of the condyle in the human fetus at 12 and 14 weeks gestation.

Materials and Methods

Surgical Procedures

Surgical procedures for tissue removal were previously considered taking into account the need for the presence of the temporal surface and the condyle of the mandible in the histological sections.

14 aborted human fetuses of 12 and 14 weeks gestation without any visible abnormality traits were selected for this study.

The zone of tissue corresponding to the area of the temporomandibular joint a square centimeter was removed. The histological sections were separated and divided by gestational ages into group A for 11 weeks and a group B for 12 weeks of gestation respectively (seven fetuses for each group).

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

Procedure for fixing histological sections

These blocks were fixed in 10% stabilized neutral formalin containing 40% formaldehyde, 4.0 g of sodium dehydrogenate phosphate, and 6.5 g of disodium hydrogen phosphate at Ph 7.0 and stored at 4◦ c. The blocks were embedded in paraffin, and these paraffin-embedded blocks were sectioned serially in sagittal planes 8 μm wide with a rotatory microtome. Ten sections were obtained for each block. Sections were deparaffinized and hydrated in distilled water.

Histochemistry

Each section was cut and mounted on a previously numbered glass slide and stained with hematoxylin-eosin. There was no calibration with respect to their rating but the two observer's reports were similar. The selected sections were photographed and digitalized.

Selection criteria

Only histological cuts that allowed us to observe a clear image of the condylar surfaces of the head or neck and temporal surfaces near the glenoid cavity were taken into account for this study.

Analysis of ossification zones

Quantitative analysis was performed with serial sections for each studied areas. The total amount of osteoid tissue was calculated for each histological section in the temporal bone and mandibular condyle by ImageJ software. This method was used to analyze and calculate the percentage of areas of osteoid tissue formation and compare them between the temporal bone and the mandibular condyle. In all the selected cases, the average of the percentage of stained of bone formation areas of the mandibular condyle and temporal in relation to the total area of photomicrography was calculated. All the images were obtained through a transmitted light microscope (Kyowa Medilux-12) with video camera (motic 2000) and motic images 2.0 Multilanguage application software for the acquisition of the differences in staining properties and morphology facilitating identification of all studied tissues. All the images of the study with a scale bar of 10 µm were obtained and analyzed in the department of morphological sciences of the University of Los Andes Venezuela

Osteoid surface signaling

The formation surfaces of osteoid tissue in the temporal bone and mandibular condyle were marked with arrows on photomicrographs.

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

Statistical analysis

Student's T was used to compare the results of the ossification areas measured with image J software. The area of osteoid tissue formation of the temporal bone was compared with the articular area of the head and neck of the condyle for the ages of 12 and 14 weeks of gestation respectively.

Ethical aspects

To carry out this study, samples of aborted human fetuses were used in accordance with procedures approved by the department of Morphological Sciences of the Medicine School of the University of Los Andes. All the conditions of Helsinki declaration related to research in human subjects were observed

Results

The identification of osteoid tissue was confirmed by the techniques hematoxylin and eosin in all histological sections performed. It was observed that the secretion of the osteoid matrix product of the activity of the osteoblasts occupied larger areas in the temporal bone in the region near the glenoid cavity than at the level of the mandibular condyle both at 12 weeks and at 14 weeks of gestation. The areas of the upper portion of the condylar head were not observed with osteoid tissue formation in the stages studied. Small scattered areas of osteoid tissue were observed in the neck of the condyles evaluated in this study. Similarly, it was observed that the areas of osteoid tissue of the temporal bone were more intensely stained than in the mandibular condyle figs (6, 9).

Percentages of ossification by areas of the temporal bone and mandibular condyle at 12 weeks of gestation

Ossification of the temporal bone at 12 weeks gestation	Ossification of the upper area of the head of the mandibular condyle at 12 weeks of gestation	Ossification of the neck of the mandibular condyle at 12 weeks gestation
4.10%	0	2.14%
4.23%	0	1.92%

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

4.16%	0	1.69%
4.55%	0	2.05%
4.29%	0	2.01%
4%	0	2.10%
4.03%	0	2.08%

Table 1. It is possible to observe that the formation of osteoid tissue is greater than that observed in the histological sections of the neck of the condyle at 12 weeks of prenatal development. No formation of osteoid tissue was observed in the articular area of the condyle head at this stage in the histological sections studied.

Percentages of ossification by areas of the temporal bone and mandibular condyle at 14 weeks of gestation

Ossification of the temporal bone at 14 weeks gestation	Ossification of the upper area of the head of the mandibular condyle at 14 weeks gestation	Ossification of the neck of the mandibular condyle at 14 weeks gestation
19.67%	0	19.01%
26.13%	0	19.59%
30.14%	0	19.53%
31.90%	0	18.55%
26.92%	0	15.29%
29.78%	0	16.92%
34.15%	0	19.20%

Table 2. The formation of osteoid tissue at 14 weeks of gestation in the area near the glenoid cavity of the temporal bone was greater than in all the cases studied in the neck of the mandibular condyle. In no case was osteoid tissue formation observed in the joint area of the condyle head at this stage of gestation.

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

International Journal of Research (IJR)
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

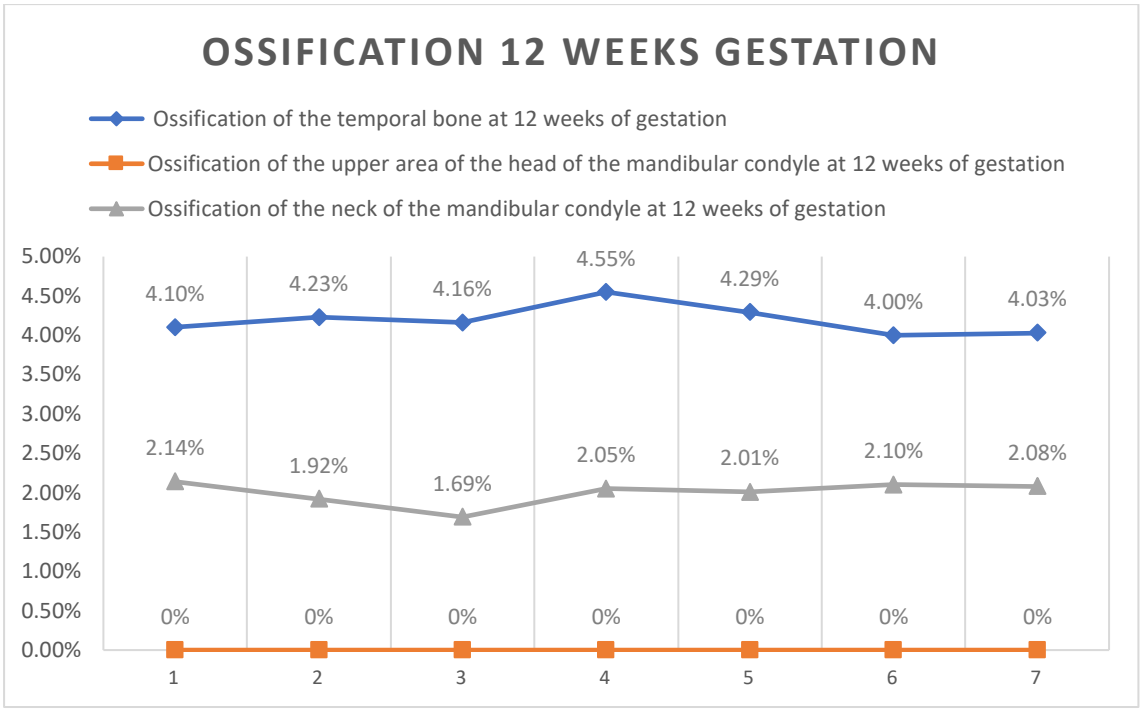

Figure 1. The comparison of osteoid tissue formation in the temporal bone, head, and neck condylar at 12 weeks of intrauterine life is observed.

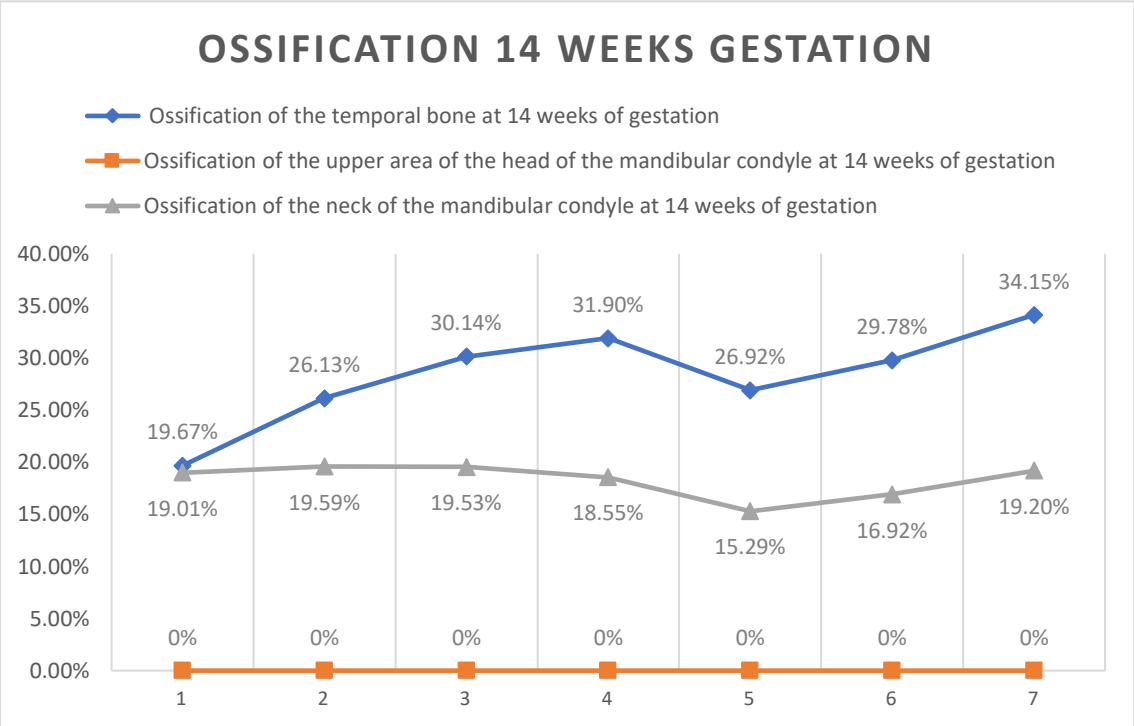

Figure 2. It shows the comparison between the formation of osteoid tissue between the articular portion of the temporal bone and condylar head and the neck of the mandibular condyle.

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

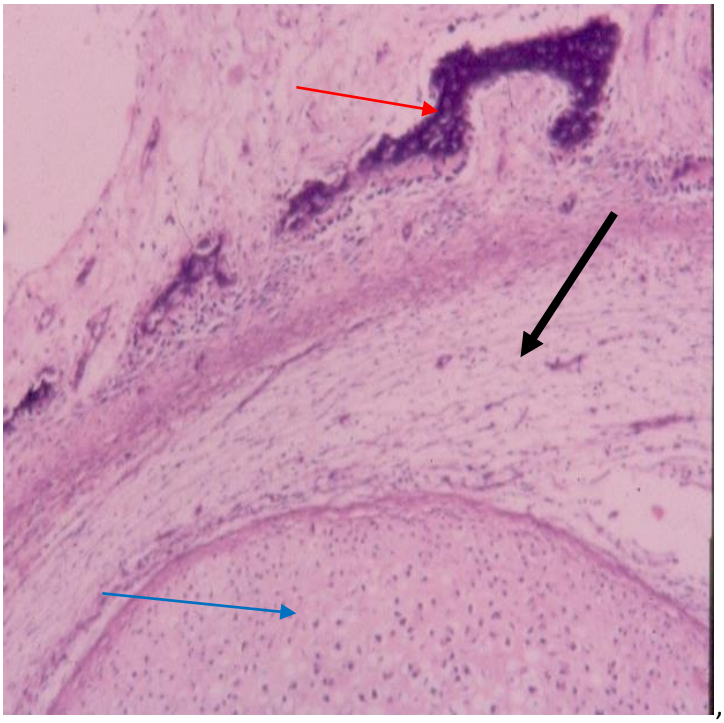

Figure 3. It shows the formation of osteoid tissue in the temporal bone at 12 weeks gestation (red arrow). The articular surface of the condyle head does not show osteoid tissue formation (blue arrow). Scale bar ——— 10 µm, articular disc (black arrow). 400x.

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

Figure 4. Photomicrography shows the formation of osteoid tissue in scattered areas of the condyle neck at 12 weeks gestation (yellow arrow), 400 X, scale bar **1**0 µm

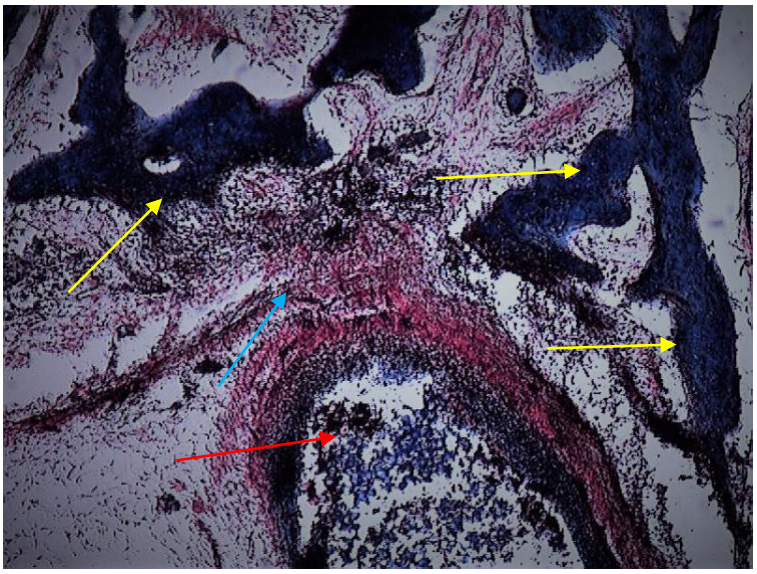

Figure 5. Histological section of human fetus of 14 weeks of gestation where osteoid tissue formation is observed that occupies a large area of the temporal bone (yellow arrows), The red arrow indicates the head of the condyle and the blue one to the glenoid cavity, 400x,Scale bar **1**0 µm

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

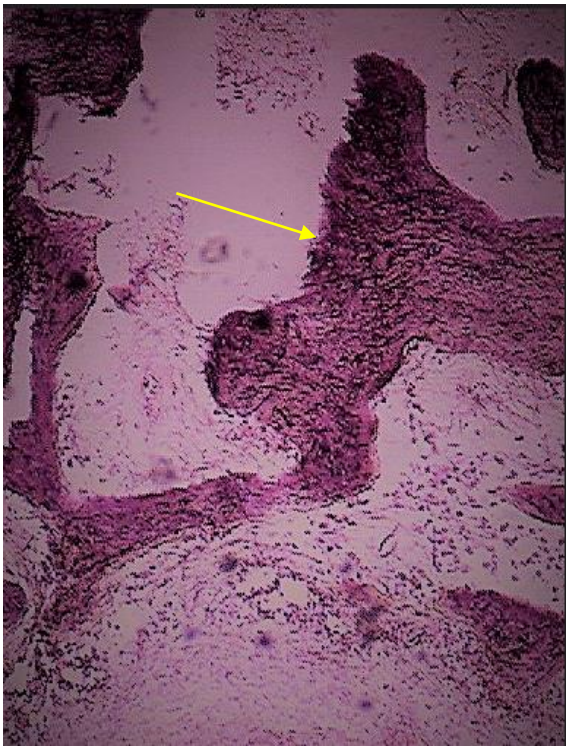

Fig 6. Microphotograph of an area of the temporal bone of a human fetus of 14 weeks of prenatal development, where osteoid tissue formation is observed in areas near the glenoid cavity (yellow arrow). 400x and scale bar **1**0 µm ▬▬▬

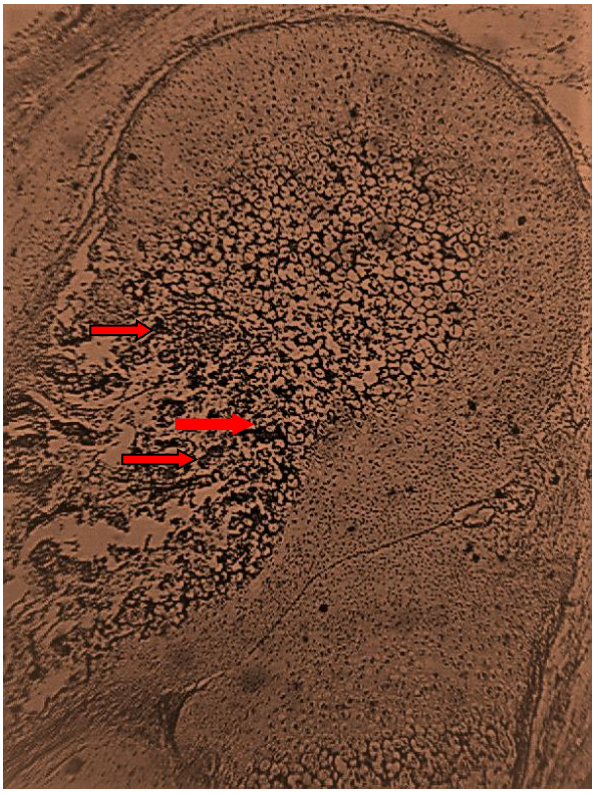

Fig 7. Human fetus 12 weeks gestation, scattered formations of osteoid tissue are observed in the neck of the condyle (red arrows).

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

International Journal of Research (IJR)
e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

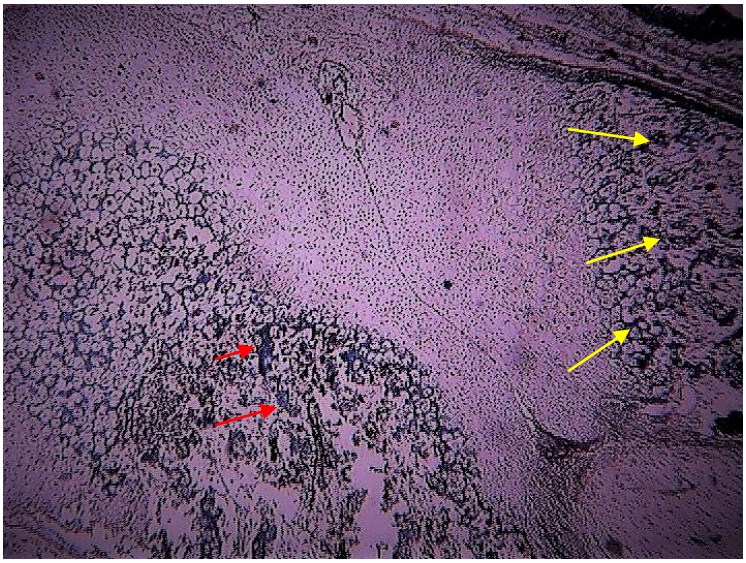

Fig 8. Human fetus at 12 weeks gestation, comparison of areas of osteoid tissue in the neck of the condyle and posterior wall of the glenoid cavity, 400x and scale bar **1**0 µm

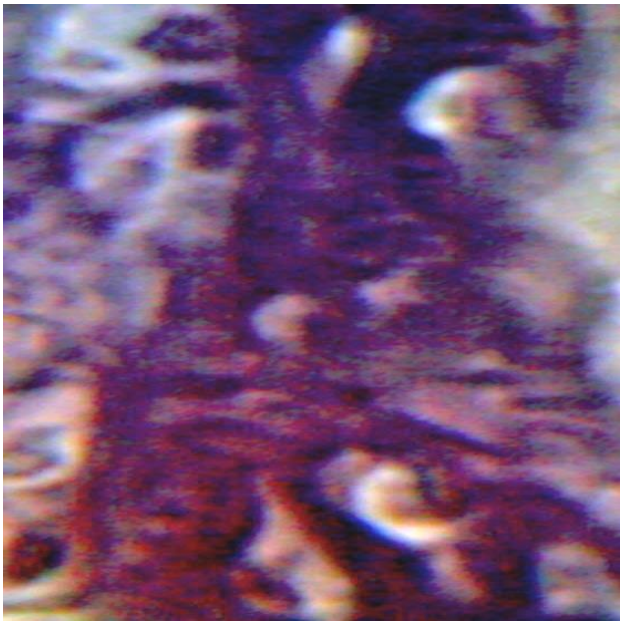

Fig 9. High-intensity osteoid tissue formation in a 14-week-old human fetus in the temporal bone

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

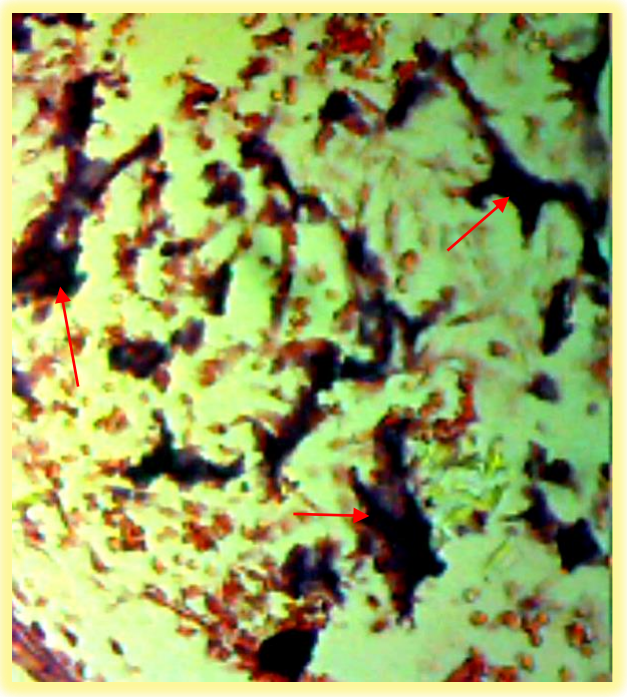

Fig 9. Formation of osteoid tissue in the neck of the condyle at 14 weeks gestation (red arrows) in the same way, it is possible to observe that the formation of osteoid tissue occurs near the blood vessels.

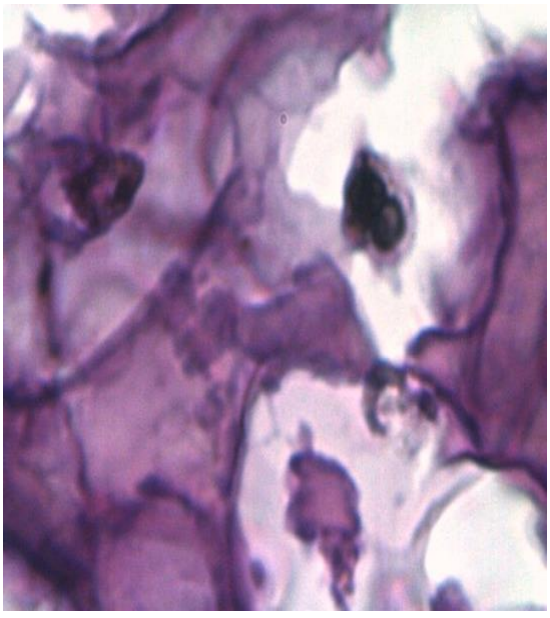

Fig 10. Formation of osteoid tissue in the neck of the condyle at 12 weeks gestation

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

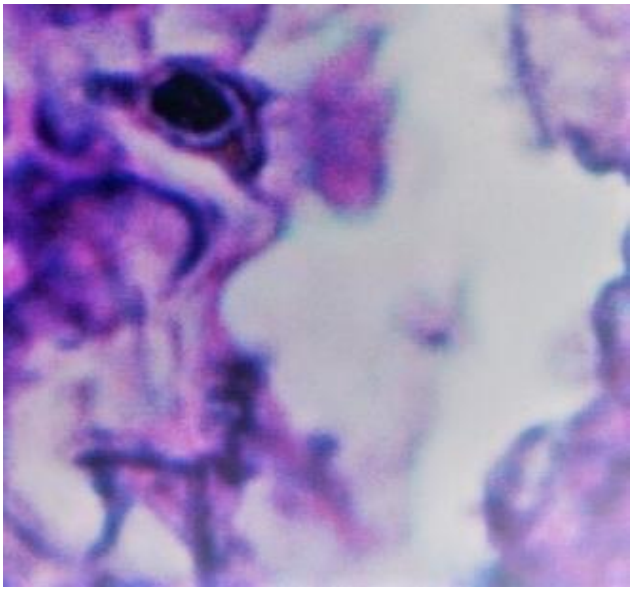

Fig 11. Formation of osteoid tissue in the temporal bone at 12 weeks gestation, scale bar **1**0 µm ——

Statistical results

Comparison between ossification of the temporal bone and the ossification of the upper area on the head of mandibular condyle at 12 weeks of gestation: P value <0.0001, this difference is consider extremely significant. T = 59.06, df = 12 and standard error of difference was 0.071.

Comparison between ossification of the temporal bone and the neck of mandibular condyle at 12 weeks of gestation: P value <0.0001. T = 23.95, df = 12, standard error = 0.092.

Comparison between ossification of the temporal bone and articular zone of the head of the mandibular condyle at 14 weeks of gestation: P value <0.0001. T = 15.90, df = 12, standard error of difference = 1.785.

Comparison between ossification of the temporal bone and the neck of mandibular condyle at 14 weeks of gestation: P value = 0.0002. T 05.34, df = 12 and standard error of difference 1.886.

Discussion

The importance of the findings of this comparative study is to show the formation of osteoid tissue of great staining intensity that occupies more areas in the temporal bone than in the mandibular condyle in these stages of intrauterine life studied. The mandibular condyle is considered an important center of growth and regulation of these processes of development is determined by genetic and epigenetic factors that modify the expression of this growth. Similarly, functional factors involved in craniofacial growth have been considered, Moss (1997) . For this reason, it is possible

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

to suggest the possible influence of the condyle on the rapid growth of temporal bone into the articular region.

Although it has been reported that in histological findings in the fifteenth gestational week, a thin intramembranous ossification of the temporal components and endochondral ossification of the condylar process has been observed, *K Morimoto, N Hashimoto, T Suetsugu (1987),* the results obtained in this study show that in weeks 12 and 14 of prenatal development, there is the greater formation of osteoid tissue in the temporal bone than in the mandibular condyle.

There is an intimate relationship between osteoid tissue secreted by osteoblasts and blood vessels in nearby areas. The findings of this study show this relationship, especially in the area of the condylar neck, and are similar to the observations of Percival and Riechtsmeier (2013).

Conclusion

Ossification is the process in which osteoblasts secrete layers of osteoid tissue. During the early stages of gestation at weeks 12 and 14, osteoid tissue was observed occupying larger areas in the zone near the glenoid cavity in the temporal bone than in the mandibular condyle. In the condyle the formation of osteoid tissue was located at the neck level and surrounded by blood vessels.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors want to thank the Pathological Anatomy Unit of Valencia Central Hospital, the Histology Department of the University of Los Andes, the Dr. Ronnie Molina and Mr. Orlando Ortiz of the University Latina of Panamá for their contribution to this work.

References

1-Suk Keun Lee,Yeon Sook Kim,Hee Soo Oh,Kyu Ho Yang,Eun Cheol Kim,Je Geun Chi, 2001, Prenatal development of the human mandible, jour Anatomical record, **https://doi.org/10.1002/ar.1110**

2-Goret-Nicaise M, Dhem A. 1984. The mandibular body of the human fetus. Histologic analysis of the basilar part. *Anat Embryol* **169**: 231– 236.

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*

e-ISSN: 2348-6848
p-ISSN: 2348-795X
Vol. 9 Issue 12
December 2022

3-Wilfredo Molina, Silvano Pino, Gisela Sosa and Luis Hernández (2005), Distribution of mucopolysaccharides and glycoprotein in the articular discs of the temporomandibular joints in human fetuses, journal of orofacial pain, 19: 325-330.

4- Wilfredo Molina Wills (2017) Los movimientos bucales, y desarrollo neuromuscular y temporomandibular en el feto humano, (Rev Esp Ortod. 2017;47(4):197-200) , *Volumen 47, Número 4*

5- Sperber GH, Sperber SM, Guttmann GD : Craniofacial Embryogenetics and Development, ed 2. Shelton, CT : PMPH-USA, 2010, pp95-118

6-Jk Avery (2001), Oral Development and Histology (Thime, 3rd Ed, New York), pp. 435.

7- Moss ML (1997), The functional matrix hypothesis revisited. 3. The genomic thesis. Am J Orthod Dentofacial Orthop, Sep;112(3):338–42. Doi: 10.14670/HH-11-858.

8-K Morimoto, N Hashimoto, T Suetsugu (1987), Prenatal developmental process of human temporomandibular joint**,** Prosthet Dent;57(6):723-30.doi: 10.1016/0022-3913(87)90372-6.

9-Percival CJ, Richtsmeier JT. Angiogenesis and intramembranous osteogenesis. Dev Dyn. 2013 Aug;242(8):909-22. [PMC free article] [PubMed]

Received: *21 Nov. 2022*
Revised: *15 Dec 2022*
Final Accepted for publication: *26 Dec 2022*