Distribution of Mucopolysaccharides and Glycoproteins in the Articular Discs of Temporomandibular Joints in Human Fetuses

Wilfredo Molina, DDS, MSc
Researcher

Silvano Pino, MD
Professor

Department of Physiology
School of Medicine
Universidad de Los Andes
Mérida, Venezuela

Gisela Sosa, MD, MSc
Professor
Pathological Anatomy Unit
Universidad de Carabobo
Valencia, Venezuela

Luis Hernández, MD
Professor
Department of Physiology
School of Medicine
Universidad de Los Andes
Mérida, Venezuela

Correspondence to:
Dr Wilfredo Molina
Apartado 109
Mérida 5101-A, Venezuela
Fax: +0058 274 2638304
E-mail: wjmw55@hotmail.com

Aims: *To evaluate the distribution of mucopolysaccharides and glycoproteins in the articular discs of temporomandibular joints (TMJs) in human fetuses at different stages of development in order to test the hypothesis that the development and histological maturation of the articular disc has already begun by the 12th week of gestation.* ***Methods:*** *Eighteen human fetuses at gestational ages 12, 14, and 16 weeks were used (6 fetuses of each age). Sections (6 μm wide) of the articular discs were stained with trichromic stain for collagen fibers, Mayer's mucicarmine for mucopolysaccharides, and Schiff's periodic acid reaction for glycoproteins. The densities of the stained zones were measured by means of Image J software. The nonparametric Kruskal-Wallis test was used to evaluate the differences among stained zones in the 3 fetus groups.* ***Results:*** *TMJ tissues of four of the six 12-week-old fetuses were stained positive for collagen fibers, mucopolysaccharides, and glycoproteins. In these fetuses the stain was localized to the articular posterior area and was denser in that area than in the middle and anterior areas. The stained areas in the 14-week-old fetuses were distributed throughout the articular discs, with isolated clear unstained areas. The stained areas of all the articular discs of the 16-week-old fetuses were more compact than those of the 12-and 14-week-old fetuses. In all the fetuses examined, the collagen fibers along the articular disc had a wavy appearance. The fossa of the temporal bone was observed in all the fetuses as a straight structure that was similar in the 3 fetus groups. The densities (mean ± SD) of the stained zones were 38.36% ± 3.39%, 59.5% ± 1.56%, and 94.04% ± 2.04% for 12, 14, and 16 weeks of gestation, respectively; these densities were significantly different (χ^2 = 15.16;* df = 2, P < .001). ***Conclusion:*** *This study indicates that mucopolysaccharides and glycoproteins as well as collagen fibers are present at 12, 14, and 16 weeks of gestation. This suggests that the histological maturation of the articular disc has already begun at the 12th week and is complete by the 16th week of gestation.* J OROFAC PAIN 2005;19:325–330

Key words: articular disc, collagen, human fetuses, mucopolysaccharides, temporomandibular joint

Studies of the physiology of the temporomandibular joints (TMJs) and their painless and frictionless functional dynamics have clarified the relationship between articular positioning and normal movements. These studies have moreover contributed to the understanding of the restrictive patterns, compression, and degeneration processes that affect the osseous elements and the soft tissues of the TMJs. The articular disc, as a means of harmo-

nization, is 1 of the structures that is most severely affected by compressive processes in the TMJ. It is therefore a structure that needs to be studied.

However, to better understand the normal functioning of the TMJs, it is not only necessary to understand their physiological processes, but it is also necessary to understand the embryologic development of the joints. TMJs develop from 2 cellular condensations: a temporal and a condylar blastema. The secondary cartilage growth in the condylar blastema appears by the 10th week of intrauterine life.[1] The premaxilla, the superior maxilla, part of the temporal bone, and the mandible develop from the first pharyngeal arch. The cells of the neural crests R_1 and R_2 move toward this arch to form the skeletal components of the face.[2] At 10 weeks of gestation, the mesenchyma between the condylar cartilage of the mandible and the developing temporal bone begins to differentiate.[3] Collagen fibers begin to turn in the postero-anterior direction during the 4th month of intrauterine life,[4] and the presence of a specific proteoglycan in the cartilage has been detected along with type II collagen in areas of craniofacial growth induction.[5]

The biomechanical properties of the articular disc depend to a great extent on the composition and organization of the extracellular matrix.[6] There is evidence of the biomechanical importance of elastic and collagen fibers and proteoglycan, especially in many collagen tissues, which are subjected to tensile and pressure processes.[7]

With regard to elastic fibers, these occur in various areas between the collagen fibers in the disc.[8] A fine fibril group located in the middle part of the TMJ disc in dogs presents a twisted and wavy aspect. This configuration is associated with articular function to absorb shock.[9] In the human TMJ disc, the elastic fibers run parallel to collagen fibers; this configuration is thought to be related to resistance and reversible deformation.[10] The distribution of elastic and collagen fibers is proportional to the disc function[11]; this may reflect the mechanical loads to which they are subjected.[12]

The orientation of collagen fibers has also been linked to areas of greatest resistance and tensile forces. These tensions are not only supported by the morphology and disposition of the fibers in the matrix, but also by the presence of proteoglycans. However, the proteoglycan composition of the human fetal disc has not been previously reported,[13] and the distribution of glycosaminoglycans in the developing disc is not clear.[14] What is clear is the lubricating role of the mucopolysaccharides and glycoproteins disbursed among the collagen fibers.[15] They allow these fibers to slide and remain separate. The amount of proteoglycan between the fibers of the articular disc has been linked to the articular compressive response. As such, the proteoglycan increases in the unilateral bite.[16] Therefore, the amount of proteoglycan should be a good indicator of articular disc maturation.

The purpose of this study was to evaluate the distribution of mucopolysaccharides and glycoproteins in the articular disc of TMJ in human fetuses and to test the hypothesis that the development and histological maturation of the articular disc has already begun at the 12th week of gestation. The disposition of collagen fibers and the presence of mucopolysaccharides and glycoproteins in the matrix surrounding the fibers in the articular disc of the TMJs in human fetuses are also discussed.

Materials and Methods

The TMJs of 18 human fetuses aborted at 12, 14, and 16 weeks of gestation were removed in 1 cm^3 blocks. These blocks were fixed by immersion in 10% stabilized neutral formalin containing 40% formaldehyde, 4.0 g of sodium dihydrogen phosphate (NaH_2PO_4), and 6.5 g of disodium hydrogen phosphate (Na_2HPO_4) 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 6 μm wide with a rotary microtome. Thirty sections were obtained for each TMJ. Sections were deparaffinized and hydrated in distilled water. Trichromic stains were used for collagen fibers, Mayer's mucicarmine was used for mucopolysaccharides and Schiff's periodic acid reaction (PAS) for glycoproteins. The protocol used was the US Armed Forces Institute of Pathology histo-technology method.[17]

Each section was cut and mounted on a previously numbered glass slide and independently assessed by 4 members of the Pathological Anatomy Unit of Valencia Central Hospital; all of the observers were specialists in pathological anatomy. There was no calibration with respect to their ratings, but the 4 observers' reports were similar. Their findings were reported according to the following criteria: presence or absence of the articular disc, presence or absence of mucopolysaccharides and glycoproteins in the articular disc, and presence of empty spaces (unstained zones). The observers did not know the fetus ages.

The selected sections were photographed and digitalized. Only those with a complete anterior, middle, and posterior area of the disc were chosen.

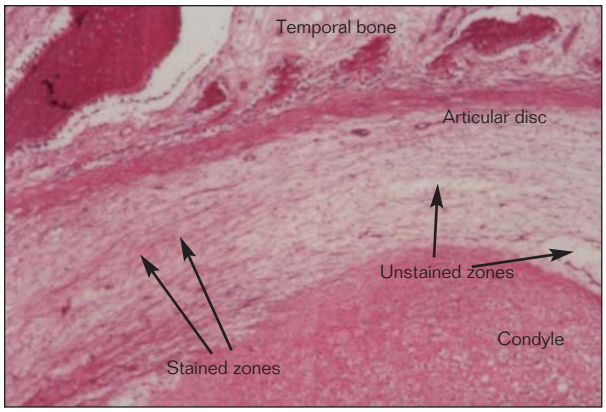


Fig 1 TMJ section of a 12-week-old fetus stained with the PAS technique. Observe the stained zones on posterior part of the articular disc that represents glycoproteins between collagen fibers. The intermediate and anterior zones are not stained.

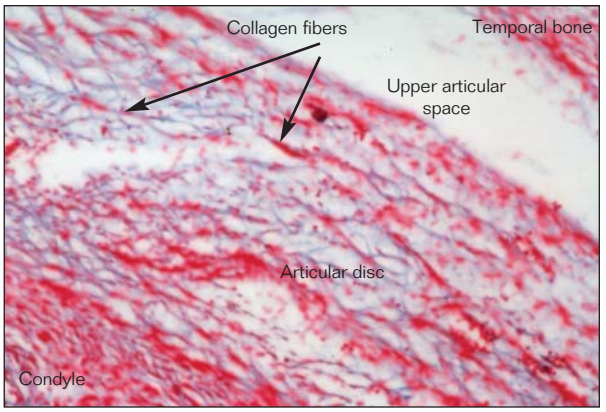


Fig 2 TMJ section of a 12-week-old fetus stained with trichromic. Black arrows indicate collagen fibers (blue) in an undulated array along the disc.

Image J software (US National Institutes of Health) was used to analyze the photographs in order to detect the percentage of the stained and unstained zones in relation to the whole area of the articular disc. In all the selected cases, the average of the percentage of the stained zone and unstained areas of the articular disc was calculated.

The presence of mucopolysaccharides and glycoproteins in the spaces between collagen fibers in the articular disc of the TMJs was then determined. The results were classified into 4 types as follows:

- Type I: Absence of mucopolysaccharides and glycoproteins between the collagen fibers of the articular disc
- Type II: Isolated presence of these elements with large, empty spaces between the collagen fibers
- Type III: Large quantities of mucopolysaccharides and glycoproteins with a few isolated empty spaces between the collagen fibers
- Type IV: A compact mass of mucopolysaccharides and glycoproteins in the articular discs, with a smaller percentage of empty spaces between the collagen fibers than what was observed in the previous types

The nonparametric Kruskal-Wallis test for independent samples was used to evaluate the difference between stained zones. The level of significance was .05.

No fetus used in this study had any visible evidence of developmental abnormalities or genetic disorders. To carry out this study, samples of naturally aborted human fetuses were used in accordance with procedures approved by the Ethical Committee of the Pathological Anatomy Unit of Valencia Central Hospital. The Pathological Anatomy Unit of the Hospital is responsible for aborted fetuses when the mothers do not request them. The Hospital thus gave written consent for the use of the fetuses in this study protocol. After the samples were taken, the fetuses were returned to the Pathological Anatomy Unit. All the issues of the Helsinki Declaration related to research in deceased human subjects were observed.

Results

Of the six 12-week-old fetuses, 2 were classified as Type I, while 4 were classified as Type II, with small, isolated stained areas showing the presence of mucopolysaccharides and glycoproteins between the fibers of the articular disc. The stain was localized to the articular posterior area and was denser in that area than in the middle and anterior areas (Fig 1). The collagen fibers along the articular discs presented a wavy appearance (Fig 2).

All six 14-week-old fetuses under study were classified as Type III. There were large orange and dark pink areas (mucicarmine stain and PAS stain, respectively). These stained areas were distributed all over the articular disc, both at the extremities and in the central portion. There were small, unstained areas scattered among the stained areas.

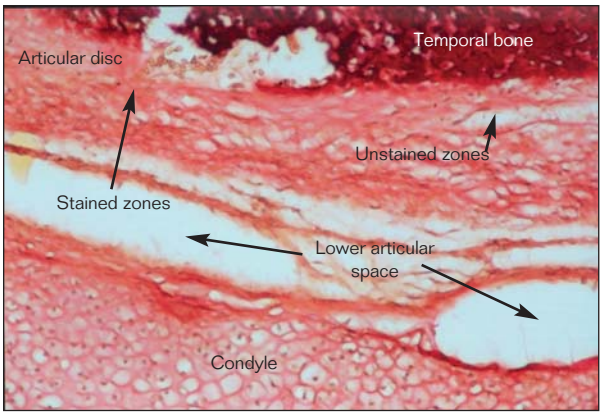
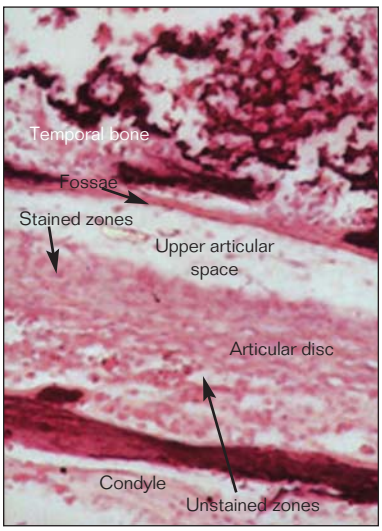
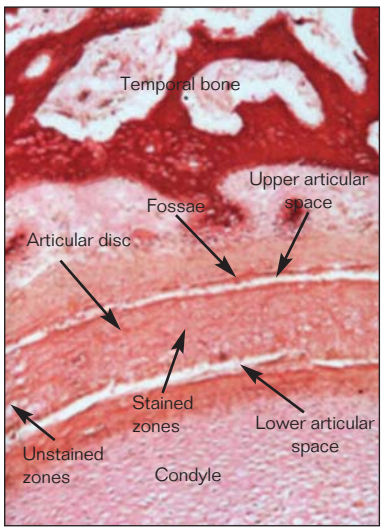


Fig 3 TMJ section of a 14-week-old fetus stained with Mayer's mucicarmine. In this section the anterior, intermediate, and posterior zones of the articular disc can be observed. These 3 zones are stained with orange. Clear spaces along the articular disc can also be observed. These clear areas are mostly located in the anterior portion of the articular disc.





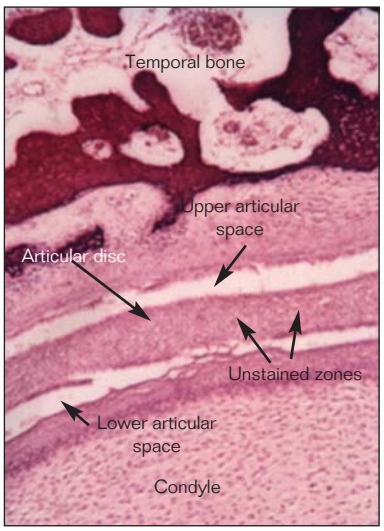


Fig 4 TMJ section of 14-week-old fetus stained with the PAS technique. Purple areas distributed along the articular disc can be observed.

Fig 5 TMJ section of a 16-week-old fetus stained with Mayer's mucicarmine. In this picture compact and uniform zones of mucopolysaccharides (orange) in the articular disc can be observed. Note the paucity of clear zones.

Fig 6 TMJ section of a 16-week-old fetus stained with PAS. Areas of the articular disc stained purple for glycoproteins. Note that there are few clear areas and that these are diffuse.

The stained areas were more uniformly distributed, and there was a reduction in the size of unstained areas when compared to those of the 12-week-old fetuses (Figs 3 and 4).

In the six 16-week-old fetuses, the stained areas on the articular disc stained with either mucicarmine or PAS were much more uniform. In all 6 fetuses, all areas of the disc, the anterior and posterior extremities as well as the central portion, had a more compact appearance compared to the 12- and 14-week-old fetuses. Additionally, fewer unstained areas were observed in the 16-week-old fetuses than in the 12- and 14-week-old fetuses, and those observed represented a smaller proportion of the articular disc than the ones seen in the 12- and 14-week-old fetuses (Figs 5 and 6). Collagen fibers stained with trichromic were interwoven with periodic undulations; however, a space was maintained between them (Fig 7). These undulations were the same in the 3 groups of fetuses.

The fossa of the temporal bone was observed in all the fetuses as a straight structure which was similar in all 3 groups.

The densities (mean ± SD) of the stained zones as measured by Image J software were 38.36% ± 3.39%, 59.5% ± 1.56%, and 94.04% ± 2.04% at 12, 14, and 16 weeks of gestation, respectively. The Kruskal-Wallis test showed that the difference between these means was statistically significant ($\chi^2 = 15.16$; df = 2, $P < .001$).

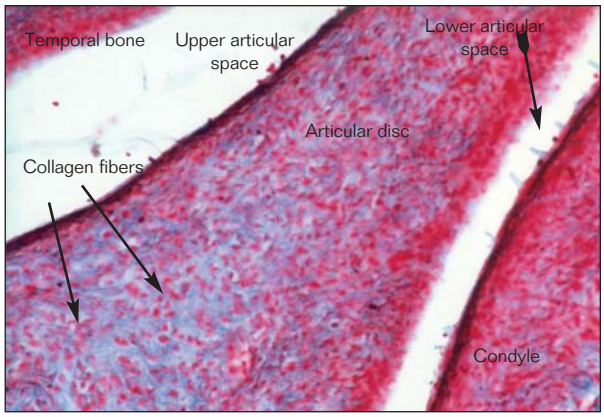


Fig 7 TMJ section of a 16-week-old fetus stained with trichromic. Uniform zones filled with cytoplasm (red areas) between collagen fibers (blue staining) can be observed. The undulated array of collagen fibers can also be seen.

Discussion

This research shows that mucopolysaccharides and glycoproteins are present in the articular disc of TMJs at the early gestational age of 12 weeks. Mucopolysaccharides and glycoproteins progressively filled the articular disc as the gestational age increased, occupying almost all the spaces between collagen fibers at the 16th week of gestation. This strongly suggests that the maturation of the articular disc is almost complete at an early stage of intrauterine life.

TMJs have been compared with similar joints in other animals, such as the rabbit.[8] The anterior and posterior bands of these joints are rich in proteoglycans. These are found not only among collagen fibers, where they play a role in the separation and movement of the fibers, but also in cartilage tissue, for example, where they provide a high degree of tissue elasticity.[18]

Collagen tissues are often subject to tensions. When these are low, tissue elasticity is high, but when tensions are high, elasticity is reduced. To withstand the tensions to which they are subjected, some collagen tissues have fibers with a non-linear disposition.[19–21] This might explain the curved disposition of collagen fibers in the articular disc of human fetuses: In the first stage after birth, this disc will be subjected to postero-anterior traction in breastfeeding. Differences between the distribution of the extracellular matrix in the embryonic and adult states could reflect functional differences between mastication and suckling.[22]

In the fetuses studied here, the development of structures such as the glenoid fossa of the temporal bone, mandibular condyle, articular capsules, and articular disc was clearly visible. In two thirds of the 12-week-old fetuses, mucopolysaccharides and glycoproteins were present. However, they were not uniformly distributed in the articular disc and were found in only approximately 40% of the section area.

In 14-week-old fetuses, there was a greater quantity of mucopolysaccharides and glycoproteins (shown by a reduction in the empty spaces in the histological section). The distribution of the mucopolysaccharides and glycoproteins in the posterior zone of the articular disc was more uniform. In the 16-week-old fetuses, mucopolysaccharides and glycoproteins were uniformly present and occupied almost all the spaces between the collagen fibers in the articular disc; fewer empty spaces were observed.

In all fetuses, the collagen fibers in the articular disc were interwoven but were kept separate from one another and had an undulating disposition. The space between the collagen fibers was filled by mucopolysaccharides and glycoproteins, and the space they occupied increased in proportion to gestational age.

The presence of mucopolysaccharides and glycoproteins between collagen fibers in the articular disc of human fetuses is a sign of histologic maturation. Since the articular disc of the TMJ at the 12th week of gestation already has the necessary anatomic elements, condylar movement may begin at that early age of gestation. However, more studies on synovial cells, TMJ muscle development, and ultrasonographic analysis are necessary to prove a link between histologic maturation and actual articular movements.

Conclusion

This study found that mucopolysaccharides and glycoproteins fill the spaces between the collagen fibers in the articular disc of TMJs in human fetuses at 12, 14, and 16 weeks of gestation. An important structural modification could also be

noted in the articular disc in 16-week-old human fetuses as compared to 12- and 14-week-old fetuses. This suggests that the development rate of the articular disc undergoes a significant increase during this period. The findings of this study thus suggest that the maturation process of the articular disc of TMJs in human fetuses may occur in the early stages of gestation. However, more research is necessary to clarify these issues.

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