Costal Cartilages Overgrowth Doesn't Induce the Deformation of the Chest Wall in Experimental Animals

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Research Article

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Abstract

Introduction: Many consider overgrowth of the costal cartilages to be the etiological factor for chest wall deformities in children. The aim of this study is to investigate if induced overgrowth of the costal cartilages leads to the deformation of the chest wall in an animal model.

Material and Methods: We injected insulin growth factor 1 (IGF1) solution directly under the perichondrium of the last three costal cartilages of rat pups. We used two different concentrations: 50 µg/ml (E50) and 100 µg/ml (E100). The procedure was repeated once per week for five consecutive weeks. Seven days from the last administration of injections, the animals were euthanized. We assessed the shape and measured the diameters of the thoracic cage. The last three costal cartilages were dissected; the samples were prepared and examined in light microscopy.

Results: In E100 the sagittal and coronal diameters of the rib cage were larger than E50 and control groups but without any deformation of the chest wall. The microscopic examinations revealed an anabolic pattern in E100.

Conclusion: Locally administered IGF1 stimulates, in a dose dependent manner, in-vivo cellular growth and multiplication in the costal cartilages. The induced overgrowth of the costal cartilages however, didn't result in the deformation of the chest wall.

Introduction

Pectus Excavatum (PE) and Pectus Carinatum (PC) are the most common malformations of the thoracic cage [1]. Till date, the etiology of both chest wall deformities (CWD) is not clearly understood. Many studies have demonstrated that patients with PE or PC present with various ultrastructural disorders of the costal cartilages [1]. Out of the several etiologies that have been proposed in various studies, the most accepted one states that the development of PE and PC is due to the overgrowth of the costal cartilages [2]. The theory suggests that the disproportionate growth of the costal cartilages forces the sternum to bend either backwards (PE) or forwards (PC) [3, 4]. Even though this theory was postulated almost a century ago, the precise reason behind the overgrowth of the costal cartilage remains unknown [3]. Moreover, recent studies have presented evidence contradicting the currently accepted theory, demonstrating that there is no difference in the lengths of the costal cartilages between the patients having CWD and healthy subjects [5, 6].

The aim of this study is to investigate if the induced overgrowth of the costal cartilages can lead to the deformation of the anterior chest wall in an animal model. Insulin Growth Factor 1 (IGF1) is the main anabolic growth factor for human hyaline cartilages [7].Because cartilage is a primary target tissue for the Insulin growth factor family, IGF1 is the optimal choice to induce the overgrowth of the costal cartilages. IGF1 regulates articular cartilage homeostasis and has been extensively used as a promoter of chondrogenesis both in vitro and in vivo conditions [7–10]. It has been proved that IGF1 increase cell proliferation, stimulate chondrocyte synthesis of proteoglycans and type II collagen [9, 10].

Materials And Methods

Forty Sprague Dowley rats were randomly selected for this experiment. The rats were two weeks' old. They were divided into four groups: two control and two experimental groups. Approval was obtained from the Ethics Committee of "Victor Babes" University of Medicine and Pharmacy, Timisoara, prior to the commencement. All experiments were performed in accordance with relevant guidelines and regulations. The entire experiment was done following the guidelines set forth by the Animal Research: Reporting in Vivo Experiments (ARRIVE). No interventions' were performed on the rats from the first control group (M0) and they were left to grow normally. The following surgical interventions were performed on the subjects from the second control group (M1) and the two experimental groups: an anterior thoracic midline incision was made, exposing the last three costal cartilages at the junction with the sternum. Using a 2 ml insulin syringe the following injectates we administered bilaterally and directly under the perichondrium of each of the last three costal cartilages. 0.02 ml of Phosphate Buffered Saline (PBS) to the rats in the group M1, 0.02 ml Insulin growth factor1 (IGF1) (Recombinant Rat IGF1 protein, ab52006, Abcam, UK) solution 50 µg/ml to the rats in the first experimental group (E50) and 0.02 ml of 100 µg/ml to the rats in the second experimental group (E100) (shown in Fig. 1). This procedure was repeated once a week for five consecutive weeks. The weight and the length of the animals included in our study were measured weekly.

Two weeks after the last injection the rats were euthanized. We isolated the rib cage including the thoracic spine and removed the soft tissues and intrathoracic organs. We inspected the rib cage for any deformities, using a ruler we measured and recorded the height of the rib cage (thoracic spine) as well as the anteroposterior and transvers diameters at the level of the lower thoracic opening.

We collected samples from all the three last cartilages of each rat. The samples were fixed in 10% buffered formalin and embedded in paraffin. Sections 3-µm thick were cut from each cartilage sample, dewaxed and rehydrated following the standard protocol and then stained using Hematoxylin – Eosin (HE). The sections were examined under light microscopy at 100 × using a Zeiss Scope A1microscope equipped with AxioCam ERc5s digital camera, one image was obtained for each section. The images were processed and analyzed using NIS elements BR 2.30 imaging software. We then assessed the following parameters: density and area of the chondrocytes; density and area of the chondroblasts; density of the chondrocytes isogenic groups and density of the chondroblasts isogenic groups.

Statistical analyses was performed using IMB SPSS Statistics v23. The unpaired t-test was used for numeric variables, with a significance threshold set at $p = 0.05$ for 95% CI. For non-numeric variables, we used chi-square test. We used Pearson's product-moment correlation to calculate if there is a correlation between the parameters.

Results

There were 21 females and 19 male rats with equal distribution among the groups ($p > 0.05$). The weight gain was similar during the entire experimental period between the groups (M0, M1 and E50). The animals from E100 group gained more weight as compared to the other three groups, in the first and second week ($p = 0.00$), after which the growth was normal and comparable among the groups. At the end of the experiment, no differences were noted in terms of weight and length among the groups ($p > 0.05$).

Despite the visible micro-trauma induced by repeated procedures at the site of surgery, none of the groups developed any deformities of the rib cage (shown in Fig. 2). All thoracic diameters were found similar between M0, M1 and E50 groups ($P > 0.05$). However, we noted that the animals from the E100 groups presented with a significantly larger sagittal and coronal diameters of the rib cage compared to those from the other groups ($p = 0.00$) (Table 1).

Table 1
Diameters of the rib cage at the end of the experiment

	Axial diameter (mm)	Sagittal diameter (mm)	Transversal diameter (mm)
M0	44.40	31.20	46.80
M1	45.70	33.90	49.00
E50	46.80	31.60	49.30
E100	45.60	40.80	59.00

M0 – Control group 1, no procedure; M1 – Control group 2, PBS injection; E50 – Experimental group 1, 50 µg/ml IGF1; E100 – Experimental group 2, 100 µg/ml

The results of the morphometric analyses are presented in Table 2. Difference was noted in the number of chondrocytes between E50 and E100 groups, 21.00 vs 27.00/ image ($p = 0.049$). While there was no statistical difference found among the other groups ($p > 0.05$). Significant difference was noted in the number of chondroblasts between M1 and E100 groups, 18.40 versus 27.00/ image ($p = 0.046$). No significant statistical differences were recorded for the other groups ($p > 0.05$). The surface area of the chondrocytes was similar between M0 and M1, M0 and E50, M1 and E50 ($p > 0.05$). However, the area of the chondrocytes was larger in M0 versus E100 group ($p = 0.031$); M1 versus E100 ($p = 0.002$) and E50 versus E100 ($p = 0.012$). The area of the chondroblasts was similar between M0 and M1, M0 and E50, M1 and E50 ($p > 0.05$). The area of the chondroblasts was higher in M0, M1 and E50 versus E100 groups 194357.00 ($p = 0.003$); 176457.80 ($p = 0.001$) respectively 159170.20 ($p = 0.00$) versus 24172.60. The number of the chondrocytes isogenic groups per image was similar in M0, M1 and E50 groups ($p > 0.05$). Similarly, there were no differences regarding the number of the chondrocytes isogenic groups per image between E50 and E100 ($p > 0.05$). However, the number of chondrocytes isogenic groups per image was significantly higher in M0 and M1 versus E100 (shown in Fig. 3), 12.40 versus 6.00 ($p = 0.010$) respectively 6.20 ($p = 0.010$). Finally, the number of the chondroblasts isogenic groups per image was similar between M0, M1 and E50 groups ($p > 0.05$). None of the rats from the E100 group presented with chondroblasts isogenic groups (Table 2).

Table 2
Results of the morphometry

	M0	M1	E50	E100
Chondrocytes	25.60 +/-11.59	25.80 +/- 6.30	21.20 +/- 9.85	28.80 +/- 5.09
Chondroblasts	24.20 +/- 13.24	18.40 +/- 9.27	21.00 +/- 6.46	27.00 +/- 8.66
Area of the chondrocytes	357741.80 +/- 217401.78	396188.80 +/- 92541.98	335276.60 +/- 75294.18	130186.80 +/- 218438.30
Area of the Chondroblasts	194357.00 +/- 159440.65	176457.80 +/- 123498.69	159170.20 +/- 35538.48	24172.60 +/- 12500.34
Chondrocytes isogenic groups	6.00 +/- 2.98	6.20 +/- 1.81	8.60 +/- 3.37	12.40 +/- 5.98
Chondroblasts isogenic groups	6.60 +/- 1.57	6.40 +/- 4.35	5.80 +/- 2.25	0

M0 – Control group 1, no procedure; M1 – Control group 2, PBS injection; E50 – Experimental group 1, 50 µg/ml IGF1; E100 – Experimental group 2, 100 µg/ml

Discussion

We designed this study with two major objectives to achieve, first to induce the overgrowth of the costal cartilages in animal model and second to evaluate if this overgrowth can lead to modifications in the structure or shape of the thoracic wall. Several factors were considered in the design of the study. First of all, we have deliberately chosen to perform this experiment on immature rats in order to assess the effects of the IGF1 onto the normal growth of the costal cartilage. We aimed to mimic the natural progression of the disease and stimulate the overgrowth of the costal cartilage during the growth phases in the animals. In most of the CWD patients, the deformity of the chest wall has a dynamic, progressive and uneven evolving pattern throughout the childhood period [2, 3, 4]. In small children, the deformity is usually absent or discreet but becomes evident and worsens during the pre-pubertal growth spurt [2]. After adolescence, the deformation usually stabilizes [2]. A similar pattern of the serum levels of IGF1 can be observed throughout childhood as well [10]. The second factor considered during the design of the study was the number of cartilages to be injected. In most of the patients with CWD the deformation is limited at the lower 3–4 costal cartilages [2, 3]. The upper costal cartilages and the upper part of the anterior thoracic wall is usually unaffected [3]. So we chose to inject IGF1 solution in the lower cartilages only, because our desire was to stay as close as possible to the natural aspect of the disease

In our series, the transversal and sagittal diameters of the rib cages, which are directly influenced by the growth pattern of the costal cartilages and of the adjacent ribs were higher in animals from the E100 group. This demonstrates that in these animals the local distributed IGF1 interfere with the normal cartilage growing pattern and stimulates the growing. This effect is dose depending because in E50

animals the dimeters of the rib cage is similar with dose in control groups. The route of IGF1 administration is extremely important, in order to achieve our aim of eliciting a local response, with minimal systemic involvement. The intravenous route was not appropriate as it could cause undesirable outcomes through relevant feedback mechanisms. The amplitude of IGF1 effects are dependent mainly on two facts: the bioavailability of IGF1 in different biological fluids and by the amount of its cellular receptors ready to interact. The IGF1 bioavailability is modulated through its binding proteins, especially IGFBP3. However, at the cellular level, the regulation mechanism is not yet well defined. In theory, the main factor limiting the effective response of IGF1 is the poor expression of its cellular receptors that is genetically determined [8]. This theory is further supported by various studies done on tumor genesis, which have demonstrated that the overexpression of IGF1R is central to the tumor genesis and proliferation [9]. The bioavailable IGF1 binds to the receptors that leads to an intracellular cascade of signaling that inhibits the further secretion of IGF1 and growth hormone (GH) by the cells. This provides a negative feed-back mechanism for GH-IGF1 axis. Hence, the IGF1 solution was administered via direct sub-perichondrium injections. As no significant differences were seen in weight and length among the groups, in our study, it can be stated that the systemic effects of IGF1 were minimal or absent.

Our study demonstrated that IGF1 can exert a dose dependent in vivo chondrogenic effect on the costal cartilages in rats. We found that the direct stimulation of the costal cartilages induces modifications in both the costal cartilages' structure and growth pattern in immature rats. The structure of the costal cartilages was also significantly influenced by the presence of the IGF1. The anabolic effect of IGF1 on the hyaline cartilage has already been demonstrated in several studies [9, 11, 12]. The results of our study revealed the same pathway, the number of chondrocytes, and chondroblasts and the area occupied by these cells were higher in E100 group compared with the control groups and E50 group. Additionally, we observed a particular pattern for the isogenic groups: the number of chondrocytes isogenic groups were increased in E100 versus control and E50 groups while chondroblasts isogenic groups were entirely absent in the E100. This is an indicator that the IGF1, in a dose dependent manner, also exerts an effect on the differentiation and maturation of the cartilage cells.

In our study, the transversal and sagittal diameters of the rib cages, which are directly influenced by the growth pattern of the costal cartilages and of the adjacent ribs, were higher in animals from the E100 group. Our experiment demonstrated that the locally distributed IGF1 interferes with the normal growth pattern of the cartilage and stimulates the growth. However, this effect is highly dose dependent as the diameters of the rib cage between the group E50, M0 and M1 were similar, and lesser than the rats from group E100. It is also noteworthy that only the transverse and sagittal diameters of the thorax were higher, suggesting that only the ribs and costal cartilages exposed to high dose IGF1 showed faster growth. However, our findings indicate that the deformation of the chest wall is independent of the overgrowth in the costal cartilages and adjacent ribs. Neither retraction nor protraction of the anterior chest wall was seen in the experimental groups. Therefore, inference can be drawn that the overgrowth is not the only factor responsible for causing CWD. Overgrowth could not be clearly demonstrated in patients with CWD as well [13]. Several other authors also support this finding, that the cartilages in

patients with CWD are no different in length when compared with non-CWD patients [5, 6]. Also, the structure of CWD patients' cartilages do not show signs of excessive or faster growth [13–16].

Conclusion

Locally administered IGF1 stimulates the in vivo cellular growth and multiplication in the costal cartilages of immature rats. The effect is dose dependent and in high doses (100 µg/ml) the results are evident on both microscopic and macroscopic levels. However, the induced overgrowth of the costal cartilages does not lead to the deformation of the anterior chest wall in experimental animals.

Declarations

Author contributions statement

Conceptualization, D.V.L.; methodology, D.V.L. and F.G.H.; validation, E.S.B.; formal analysis, E.S.B.; investigation, D.V.L. and M.C.S.; resources, D.V.L.; data curation, B.C.; original draft preparation, D.V.L.; writing, review and editing, S.A. and N.R.K.; visualization, N.R.K.; supervision, M.C.P. and E.S.B. All authors reviewed the manuscript.

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Competing interests

The authors declare no competing interests.

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Figures

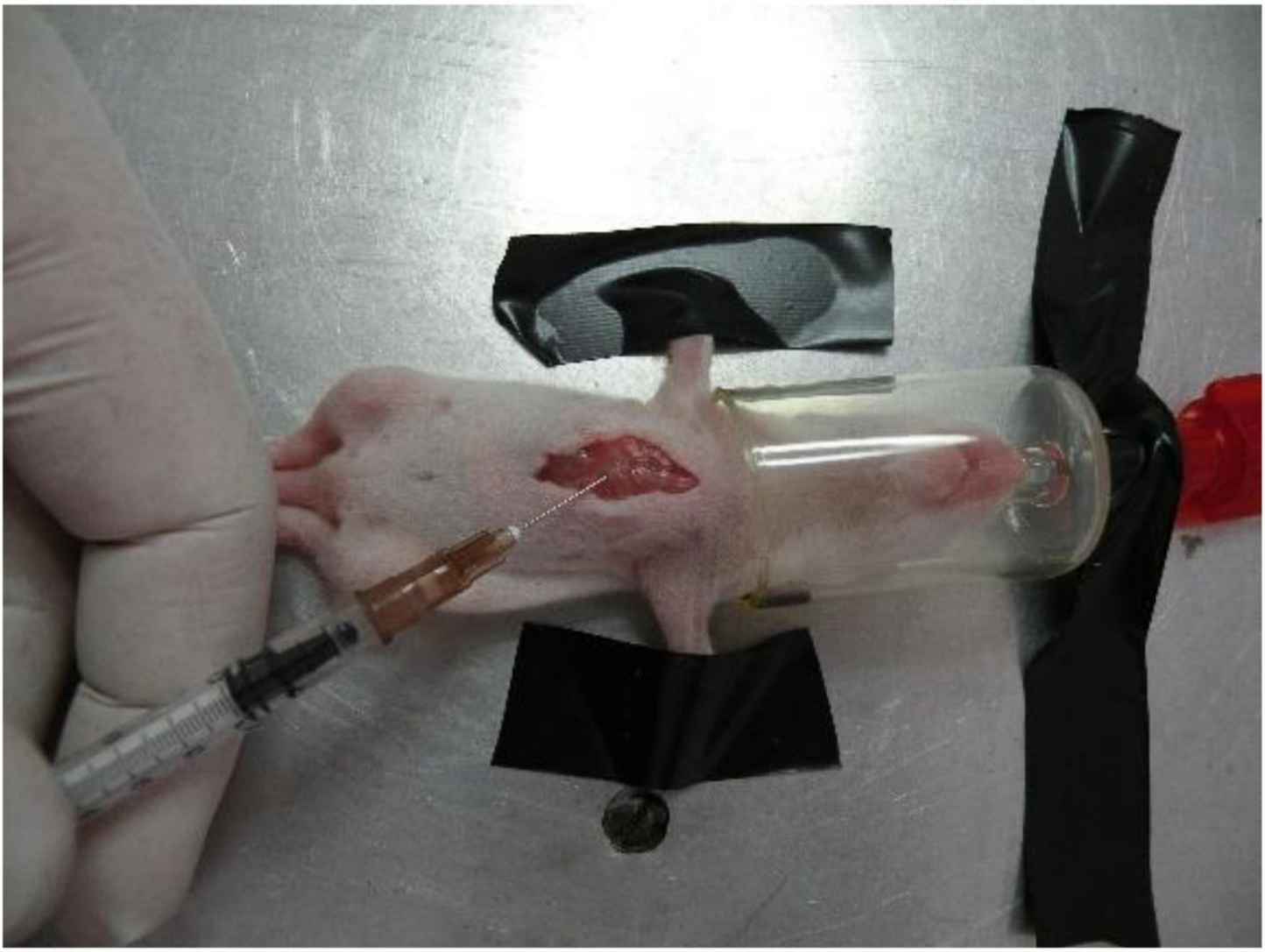

Figure 1

Injection of IGF1 in the costal cartilages of Sprague-Dowley rats

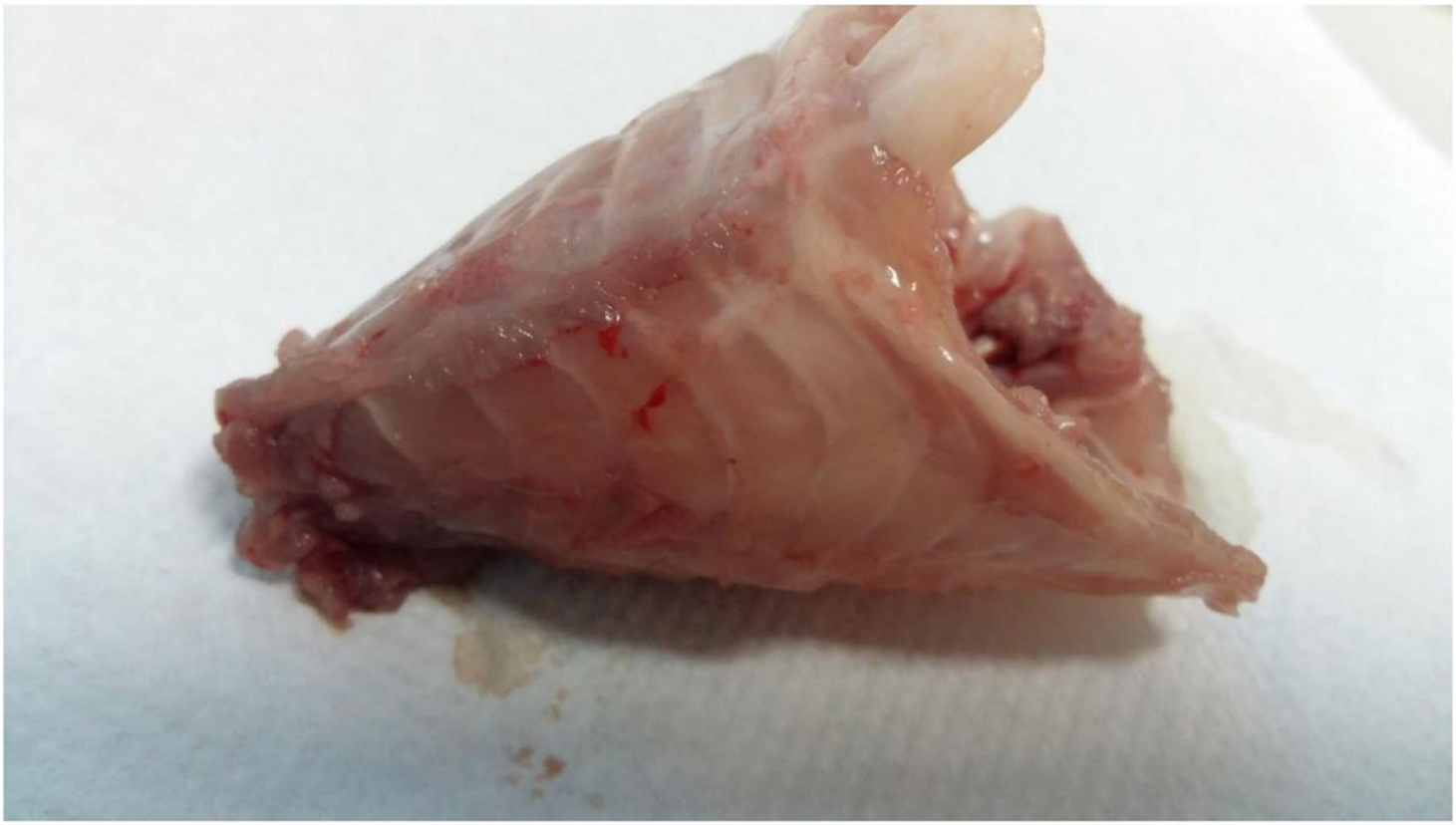

Figure 2

Normal shaped rib cage from E100 group rat.

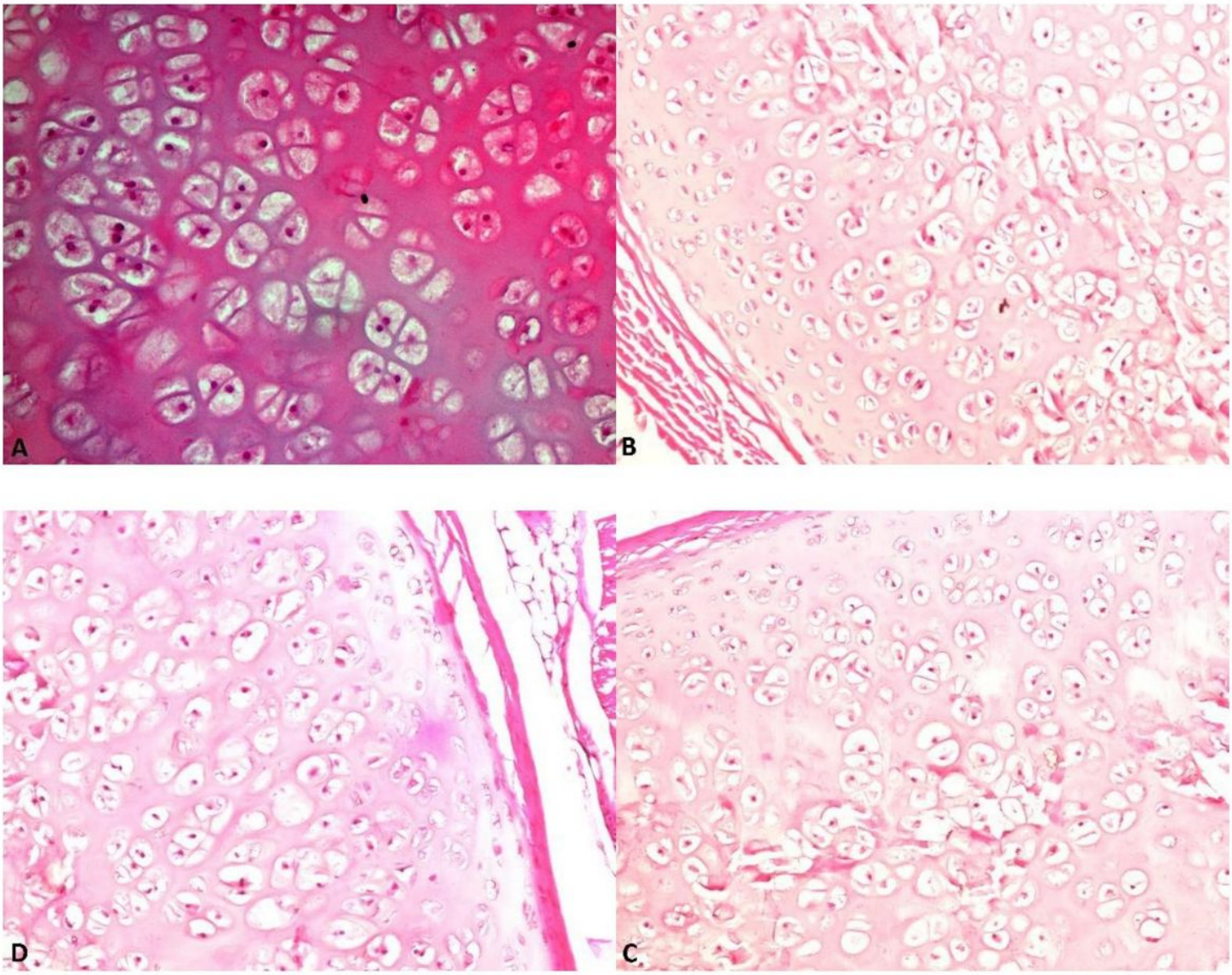

Figure 3

Isogenic groups E 100(A) vs E 50 (B), M1 (C) and M0 (D)– HE; 20X