## AURICULAR CARTILAGE AS A TOOL FOR POSTMORTEM INTERVAL ESTIMATION IN HUMAN

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#### **ABSTRACT**

Background: Accurate estimation of the postmortem (PM) interval, despite being a routine daily forensic expert work, is still a challenging practice. Science continues to grow in this topic. Cartilage is a specific avascular, non-lymphatic, and non-nervous specific connective tissue. Objectives: This work aimed to assess the use of auricular elastic cartilage as a tool for estimation of late PM interval in human. Methods: Biopsies were taken from ear cartilages of 43 victims how died from suspicious criminal causes. Routine haematoxylin and eosin (H&E) staining was performed. The following histopathological parameters were examined. perichondrium, the number of lacunae with chondrocytes, nuclear material, and extracellular matrix. A scoring system was used for each parameter in different postmortem (PM) intervals. Results: Cases were classified into 3 groups (1-7, 8-14-, and 15-21-days PM). The examined histological parameters give statistically significant changes across the tested PM intervals. The correlation coefficient between the tested parameters and PM intervals gives significant results. The r-value was highest with the percentage of lacunae without chondrocytes and lowest with perichondrium loss (r = 0.62 and 0.35 respectively). Conclusion: Auricular cartilage showed remarkable changes that are correlated with PM interval in human. In the future, a large sample should be investigated with studying the impaction of environmental factors on these changes.

Keywords: postmortem interval, cartilage, auricle, human, histopathology.

#### **INTRODUCTION**

Accurate estimation of the postmortem (PM) interval, despite being a routine daily forensic expert work, is still a challenging practice (Amar et al., 2017). Science continues to grow in this topic. Extensive techniques are used and continuously evaluated (Zhang et al., 2020). Over 2000 years, precise estimation of PM interval has questioned forensic professionals. The initial work was investigated by the Egyptians and Greek scientists during the third and fourth century BC (Donaldson and Lamont, 2014).

Multiple complicated factors affect PM interval estimation, making its precise

assessment is problematic. Factors may be exogenous as ambient temperature, humidity, oxygen saturation, precipitation, clothing, and arthropods (Maker et al., 2020). Endogenous factors of the cadavers include the cause of death, body builds, medical conditions as sepsis and diabetes mellitus, and trauma (Zhou and Byard, 2011).

Well-established methods as hypostasis, rigor mortis, and algor mortis give accepted estimates within 1-3 days postmortem (**Iqbal et al., 2020, Madea and Knight, 2016, Khartade et al., 2017, Maile et al., 2017**). However, with increased PM interval, the accuracy of

estimation of time since death decreases markedly (Shaaban et al., 2017). Bone marrow DNA degradation was used in the estimation of time of death up to 1 week (Chen and Cheng, 2002). DNA degradation was used for up to 3 weeks in the brain, kidney, and liver (Itani et al., Trimethylamine-nitrogen 2011). was detected in tissues for up to 8 days (Liu et al., 2008). In addition, oral bacteriological activity was tested for the late PM interval (Dong et al., 2019). However, most of the research was done on animals, and pilot studies.

Histopathological analysis in forensic pathology has highly specific aspects in clinical pathology. It has wide-ranging applications in forensic science. Determination of the cause of death is an important one. Trauma, drug abuse, burn, hypothermia, wound age, and identification are a few of many that histopathological examination may play a role in their examination. Estimation of PM interval is a highly vital part that histopathological examination may have a crucial role (Dettmeyer, 2018). In a study of 428 autopsies, histopathological examination established the cause of death in 40% of without apparent macroscopic cases findings. In addition, histopathology documented traumatic lesions well in 22% of Routine histopathological cases. examination of the cadavers should be advised (De La Grandmaison et al., 2010).

Cartilage is a specific avascular, nonand non-nervous lymphatic. specific connective tissue. Its cellular component is formed of chondrocytes that occupy space called lacunae and surrounded by an extracellular matrix. Chondrocytes receive nutrients by diffusion from surrounding connective tissues. Three types of cartilage are present, hyaline, elastic, and fibrocartilage. Hyaline present in joints, fibrocartilage in intervertebral disks, while elastic cartilage present in the epiglottis, ear, and larynx (Slípka and Tonar, 2017). Hyaline and fibrocartilage were studied as tools for estimation of PM interval by

different techniques (Li et al., 2019, Rogers et al., 2011). Elastic cartilage of the ear was investigated previously in the animals and gave promising results (Paulis et al., 2016). This work aimed to assess the use of auricular elastic cartilage as a tool for estimation of late PM interval in human.

### **SUBJECTS & METHODS**

This study was conducted on 43 victims who died due to suspicious criminal causes. Victims were dissected by the Medicolegal Authority Department. Histopathological examination was done in the Forensic and Toxicology Department, Faculty of Medicine, Minia University. Cases were collected in the period from January 2018 to December 2019. Cases included in the study were only those with a known time of death. Victims with severe injuries in the face interfering with tissue sampling from the auricles were excluded from the study.

Cases included 29 males (67%) and 14 females (33%). Postmortem interval ranged from 1 to 21 days. Table (1) illustrates the different causes of death in the examined cases. The age of victims ranged from 13 to 64 years old (mean  $42\pm16$ ). Cases were divided into 3 groups according to the postmortem interval (1-7, 8-14, and 15-21 days).

Valid informed consent was taken from the legal heir of the victims involved in the study. All procedures of the study were approved by the ethical committee of the Faculty of Medicine, Minia University.

Four millimeters biopsies were taken from the ear cartilages of the victims. Cartilage biopsies were kept in -20 C° until the time of examination. Tissues were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin-eosin (H and E).

Tissue sections were examined with an Olympus light microscope (Japan). Images were captured using a digital camera installed on the microscope and connected to a computer. ImageJ (free software, National Institutes of Health, Bethesda, MD, USA) was used to examine and measure different histopathological parameters.

Five sections of 4-  $\mu$ m thickness were prepared from each tissue sample. Three adjacent non-intersecting fields were analyzed for each slide. An average score of every examined parameter was calculated for each sample.

The following histopathological parameters were examined, perichondrium, the number of lacunae with chondrocytes. nuclear material, and extracellular matrix. The grading system of (Powell, 2015) was used to classify the examined parameters with some modifications. The was perichondrium classified 4 into categories according to the degree of affection. One for normally preserved tissue, 2 for mild, 3 for moderate, and lastly 4 for severe affection.

The assessment of lacunae with chondrocytes was done by calculating the number of lacunae with chondrocytes and divided by the number of total lacunae for the examined visual field and multiplied by 100.

 $\frac{100}{100}$ The number of lacunae with chondrocytes  $\times 100$ 

The total number of lacunae The average for every case was obtained and each case was given a score according to its average percentage. One, 2,

3, 4, is given for percentage  $\geq$  75, 50-74, 25-49, and  $\leq$  24, respectively.

Similarly, the nuclear material of chondrocytes was classified. A score was given for each category, 1, 2, 3, and 4 for normal, mild, moderate, and severe, respectively according to the nuclear material in each visual field. The extracellular matrix surrounding chondrocytes were classified into 4 categories normal, mild, moderate, and severely affected, and given a score of 1, 2, 3, and 4, respectively.

Statistical analysis was done using SPSS V.23 (IBM Corp., Armonk, NY, USA). Kruskal-Wallis Test was used to test the significant difference of each variable between different PM intervals. Spearman's nonparametric correlation was done to test the correlation between each variable and PM interval. The correlation was considered weak, fair, moderate, or strong if r was  $\leq 0.24$ , 0.25 to 0.49, 0.5 to 0.74, or  $\leq 0.75$  to 1.0, respectively. A p-value of less than 0.05 was considered statistically significant.

### **RESULTS**

Table (2) shows the distribution of victims according to the postmortem interval at the time of examination. Figure 1 depicts the mean of scores of perichondria, percentage of lacunae with chondrocytes, nuclear material, and extracellular matrix. The 4 histopathological parameters showed a significant difference in different PM intervals (P < 0.05). Figures 3, 4, and 5 show photomicrographs of elastic auricular cartilages from victims who died 1-, 8-, and 17-days postmortem, respectively.

The perichondrium examination showed that in the first-week postmortem, 76% of cases were mildly lost (score 2) and 24% were normal (score 1). In the second week postmortem, 77% of cases showed moderate lost perichondrium, while 15% showed mildly lost and 8% showed normal perichondrium. The 3<sup>rd</sup> group (15-21 days PM) showed 89% of cases severe lost perichondrium and 12% moderately lost.

According to the number of lacunae within chondrocytes, during the first week PM. 86% of cases had more than 75% of its lacunae with chondrocytes (score 1), while only 14% of cases showed 50-74% of its lacunae with chondrocytes. In the  $2^{nd}$  week PM, 62% of cases showed that 50-74% of lacunae have chondrocytes, and 31% of cases have 25-49% of their lacunae contain chondrocytes, and 7% of cases have their lacunae with chondrocytes more than 75%. With increased PM interval, more than 15 days, 56% of cases their auricular cartilages lacunae with chondrocytes 25-49% and 46% showed decreases lacunae with chondrocytes less than 24%.

The nuclear materials of chondrocytes showed that 62% of the victims got a score of 1 during the 1<sup>st</sup> week PM while 38% of cases have a score of 2. In the second week PM, 54% of cases have nuclear materials of score 2 and 46% of score 3. In the 3<sup>rd</sup> week PM, 78% of cases showed score 4 nuclear materials while 11% showed score 3 and 11% showed score 2.

The extracellular matrix was preserved (score 1) in 95% of cases during the  $1^{st}$  week PM and only 1 case (5%) showed that its matrix got a score 2. In the  $2^{nd}$  week PM, 62% of cases showed a matrix of score 2 while 31% of cases showed a matrix of score 3. In the  $3^{rd}$  week PM, 89% of cases showed a

matrix of score 4 while 11% of cases showed a matrix of score 3.

To examine the relation between auricular cartilage histological changes and PM interval, correlation was done. Table (3) shows a correlation between perichondrium loss, the lacunae without chondrocytes, nuclear materials, and the extracellular matrix. The r-value was highest with the percentage of lacunae without chondrocytes and lowest with perichondrium loss (r = 0.62 and 0.35 respectively).

Cause of death	Male	Female	<b>Total Number</b>	Percentage %
Firearm	11	4	15	34
Traumatic injuries	10	3	13	30
Burn	2	3	5	12
Electrocution	2	0	2	5
Poisoning	4	4	8	19
Total	29	14	43	100

**Table (2):** Distribution of cases according to the postmortem interval in days.

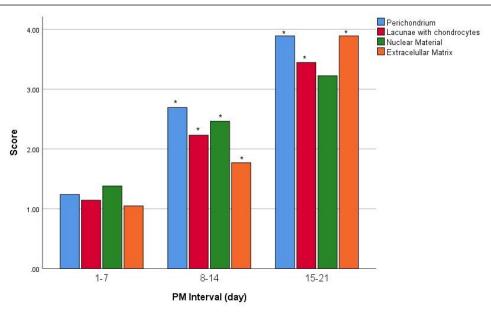
PM intervals in	1-7	8-14	15-21
days			
Male	14	8	7
Female	7	5	2
Total	21	13	9

**Table (3):** Spearman's correlation between postmortem histopathological changes of human auricular cartilage and PM intervals

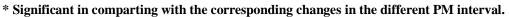
	Perichondrium	Lacunae with	Nuclear	Extracellular
	changes	empty	materials	matrix
		chondrocytes		
r <sup>@</sup>	0.35	0.62	0.41	0.37
p*	0.01	0.02	0.01	0.001

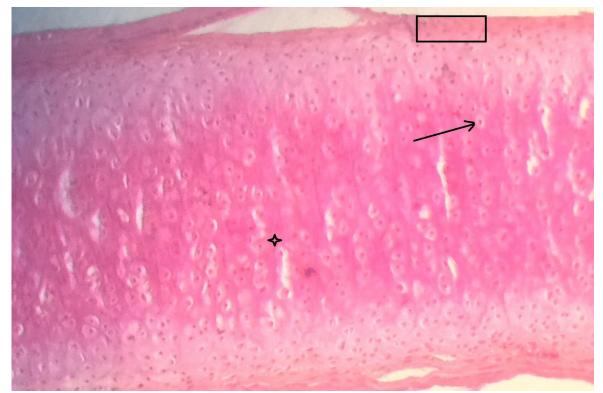
<sup>(a)</sup>  $r \le 0.24$ : week correlation, 0.25 to 0.49: fair correlation, 0.5 to 0.74: moderate correlation, or  $\le 0.75$ : strong correlation.

\* p less than 0.05 is considered significant.

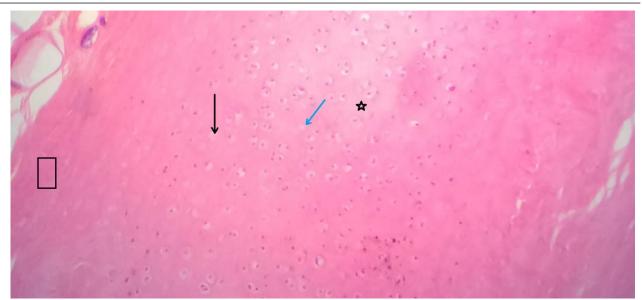


**Figure (1):** shows the score of different examined auricular cartilage histopathological changes and PM intervals.





**Figure (1):** shows a photomicrograph of human auricular cartilage of a victim 1-day PM. It depicts almost normal elastic cartilage. normal perichondrium (rectangle), lacunae with chondrocytes (score 1, arrow), normal nuclear materials (arrow), and extracellular matrix (star). Hematoxylin and eosin (H&E): X 200.



**Figure (2):** photomicrograph of human auricular cartilage of a victim 8-day PM. The perichondrium is mildly lost (score 2, rectangle), lacunae without chondrocytes (score 2, blue arrow), moderate lost nuclear materials (score 3, black arrow), and mild lost extracellular matrix (score 2, star). Hematoxylin and eosin (H&E): X 200.

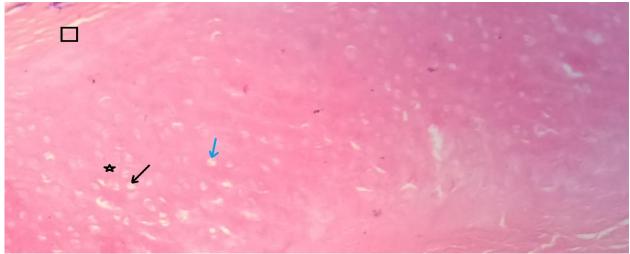


Figure (3): photomicrograph of human auricular cartilage of a victim 17-day PM. It reveals moderate perichondrium (score 3, rectangle), lacunae without chondrocytes (score 3, black arrow), moderate lost nuclear materials (score 3, black arrow), and moderate lost extracellular matrix (score 3, star). Hematoxylin and eosin (H&E): X 200.

#### **DISCUSSION**

Cartilage is an avascular specialized connective tissue of low cellular component and excess extracellular compartment. Auricular cartilage, in addition to type II collagen, has elastic fibers in its matrix to make it more flexible. The cellular component of cartilage is chondrocytes which less than 10% of cartilage mass. The perichondrium, the outer part of the cartilage, is vascular having an outer fibrous layer and inner cellular layer (Gartner, 2015). These characters make cartilage, perhaps, the most important compartment in the estimation of the PM interval (Alibegović and Martinez, 2019). Chondrocyte vitality, aggrecan destruction, and water component of the cartilaginous tissues imply that this tissue is considerably stable postmortem as well as during life.

Contrasting earlier investigations that performed were in manipulated or controlled settings in animal models, this work was conducted in human cartilage exposed to different conditions (Rogers et al., 2011, Paulis et al., 2016). In this study, authors used a simple the H&E histopathological technique which is a routine daily forensic expert work that needs no expensive complex equipment. In contrast, other studies used costly and sophisticated methods (Li et al., 2019, Ramírez et al., 2019).

In this study, 4 parameters were assessed quantitively for better estimation of the PM interval. Perichondrial loss, percentage of lacunae without chondrocytes, nuclear material. and extracellular matrix, all showed significant changes with increased PM interval. These findings are in agreement with a controlled work done in the auricular cartilage of rabbits (Paulis et al., 2016). Similar work in chondrocyte viability found that 30% of chondrocytes remain viable after 21 days PM in 35 C (Alibegović et al., 2014). In contrast, Lasczkowski et al. observed that chondrocyte viability dropped from 70% (2 days PM) to 20% (7 days PM) in summer times (Lasczkowski et al., 2002). However, this work was conducted in 2 cases only, which was not examined statistically. Moreover, in laboratory conditions chondrocytes could survive for a longer time. Hicks et al., 2006 found that 70% of chondrocytes in human cartilage outlasted live for 1 month and 38% was viable for 2 months in bacteriostatic saline (Hicks et al., 2006). In aseptic conditions, Alibegović et al. found that there was a relation between chondrocyte viability and PM interval. All these works proved that the vitality and PM changes of chondrocyte could have a crucial role in the estimation of PM interval.

In the comparison of correlation of different examined parameters with PM interval, the percentage of lacunae without chondrocytes give the highest r-value, while the perichondrium loss was the least. This was expected from the characters of chondrocytes being resistant to anaerobic conditions. In contrast, the perichondrium is being superficial which is more exposed to bacterial invasion (Alibegović, 2014).

In this study, due to the small sample size, the external factors affecting PM changes as the temperature was not included in the study. However, Bolton et al., found that environmental factors have a slight effect on the postmortem changes of cartilage (**Bolton et al., 2015**). In addition, they stated that different soil types have no observed effect on the biochemistry of the cartilage. The explanation was the lower cell density character of the cartilage tissue and the surrounding protection of other tissues.

Although to the knowledge of the authors, this is the first study to examine auricular elastic cartilage as a tool for estimation of the PM interval, few limitations are present. The small sample size is the first. This makes studying the effect of external factors that affect PM changes like climate, soil, and the cause of death is difficult. Another restriction is related to the auricular cartilage, being easily accessible to postmortem predation.

However. cartilage has unique histopathological and chemical characters that make it of slow degradation rate than other soft tissues and body fluids and much higher than that of bone. The nature of chondrocytes being encapsulated within lacunae and receive nutrients and oxygen from the extracellular matrix enables it to survive for days after death. In addition, the solid properties of the extracellular matrix of dense fibers and lowest chondrocytes requirements for nutrients and oxygen, provide an additional defense against postmortem bacterial invasion and putrefaction (Li et al., 2019). This character makes cartilage tissue has advantageous properties in the estimation of late PM intervals. In this work, auricular cartilage remarkable changes that showed significantly correlated with PM interval in human.

### **CONCLUSION**

Auricular elastic cartilage of the human ear could play an important role in the estimation of the PM interval. This prominence is crucial in late PM intervals where the ordinal tools as hypostasis, rigor mortis, and algor mortis could not be used.

#### **RECOMMENDATIONS**

• Histological examination of auricular cartilage should be added to routine forensic histopathological examination in estimating PM interval.

• Future studies with a large sample size are required to examine the effect of temperature, other endogenous, and exogenous factors that might affect the rate of PM changes of elastic cartilage.

• Additional work may assess other elastic cartilage in the body as epiglottis or laryngeal cartilage.

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No conflicts of interest.

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# الغضروف الأذنى كأداة لتقدير زمن الوفاة في الانسان

لا يزال التقدير الدقيق لزمن الوفاة، على الرغم من كونه عملًا روتينيًا يوميًا من خبراء الطب الشرعي، ممارسة شاقة. فالعلم يواصل التطور في هذا الموضوع. الغضروف هو نسيج ضام لا وعائي، لا ليمفاوي، وغير عصبي. يهدف هذا العمل إلى تقييم استخدام الغضروف المرن الأذني كأداة لتقدير زمن الوفاة المتأخرة في الإنسان.

تم أخذ خزعات من غضروف الأذن من 43 ضحية ماتوا لأسباب إجرامية مشتبهة بها. تم إجراء فحص الانسجة بواسطة صبغة هيما توكسلين وإيوزين (H & E). التغيرات النسيجية التالية تم فحصها ميكروسكوبيا، غشاء الغضروف، عدد الثغرات التي تحتوي علي الخلايا الغضروفية، والمواد النووية، والشبكة خارج الخلية. تم استخدام نظام التسجيل لكل تغير مع ازمنة الوفاة المختلفة.

تم تصنيف الحالات إلى 3 مجموعات (1-7، 8-14، 15-25 يومًا بعد الوفاة). اظهرت التغيرات النسيجية الأربعة التي تم در استها نتائج مختلفة حسب زمن الوفاة. كما أن معامل الارتباط بين التغيرات الأربعة وزمن الوفاة يعطي نتائج ذات دلالة احصائية. كانت قيمة r أعلى مع وجود نسبة من الثغرات بدون خلايا غضروفية وأقلها مع فقدان غشاء الغضروف.

أظهرت هذه الدراسة ان الغضروف السمعي يظهر تغيرات ملحوظة مرتبطة بزمن الوفاة في الإنسان. توصي الدراسة بإدراج الغضروف الاذني في تحديد زمن الوفاة. كما توصي باستخدام عينة كبيرة لدراسة تأثير العوامل البيئية على هذه التغييرات.