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## Research Article

# E-Cadherin in Age-Related Mammary Gland Changes in Women: Aperio Image Analysis

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**Abstract**

***Background***: E-cadherin is a transmembrane protein that is essential for cell-cell adhesion and is primarily expressed in mammary glands and epithelial cells, particularly at intercellular junctions of ducts and lobules. It helps maintain structural integrity and prevents pathological conditions such as unregulated cell growth. Immunohistochemical analysis shows strong labeling at intercellular borders, emphasizing its role in preserving epithelial structure. ***Objective***: To analyze age-related changes in E-cadherin expression at epithelial cell junctions using histochemical and immunohistochemical techniques. ***Methods***: This study, conducted from April to September 2024, analyzed 60 mammary gland samples from forensic medicine cadavers. Samples from female mammary glands were categorized into three groups: group 1 (ages 15–25), group 2 (ages 26–45), and group 3 (ages 46 and older). E-cadherin was used to analyze epithelial cell interactions. Samples were processed histologically and immunohistochemically, and interactions were evaluated using the Aperio Algorithm program. ***Results***: E-cadherin was consistently expressed on the epithelial cell surface, facilitating adhesion through tight junctions. Expression levels were higher in group 2 but decreased in group 3 compared to group 1. Statistical analysis confirmed a significant difference. ***Conclusions***: E-cadherin plays a crucial role in signaling, proliferation, differentiation, regeneration, and maintenance of epithelial balance. Age-related changes in expression may contribute to altered epithelial integrity, especially in older individuals.

***Keywords***: Alveoli, Aperio algorithm program, E-cadherin**,** Mammary glands.

**دور كادهيرين في تغيرات الغدة الثديية المرتبطة بالعمر عند النساء: تحليل صور أبيريو**

**الخلاصة**

**الخلفية**: E-cadherin هو بروتين عبر الغشاء ضروري لألتصاق الخلايا ويتم التعبير عنه بشكل أساسي في الغدد الثديية والخلايا الظهارية، خاصة عند التقاطعات بين الخلايا للقنوات والفصيصات. يساعد في الحفاظ على السلامة الهيكلية ويمنع الحالات المرضية مثل نمو الخلايا غير المنظم. يظهر التحليل الكيميائي المناعي وضع علامات قوية على الحدود بين الخلايا، مما يؤكد دوره في الحفاظ على البنية الظهارية. **الهدف**: تحليل التغيرات المرتبطة بالعمر في تعبير الكاديرين الإلكتروني عند تقاطعات الخلايا الظهارية باستخدام التقنيات الكيميائية النسيجية والكيميائية المناعية. **الطرائق**: التي أجريت هذه الدراسة في الفترة من أبريل إلى سبتمبر 2024 ، وتم تحليل 60 عينة من الغدد الثديية من جثث الطب الشرعي. تم تصنيف عينات من الغدد الثديية الأنثوية إلى ثلاث مجموعات: المجموعة 1 (الأعمار من 15 إلى 25 عاما) ، والمجموعة 2 (من 26 إلى 45 عاما)، والمجموعة 3 (من سن 46 عاما فما فوق). تم استخدام E-cadherin لتحليل تفاعلات الخلايا الظهارية. تمت معالجة العينات نسيجيا وكيميائيا مناعيا، وتم تقييم التفاعلات باستخدام برنامج خوارزمية Aperio. **النتائج**: تم التعبير عن E-cadherin باستمرار على سطح الخلية الظهارية، مما يسهل الالتصاق من خلال التقاطعات الضيقة. كانت مستويات التعبير أعلى في المجموعة 2 ولكنها انخفضت في المجموعة 3 مقارنة بالمجموعة 1. أكد التحليل الإحصائي وجود فرق كبير. **الاستنتاجات**: يلعب E-cadherin دورا مهما في الإشارات والانتشار والتمايز والتجديد والحفاظ على التوازن الظهاري. قد تساهم التغيرات المرتبطة بالعمر في تكوين هذا البروتين في تغيير السلامة الظهارية، خاصة لدى كبار السن.

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## INTRODUCTION

E-cadherin is the first identified member of the cadherin superfamily, a group of calcium-dependent cell adhesion molecules. Like other classical cadherins, it consists of five extracellular domains and a conserved intracellular region that binds to catenins [1, 2]. During the early stages of embryonic development, both maternal and zygotic E-cadherin regulate compaction during the morula stage and blastocyst formation [3]. The E-cadherin-catenin adhesion complex is vital for maintaining the polarization, function, and structural integrity of epithelial cells [4,5]. In mammary epithelial cells, E-cadherin establishes strong intercellular connections and anchors to components of the adherent junctions. Within these junctions, the cytoplasmic tail of E-cadherin serves as a binding site for β-catenin, p120, and γ-catenin, linking the actin cytoskeleton to various signaling pathways [6,7]. E-cadherin, the primary cadherin expressed in epithelial cells, is localized at cell-cell contact points known as adherent

junctions. It plays a pivotal role in maintaining epithelial integrity by interacting with other E-cadherin molecules. The cytoplasmic tail of E-cadherin binds directly to catenins, forming a dynamic complex that regulates various intracellular signaling pathways, including epithelial-mesenchymal transition (EMT) [8,9]. Each breast comprises 15-20 secretory lobes embedded within fat tissue. Functionally, the mammary gland was a specialized sweat gland, with each lobe classified as a complex tubular-acinar gland. The acini release their secretions into ducts lined with low columnar or cuboidal epithelial cells, which are surrounded by myoepithelial cells. These ducts from each lobule converge into a lactiferous duct that opens onto the nipple surface. The ducts surrounding the nipple are encircled by smooth muscle, and when this muscle contracts, it causes the nipple to become erect [10,11]. This study aims to investigate changes in protein expression in the basement membranes of epithelial cells across different age groups using histochemical reactions.

## METHODS

### *Study design and setting*

This descriptive and analytical study was conducted on 60 mammary gland tissue samples obtained from cadavers at the Forensic Medicine Department in Baghdad. The samples were collected between April and September 2024 based on an official letter from the ethics committee and with consent from the families of the participants. The experiment was conducted in the Anatomy and Histology Laboratory at the Department of Human Anatomy, College of Medicine, Tikrit University.

### *Inclusion and exclusion criteria*

The study included mammary gland samples from women aged 15 years and older. The samples were divided into three age groups as follows: group 1: 15 to 25 years; group 2: 26 to 45 years; group 3: 46 years and older. Out of the initial 60 samples, 54 were selected to ensure an equal distribution across the groups, with 18 samples in each. The excess samples from Group 3 (46 years older) were excluded due to the higher number in this group compared to the other groups.

### *Sample preparation and fixation*

The mammary gland samples were collected from deceased women in the Forensic Medicine Department in Baghdad, based on official approval from the ethics committee and consent from the participants' families. The samples were categorized according to the specified age groups to ensure a balanced representation across different life stages. The samples were fixed in 10% neutral buffered formaldehyde (NBF) and processed for paraffin embedding following standard protocols [12]. Tissue sections were prepared using standard techniques to ensure accurate and reliable results for histological analysis.

### *Immunohistochemical analysis*

Immunohistochemical staining was used to evaluate the expression of E-cadherin in the mammary glands. Specific antibodies against E-cadherin were employed, and the staining procedure followed established laboratory protocols. Observations were made under a light microscope [13,14].

### *Outcome measurement*

Images captured using the light microscope were processed in the Aperio Image Scope program to quantify E-cadherin expression. This software analyzed staining intensity and extent, generating objective classifications for each tissue section and categorizing positivity into the following classifications [15]: brown color, strong positivity; orange color, moderate positivity; yellow color, weak positivity; and blue color, negative.

### *Ethical considerations*

The study was conducted according to ethical standards for medical research. An official letter of approval was obtained from the Forensic Medicine Department in Baghdad, and the institutional ethics committee granted ethical clearance. All protocols concerning using human tissue samples were strictly adhered to during the study.

### Statistical analysis

The Statistical Package for the Social Sciences (SPSS) was utilized to analyze the parameters of the study. Differences in the mean percentage of E-cadherin expression in mammary gland epithelial cells across age groups were assessed for statistical significance. The results were considered as the mean positivity ± standard deviation (SD) among the groups and the mean number of positive pixels representing the immunohistochemical reactivity of E-cadherin [16].

## REULTS

The results revealed a strong positive immunohistochemical reaction, indicated by a brown color, in group 2. This strong reaction may be associated with the higher differentiation grade observed in this group (Figure 1, Figure 2 A and B, and Figure 3 A and B).

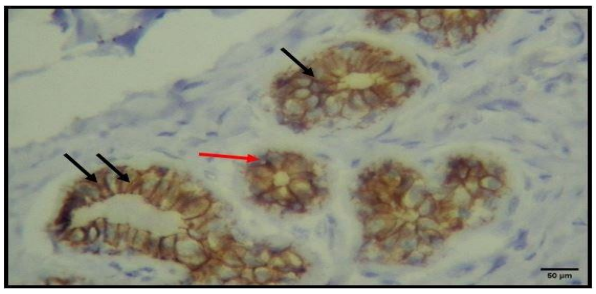

**Figure 1**: Mammary gland, strong immunolabelling in the intercellular border of epithelial cells (black arrows), myoepithelial cells show a weaker positive (red arrow)(E-cadherin, 40X).

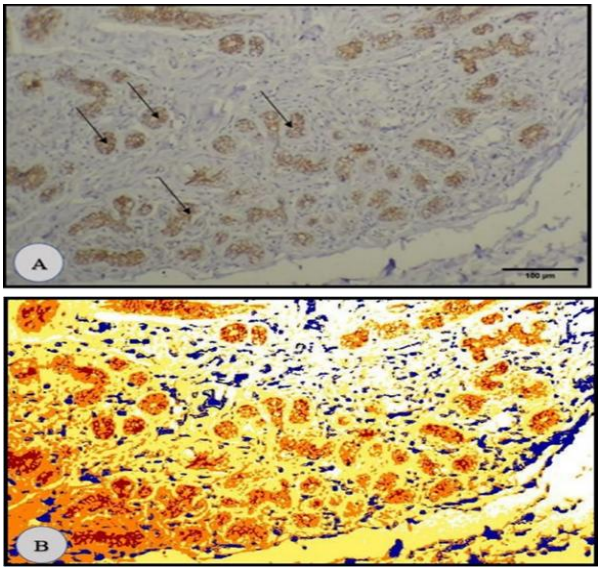


**Figure 2**: **A)** Group 1: Strong immunolabelling is seen at the intercellular borders of ductal cells (black arrows) (E-cadherin, 4X). **B)** Group 1: Marked image illustrates a strong positive reaction in brown, a faint positive reaction in yellow, and a negative reaction. was represented by the blue.

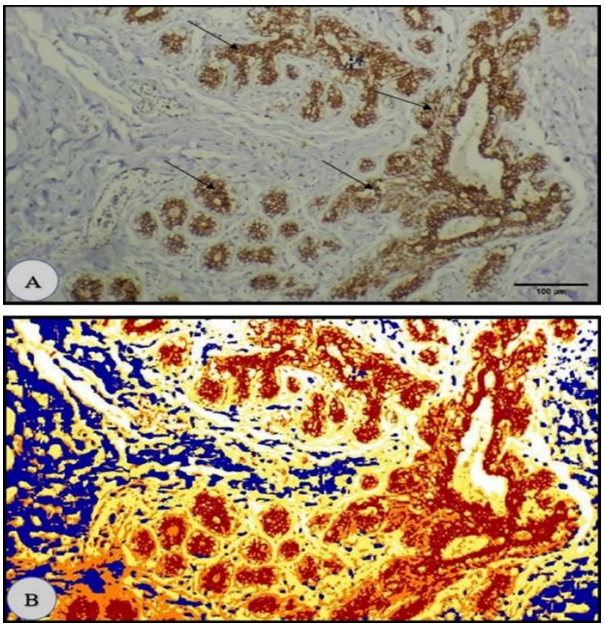


**Figure 3**: **A)** Group 2: Strong immunolabelling is seen at the intercellular borders of ductal cells ( black arrows) (E-cadherin, 4X). **B)** Group 2: Markup image shows the positive reaction represented strongly in brown color, weakly positive in yellow color, and negative was represented by blue.

In contrast, group 1 exhibited yellow staining, indicating a weakly positive reaction. Furthermore, the connective tissue, which did not react with E-cadherin, stained blue, as shown in Figure 4A and B.

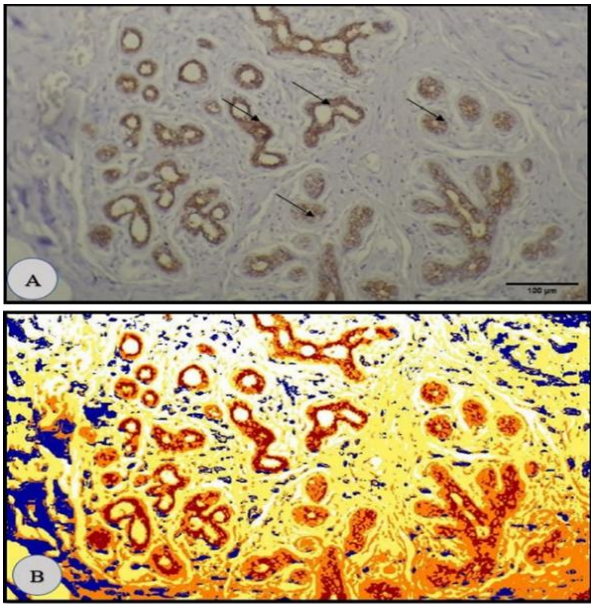


**Figure 4**: **A)** Group 2: Strong immunolabelling is seen at the intercellular borders of ductal cells (black arrows). (E-cadherin, 4X). **B)** Group 2: The markup image shows the positive reaction, represented strongly in brown. The weak positive was represented by yellow, and the negative was represented by blue.

This phenomenon was particularly prominent in group 3, where increased connective tissue replaced epithelial tissue with age, as depicted in Figure 5A and B. Positivity levels assessed by the Aperio program (Image Scope) varied in intensity and were represented by different colors. E-cadherin expression was significantly higher in group 2.

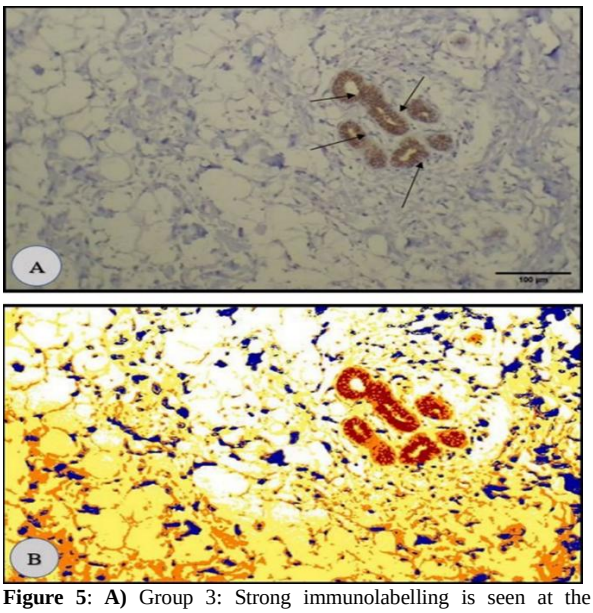


**Figure 5**: **A)** Group 3: Strong immunolabelling is seen at the intercellular borders of ductal cells (black arrows) (E-cadherin, 4X). **B)** Group 3: The markup image shows the positive reaction represented strongly in brown color, the weak positive represented in yellow color, and the blue represented the negative.

Statistical analysis revealed a significant difference in the mean number of strong positive immunohistochemical reactions for E-cadherin between the groups. The mean of the positive responses (µm) was as follows: group 1 (0.573±0.15) µm, group 2 (0.736±0.11 µm), and group 3 (0.239±0.09 µm), with a *p*-value of 0.01 (Figure 6).

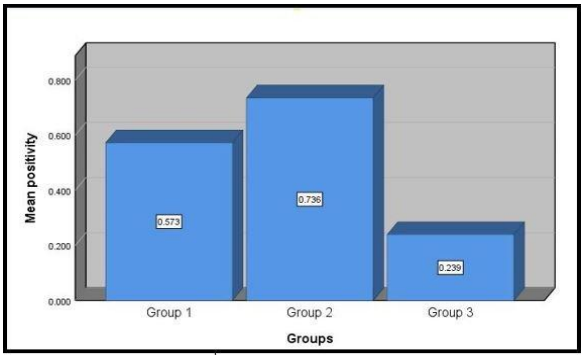


**Figure 6**: The different immunohistochemical reactivity of E-cadherin protein for age groups.

## DISCUSSION

E-cadherin expression in mammary gland tissues exhibited distinct patterns across the three studied groups, which correlated with age and tissue differentiation. Group 2 showed a strong positive response, characterized by intense brown staining at the intercellular junctions of epithelial cells. This robust expression indicates enhanced epithelial differentiation and structural integrity in this age group. These findings align with studies that emphasize E-cadherin plays a crucial role in maintaining the structure of epithelial tissue and promoting cell adhesion. [16,17]. Yellow staining, on the other hand, indicated a less pronounced positive response in Group 1. This reduced expression may reflect a transitional epithelial state or early developmental dynamics of cellular adhesion mechanisms. This observation is consistent with findings highlighting the sensitivity of E-cadherin expressions to developmental and hormonal variations within the mammary gland [18, 19]. Group 3 showed a significant decline in E-cadherin expression with an increased connective tissue replacing epithelial components. The blue staining, indicative of minimal E-cadherin reactivity, underscores tissue remodeling processes and epithelial deterioration associated with aging. These results corroborate earlier research documenting age-related decline in E-cadherin expression, leading to weakened epithelial cohesion and increased susceptibility to tissue dysfunction [20,21]. Quantitative analysis using the Aperio Image Scope program revealed statistically significant differences in E-cadherin expression between the groups. Group 2 exhibited the highest mean positive response (0.74±0.11 µm), followed by group 1 (0.57±0.15 µm) and group 3 (0.24±0.09 µm). The significant *p*-value ($p<0.01$) confirmed the reliability of these differences and highlighted the gradual decline in E-cadherin expression with advancing age. The spatial localization of E-cadherin, primarily at intercellular junctions, emphasizes its vital role in preserving epithelial cell-cell contact. The observed variation in staining intensity across the groups underscores the dynamic regulation of E-cadherin expression by hormonal, developmental, and aging factors. These findings align with the literature, suggesting that E-cadherin is subject to modulation by extracellular signals and intrinsic cellular pathways [22,23].

### Study limitations

This study utilized 54 breast tissue samples obtained from deceased individuals through the Forensic Medicine Department in Baghdad. The sample was reduced from 60 to 54 by excluding 6 samples from an overrepresented group, resulting in balanced groups of 18 for improved comparability and statistical reliability. The samples were carefully selected from individuals who had passed away due to accidents, fires, and other non-medical causes, ensuring that there were no underlying pathological conditions present. All procedures follow strict ethical guidelines approved by the relevant authorities. While postmortem samples provide a controlled environment for analysis, physiological differences from living tissue may limit the generalizability of the findings. Additionally, the regional diversity of samples minimizes bias and offers a broader perspective on tissue characteristics of the studied tissues.

### Conclusion

Quantitative analysis revealed significant variations in E-cadherin expression across the age groups, with a clear reduction observed with advancing age. These findings underscore the essential role of E-cadherin in sustaining epithelial integrity. and its vulnerability to age-related changes.

### Recommendations

We recommend Conducting future research to explore the impact of hormonal fluctuations on E-cadherin regulation, aiming to deepen the understanding of tissue remodeling processes. Additionally, development strategies to sustain E-cadherin expression are also suggested to mitigate the adverse effects of aging on epithelial tissues and potentially reduce the risk of related diseases.

#### Conflict of interests

No conflict of interest was declared by the authors.

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The authors did not receive any source of funds.

## Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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