**Evaluation of Fibrosis and Histopathological Changes in The Psoas Muscle with E- Cadherin, Claudin-5 Expression and Demographic Data**

Running Title: Evaluation of Fibrosis and Histopathological Changes in The Psoas Muscle with Demographic Data

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**Abstract**

**Background:** It is known that muscle mass decreases with age. Sometimes it may be possible to adversely affect this reduction. In this study, it was aimed to investigate the contribution of claudin-5 (C) and e-cadherin (E) to this process.

**Material and methods:** In this study, samples were taken from the psoas muscle of 55 cases autopsied for different reasons between 2018-2019.

Age, gender, weight, height, chronic disease, and addiction were recorded. Histopathological degeneration parameters were evaluated.

In addition to, samples were prepared for immunohistochemical study. Evaluation for E and C; no staining, weak staining, moderate staining and severe staining. Fibrosis was evaluated with Masson trichrome ( yes/no).

**Results:** It was observed that there was a very strong and statistically significant inverse relationship between acidophilic sarcoplasma and staining with C ( p <0.01). There was also a strong positive correlation between cellular fatty cell degeneration status and staining with C (p <0.05). No correlation could be established between sociodemographic characteristics of the cases and staining with C and E, nor could there be a correlation between staining with E and staining with C (p> 0.05).

**Conclusion:** As a result, C tends to decrease in striated muscle tissue in degeneration and atrophy.

**Keywords:** claudin-5, e-cadherin, sociodemographic characteristics, histopathological degeneration, striated muscle.

**Introduction**

Muscle mass in humans is observed at maximum values at the ages of 25-30. This mass decreases by about 25-30% by age 65. This decrease in muscle mass is accompanied by an increase in non-contractile structures such as adipose tissue and connective tissue. In addition to the causes of age-related decrease in muscle mass, nutrition, sedentary lifestyle and chronic diseases can also affect this situation. The opposite situation may also have a protective effect [1].

The intersection complex consists of two main components. These components are: Tight junctions (TJ) and adherens junctions. Cadherins, the main component of adhesive junctions, provide a molecular bond between cells and connect the plasma membrane with the intracellular actin cytoskeleton through catenin family proteins. It belongs to the family of adhesion molecules [2]. Tight junctions are responsible for the barrier function of the epithelium and for maintaining polarity. TJs play a role in tissue differentiation and homeostasis of epithelial tissues [3].

Claudines (Cs) are the main transmembrane proteins of TJ. So far, 24 different types have been identified in human cells. Epithelial cells contain more than one protein C, but some family members exhibit tissue-specific expression patterns [3,4].

Cs proteins appear to be important for TJ formation and play an important role in controlling the paracellular permeability of ions. Indeed, Cs gene expression is sufficient to induce the formation of TJ fibrils in fibroblast cells [5].

TJs play important roles in tissue homeostasis and inflammation through their role in controlling paracellular transport and barrier function. There is evidence that these functions are compromised in aged organisms, but the mechanisms that lead to TJ degradation are not fully understood [6].

Cadherins, Ca-2-dependent cell adhesion molecules, are central morphogenetic regulators that act by imparting selective adhesion to cells. Classical cadherins are associated with catenins, a highly conserved cytoplasmic domain and containing the α-catenin, b-catenin, plaoterglobin p120 protein. The formation of cadherin-catenin is associated with the actin cytoskeleton, where the cadherin family of the Ca2 / -dependent complex is required for Cadherin-mediated cell adhesion and binds to cadherin-b-catenin or cadherine-plaoglobin complexes. As with beta catenin, it has been reported that the p20 protein and the COOH-terminal region of the E-cadherin (E) are combined by a different mechanism [7].

It has been observed that different expression patterns and dynamic changes of cadherins during development affect the function of cadherin in various morphogenetic events, such as mesenchymal organization, cell migration and regionalization of the nervous system [7].

In this study, it was aimed to reveal the expression differences of Claudin-5 (C) and E which are components of the junction complex, in the psoas muscle and the relation of these differences with the histopathological changes in the muscle with aging, fibrosis, adipose tissue as well as demographic data.

**Material and method**

This study was ethically approved by the ….University clinical research ethics committee (217/2020).

In this study, samples were taken from the psoas muscle of 55 cases autopsied for different reasons between 2018-2019. Age, gender, weight, height, chronic disease (K), and addiction were recorded. Histopathological fatty cell degeneration (Y), fibrosis (F), acidophilic sarcoplasma (A), streaking (D), vacuolization (V), disruption in fibers (L), fragmentation (P), cellular infiltration (H), lipofuscin pigment accumulation ( Parameters such as LP) (graph-1) indicating tissue degeneration and injury were evaluated.

Paraffin blocks were prepared using manual microarray method from existing samples. 5 µm thick sections were taken from these muscle samples. Sections were stained with H&E stain. Light was evaluated microscopically. Evaluation was done under a light microscope (Nikon eclipse Ni-U Tokyo / Japan) at different magnifications. Histopathological evaluation was graded as yes / no (figure-1) [8].

In addition, the scores of these parameters were summed up and the degeneration score was obtained. Average score was obtained according to age groups. The score belonging to the degeneration was collected as (yes: 1, no: 0 accepted) and averaged. The score for Claudin and E; no staining: 0, light: 1, moderate: 2, severe: 3) and averaged.

**Immunohistochemical study**

Samples were taken from the tissues and then sections with a thickness of 5μ were taken on the poly- laminated slide. Prepared for immunohistochemical study. A Leica Bond-Max IHK staining device (Vision Biosystems, Melbourne, Australia) was used for the immunohistochemical study. It was stained with C (Epitomics (AC-0212A), 0.1ml (1: 100). Slides were evaluated with light microscope. Cytoplasmic membrane staining was considered positive. Four grades were evaluated. 0 staining (no staining), 1 + (waek) (1% to 10%) staining, 2 + (moderate) staining (11% to 50%), 3+ (severe) staining (over 50 %) were evaluated [9].

The sections were kept at 60 ° C for 1 hour, then xylol and alcohol steps were applied. The sections were incubated in 3% hydrogen peroxide solution for 10 minutes, then washed in distilled water for 5 minutes. Antigen retrival stage was applied. Immunohistochemical staining was performed using the avidin-biotin complex technique. The sections were then washed three times for 2 minutes with PBS (phosphate buffer solution). E (mouse monoclonal antihuman antibody, Biogenex (10 microliters diluted per 1 ml)) was applied. The sections were rinsed in 3-amino-9-ethyl carbazole and chromogen substrate (10 minutes). Washed in water. It was stained with hematoxylin (3 minutes) and sealed with balsam respectively. The stained slides were examined with a Nikon Eclipse Niu microscope and photographs were taken. Grading of immunohistochemical slides was done semiquantitatively.

Evaluation for E; 0 no staining; 1 (less than 10%) weak staining; 2 (10% to 50%) moderate staining; and 3 (more than 50%) were rated as severe staining [10].

**Histochemical study**

Masson's Trichrome Stain kit was used to evaluate fibrosis. It was evaluated as yes/no fibrosis.

**Statistical evaluation**

The data were collected and analyzed using Statistical Package for software. In addition to descriptive statistics, chi-square test was used in classifying categorical data. Spearman Correlation analysis was performed to investigate the relationship between staining and sociodemographic data and histopathology findings. In addition, the quality of the relationship was revealed by performing multiple regression analysis. In all types of analyzes, 5.0 % significance level was used.

**Results**

89.1% (n = 49) of 55 patients were male and 10.9% (n = 6) were female. The mean age of the cases was 60.67 ± 13.38 and the youngest case was 35 and the oldest case was 90 years old. The sociodemographic data obtained from the cases according to their BMI (body mass index) are presented in Table 1.

As a result of staining the muscle samples of the cases with E, it was observed that 92.7% (n = 51) of the samples were not stained, but only 7.3% (n = 4) were stained. As a result of staining the psoas muscle samples of 55 cases with C, 29.1% (n = 16) were not stained, and 70.9% (n = 39) were stained. 5 (9.1%) of the stained samples were strongly stained with C.

No correlation could be established between sociodemographic characteristics of the cases and staining with C and E, nor could there be a correlation between staining with E and staining with C (p> 0.05). In addition, there was no correlation between E staining and histopathological features (p> 0.05).

In the correlation analysis made to evaluate the relationship between histopathological findings and staining; It was observed that there was a very strong and statistically significant inverse relationship between A and staining with C (r = - 0.41, p <0.01). There was also a strong positive correlation between cellular Y status and staining with C (r = 0.28, p <0.05) (graph-2). In the multiple regression analysis; Staining with C in non-fatty cells is statistically significant 0.25 times higher. In addition, staining with C in cells with A is statistically significantly higher than 7.73 times. The relationship between histopathological findings and age groups was demonstrated by graph-1, and when age groups were grouped as 35-45,46-55,56-65, 66 and above, it was observed that degeneration increased as age progressed. The same relationship with claudin is shown in the table 2,3.

**Discussion**

There are many publications stating that myofibrillary decline starts around the age of 25 and the rates of this decline increase with age. In some of these publications, it has been noted that the decrease between the ages of 30-50 is approximately 15% and after this age it is 30% every ten years. In addition, there are determining factors such as changes in the nervous system with age, changes in the structure and function of the neuromuscular junction, fat infiltration, cellular and molecular modifications of the muscle. Again, lipofuscin (age-related pigment) and fat settle into the muscle and promote muscle tissue loss. This ensures less tone and ability to contract [11]. Although adult skeletal muscle is fully differentiated, fibers retain the ability to regenerate and replace it in response to an injury. However, in pathological conditions or during the aging process, functional muscle damage may result in the formation of fibrotic tissue regeneration [11].

One of the parameters evaluated in this study was the association of psoas muscle fibrosis with age and chronic diseases. In this evaluation, age was evaluated individually as well as in age groups. They had chronic cardiovascular disease, allergy and psychiatric diseases in terms of chronic diseases. The other two diseases were excluded from the group and their relationship with the more common chronic cardiovascular disease was examined. It was observed that this disease increased with age, and fibrosis increased with age but it was not statistically significant (P>0.005). This is probably because the sample is small.

Atrophy and degeneration often occur for different reasons. They may occur due to mechanical reasons or immobility. Degeneration can also occur by many factors such as inflammation, abnormal mechanical strength, and altered vascularization [12].

In animal models of muscle injuries, atrophy characterized by fat accumulation has been found to be effective in early and middle stage diseases. It has also been shown that high levels of inflammation exhibit an active cycle of degeneration and regeneration. In this study by Gibbons et al, muscle loss was so severe that in the vast majority of samples, muscle tissue was reported to be replaced by an irregular, vascular connective tissue network with high macrophage density. It has been stated that in clinical imaging of such tissues, this appearance resembles a muscle, which may prevent the detection of true loss [11].

Although there is no muscle injury known in this study, parameters such as BMI as well as age and chronic diseases are associated with degeneration and atrophy.

In this study, it was observed that fatty degeneration, histopathological signs of degeneration and chronic diseases tended to increase with age, and this trend was accompanied by loss of C (table-3).

Inflammation is prominent in an increasing number of disease states where changes in TJ have been observed. Changes in TJs have been associated with collagenous colitis, psoriasis, multiple sclerosis / encephalomyelitis, Crohn's disease, ulcerative colitis, and perhaps arthritis. In addition, proinflammatory cytokines have been shown to alter epithelial barrier permeability by inducing apoptosis in individual cells and acting directly on TJ structure and composition [12].

In a study on changes in claudin expression profiles, it was reported that it contributed to epithelial lung dysfunction during infection and inflammation [13].

It has been reported in the literature that the change in klaudin levels contributes to the disruption of the blood barrier. In several diseases related to barrier dysfunction, various pathologies of the nervous system characterized by a pronounced neuroinflammatory component such as Alzheimer's disease, multiple sclerosis, diabetic retinopathy and retinopathy of prematurity, the dysfunction of the brain and retinal barriers contributes to the pathogenesis of these diseases and even to the pathogenesis of these diseases [14].

Recent reports have also shown that C is reduced in retinal endothelial cells due to endoplasmic reticulum (ER) stress, which also plays a role in vascular dysfunction in diabetic retinopathy [14].

In this study, a statistical relationship was not found when evaluated in terms of chronic diseases. When it is divided into age groups and evaluated, it has been observed that it tends to decrease (muscle mass). Since the youngest adult case was 35 years old, no comparison was made with muscle tissue under 35 years old. Evaluation was made with C in the form of muscle expression, and the blood level was not measured. However, when the degeneration score was compared with the claudin score, an inverse relationship was observed. No relationship was found between E and C. This may be due to the rather epithelial localization of E and the endothelial location of C. In previous references, the increase in fat tissue and fibrosis in muscle tissue with age is indirectly indicative of atrophy. It can also indicate degeneration. In this study, an inverse relationship was observed between the increase in adipose tissue and C, and it was observed that the increase in adipose tissue increased with age. Based on this result, we can say that atrophy and degeneration occur with age, and as a result, claudin expression decreases. Although a positive correlation was observed between A and C, it was seen that this parameter showed a homogeneous distribution when the age distribution was examined. When the degrees of degeneration including A were scored, we observed that claudine expression decreased with age and the score of the degeneration increased.

In a study by Ozawa, it was shown that expression of the DsRed-labeled E cytoplasmic domain (DECT) in C2C12 myoblasts inhibits myoblast fusion as well as the transport of endogenous cadherins, including N-cadherin and M-cadherin [15].

Hollnagel et al. in their study, they analyzed the known components of adhesive plaques on Western blot of proteins from regenerated muscle to investigate alternative molecules that could mediate cell adhesion in the absence of M-cadherin. Interestingly, heterozygous and homozygous animals reported that they contained levels of N- and E-cadherin, both of which were regulated by a significantly significant increase during degeneration [16].

In this study, since the expression of E was very low, no relationship was found with age groups when evaluated in terms of the trend in expression of E. Likewise, no relationship was found with histomorphological findings. As stated above, the very low expression level could possibly be the cause.

**Conclusion**

As a result, C tends to decrease in striated muscle tissue in degeneration and atrophy. Therefore, it can be a target protein that can be used in the detection and prophylaxis of degeneration and atrophy in striated muscles. No relationship was found between E and striated muscle degeneration.

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**Figure legend**

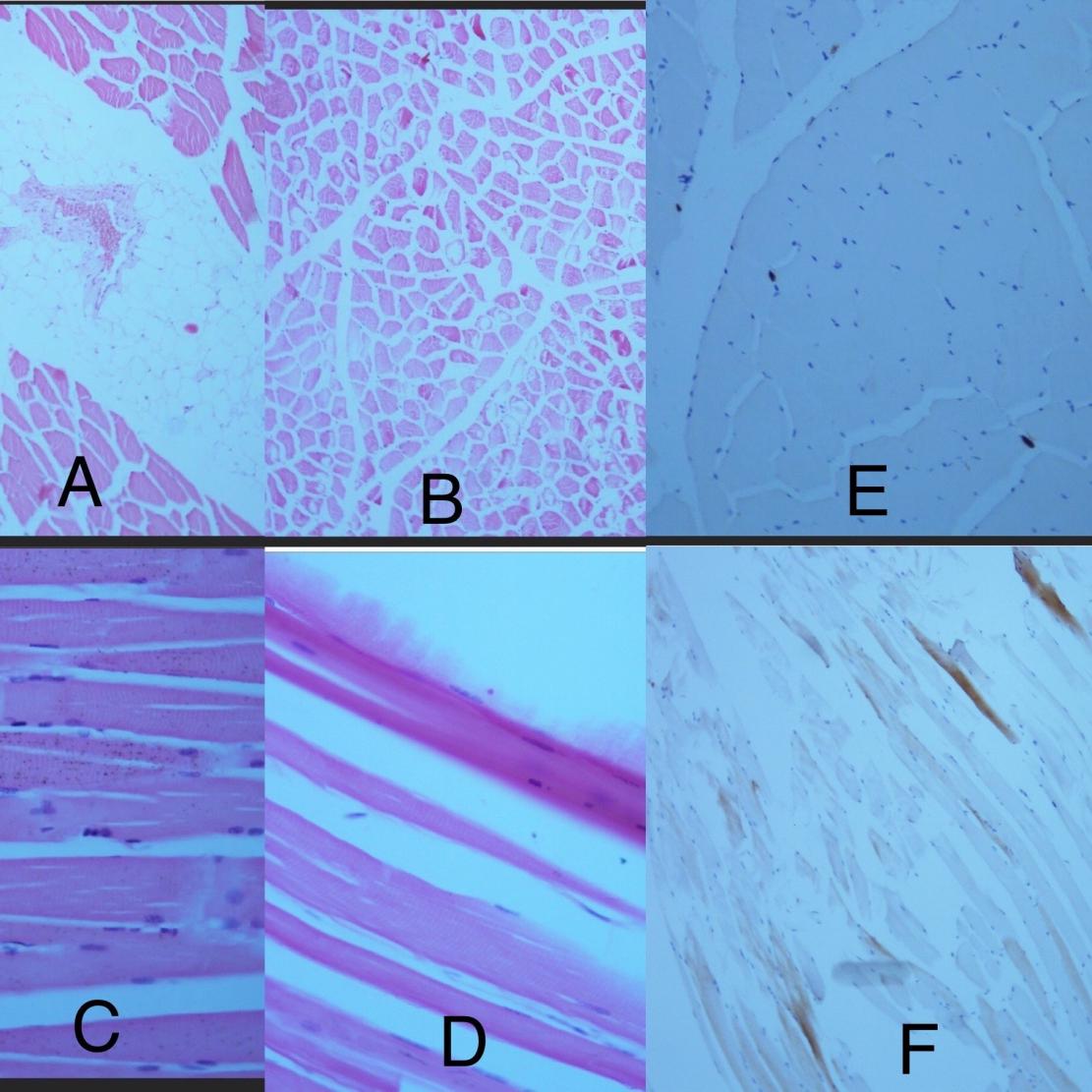
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Figure 1 (A-E): A- Adipose cell aculumulation (fatty degeneration) of muscle tissue (H&EX100), B- Vacuolisation and breakdown in muscle tissue (H&EX40), C- Accumulation of lipophuscin pigments in muscle tissue (H&EX400), D- Acidophilic-like changes in muscle tissue (H&EX400), E- Claudin-5 expression in muscle tissue (grade-1) (x100), F- Claudin-5 expression in muscle tissue (grade-2) (x100).

Graph-1: Distribution of histopathological parameters of degeneration by age groups

Table 1: Distribution of sociodemographic data according to BMI index

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | |  |  | |  | | |  | | |  | |
|  |  | BMI< 18.5 |  | 18.6< BMI <24.9 | |  | 25.0< BMI <29.9 | |  | 30.0< BMI <39.9 |  | P value | |
|  |  |  |  |  | |  |  | |  |  |  |  | |
|  |  | n (%) |  | n (%) | |  | n (%) | |  | n (%) |  |  | |
| gender |  |  |  |  | |  |  | |  |  |  | p=0.46 | |
|  |  |  |  |  | |  |  | |  |  |  |  | |
| female |  | - |  | 1(% 1.81) | |  | 3(5.45) | |  | 2(3.63) |  |  | |
|  |  |  |  |  | |  |  | |  |  |
| male |  | 4 (7.27) |  | 19(34.54) | |  | 19 (34.54) | |  | 7(12.72) |  |  | |
|  |  |  |  |  | |  |  | |  |  |  |  | |
| age |  |  |  |  | |  |  | |  |  |  | p=0.86 | |
|  |  |  |  |  | |  |  | |  |  |  |  | |
| 35-65 |  | 2(3.63) |  | 12 (21.81) | |  | 16(29.09) | |  | 7(12.72) |  | | |
|  |  |  |  |  | |  |  | |  |  |
| 66-85 |  | 2(3.63) |  | 7(12.72) | |  | 5(9.09) | |  | 2(3.63) |  | | |
|  |  |  |  |  | |  |  | |  |  |
| 85 and above |  | - |  | 1(% 1.81) | |  | 1(% 1.81) | |  | - |  |  | |
|  |  |  |  |  | |  |  | |  |  |  |  | |
| Alcohol |  |  |  |  | |  |  | |  |  |  | P=0.62 | |
| using |  | 3(5.45) |  | 3(5.45) | |  | 4(7.27) | |  | 2(3.63) |  |  | |
| Not using |  | 1( 1.81) |  | 17 (30.90) | |  | 18(32.72) | | 7(12.72) | 1( 1.81) |  |  | |
| cigarette |  |  |  |  | |  |  | |  |  |  | p=0.16 | |
| using |  | 4(7.27) |  | 15 (27.27) | |  | 13(23.63) | | 4(7.27) |  |  |  | |
| Not using |  | - |  | 5 (9.09) | |  | 9(16.36) | | 5(9.09) |  |  |  | |
| Disease state |  |  |  |  | |  |  | |  |  |  | p=0.67 | |  |
| No known disease |  | 1( 1.81) |  | 7 (12.72) | |  | 8 (14.54) | | 2 (3.63) |  |  |  | |
| CAD |  | 3(5.45) |  | 10 (18.18) | |  | 10 (18.18) | | 6(10.90) |  |  |  | |
| CAD and COPD |  |  | - |  | | 1(1.81) | 1( 1.81) | | - |  |  |  | |
| Psychiatric disease |  | - |  | - | |  | 3(5.45) | | - |  |  |  | |
| Coronary artery disease (CAD), (COPD) chronic obstructive pulmonary disease | | | | | | | | | | | | | |

Table 2: Results of multiple regression analysis of factors associated with claudin staining

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | β | S.E | p | OR |  | 95 % CI | |
|  |  |  |  |  |  | Lower | Upper |
| Y | -1.35 | 0.66 | 0.04 | 0.25 |  | 0.07 | 0.94 |
| A | 2.04 | 0.72 | p<0.01 | 7.73 |  | 1.87 | 31.87 |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Table-3: Relationship between age groups and degeneration score, claudin score, chronic disease | | | | | | | | |
| age |  | 35-45 | 46-55 | 56-65 | 66-90 | 65 age below | 66 age above |
| Degeneration score | mean | 3.5 | 3.57 | 3.52 | 4 | 3.56 | 4 |
| Claudin score | 0.8 | 1 | 0.75 | 0.77 | 0.81 | 0.77 |
| Chronic Disease | 0.2 | 0.57 | 0.85 | 0.94 | 0.62 | 0.94 |

Graph 2: Graph showing the relationship between staining with Claudin-5 staining degree and acidophilic sarcoplasma (A) and fatty changes (Y)

Blue line: Y, red line: A