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RELATION OF APELIN, TUMOR NECROSIS FACTOR ALPHA AND CLAUDIN-5 TO BODY MASS INDEX IN CHOLECYSTECTOMIES

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Abstract

Objective. The aim of this study was to investigate the relationship of Claudin-5, Apelin, Tumor Necrosis Factor Alpha (TNF-α) expression, and body mass index (BMI) of cholecystectomies.

Materials and methods. Sixty-eight paraffin embedded cholecystectomy specimens diagnosed as chronic cholecystitis were collected in the Pathology Department of the Training and Research Hospital between 2015-2017. The samples were stained with Apelin, Claudin-5 and TNF-α. The immunohistochemical study was carried out using the system in an automatic staining machine.

Results. There was a significant positive correlation between BMI and TNF-α staining (p=0.010). This result indicated that the degree of staining increased together with BMI. When age, BMI, and the other biochemical parameters were evaluated, a significant correlation was found between BMI and blood glucose only (p=0.029); correlations of BMI with the other parameters were not statistically significant.

Conclusion. Although there is no relationship between inflammation and BMI with Claudin-5 and Apelin in this study, there is a significant relationship between BMI and TNF-α.

Keywords: Body Mass Index, Apelin, Claudin, TNF-α.

INTRODUCTION

Tight junctions (TJ) are important cell contact points in the apical region of the epithelial and endothelial cell layers. They have many functions, and two functions in particular are highly important (1); the barrier function regulates the passage of ions, water, various macromolecules, and inflammatory cells through the paracellular cavities, while the fence function protects the cell's polarity (1).

The main integral membrane proteins that always constitute TJ fibers are the junctional adhesion molecules known as occludin and claudins (2). The main cytoplasmic structural proteins associated with TJs are zonula occlusions-1 (ZO), ZO-2, and ZO-3, which bind to the cytoplasmic tails of occludin and claudins (3). Together, these proteins are thought to preserve the integrity of the TJs. It is assumed that the expression and localization of these proteins play an important role in the regulation of paracellular permeability (2,3).

Studies have also shown that TJ proteins are commonly expressed in normal human bile duct epithelium (4).

The main peptide hormones secreted by adipose tissue are adipokines, including adiponectin, leptin, resistin, visfatin, and Apelin. These hormones are associated with the metabolic syndrome (5).

Studies have shown that adipokines act not only in metabolic syndrome but also in inflammatory and preneoplastic process. They have also been shown to affect cell growth and proliferation (6,7).

It has been demonstrated that Apelin level changes depend on nutritional habits, and that Apelin mRNA level increases in rats fed with a high-fat diet (8,9). Plasma level of Apelin is high in obese humans and experimental animals, and that Apelin gene expression in the adipose tissue shows increase through insulin and tumor necrosis factor-alpha (TNF-α) (8,9).

The plasma level of Apelin is known to increase in parallel with increased body fat content and hyperinsulinemia in obese people (10).

Obesity is an established risk factor for gallstones, and several epidemiologic studies have shown a positive relationship between the body mass index (BMI) and the risk of gallstones (11).

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TNF-α is one of the first identified cytokines in the link between obesity and insulin resistance. Studies have shown that TNF-α is secreted by macrophages and adipocytes in glucose uptake, metabolism, and the signaling and induction of insulin resistance (12).

After binding to TNF-α, the receptor triggers two pathways in particular. These are cytosolic inflammatory pathways leading to c-Jun Kinase activity, and the nuclear Kappa Kinase inhibitor (12-14).

The aim of this study is to investigate the relationship between Claudin-5, Apelin, TNF-α expression, and BMI in patients with cholecystectomies, which were diagnosed with cholecystitis and cholelithiasis (some cases).

Additionally, we aimed to investigate the relationship between inflammation and liver function biochemical markers (such as; hemoglobin, white blood cell, platelet, neutrophils, lymphocyte, monocyte, mean platelet volume, red cell distribution with width, blood glucose, blood urine nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, bilirubin (direct, indirect), and C-reactive protein) with Claudin-5, Apelin, TNF-α.

MATERIALS AND METHODS

This study was discussed by the local ethics committee for clinical research and was approved by decision 2017/85.

This study was conducted on a total of 68 paraffin-embedded cholecystectomy samples, which were histopathologically diagnosed at the Department of Pathology of Training and Research Hospital between 2015 and 2017. Gender distribution was 48 female and 20 male. These cases consisted of cases with chronic cholecystitis and cholelithiasis (some cases, macroscopically).

The paraffin-embedded samples, measuring 3 μm in thickness, were then cut. The slides were stained with Apelin, Claudin-5 and TNF-α. An immunohistochemical study was carried out using the system in an automatic staining machine (Leica Autostainer XL).

Apelin Scoring of Immunohistochemical Staining

Staining in the bronchial-covering epithelium and glandular epithelial tissue were used as a positive control.

Cross-sections where the primary antibody could not be instilled were taken as negative controls. Apelin immunoreactivity was graded using the method proposed by Berta *et al.* (15). The proportion of cells with cytoplasmic positivity was taken into account. Accordingly, 0 staining, 1 + staining, 2 + staining, or 3 + staining categories were defined on the basis of no staining, 1% to 10% staining, 11% to 50% staining, and greater than 50% staining, respectively (Figs 1,2).

Claudin-5 Scoring of Immunohistochemical Staining

The Claudin-5 positive tissues were quantified based on the percentage of positive cells (Fig. 1A). Scoring was performed as follows: negative (-), for 1% to 25% positive cells; positive (+), for 26% to 50% positive cells; positive (++), for 51% to 75% positive cells; positive (+++), positive for 76% to 100% positive cells; positive (++++) (16).

TNF-α Scoring of Immunohistochemical Staining

Immunohistochemical staining was evaluated in the cytoplasm of epithelial cells (Fig. 1C). The intensity of staining for each section was evaluated subjectively by three separate observers (who were blinded to the experiment) using the following designations: 0-10% of cells stained, score 0; 11-25 % of cells stained, score 1; 26-50% of cells stained, score 2; 51-100% of cells stained, score 3 (17).

BMI classification was determined as follows: 0 - 18.4: Underweight; 18.5 - 24.9: Normal range; 25.0 - 29.9: Overweight; 30.0 - 34.9: Obese – Class I; 35.0 - 44.9: Obese – Class II;≥45.0: Extreme Obese – Class III (18).

In addition, several biochemical parameters were evaluated in the patients including hemoglobin, white blood cell, platelet, neutrophils, lymphocyte, monocyte, mean platelet volume, red cell distribution with width, blood glucose, blood urine nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, bilirubin (direct, indirect), and C-reactive protein.

Statistical analyses

Descriptive statistics were presented as mean and standard deviations for continuous variables, and as frequencies and percentiles for categorical variables. Kruskal-Wallis test was used to evaluate differences between Apelin, Claudin-5, TNF-α and

for BMI. Fisher-Freeman-Halton exact test was used to detect statistical differences between those groups for BMI. A p-value of $p<0.05$ was assumed to be statistically significant. SPSS (ver. 18) software was used for analyses.

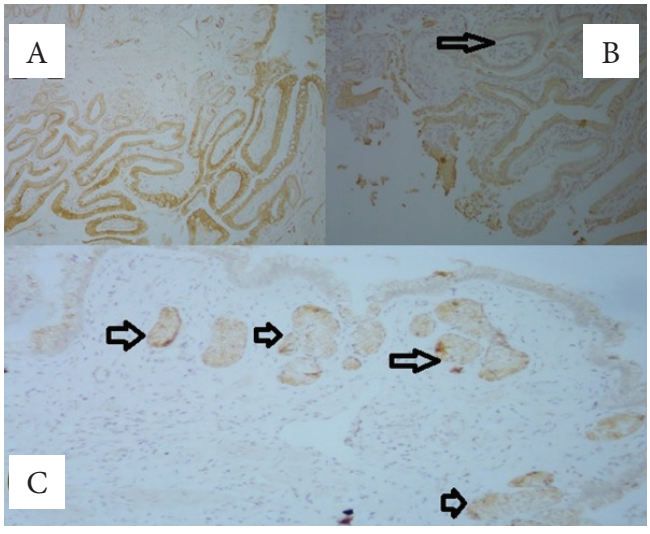


Figure 1. Claudin-5, Apelin, TNF-α expression were observed in the cell membrane (Single layer of uniform, tall columnar cells and slightly chronic inflammatory cells) (BMI: 26). A. 2+ expression of claudin 5 in cell membrane of columnar cells (Claudin-5 X 100). B. 2+ expression of Apelin in cell membrane of columnar cells (apical membran positivity higher than Rokitansky-Aschoff sinuses (arrow) positivity)(Apelin X 200). C. 1+ expression of TNF-α in cell membrane of Rokitansky-Aschoff sinuses (arrow) positivity higher than apical membran positivity (TNF-α X 200).

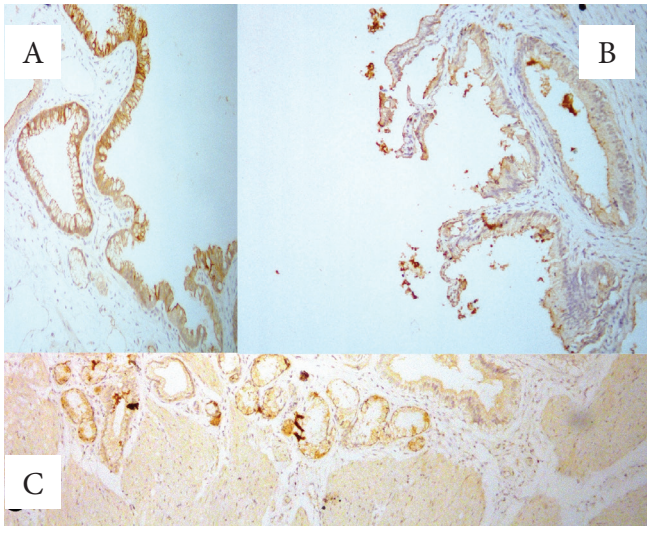


Figure 2. Claudin-5, Apelin, TNF-α expression were observed in the cell membrane (A,B,C) A. 3+ expression of claudin 5 in cell membrane of columnar cells (Claudin-5 X 200). B. 3+ expression of Apelin in cell membrane of columnar cells and Rokitansky-Aschoff sinuses positivity (Apelin X 200). C. 2+ expression of TNF-α in cell membrane of Rokitansky-Aschoff sinuses and apical membran positivity (TNF-α X 100).

RESULTS

Distribution of the staining outcomes is given in the following tables respectively (Tables 1-5). Gender distribution was 48 female (70.6%) and 20 male (29.4%). Twenty of the cases were male and 48 were female. The age range of men ranged from 20 to 86 (mean age: 51). The age range of female ranged from 24 to 72 (mean age: 48).

Distribution of BMI was as follows: 15 (22.1%) normal, 34 (50%) overweight, 13 (19.1%) obese (1^{st} class), and six (8.8%) obese (2^{nd} class).

Descriptive statistics of age, height, and biochemical parameters are given in Table 4.

When the correlation between the degrees of staining and other characteristics were evaluated (Table 5).

Mean and standard deviation values of BMI measurements according to the staining degrees of Apelin, Claudin-5, TNF-α are shown in Graphs 1-3; the only significant, positive correlation was between BMI and TNF staining ($p=0.010$). This result indicated that the degree of staining increased together with BMI. The relationship of apelin and claudin-5 with other parameters was evaluated. Statistically, there was no significant relationship (Table 5). Apart from this, no significant correlation was found between BMI and the other biochemical markers (hemoglobin, white blood cell, platelet, neutrophils, lymphocyte, monocyte, mean platelet volume, red cell distribution with width,

Table 1. Frequency and degree of staining of apelin in cases

		Frequency	Percent
Apelin	0	27	39.7
	1	30	44.1
	2	10	14.7
	3	1	1.5
	Total	68	100.0

Table 2. Frequency and degree of staining of TNF-α in cases

		Frequency	Percent
TNF-α	0	32	47.1
	1	30	44.1
	2	6	8.8
	Total	68	100.0

Table 3. Frequency and degree of staining of Claudin-5 in cases

		Frequency	Percent
Claudin-5	0	4	5.9
	1	21	30.9
	2	20	29.4
	3	23	33.8
	Total	68	100.0

blood glucose, blood urine nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, bilirubin (direct, indirect), and C-reactive protein).

When age, BMI, and the other biochemical parameters were evaluated, a significant correlation was found between BMI and only blood glucose (p=0.029); correlation of BMI with the other parameters were not statistically significant.

Table 4. Distribution of age and biochemical markers in colecistectomysed patients (N=68)

	Minimum	Maximum	Mean	Std. Deviation
Age	20	86	48.68	14.205
Height	1.48	1.85	1.6282	0.08117
Weight	50.0	110.0	75.074	13.0845
BMI	19.53	42.97	28.3239	4.62693
Hemoglobin (Hb)	9.16	16.60	12.6318	1.81979
White blood cell (WBC)	2.11	14.50	7.7024	2.61926
Platelet (PLT)	116	455	272.37	84.535
Neutrophile (Neu)	42.60	87.70	61.0779	10.14792
Lymphocyte (Lym)	5.90	51.20	29.9382	9.10789
Monocyte	.56	14.50	6.3455	2.26788
Mean platelet volume (MPV)	6.51	12.80	8.7643	1.51652
Red cell distribution widht (RDW)	10.80	41.40	16.2912	4.19263
Blood glucose (Bg)	80.0	397.0	111.976	42.3283
Urea BUN ratio (U/B)	5.00	82.00	14.6569	11.56444
Creatinin (Cre)	.55	1.13	.7530	.13689
Aspartate aminotransferase (AST)	10.30	721.00	36.2397	85.79271
Alaninaminotransferase (ALT)	8.0	797.0	45.918	98.4870
Gamma-glutamy ltransferase (GGT)	10	1186	80.97	202.934
Alkaline-phosphatase (ALP)	49	259	84.61	40.864
Total bilirubin (T.Bil.)	.14	5.29	.7415	.67168
Direct bilirubin (D.Bil.)	.08	3.67	.3248	.45264
Indirect bilirubin (I.Bil.)	.06	1.62	.4292	.28636
C-reactive protein (CRP)	.00	17.39	2.6385	3.83985

Table 5. Relationship between TNF-α, claudin and apelin with biochemical markers

		Apelin			TNF-α			Claudin-5		
		R	P	N	R	P	N	R	P	N
Spearman'srho	Age	0.127	.304	68	0.150	.223	68	0.085	.489	68
	BMI	.101	.412	68	.309	.010	68	.055	.658	68
	Hb	0.106	.391	68	0.037	.765	68	0.087	.480	68
	WBC	0.013	.919	68	.024	.843	68	0.013	.915	68
	PLT	0.057	.644	68	0.124	.315	68	0.127	.302	68
	Neu	.033	.792	68	.040	.748	68	.090	.465	68
	Lym	.004	.974	68	0.038	.757	68	0.036	.773	68
	Monocyte	0.115	.349	68	0.002	.988	68	0.162	.187	68
	MPV	0.078	.528	67	.131	.291	67	.039	.752	67
	RDW	0.004	.976	68	0.033	.792	68	0.034	.782	68
	Bg	.030	.810	67	0.087	.485	67	.032	.798	67
	U/B	.097	.441	65	.018	.886	65	.017	.892	65
	Cre	0.030	.809	67	.050	.690	67	.005	.967	67
	AST	.049	.691	68	0.006	.962	68	.015	.903	68
	ALT	0.110	.374	67	0.056	.653	67	.010	.935	67
	GGT	0.097	.581	35	0.232	.181	35	.055	.755	35
	ALP	.243	.173	33	.196	.275	33	.281	.114	33
	T.Bil.	.072	.571	65	0.116	.359	65	.026	.837	65
	D.Bil.	.040	.749	65	0.121	.336	65	.055	.666	65
	I.Bil.	0.016	.905	59	0.162	.221	59	0.045	.733	59
	CRP	.049	.724	54	.033	.811	54	.026	.852	54

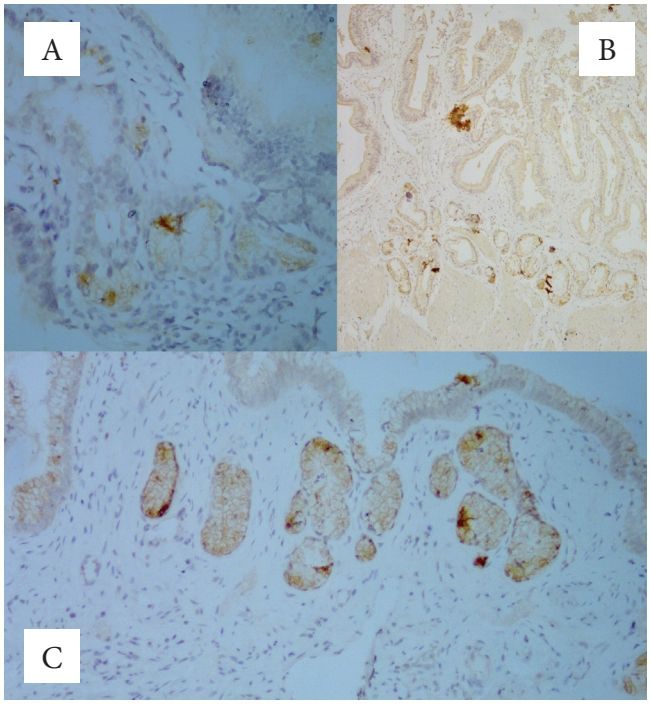
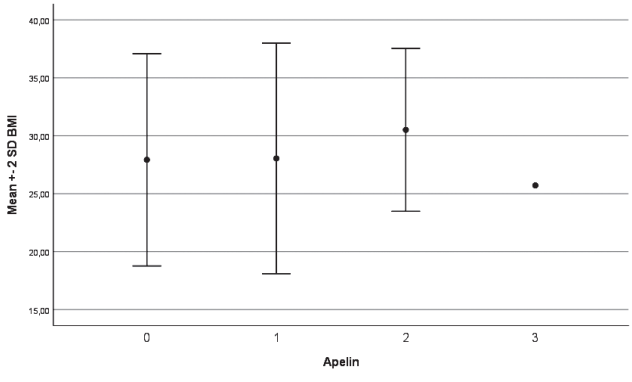
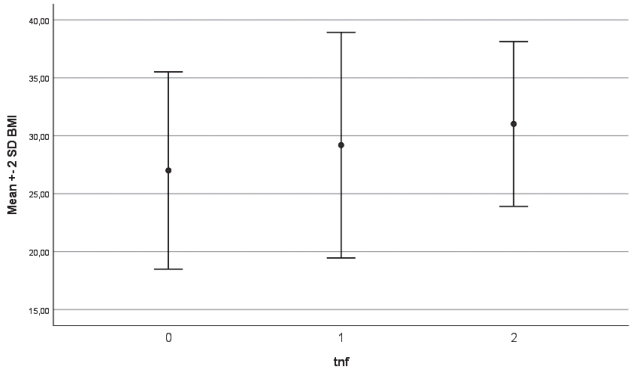


Figure 3. Staining of different degrees of TNF-α (A:X400 (grade 1), B:X 100 (grade1), C:X200 (grade 2)).



Graph 1. Mean and standard deviation values of BMI measurements according to staining degrees (Apelin).



Graph 2. Mean and standard deviation values of BMI measurements according to staining degrees (TNF-α).

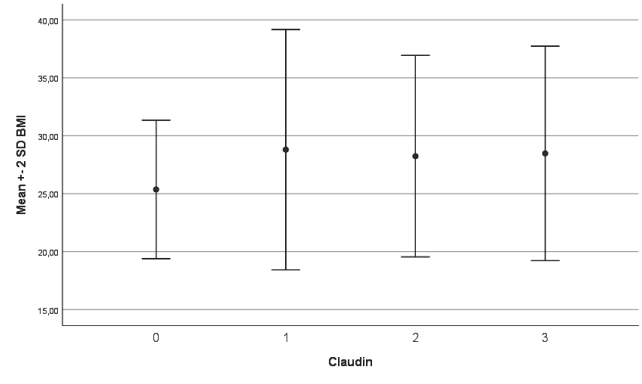
DISCUSSION

The relationship between elevated BMI and 13 different anatomical region tumors has been proven. One of these regions is the gallbladder. Recently, a number of studies have examined the biological mechanisms of insulin resistance and abnormalities that reveal the relationship between cancer and obesity (19). In these studies, sex hormones biosynthesis and pathway, changes in the pathophysiology of adipokine, factors related to ectopic fat accumulation, chronic low-grade inflammation, oxidative stress, vascular disturbances, epithelial-mesenchymal transition, endoplasmic reticulum stress and cellular disturbances including migrating adipose progenitors and microenvironment, disorders in circadian rhythms, diet nutrients and intestinal microbiome changes were noted (19).

In this study, Apelin, Claudin-5, TNF-α markers were stained and evaluated in gallbladder tissue in order to reveal the relationship between BMI and inflammation and obesity. The obtained results are examined with the literature. There is no article on the study of claudin-5 and apelin in gallbladder in current scientific article search (with accessible scan engine).

Studies have shown a change in organ- and tissue-specific Claudin as a potential cause in the restructuring of tight joints in obesity and in the deregulation of the mucosal barrier. There are studies of obesity-related secretions that induce changes in Claudin expression, although intestinal epithelial cell-cell integrity is similarly disordered. These studies suggest that claudin proteins play a more important role in barrier deregulation in the pathobiology associated with obesity (20).

In general, the findings of Rizwan *et al.* emphasize that Claudin-2 is particularly important in the clinical setting in the restructuring of tight joints to cope with obesity (20).



Graph 3. Mean and standard deviation values of BMI measurements according to staining degrees (Claudin-5).

Claudin-5 has been reported to be expressed in the TJ of endothelial cells, including pancreatic cells, alveolar cells in the lung, epithelial cells in the colon and the blood-brain barrier and endoneural blood-nerve barrier. It is especially associated with parasellular permeability in colonic regions (21). Active inflammatory diseases and barrier function disorders of TJs have been shown to be effective in the regulation of Claudin-5 distribution (21).

In this study, a mild inflammation was observed when interpreted in terms of degree of inflammation. In terms of weight, most of the cases were not obese. Probably, there was no statistical significance between claudin-5 expression and biochemical parameters (Table 5).

One of the first Apelin effects observed on glucose metabolism was insulin secretion. In addition, it was found to have a glucose-lowering effect both during fasting conditions and during the glucose tolerance test in standard mice (22,23). Apelin may stimulate glucose transport and its effect may contribute to insulin.

The aim of a recent study by Krist *et al.* was to determine whether the changes in plasma Apelin are due to reduced body fat mass or improved insulin sensitivity. Primarily, in all the different weight loss intervention studies, the high serum Apelin concentration determined in different cohorts of previously reported obese and diabetic patients was reduced (24-26).

Subsequently, there was a significant independent correlation between reduced Apelin levels and improved insulin sensitivity and BMI (26).

For this reason, it can be assumed that increased plasma Apelin observed in type 2 diabetic patients, such as type 1 diabetes, has a mechanism for directly reducing insulin resistance. When insulin resistance decreases, this may lead to a decrease in Apelin levels. For this reason, it has been suggested that low Apelin serum concentrations in healthy subjects may be largely a consequence of normal insulin sensitivity (26).

There are reports that apelin plasma level is increased in obese individuals (10). In this study, there was no significant relationship between weight and Apelin (p =0.412) and blood glucose (p=0.810). This may be due to the fact that obese group (1st class and 2nd class) is low (% 27.9) (Table 5).

In this study, BMI rates in normal and overweight categories might be affected by the association between Apelin and Claudin-5. When the study group was formed, the patients were not selected according to BMI. We studied those cases that were archived between 2015 and 2017.

There is no article on the study of claudin-5 and apelin in gallbladder in current scientific article search.

In this study, the mean blood glucose level was 111.9, with the minimum being 80 and maximum 397. As expected, blood glucose level was correlated with BMI (p=0.029). Since this study was evaluated retrospectively, it could not be evaluated with HbA1c, and could not be associated with insulin resistance.

Nevertheless, the correlation between BMI and blood glucose may be considered a clinical indication of insulin resistance. No positive or negative correlation could be established between blood glucose level and Apelin. The assessment was made in the form of immunohistochemical evaluation.

Since this was a retrospective study, serum levels of Apelin could not be studied. Its correlation with BMI was evaluated for all groups. The majority (72.1%) of the groups consisted of normal and overweight persons, while the proportion for the obese group was only 27.9%. There was no significant correlation because the rate of obesity was not high.

In a recent study of apelin, it was claimed that hepatic apelin acts as a proinflammatory and neoangiogenic factor and therefore can play an important role in initiating and maintaining inflammatory and hyperplastic processes occurring in acute liver injury (27).

In this study, evaluating in terms of chronic inflammation; there was a significant correlation between TNF-α and BMI, although no correlation was observed between Claudin-5 and Apelin. A remarkable point was that white blood cell (mean: 7.7 μL, N: 3.6 – 10.0 μL) and CRP (mean: 2.6 mg/L, N: 1 mg/L) were not very high. These parameters might be the reason for the absence of any significant correlation.

It has been suggested that apelin gene expression in adipose tissues of obese people increases with insulin resistance and tumor necrosis factor-alpha (TNF-α) (8,9). In one study, a significant correlation was proposed with TNF-α in patients with a low BMI. In another study, phlegm and serum levels were measured in patients with chronic obstructive pulmonary disease (COPD). It was shown to be correlated with BMI (26-29). While there was no correlation between BMI and serum-sputum TNF-α levels, BMI was significantly associated with duration of illness as well as smoking history in patients with COPD. Also, there was no relationship between TNF-α and asthmatic obese patients (28).

Emaduldin Seyam *et al.* investigated the correlation between TNF-α serum levels and inflammatory biomarkers in obese women with the lean polycystic ovarian syndrome (PCOD) and with clomiphene citrate-resistant polycystic ovarian syndrome (CCOD). TNF-α serum levels were evaluated in women with PCOD, and also in the group receiving clomiphene citrate (CC) treatment. It was reported that TNF-α serum levels were significantly higher in all women with PCOD, particularly those with CCOD (29).

Androgenic obesity, with a higher W / H ratio, showed a positive correlation with TNF-α serum levels. This level can be thought of as a good predictor of PCOD status and an informative predictor of the use of clomiphene citrate-resistant (29).

In this study, a relationship was found between TNF and BMI (Graph 2) and blood glucose, showing similar results with literature (8, 9).

In a study, the relationship between acute cholecystitis and some antimicrobial toxins (HNP-α, BPI, endotoxin, neutrophil elastase, lactoferritin, hepcidin) and cytokines (TNF-α, IL-6, 8, 10) was investigated and a significant correlation was found between the severity of inflammation (30).

In another study, TNF-α was evaluated in chronic and acute cholecytitis. TNF-α was thought to play a role in the pathogenesis of cholecystitis. It is concluded that TNF-α may play a role in the inflammation mechanism of chronic cholecystitis, which is more prominent in acute cholecystitis (31).

Microscopically, the degree of inflammation was mild in this study. Chronic inflammation with lymphocyte predominant inflammatory cells was observed. Acute cholecystitis and healthy gallbladder group were not included in this study. There were not enough cases of these two groups in our department archive.

In the present study, although biochemical inflammatory parameters are not very high, the correlation between TNF- α and BMI seems to result from inflammation. This study was conducted on cholecystitis, because it was associated with inflammation and obesity.

In conclusion, it was found in the present study that TNF-α tends to significantly increase in patients with chronic cholecystitis, there was a tendency toward obesity, but half of the patients were in the overweight group. No significant correlation was observed between Claudin-5 and Apelin. Further studies containing blood levels of biomarkers (TNF-α, Claudin-5, Apelin) and including obese groups could be more enlightening.

Conflict of interest

The authors declare that they have no conflict of interest.

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