A Morphometric Comparison of the Elastic Fibers Found in the Serosal-Subserosal and Submucosal Layers in the Corpus, Antrum and, Pylorus Sphincter of the Stomach

MİDENİN KORPUS, ANTRUM VE PYLOR SFİNKTER BÖLGELERİNDE SEROZA-SUBSEROZA VE SUBMUKOZA TABAKALARINDA BULUNAN ELASTİK LİFLERİN MORFOMETRİK KARŞILAŞTIRILMASI

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Summary

Purpose: The purpose of this study is on the relation to pylor sphincter of duodenal reflux (bouncing-drawing back into the stocmach from the duodenum), which hasn't been understood well yet from the morphological point of view, and what role these elastical fibers in the form of sphincter have on this mechanism. This study was done by Anatomy Department at the Faculty of Medicine of Karadeniz Technical University.

Materials and Methods: Aproximatey, 30 fresh stomachs in human cadavers obtained from different university were studied in this study. The density and lengths were compared between elastic fibers of serosa-subserosa and submucosa layers in the antrum area and the ones in pyloric sphincter of these stomachs. For these measurements, visopan type microscope, mostly used in forestry engineering, were used.

Result: As a result of this study, elastic fibers in the layers of serosa-subserosa and submucosa in pylor sphincter were found to remarkably be both longer and denser than the ones in serosa-subserosa and submucosa in the corpus and antrum area.

Conclusion: Acording to the data obtained in this study, we came to a conclusion it was essential that it should be investigated in humans whether these morphological formation characteristics of the stomachs would cause some functional disorder in clinics and what effects these elastic fibers have on the mechanism of bouncing back into stomach from duodenum.

Key Words: Serosal-subserosal layer, Submucosal layer, Elastic fiber, Pylorus, Stomach

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Özet

Amaç: Bu çalışmanın amacı morfolojik yönden henüz tam olarak anlışılamamış olan duodenal reflünün pylor sfinkteri ile ilgisi ve bu sfinkter yapısında elastik liflerin ne kadar etkisi olduğudur. Çalışma Karadeniz Teknik Üniversitesi Tıp Fakültesi Anatomi Anabilim Dalı'nda gerçekleştirilmiştir.

Materyel ve Metod: Çalışma için farklı üniversitelerden elde edilen 30 adet insan midesi incelenmiştir. Bu midelerde pylor sfinkteri'nde seroza-subseroza ve submukoza tabakalarında bulunan elastik lifler, midenin korpus ve antrum bölgesindeki seroza-subseroza ve submukoza tabakalarındaki elastik lifler ile yoğunluk ve uzunluk bakımından karşılaştırılmıştır. Ölçümler için daha çok orman endüstri mühendisliğinde kullanılan Vizopan tipi mikroskop kullanılmıştır.

Bulgular: Yapılan çalışma sonucunda pylor sfinkterindeki seroza-subseroza ve submukoza tabakasındaki elastik lifler, korpus ve antrum bölgesindeki seroza-subseroza ve submukoza bölgelerinde bulunan elastik liflerden istatistiksel olarak anlamlı derecede hem uzun hemde yoğun bulunmuştur.

Sonuç: Bu çalışma sonucunda elde edilen verilerle, midenin bu morfolojik yapı özelliklerinin klinikte çeşitli fonksiyon bozukluğu sebebi olup olmayacağının canlılarda araştırılması gerektiği ve böylece duodenumdan mideye geriye kaçış mekanizmasında elastik liflerin etkilerinin açıklanabileceği fikrine varılmıştır.

Anahtar Kelimeler: Seroza-subseroza tabakası, Submukoza tabakası, Elastik lif, Pilor sfinkteri, Mide

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Material and Methods

Thirty human stomachs taken from fresh cadavers collected from different University Hospitals in Turkey were examined. None of the stomachs had had any pathology.

The samples were taken from individuals 25 to 55 years of age, without any consideration to sex.

The samples were fixed in 10% formaldehyde. It was carefully noted that the preparates taken from the stomach contained all layers and followed up in accordance to the parafin imbedding technique. Cross sections of gastric wall were stained according to the Verhoeff van Gieson technique and followings were measured:

- length of the elastic fibers in the serosa-subserosa layer (Figure 2).

- density per unit area of the elastic fibers in the serosa-subserosa layer (Figure 2).

- length of the elastic fibers in the submucosa layer (Figure 3).

- density per unit area of the elastic fibers in the submucosa layer (Figure 3).

Slides were evaluated on the screen of a Vizopan microscope in the Department of Forestry Industry Engineering Laboratory of the Karadeniz Technical University. The length of the elastic fibers, and their density per unit area were evaluated. The highest magnification of the Vizopan (X 570) was used for the measurements. To determine the units of magnification from milimetric values, the Carl-Zeiss scale placed in the Vizopan plate, was converted into micron units by fixing the values to a marked ruler where the millimeter was divided every 1/100 of the unit space. In this case a value of 1mm on the screen was determined to be as 1.76 micron and the gross value was multiplied by 1.76 and the value was obtained in microns.

A matrix of millimetric measure was placed on the transparency, on which the 80mm radius screen of Vizopan's millimetric fiber density. 25 mm^2 squares were used for counting. The area of the circle was found according to the p.r^2 formula as 3.14 x 802 = 20096. When this was divided into 25 mm^2 squares, 20096/25 mm^2= 804 squares were obtained. Their fiber content was observed and the density per unit area of the fibers was thus obtained.

Since the vizopan microscope with a screen does not have a photograph device technics, the preparates were photographed according to the X 400.

The statistical evaluvation of data was performed by SSPS. Since the data for density per unit area of elastic fibers fitting independence and parametric assumptions, one way post hoc. Comparison on Newman Keuls test were used on comparing of 5 different measured regions.

The length of elastic fibers have not fitted on parametric assumptions, Kruskal Wallis variance analysis, Mann Whitney-U test on post hoc comparisons were used.

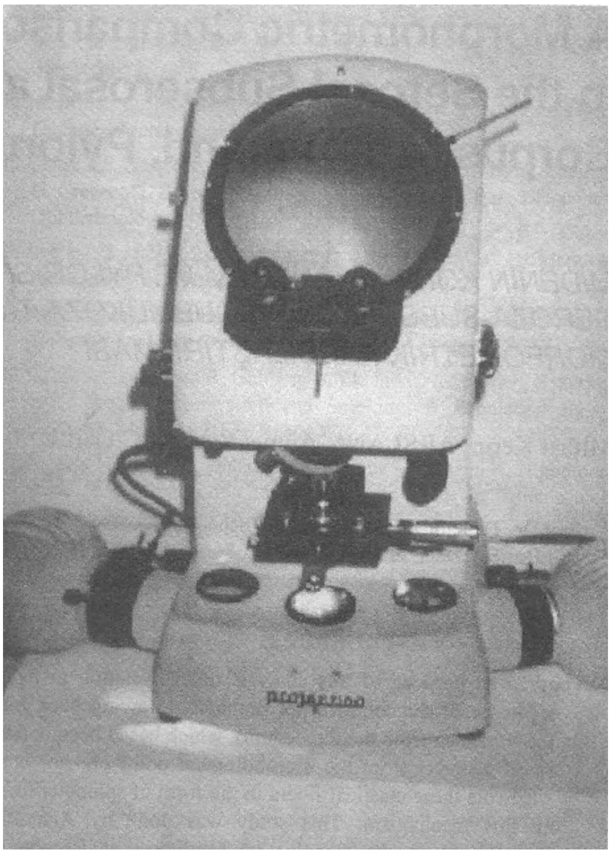

Figure 1. Photograph of Visopan microcope.

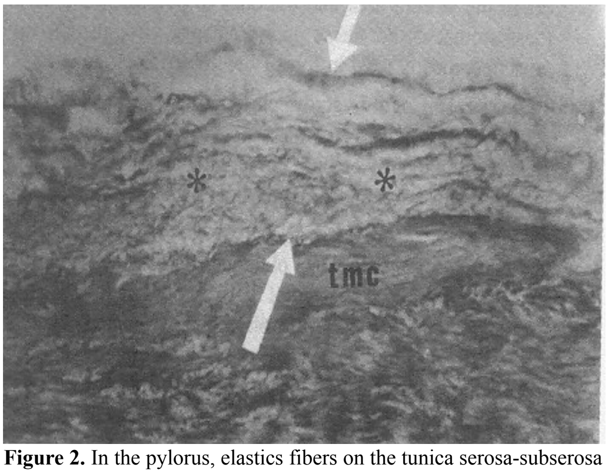

Figure 2. In the pylorus, elastics fibers on the tunica serosa-subserosa layer (X 400)
*: elastics fibers
tmc : tunica muscularis layer

Results

In the research, it was possible to see the elastic fibers in the tunica serosa and tunica submucosa of the stomach. The elastic fibers in the preparates stained by Verhoeff van Gieson elastic stain were differentiated by the black purplish thread-like appearence. The mucosa layer was stained brown, muscular layer was stained red and light brown colours, it was also noted that the submucosa sometimes extends into the depths of the muscular layer.

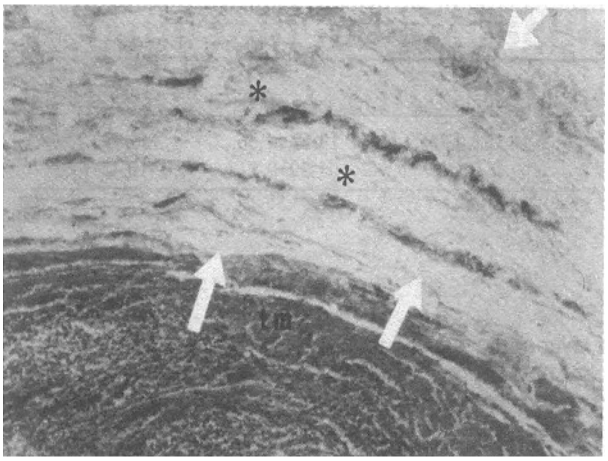

Figure 3. In the pylorus, elastics fibers on the tunica submucosa layer (X 400)
*: elastics fibers
tm : tunica mucosa layer

It was found that the length of the elastic fibers within the tunica serosa-subserosa was greatest at the pylorus and shortest at the antrum (Table 1).

*According to the post hoc Mann Whithney U test, the elastic fiber length were significantly greater in pylorus than in the other areas (P = 0.0125).

The density per unit area of the elastic fibers in the tunica serosa-subserosa was found to be greatest at the pyloric region and least in the antrum region. The elastic fiber density per unit area in the pylorus was found to be statistically greater in comparison to that of the other areas (P< 0.001) (Table 1).

**According to the post hoc Newmann Keuls tests the layer density of the fibers in pylorus found to be significantly more than in the other areas, (P = 0.05)

The length of the elastic fibers in the tunica submucosa was greatest in the pylorus and shortest in the antrum region. The length of the elastic fibers in the pylorus was found to be significantly greater than the length of the elastic fibers in the other regions (P<0.001) (Table 2).

*The length of the elastic fibers in the pylorus according to the post hoc. Mann Whithney-4 test was found be to significantly greater than in the other regions (P = 0.0125).

The density per unit area of the elastic fibers in the tunica submucosa was found to be greatest at the pyloric region and least in the corpus region. The elastic fiber density per unit area in the pylorus was found to be statistically greater in comparison to that of the other areas (P = 0.0000) (Table 2).

**The density Per unit area of the fibers in pylorus, according to the post hoc. Newmann Keuls test was found to be significantly greater than of the other areas (P = 0.05)

Discussion

The examination of elastic fibers goes back to the end of the 19th century. Some writers have carried out studies on elastic fibers in a number of organs. The most recent results have been reported by Meinel in 1902, who demonstrated the presence of elastic fibers in the stomach. Storhr in 1919, Mosken and Mc Gregor in 1928 demonstrated the presence of elastic fibers in the tunica submucosa of the cardia. In 1907, Schutz demonstrated the increase of the elastic fibers in the pylorus. In 1974, Ferraz de Carvalho studied the elastic fibers of the terminal ileum (2). In 1995, Ferraz de Carvalho et al. described, elastic fibers at hepatoduodenal ligament and subserosa and adventitia of the duodenum (3).

In humans, the digestive tract is a continuation of one part to the next, although each part is morphologically different from the other. This tract has the form of a canal, starts from the mouth and ends at the anal canal by exhibiting different morphologic forms. These different parts manifest various physiologic functions in a continous manner. These functions are especially dependent on the regular pattern of movements of the digestive tract (4,5). The presence of the sphincters separating the morphologically different parts allow the proper functioning and prevent the reflux of the contents and their mixing (4,5). The presence of reflux has not been demonstrated due to decrease in intraluminal pressure in the pylorus ring (6,7). The most important role of the pylorus sphincter is the prevention of a return of the chyme into the stomach from the duodenum (8,9). When reflux occurs , and the histology of the former position is not appropriate, resulting histology and physiology can be disordered (10). The cause of the reflux is the result of sphincter dysfunction. Although the functions of the sphincter may be different from each other, their effects are generally regulated by neurologic, hormonal, intraluminal material and substances effective via the hematogenous route (11). All these components affect the motility of the sphincter muscle and thus the sphincters display their controlling junctions (12-14). In this study, the effects of the elastic fibers in the serosa-subserosa and submucosa layers on the function of the sphincter was investigated.

The pyloric muscle of the stomach has continuous contraction during resting but it has an open space of 3-4 mm . The antro-pyloric area also is under high pressure during resting. As a result, a high pressure difference occurs between the corpus of stomach and duodenum (4,5). Therefore, the contents of the stomach pass into the duodenum in the form of a bolus, with the strong prepulsive movements of the stomach. The contents of the duodenum, however, can not move back to the stomach. The above specificity also prevents a passive liquid reflux. Some authors reported that in dogs, elongated ligament may increased stomach mobility and predispose, partial or complete gastric volvulus (15).

Table 1. The length and density of elastic fibers in the serosa subserosa layer of the pylorus, antrum-1, antrum-2, corpus-1 and corpus-2

	Pylorus	Antum 1	Antrum 2	Corpus 1	Corpus 2
Length of the fibers	45.68± 1.48	16.00± 1.29	16.38± 0.86	19.12± 1.36	20.53± 1.37
Density of the fibers	17.78+0.80	10.47+0.74	9.46+0.59	11.39+0.57	10.42+0.63

P<0.0001

Table 2. The length and density of elastic fibers in the submucosa layer of the pylorus, antrum-1, antrum-2, corpus-1 and corpus-2

	Pylorus	Antum 1	Antrum 2	Corpus 1	Corpus 2
Length of the fibers	44.17± 2.25	24.39± 1.68	22.77± 1.31	27.27± 1.31	25.92± 1.84
Density of the fibers	15.36+0.48	11.93+0.67	11.50+0.07	9.95+0.49	9.99+0.46

P<0.0001

The difference between the anatomic and physiologic sphincters of the digestive system have for a long time been discussed at the level of the light microscopy. The terminal ileum in particular has been studied in relation to this topic and it was found that the number of elastic fibers increase as the terminal ileum is approached (2,16). In one study, the distal portions of oesophagus in relation to the stomach have been shown to have increased elastic fibers (13). In the same studies, the elastic fibers in the muscular areas but without sphincteric type have been supported to show physiologic functions (2,13,16).

In this study, the morphometric comparison of the elastic fibers in the serosal-subserosal and submucosal layers in the corpus, antrum and, pylorus sphincter of the stomach has been investigated. Our results indicate that, elastic fibers may strength pylorus sphincter and may have a role in duodenal reflux. A detailed in vivo research may be required to demonstrate whether morphological properties of elastic fibers in the pylorus obtained in this study may contribute to clinical dysfunction or not. In the treatment of clinical dysfunction originating in at this region, a pharmacological agent supporting or strengthening elastic fibers and collagen tissue might be used for further investigations.

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