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ESTERATIC ACTIVITY DEMONSTRATION ON HUMAN GASTRO-ESOPHAGEAL REGION

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ABSTRACT

Background: Histochemical demonstration of the smooth muscle fibers in the gastro-esophageal region, esteratic activity was used to demonstrate the muscle fibers activity and differentiation. **Objectives:** To identify the site of activity of muscle fiber in the gastro esophageal region. **Material and Method:** Full thickness specimens was taken from GEJ of three different adult male human cadavers from the central forensic institute to demonstrate the esteratic activity by aso-dye coupling method then the optical density was measured. **Result:** Different areas were chosen to show that the activity of the muscle fiber located in Z-line region and the region above and below by four cm. Staining of muscle sections is simultaneous azo-dye coupling method in which α-naphthyl acetate act as substrate for esterase group of enzyme. This staining with ANAE method showed clear increased muscle activity in the region of lower esophagus (above Z-line) more than the region below Z-line and the least in the Region of Z-line. Full automatic computerized software was used to specrtophotomorphometric evaluation (global lab image/2). **Conclusion:** Depending on esteratic activity the muscular layer of the gastro-esophageal junction shows that the sphinecteric action in the lower esophagus related with the high esterase activity of smooth muscle fibers in this region which is apparently clear in histochemical esteratic activity when the different sections dealt with non specific ANAE and the transitional Z-region area just rooming area for food stuff. The Z-area smooth muscle had the least activity after studying the morphometry of the biopsies taken from these areas.

KEYWORD: Z-line, optical density, ANAE, polyvinyl pyrrolidone (PVP).

INTRODUCTION

Reflux of gastric contents into the abdominal and lower thoracic esophagus as a result of transient relaxation of the lower esophageal sphincter occurs as a normal event in most individuals for a small percentage of their daily life. It also occurs as a result of a weak lower esophageal sphincter, or of hiatus hernia which disrupts the normal anatomical barriers.[1]

Several anatomical and physiological factors normally prevent gastro-esophageal reflux. The folds of gastric mucosa present in the gastro-esophageal junction, the mucosal rosette, contribute to the formation of a fluid-and gas-tight seal. They also help to ensure that even low levels of tone within the lower esophageal wall muscles may occlude the lumen of the junction against low pressures of gastric gas. The angle of the cardiac orifice may help to form a type of 'flap valve' and the length of abdominal esophagus is buttressed externally by pads of adipose connective tissue at and below the level of the diaphragmatic hiatus.[2]

The major anti-reflux mechanism is the tonic contractions of the lower esophageal musculature, which forms an effective high pressure zone (HPZ).[1] The tonic contraction of this sphincter prevents the stomach contents from regurgitating into the esophagus. The closure of the sphincter is under vagal control.[3] The specialized smooth muscle of the wall of the lower esophagus and the encircling fibers of the crural diaphragm exert a radial pressure that can be measured by a sensing device as it is withdrawn from the stomach into the esophagus.[4]

Smooth muscle is derived from loose mesenchyme, as most massively manifest in the splanchnic mesoderm surrounding the endodermal primitive gut epithelium (including the esophagus) and its appendages.[5] The esophageal wall has internal circular and external longitudinal layers of muscle.[6]

At the distal end of the esophagus, the muscular layer consists of only smooth muscle cells that is close to the stomach; in the mid portion, a mixture of striated and smooth muscle cells; and at the proximal end, only striated muscle cells.[7][8] Only the distal portion of the esophagus that is in the peritoneal cavity is covered by serosa. The rest is covered by a layer of connective tissue, the adventitia that blends into the surrounding tissue.[8]

Internal appearance of the lower esophagus

Internally, the transition between esophagus and stomach is difficult to be defined because mucosa of gastric fundal pattern extends a variable distance up into the abdominal esophagus forming a 'zig-zag' squamo-columnar epithelial junction this called Z-line.[1]

The transition between the squamous esophageal and columnar gastric epithelium is an objectively recognizable reference point. This abrupt, serrated line has "four to six small, long or short tongues – like projections.[9] The Z-line is normally located near the gastric orifice or just above it.[10].[11]

Endoscopists thus base their determination on differences in color, the degree of transparency of the epithelium, mucosal structure, and epithelial thickness.[12]

No anatomic sphincter exists at the lower end of the esophagus. However, the circular layer of smooth muscle in this region serves as a physiologic sphincter.[13]

The Z-line often referred to as the gastro – esophageal junction, for histological and endoscopic purposes. A sling of longitudinal gastric muscle forms a loop on the superior, left, side of the gastro-esophageal

junction between the esophagus and the lesser curvature, and this is taken as the external boundary of this junction.[1]

Ontogeny of gastro – esophageal wall

Both endoderm and mesoderm participate in the formation of the esophageal wall. The endoderm produces the esophageal epithelium and glands, and the mesoderm produces the connective tissue, muscular coat of the esophagus. The developmental event of the esophageal wall formation takes place at approximately the 34th day.[12]

Peristalsis along the esophagus and at the lower esophageal sphincter is immature at birth, which probably accounts for the frequent regurgitation of food that occurs in the newborn period. The pressure at the lower esophageal sphincter approaches that of the adult at 36 weeks of age.[1]

Aims of the study

This research had been designed to be descriptive, histochemical and quantitative evaluation

study on the smooth muscular layers at the sphincteric region of the lower end of esophagus using esteratic activity.

Subjects and Methods

The biopsies were prepared from three different adult male human fresh cadavers at the institution of forensic medicine in Baghdad. The subject was apparently having a normal gross morphology of the gastro esophageal region. Full thickness biopsies of the gastric-esophageal junction (GEJ) region were taken at the Z-line, 4cm above the Z-line, and 4cm below the Z-line. Each of these three regional biopsies was of about 0.5cm length.

Fresh frozen section was done on specimens by using freezing microtome (Reichert – Jung Biocut stage microtome attached to Frig Mobil freezing unit) model 1205 with a stage hand a type C (wedge) cutting knife (Gorden & Bradbury 1982).[13]

The specimens were oriented on the stage of the freezing microtome. The pieces are held together with a drop of Gum Arabia (Fluka) as an adhesive substance on the cryostat chuck. The freezing of the specimen on the stage was conducted via a thermo-electrical module at -40^{0}C for a total time of 60 seconds, and then controlled freezing at -20^{0}C was monitored throughout the sectioning procedure.

Ten sections from each of the regional biopsies specimen where taken on cover slips, a total of 90 sections from all the specimens were obtained. Sections are cut at 10µm thickness and picked up on 22x22mm #2 thickness cover slip.[14] The slide was kept for a night in the freezer.

Azo-dye coupling method was used to demonstrate the esteratic activity on these sections. The histochemical demonstration of a-naphthyl acetate esterases achieved by the modified method of Nachlas and Sligman 1949 By Oliver *et al.,* (1991) was employed. The a-naphthyl acetate was used as the substrate while freshly prepared hexazotized.

pararosanaline used as the diazonum salt which posses' high efficiency of its coupling with the nephthol.[15]

The incubation media was containing (in order): 0.2 phosphate buffer pH7.4 (7.5ml), a-naphthyl acetate (10mg in 1ml acetone) and distilled water (2.5ml) after shaking well then (0.8ml) of freshly prepared hexazotized pararosanaline from equal size of pararosanaline

added to sodium nitrate. This is a simultaneous azo-dye coupling method in which a-naphthyl acetate acts as a substrate for the esterase group of enzymes.[16]

The sections were incubated in the incubation solution at 37°C for 5 minutes, and then washed in distilled water for one minute. When histochemical reactions and stained the cover slips are mounted on slides with polyvinyl pyrrolidone (PVP).

Reichert-Jung polyspec microspectrophotometry was used for studying quantization of GEJ esterase histochemistry by measuring the optical density. Basically it consists of a photometer and a central processing unit attached to a Polyvar microscope (Reichert) through a dual reflex model.

Extension mode of measurement with wavelength of maximal absorption 425nm (Al-salihi 2002) was employed in this study.[17] The optical density of final reaction product i.e. extinction measurement is done through the measurement of transmittance according to the following formula:
Optical density=2-logarithm to base 10 (Transmittance).

RESULT

The positive esteratic reaction was showed in the smooth muscles of the three chosen lower esophageal regions. The esteratic activity in GEJ demonstrates a positive homogenous brown-reddish colored reaction within the cytoplasm (figure 1-A, 1-B, and 1-C).

Assessment of the optical density of the ANAE final reaction product in the cytoplasm of the smooth muscles at the gastro-esophageal regions was investigated. The mean of the optical densities of the reactivates at the region above the Z-line was (2.121±0.19). The mean optical density at the Z-line was (1.251±0.12), the region below Z-line showed a mean of optical density (1.864±0.11).

The measurements of optical density showed statistical significant differences between these three regions studied. The region at the Z-line had the lowest esteratic activity, while the highest reactivity was seen in the smooth muscles at the region above the Z-line (table 1-1).

Table (1-1) show the optical density of esteratic activity.

Optical density	4cm above Z-line	Z-line Region	4cm below Z-line
Mean (SD)	2.121(0.19)	1.251(0.12)	1.864(0.11)

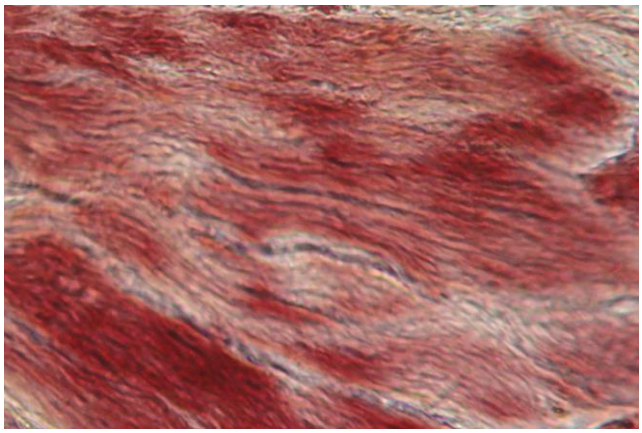

Figure (1-A): sections demonstrating the non-specific esteratic activity in smooth muscles at the region above Z-line. (X400).

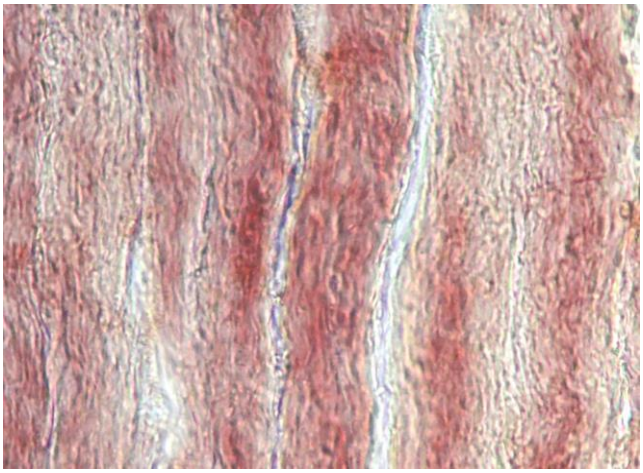

Figure (1-B): sections demonstrating the non-specific esteratic activity in smooth muscles at the region of the Z-line. (X400).

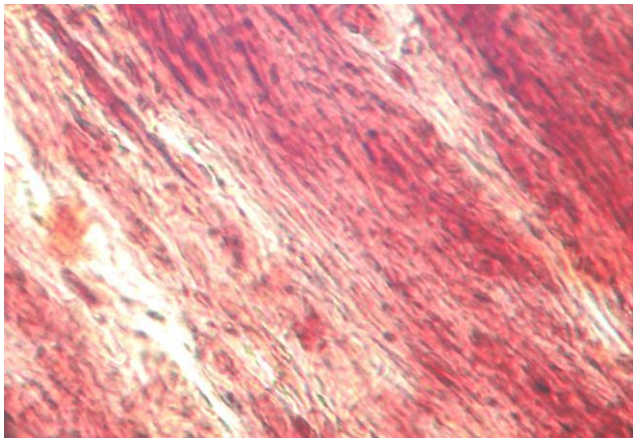

Figure (1-C): sections demonstrating the non-specific esteratic activity in smooth muscles at the region below Z-line. (X400).

DISCUSSION

The usage of hexazotized pararoseaniline as coupling agent and alpha naphthyl acetate as a substrate in simultaneous azo coupling reaction is recommended for correlative quantitation of esterases hitochemically.[17]

The hexazotized pararoseaniline provides the least inhibitory action on the enzymatic activity and thus it is suitable for enzymatic assay (Lam *et al.* 1985).[18] This quantitative method has been also used successfully to classify the type of skeletal muscle fibers in human muscle biopsy material and murine muscles.[19]

The results of this study supported the conclusion of Pugh et al (1983) that reported a positive esteratic activity in smooth muscle fibers, this reactivity were unclear to differentiated and identify types of activity of smooth muscles.[20]

Abd and Al-Salihi (2007) established a confirmation to the fact that microspectrophotometric evaluation using the optical density of final reaction product for α-naphthyl acetate esterases seems to give a good mean for evaluating the quantitative histochemistry of esterases.[21] The results of this study supported the view of evaluating the quantitative histochemical esteratic activity by using the method of microspectophotometery.

Al-Kabee and Al-Salihi (2005) reported that the smooth muscles of the gastrointestinal tract showed positive esteratic activity with a variable intensities of α-naphthyl acetate esterase reactivity.[22]

The final reaction product of ANAE enzymatic reaction in this study was brown reddish in color. It was homogenous in distribution, although the distribution wasregular within the area of cytoplasm of smooth muscle fibers.

The results of this study were designed to be conclusive for the esteratic activity specifically in the gastroesophageal region apart from other subdivisions of the gastrointestinal tract. The esteratic activity demonstrated in this study was correlated with the sphincteric function at the gastroesophageal junction. This correspondence gives a hint to elaborate a fact that the region above the Z-line which is considered as region the physiological gastroesophageal sphincter.[8] This region contains smooth muscular wall with high esteratic activity (table -1-).

The tracheoesophageal septum gradually partitions the respiratory diverticulum from the dorsal part of the foregut. In this manner the foregut is divided into a ventral portion namely the respiratory primordium, and a dorsal portion which is the esophagus.[23]

Most of the smooth muscles arise from the mesoderm. Differentiation of various regions of the gut tube wall and its derivatives is dependent upon a reciprocal interaction between the endodermal epithelium of the gut tube and the surrounding splanchnic mesoderm.[24]

The muscular coat of the esophagus, which is formed by surrounding splanchnic mesenchyme, is striated in its upper two-thirds and innervated by the vagus; the muscle coat is smooth in the lower third and is innervated by the splanchnic plexus.[1]

McMinn (1998) reported that the innervations of a muscle are an indicator of the embryonic origin of that muscle. The difference in estratic activity with higher reactivity above Z-line and lower activity at Z-line possibly indicate the different embryonic trend at the esophageal muscular wall ontogeny in these regions. The weaker reactivity at the Z-line could be possibly explained that the smooth muscles at the Z-line may originated by a dynamic interaction of the embryonic mesodermal precursor that is different from that smooth muscles at other regions of the esophagus.[2]

The crural fibers are developed from the mesentery of the esophagus that is a derivative of the visceral mesoderm.[23] Anatomically the fibers from the right crus of diaphragm surrounded the GEJ.[12] The mesoderm forming the diaphragmatic crura has the potentiality to be differentiated into muscular tissue.[24] The weaker reactivity at the Z-line may possibly

point out an embryonic interaction between the mesoderm of the esophageal mesentery (forming the diaphragmatic crura) that surround and thus influence the visceral mesoderm forming the smooth muscles at the Z-line.

The esophageal Z-line is surrounded by inferior phreno-esophageal ligaments and adipose tissue.[1] The origin of fibrous tissues of ligaments and adipose tissues was confirmed to be from a mesodermal precursor.[23] The visceral mesoderm that will differentiate into smooth muscles at the Z-line is the logical possible source for the fibrous tissues and the adipose tissues surrounding the Z-line. Therefore; the interaction suggested in this study between the mesodermal precursor of the diaphragmatic crura and this visceral mesoderm may also contributes to differentiation of the adipose and fibrous tissues surrounding the Z-line in addition to its effect in minimizing the esteratic activity of the smooth muscles.

Cytochemical demonstration done in this study for the carboxyl ester hydrolases in smooth muscle fibers of GEJ sections with α-Naphthyl acetate esterase (ANAE) showed variable reactivity. The simultaneous coupling azo-dye method used for the demonsration of these classes of hydrolyzing naphthyl acetate was suggested to be very efficient. Adequate localization of the enzymatic reaction of ANAE could be noticed; moreover the color characteristic is more easily identified and demonstrated at light microscope study.[17][25][26]

Conclusively, the results of this study suggested that the esteratic activity could be used as an indicator of the embryonic interaction of the mesoderm forming the smooth muscles. Furthermore, the highest esteratic activity in the smooth muscle of the gastro-esophageal sphinecteric region (the region above Z-line) probably giving a clue to sphincteric function.

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