Morphology of the esophagus using formalin-fixed samples

Rodolfo Pessato Timóteo¹, Carlos José Freire Oliveira², Sebastião Tostes Júnior¹, Sheila Jorge Adad¹

ABSTRACT

Introduction: The esophagus is a muscular tube connecting the pharynx to the stomach, which carries food and liquid from the mouth into the stomach. Previous studies have not provided detailed descriptions of the conditions of the esophagus, as measured using standards prepared in formalin. Material and Methods: Post-mortem measurements can be important to assist in diagnosis of the pathology of the esophagus. In this work, measurements were made of 73 formalin-fixed esophagi obtained from autopsied patients who did not present any evidence of pathologies in this specific organ. The measurements of the esophagus were made after transverse section at the level of the lower border of the cricoid cartilage. Measurements of the organ were standardized as follows: esophageal length, external perimeter in the cardia, lower external perimeter, average external perimeter, and upper external perimeter. Results: The average height of the men in the study was 9.5 cm greater than the average height of the women. Correlation between the height of the individual and the esophagus measurements showed that the greater the height, the greater the length and upper external perimeter of the esophagus. A greater length was associated with a greater upper external perimeter. The cardia was larger than all the perimeters. The data showed that the esophagi were tubular in shape, except at the lower end, where the diameter was larger than in the other regions. Conclusion: These measurements should assist in the identification and elucidation of chagasic megaesophagus, idiopathic megaesophagus, and other esophagus-related disorders.

Keywords: esophagus, formalin-fixed, megaesophagus, esophagus measurements

INTRODUCTION

The esophagus is an organ of the digestive system with elongated tubular morphology. It starts behind the trachea and the heart, in front of the spine, and extends to the stomach through the esophageal hiatus in the diaphragm. In adults, it measures about 23 to 25 centimeters (cm) in length [1, 2]. Anatomical studies evaluating the normal esophagus indicate that the esophageal diameter varies between 1.3 and 3.0 cm, while the length is between 22 and 30 cm [3, 6]. The length is 1-2 cm shorter in women [7].

In addition, the esophageal length usually shows a direct relationship with height and gender [3]. The male esophagus was relatively longer than female with a mean length of 22.7 cm (standard deviation 1.84 cm) compared to a female mean length of 21.6 cm (standard deviation 3.32 cm) [6]. Despite the importance of the description of these anatomical parameters, many studies have not provided details of the measured conditions of the esophagus or the standards on which the measurements were based.

Until now, there has been only one study, undertaken 3 decades ago, in which measurements were made of the external perimeters, at 5 cm from...
the cardia, of 26 pathology-free esophagi fixed in formaldehyde. The diameters at this point of the esophagus ranged from 1.3 to 2.2 cm (1.64 ± 0.21 cm) [8]. Together with functional evaluation, this esophageal measurement is essential in diagnosis of diseases of this organ. Pathological conditions such as chagasic megaesophagus and idiopathic megaesophagus, as well as physiological conditions such as aging, can alter the size, position, and function of the esophagus [9, 10].

These morphofunctional changes are usually confirmed in vivo by complementary examinations, such as contrast radiography and manometry tests. These examinations enable assessment of the degree of dilation and functional alterations such as abnormal or absent peristaltic waves, as well as the time for emptying of the esophageal contents into the stomach [11]. In chagasic megaesophagus, the radiographic results are classified only as small, moderate, or large dilation, according to the classification proposed by Rezende et al., in 1982 [12, 13]. Another classification that is used was proposed by [14] and is based on two parameters, namely the transverse diameter of the esophageal contrast image in anteroposterior incidence and the stasis time. Despite the advances in radiology, cadaver anatomical studies are still important for learning [15] and the use of organic parts treated in formalin is essential for the purposes of visualization and dissection [16].

Morphological measurements by imaging are used in the diagnosis of chagasic megaesophagus and idiopathic megaesophagus, although there is still no consensus in the literature regarding a comprehensive and updated classification for these esophagopathies [17]. Moreover, there are no reliable measures to determine cases of discrete dilatation in megaesophagus close to the normal condition, whether in vivo or port-mortem [8, 9]. Due to the variations and lack of standard post-mortem esophageal measurements, the aim of the present study was to measure formalin-fixed esophagi obtained from autopsied patients who did not present any evidence of pathologies in this specific organ.

MATERIAL AND METHODS

Obtaining the esophagi

The esophagi, fixed in 4% formalin, were obtained from 73 necropsies at the Surgical Pathology Section of the Clinical Hospital at the Federal University of Triângulo Mineiro (HC-UFTM). Based on the necropsy records available in the archive, cases that presented positive serology for Chagas disease, HIV, or any other disease correlated to esophageal effects were excluded. The esophagi were sectioned transversely at the level of the lower border of the cricoid cartilage. Fragments of adipose, muscle, and diaphragm tissues were removed from the outer surface of the organ, which was then opened longitudinally. The external and internal surfaces were examined and cases with esophageal alterations were also excluded. When an esophageal abnormality was found, the organ was discarded from the study.

Esophageal measurements

The esophageal measurements were made using a sewing thread (extra strong, thickness number 0, Corrente® brand). The esophagus and the thread were wetted with water prior to the measurements, in order to improve contact between the materials. The stretched line was laid on the organ area to be measured and the surplus at the ends was cut and discarded. The line segment was then placed on a ruler and the length was measured (in cm). The standardized measurements of the organ were as follows: esophageal length (EL), external perimeter at the cardia (EPC), lower external perimeter (LEP), average external perimeter (AEP), and upper external perimeter (UEP). For all measurements, the organ was positioned on a flat surface, maintaining its natural anatomical shape as much as possible, so that there was no interference in the technique used to make the measurements.

The esophageal length was measured from the upper end to the lower end of the Z line through the longitudinal section. For measurement of the external perimeters, the organ was maintained as a tube and was fixed in its natural position. The sewing thread was placed on the outer surface,
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surrounding the entire periphery of the organ, and was then cut with angled fine-bladed scissors. The EPC was measured in the middle portion between the highest and lowest levels of the Z line. The LEP was measured five centimeters (5 cm) from the lower level of the Z line. The AEP was measured twelve centimeters (12 cm) from the lower level of the Z line. The UEP was measured 18 cm from the lower level of the Z line (Figure 1).

The outer perimeter of the esophagus was considered as the circumference of a circle, so the measurements were transformed into diameters using the formula: \( C = \pi d \), where \( C \) is the circumference of the circle, \( \pi = 3.14 \), and \( d \) is the diameter.

**Statistical analysis**

All the measurement data were analyzed considering the distribution and the variance. The D'Agostino-Pearson test was used to evaluate the normality of the data groups. Analysis of variance, using the F test, was then applied to the data that showed normality.

The nonparametric Mann-Whitney test was applied in cases of non-Gaussian distribution, while the parametric t-test was used in cases of Gaussian distribution. Multiple comparisons were performed using the Kruskal-Wallis test with the Dunn post-test. Pearson’s test was used for Gaussian distribution correlations.

![Figure 1: Illustration of the method described for measurement of the esophagus. (A) esophageal opening longitudinally; (B) esophageal length (EL) measured from the upper end to the lowest level of the Z line; (C) esophagus maintained in position as a tube, naturally in its fixed position. The seam line was superimposed on its outer surface around the periphery of the organ in the lower external perimeter (LEP); (D) The line overlapped to the full extent was measured and cut at the ends with thin-tip angled type scissors; (E) superimposed line on LEP after cut at the ends. (F) Part of the stitching line that is the product of the cut was superimposed on a ruler featuring the centimeters and millimeters of the evaluation (in this photo equivalent to 4.1cm).](image)
For the correlations in the cases of non-Gaussian distribution, the Spearman test was used. The observed differences were considered significant when $p < 0.05$ (5%). Statistical analysis and graphing were performed using GraphPad Prism 6.0 software (GraphPad, La Jolla, CA, USA).

**RESULTS**

A total of 73 esophagi from necropsied individuals were analyzed, 17 of them from women and 56 from men who died in the units of the UFTM clinical hospital. The necropsied individuals were between 18 and 95 years of age. The medians and percentiles of the perimeters (EPC, LEP, AEP, and UEP) were transformed into diameters, as shown in Table 1. The perimeter and esophageal length (EL) values were used in comparative and correlation analyses to determine associations between these measures and parameters including age, gender, and height. The diameters were used for comparison with literature data.

The mean ages of the women and men were 47.5 (±21.5) and 51.11 (±21.11) years, respectively. The difference in mean age between the genders was not significant ($p = 0.556$, t-test). Evaluation of the correlation between age and the esophagus perimeter measurements revealed a positive correlation between age and the EL and UEP measurements. The greater the age, the higher the EL ($p = 0.004$, $r = 0.352$, Spearman test) and UEP ($p = 0.007$, $r = 0.320$, Spearman test).

The mean height of the men in the study was 9.5 cm greater than the mean height of the women, with a significant difference between them ($p = 0.0016$, t-test). Analysis of the correlation between height and the esophagus measurements showed that the greater the height, the greater the EL ($p = 0.019$, $r = 0.307$, Spearman test), AEP ($p = 0.03$, $r = 0.283$, Spearman test), and UEP ($p = 0.005$, $r = 0.357$, Spearman test) (Figure 2). Evaluation of the relation between the esophageal length and the perimeters showed that higher EL was associated with higher UEP ($p = 0.0005$, $p = 0.407$, Spearman test). Since the heights of the men and women were different, comparison was also made between the esophagus measurements for these two groups. It was found that only the AEP and UEP values were higher for the men ($p < 0.05$, Mann-Whitney test). No significant differences were observed for the other measurements (Table 2).

The perimeter measurements (EPC, LEP, AEP, and UEP) were compared to each other, in order to identify possible differences between the esophageal regions (Kruskal-Wallis/Dunn's post-test, $p < 0.05$) (Table 3). These data showed that the perimeter of the cardia was larger than the perimeters of all the other regions, while the perimeter of the upper end, on the other hand, was smaller than the perimeters of the other regions.

Considering the differences between the external perimeters, correlation tests were used to evaluate the morphological dimensions of the esophagus. Based on the results of the Spearman test, most of the measurements were correlated with each other, with the exception of EPC and UEP (Table 3).

**DISCUSSION**

Despite technologies being available, there is a lack of epidemiological data concerning the frequency of diseases affecting the esophagus, such as chagasic megaesophagus and idiopathic megaesophagus, which can cause changes in the shape and/or size of this organ. This can be explained by the difficulty in detecting these diseases in their early stages, or even postmortem. Depending on the case, it is also possible that esophageal alterations may become stabilized in the early stages of the disease, making it difficult to classify or diagnose the changes, even in necropsies [11,18].

In order to improve evaluation of diseases that affect the esophagus, such as chagasic megaesophagus and idiopathic megaesophagus, it is essential to perform detailed anatomical measurements, in order to obtain reliable data characterizing normal organs within the population.
Table 1. Diameter of external esophageal perimeters (P).

<table>
<thead>
<tr>
<th></th>
<th>EPC</th>
<th>LEP</th>
<th>AEP</th>
<th>UEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (25-75%)</td>
<td>5.7 (5.0-6.0)</td>
<td>4.4 (4.0-4.7)</td>
<td>4.1 (3.8-4.5)</td>
<td>4.0 (3.6-4.3)</td>
</tr>
<tr>
<td>Diameter</td>
<td>1.8 (1.6-1.9)</td>
<td>1.4 (1.3-1.5)</td>
<td>1.0 (1.2-1.4)</td>
<td>1.27 (1.1-1.4)</td>
</tr>
</tbody>
</table>

The median and percentile values (25-75%) of the table are expressed in centimeters (cm). The respective perimeters were transformed into diameters by means of the calculation $P = \pi D$ and posteriorly expressed in cm and their respective percentiles (25-75%).

Table 2. Comparison of esophageal measurements from men and women in the study.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Women</th>
<th>Men</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL</td>
<td>21.4 (20.0-22.8)</td>
<td>22.2 (21.0-25.0)</td>
<td>0.135</td>
</tr>
<tr>
<td>EPC</td>
<td>5.5 (4.7-5.9)</td>
<td>5.8 (5.1-6.1)</td>
<td>0.217</td>
</tr>
<tr>
<td>LEP</td>
<td>4.2 (3.8-4.5)</td>
<td>4.5 (4.0-4.9)</td>
<td>0.078</td>
</tr>
<tr>
<td>AEC</td>
<td>3.9 (3.6-4.3)</td>
<td>4.2 (3.8-4.5)</td>
<td>0.033*</td>
</tr>
<tr>
<td>UEP</td>
<td>3.7 (3.5-4.0)</td>
<td>4.0 (3.7-4.3)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

The median values (25-75%) in the table are expressed in centimeters (cm). The * are representative of the significant values (Mann-Whitney test, or parametric T test, $p<0.05$). Esophageal length (EL); external perimeter in the cardia (EPC); lower external perimeter (LEP); mean external perimeter (AEP); upper external perimeter (UEP).

Table 3. Comparison between esophageal perimeters/diameters and their correlations.

<table>
<thead>
<tr>
<th></th>
<th>Median (25-75%)</th>
<th>Diameter</th>
<th>Correlation (r/p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPC X LEP</td>
<td>5.7 (5.0-6.0) X 4.4 (4.0-4.7)*</td>
<td>1.8 X 1.4*</td>
<td>$r=+0.452/p&lt;0.0001*$</td>
</tr>
<tr>
<td>EPC X AEP</td>
<td>5.7 (5.0-6.0) X 4.1 (3.8-4.5)*</td>
<td>1.8 X 1.3*</td>
<td>$r=+0.299/p=0.013*$</td>
</tr>
<tr>
<td>EPC X UEP</td>
<td>5.7 (5.0-6.0) X 4.0 (3.6-4.3)*</td>
<td>1.8 X 1.2*</td>
<td>$r=+0.192/p=0.109$</td>
</tr>
<tr>
<td>LEP X UEP</td>
<td>4.4 (4.0-4.7) X 4.0 (3.6-4.3)*</td>
<td>1.4 X 1.2*</td>
<td>$r=+0.419/p=0.0002*$</td>
</tr>
<tr>
<td>LEP X AEP</td>
<td>4.4 (4.0-4.7) X 4.1 (3.8-4.5)</td>
<td>1.4 X 1.3</td>
<td>$r=+0.429/p=0.0002*$</td>
</tr>
<tr>
<td>AEP X UEP</td>
<td>4.1 (3.8-4.5) X 4.0 (3.6-4.3)</td>
<td>1.3 X 1.2</td>
<td>$r=+0.553/p&lt;0.0001*$</td>
</tr>
</tbody>
</table>

The median and percentile values (25-75%) of the table are expressed in centimeters (cm). The * are representative of significant values (Kruskal-Wallis/Dunn’s post-test, or Spearman test, $p<0.05$). All correlations for the Spearman test were positive (+). Esophageal length (EL); external perimeter in the cardia (EPC); lower external perimeter (LEP); mean external perimeter (AEP); upper external perimeter (UEP).

Figure 2: Correlation between the height (cm) of the esophagus of necropsied individuals and (A) EL, (B) EPC, (C) LEP, (D) AEP, (E) UEP. The black dots represent each individual sample (Spearman test). Significant values considered $p < 0.05$. 
Despite advances in radiology and alternative methods for anatomical studies, cadaver studies are still important for learning [15]. Therefore, in this study, simple and direct measurements were made of the length and circumferential perimeters of macroscopically normal esophagi. For practical purposes, the perimeter measurements were transformed to diameters, enabling comparison with data reported in the literature. In the work by Adad et al. (1991) [8], a similar method was used to determine the perimeter and diameter at a specific point of normal and chagasic megaesophagus esophagi. For the normal esophagi, the diameters measured at 5 cm from the cardia ranged from 1.3 to 2.2 cm (1.6 ± 0.2 cm), which were close to the values obtained in the present study, which ranged from 1.0 to 2.1 cm (1.4 ± 0.2 cm) (data not shown).

Chagasic megaesophagus can occur in any age group, but the diagnosis is more frequent for individuals between 20 and 40 years of age, predominantly in men [19]. Other recent work showed a predominance of cases in 55-year-old male patients [20]. Cases of idiopathic megaesophagus are generally identified in individuals between 30 and 60 years of age [21]. The esophagi used in this study were mostly from men in the fifth decade of life, so the sample was representative, in terms of gender and age, of individuals usually diagnosed with these two diseases.

Another important consideration concerning age and esophagus measurements is related to esophageal dysfunction associated with aging. One such dysfunction is the lack of contraction of the organ, leading to esophageal reflux, which may be the cause or consequence of morphological changes that become aggravated with age [22]. Although controversial [23], it has been reported that most of these age-related dysfunctions can be directly attributed to neuron reduction, even in normal esophagi [24]. For the esophagi analyzed in this study, age was positively correlated with EL and UEP. Increases of EL and UEP may occur due to the impairment of esophageal transit [25] and chagasic megaesophagus [26]. However, the method adopted in the present study was only descriptive and did not allow correlations to be made with data reported elsewhere. In order to confirm such associations, it would be necessary to undertake a morphological study (as performed here), together with the counting of neurons in esophagi presenting dysfunctions.

As expected, the mean height of the males was greater than that of the females. The height of the individuals was correlated with EL, so the median length of the esophagus was greater for men than for women, although the difference was not statistically significant. There is no description available of the craniocaudal development of the esophagus, but it can be assumed, based on the current literature, that the vertical growth of the esophagus should accompany thoracic extension and, consequently, the posterior mediastinal length [27-29]. For this reason, taller people may have longer esophagi. However, it is important that further studies should be undertaken to determine whether the esophageal length is also proportional to the thoracic length, or only to the height of the individual.

Another finding in relation to height and esophageal length was a positive correlation between EL and UEP. In addition, UEP was greater in the male group. These data suggest that the perimeter of the upper esophageal region may be proportional to the height of the individual. However, the technique employed in this study measured fixed points, so the UEP measurement (always measured at 18 cm from the cardia) would be in different regions of the upper part of the esophagus, depending on the esophageal length. Therefore, additional studies are required for detailed investigation of the development of the posterior mediastinum and esophagus, together with their correlations with gender, height, and age.

Comparison of the esophageal measurements for men and women showed that the men presented greater UEP and AEP values. Although the men were mostly taller, compared to the women, there was no proportional relation between AEP and the esophageal length or the height of the individual. This showed that a taller individual would not necessarily have an esophagus
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with higher AEP, LEP, or EPC values, compared to a shorter individual.

Consideration of the esophageal shape and the differences between the measurements evaluated in this study provided important information about the esophageal form. For example, determination of the external perimeters (EPC, LEP, AEP, and UEP) revealed, for the first time, that the esophagus is not a perfect tubular organ. The AEP did not present significant variation, relative to the UEP and LEP. However, the esophageal UEP was smaller, compared to the other regions, while the median EPC value was 1 cm greater than the values for the other perimeters.

It is known that the esophagus parameters measured in this study, including the AEP and EPC, may be influenced by thoracic organs or muscles such as the diaphragm. The diaphragm may also contribute to changes in the lower esophageal regions, since the esophagus passes through the esophageal hiatus, which could influence LEP and EPC [30, 31]. Further investigation of such effects was not possible in the present study, since this would require consideration of the entire thorax, a greater number of organs, and exclusion of the height, gender, and age variables.

CONCLUSIONS

In this study, analysis was made of external perimeter measurements obtained for different regions of esophagi fixed in formalin, in order to obtain a deeper understanding of the structure and shape of the esophagus, an organ of the digestive tract important for food intake, among other functions.

The results showed correlation between the height of the individual and the length of the normal esophagus. It was also found that the esophagus is tubular in shape, except at the lower end, where its diameter is greater than in the other regions.

These measurements contribute to elucidation of the properties of the esophagus and should be useful in future evaluations of chagasic megaesophagus, idiopathic megaesophagus, and other esophageal disorders.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

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RESUMO

Morfologia do esôfago usando amostras fixadas em formol

Introdução: O esôfago é um tubo muscular que liga a faringe ao estômago, que transporta comida e líquido da boca para o estômago. Estudos anteriores não forneceram descrições detalhadas das condições do esôfago, medidas usando padrões preparados em formalina. Material e Métodos: As medições post-mortem podem ser importantes para auxiliar no diagnóstico da patologia do esôfago. Neste trabalho, foram realizadas medidas de 73 esofagias fixadas em formol obtidas de pacientes autopsiados que não apresentaram evidências de patologias neste órgão específico. As medidas do esôfago foram feitas após a secção transversal ao nível da borda inferior da cartilagem cricóide. As medidas do órgão foram padronizadas da seguinte forma: comprimento esofágico, perímetro externo na cárdia, perímetro externo inferior, perímetro externo médio e perímetro externo superior. Resultados: A altura média dos homens no estudo foi 9,5 cm maior que a altura média das mulheres. A correlação entre a altura do indivíduo e as medidas do esôfago mostrou que quanto maior a estatura, maior o comprimento e o perímetro externo superior do esôfago. Um comprimento maior foi associado a um maior perímetro externo superior. A cárdia era maior que todos os perímetros. Os dados mostraram que os esôfagos eram de forma tubular, exceto na extremidade inferior, onde o diâmetro era maior do que nas outras regiões. Conclusão: Essas medidas devem auxiliar na identificação e elucidação do megaesôfago chagásico, do megaesôfago idiopático e de outros distúrbios relacionados ao esôfago.

Palavras-chave: esôfago, esôfago fixado em formalina, megaesôfago, mensurações do esôfago