

Prevalence Study of Esophageal HPV Infection in Patients with Megaesophagus and Correlation with *in situ* and 24-Hour PH Measurement

Estudo da Prevalência do HPV em Esôfago de Portadores de Megaesôfago e a Correlação com o PH *in situ* e de 24 Horas

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ABSTRACT

Introduction: Studies have clearly demonstrated an association between infection with human papillomavirus (HPV) and malignant epithelial lesions. This relationship seems to be well established for squamous cell carcinoma of the uterine cervix, for which high- and medium-risk HPV types are associated with more than 95% of cases. A literature review correlating esophageal carcinoma with the presence of HPV demonstrated an association in 22.9% of cases when *in situ* hybridization was used and in 15.2% of cases when PCR was used for detection of the virus. The incidence of esophageal cancer has been shown to be 100 to 150 times higher among patients with megaesophagus compared to the general population. **Methods:** Fifty patients with megaesophagus ranging in age from 27 to 68 years (mean: 58.0) and 20 patients without esophageal pathology ranging in age from 52 to 70 years (mean: 59.9) were studied prospectively. In the study group, 29 patients were males and 21 were females. *Primers* specific for detection of HPV 16 and 18 used for the amplification of viral DNA by PCR. The measurement of *in situ* pH was performed using a SENTRON digital pH meter, with a scale of 1 to 14. 24-hour pH monitoring was conducted using the DIGITRAPPER MKIII, with the sensor probe positioned 5cm above the lower esophageal sphincter (LES). **Results:** HPV was detected in 19 (63.3%) of the 30 patients with megaesophagus studied, but in only three (16.7%) of the 18 controls, a difference that was statistically significant. HPV subtype 16 was detected in 15 cases. Positivity for HPV was significant in patients with megaesophagus and presented more percentage of time of pH in 24 hours between 5 and 6. **Conclusions:** These results demonstrate that the pH of the esophageal fluid ranges from 4 to 6 in patients with megaesophagus and shows a direct relationship with the presence of HPV. Subtype 16 was the most frequent HPV type.

Key words: 24 hour pH measurement; HPV; esophageal neoplasm.

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INTRODUCTION

Esophageal cancer is among the highest incident cancers in Brazil, with an estimated 10,630 new cases in 2010. This cancer is associated with heavy consumption of alcoholic beverages and tobacco

products and other conditions such as nutritional deficiencies, achalasia, caustic lesions, and HPV infection.¹

Many publications have irrefutably demonstrated an association between human papillomavirus (HPV) infection and malignant epithelial

lesions. For squamous cell carcinoma of the cervix this relationship appears to be well established,² and medium and high risk types of HPV are associated with more than 95% of the cases of cervical carcinoma globally.³

A review of the literature seeking to establish if there is a correlation between the presence of HPV and esophageal carcinoma, found an association in 22.9% of cases when *in situ* hybridization was used and in 15.2% of cases when PCR was used.⁴

There are currently 15 types of oncogenic HPV described as directly associated with genital and extra-genital neoplasias, with subtypes 16 and 18 predominating in 80% of cancers of the cervical canal⁵ and 60 to 70% of cancers of the esophagus.⁶

In the literature there is geographic variation in this association; it is most characteristic of areas where squamous cell carcinoma of the esophagus is most prevalent, principally in South Africa, where the association was present in 46% of cases⁷, and in China, where it occurred in 34.4%⁸ to 65.5%⁹ of cases.

In a study exploring the relationship between the presence of vaginal bacteria and HPV infection in pregnant women, a higher prevalence of vaginal pH exceeding 4.5 was noted in women infected with HPV, suggesting a relationship between an increase in vaginal pH and the presence of HPV.¹⁰

According to Pajecki *et al.*, 2003, patients with Chagasic megaesophagus have a 33-fold greater risk of developing esophageal carcinoma, due to the production of N-nitrous compounds in the lumen of the organ that is mediated by bacteria in suspension in the stasis liquid causing chronic contact of these carcinogens with the esophageal mucosa.¹¹

Several studies have found high rates of prevalence of HPV in squamous cell carcinomas of the esophagus.^{12,13,14} It is known that the likelihood of developing this neoplasm is much greater in the presence of megaesophagus; however, the prevalence of HPV in this esophageal pathology has not been investigated. The objective of the present study is to evaluate the prevalence of **high risk** HPVs in the megaesophagus and their relationship with an increase in cases of neoplasm of the megaesophagus, and also correlate the esophageal pH with the presence of HPV.

METHODS

In a prospective study, 50 patients with megaesophagus followed by the Gastrointestinal

Surgery Service of UFTM and 20 patients without esophageal pathology that constituted a control group were studied.

All patients underwent contrast radiography of the esophagus, stomach and duodenum (Upper GI series) and Upper Gastrointestinal Endoscopy (UGE).

The measurement of *in situ* pH was performed using a SENTRON digital pH meter, with a scale of 1 to 14.

24-hour pH monitoring was conducted using the DIGITRAPPER MKIII, with the sensor probe positioned 5cm above the lower esophageal sphincter (LES).

Biopsy fragments of the esophagus collected were immersed in 1.0 ml of TRIZOL® (Invitrogen™, Life Technologies, Carlsbad, California, USA), according the manufacturer's protocol and stored in a freezer at -70°C for later extraction of RNA. HPV was investigated using **Polymerase Chain Reaction** (PCR). Primers specific for HPV 16 and 18 were used to amplify the viral DNA. The amplified products were subjected to electrophoresis in a 10% polyacrylamide gel and stained with silver.

For the statistical analysis, a non-parametric analysis of variance (ANOVA) using Kruskal-Wallis and Mann-Whitney tests was performed. Results were considered statistically significant when $p < 0.05$.

RESULTS

The prospectively studied cohort included 50 patients with megaesophagus age 27 to 68 (mean age 58.0) and 20 patients without esophageal pathology age 52 to 70 (mean age 59.9). The study group included 29 men and 21 women; in the control group 10 subjects were women. Two patients had to be excluded from this group because they had alterations in their 24 hour pH monitoring. The most relevant clinical findings in the study group were concomitance of Chagasic megacolon in 19 and cholelithiasis in 15 patients, and the presence of cholelithiasis in 8 subjects in the control group. An analysis of the detection of HPV in both of the groups found no significant different with regard to gender, age, and the presence of cholelithiasis. The mean *in situ* esophageal pH was 6.5 in the study group (range 5.3 to 7.3) and 5.8 in the control group (range: 5.0 to 6.5). In relation to 24 hour pH monitoring, in all 50 cases of megaesophagus, it was noted that the percentage of the time the pH > 5 and < 7 (minimum 64% and maximum 99.5%) demonstrated a

acidification of the esophageal fluid, probably stemming from the fermentation of liquid residues in the setting of esophageal stasis with the formation of weak acids. In all of the 18 cases of the control group the pH > 6.3. In analyzing the 70 patients with regard to the pH range, the *in situ* pH value fell within the range measured during 24 hour pH monitoring in only 31 patients (44.3%). 24 pH monitoring is more sensitive and reliable for the analysis of the pH of esophageal liquid.

Of the 50 patients with megaesophagus, 30 patients were analyzed for the presence of HPV, and 19 (63.3%) were found to have HPV. Among the 18 controls analyzed, HPV was detected in three (16.7%). The presence of HPV was significantly greater in the group with megaesophagus than the control group. The presence of HPV was greater in grades III and IV than in grade II, a difference that was statistically significant (Figure 1).

Of the 22 cases where HPV was detected, 15 were HPV subtype 16. Subtype 18 was detected in 4 cases, including two of cases with subtype 16.

The correlation of a pH measurement < 4 and the detection of HPV in the megaesophagus was not statistically significant (Figure 2). However, the detection of HPV in the megaesophagus was significantly greater when the pH of esophageal fluid was in the range of 5 to 6 (Figures 3 and 4).

DISCUSSION

Infection by the Human Papillomavirus (HPV) is directly associated with cervical cancer, carcinoma of the esophagus, anal cancer and cancer of the penis.¹⁵

In a meta-analysis of 15 articles with 980 specimens, the presence of HPV was detected in 46.9% of cases. Subtypes 16 and 18 were the subtypes most commonly associated with carcinoma of the esophagus.¹⁶

In this study, the presence of HPV was significantly greater in patients with megaesophagus (63.3%) than in controls (16.7%). In the literature there are no articles which investigated the presence of HPV in patients with megaesophagus without neoplasia.

Birgisson *et al.*, 1997, studying the cause of achalasia analyzed myotomy muscular fragments (HELLER) in patients with idiopathic megaesophagus and did not detect the presence of HPV, Herpes virus, or measles.¹⁷

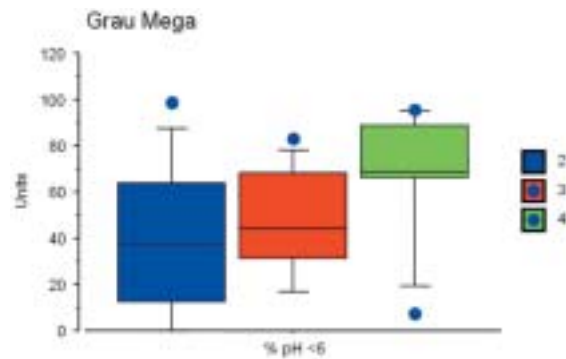


Figure 1 – Relationship between the presence of HPV and the degree of megaesophagus ($p=0.020$).

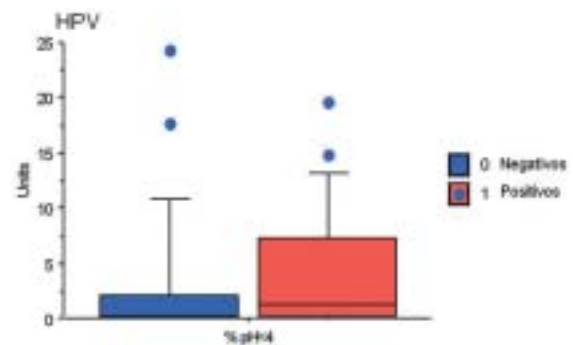


Figure 2 – Relationship between the presence of HPV and period of time with pH < 4 ($p=0.14$).

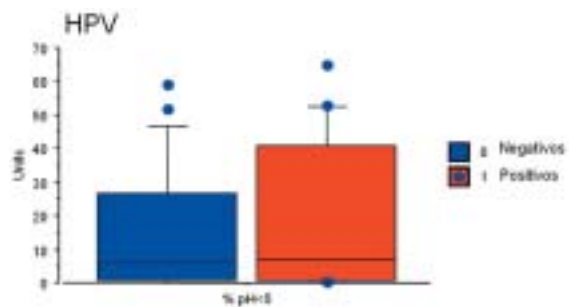


Figure 3 – Relationship between the presence of HPV and the period of time with pH < 5 ($p=0.01$).

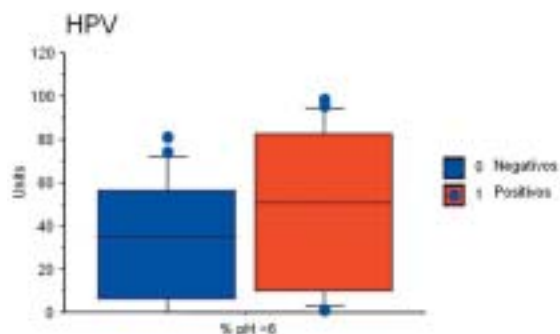


Figure 4 – Relationship between the presence of HPV and the period of time with pH < 6 ($p=0.014$).

In this study, subtype 16 was the most commonly detected, and subtype 18, when found was associated with concomitant presence of subtype 16 present in two cases. These data corroborate the study of Zhao *et al*, 2009 that detected the presence of HPV in 88.1% of samples of esophageal carcinoma analyzed, with 19 specimens (45.2%) positive for HPV 16 and eight (19.9%) samples positive for HPV 18.¹⁸

Patients with megaesophagus have a rate of association of with esophageal cancer that varies from 100 to 150 times that encountered in the general population, probably from the chronic irritation of stasis liquid with weak acids from fermented food debris. Crema *et al*, 2002, detected an increased presence of bacteria in 91.4% of biopsy fragments of the esophagus in patients with megaesophagus.¹⁹

Da Silva *et al*, 2004, observed that the acidification of the vagina was associated with the presence of HPV.¹⁰ Given that patients with

megaesophagus have stasis, proliferation of bacteria with fermentation of food debris, production of weak acids, and consequently acidification of esophageal fluid, it was observed in this study that the *in situ* pH measurement was not as sensitive as 24 hour pH monitoring, which demonstrated acidification of the esophagus in all of the cases, with a pH in the range of 5 to 6 the most common. It was also noted that the presence of HPV can be correlated with a pH of esophageal fluid in the range of 5 to 6.

CONCLUSIONS

Based on these results, it can be inferred that esophageal liquid in patients with megaesophagus has a pH between 4 and 6. Detection of HPV is greater in patients with megaesophagus; the pH range with the highest rates of HPV detection was between 5 and 6. HPV Subtype 16 was the most frequently encountered.

RESUMO

Introdução: Diversas publicações têm demonstrado de maneira irrefutável a associação entre infecção pelo papilomavírus humano (HPV) e lesões epiteliais malignas. No carcinoma cervical uterino de células escamosas essa relação parece estar bem estabelecida, e os tipos de HPV de alto e médio risco estão associados a mais de 95% dos casos. Em estudo de revisão da literatura, procurou-se correlacionar o carcinoma esofágico e a presença do HPV, detectando uma associação de 15.2 a 22.9% na dependência da técnica utilizada. Nota-se que a incidência de neoplasia de esôfago associada ao megaesôfago é de 100 a 150 vezes maior que na população geral. **Metodologia:** Foram estudados prospectivamente 50 portadores de megaesôfago com idades variando de 27 a 68 anos (média de 58,03) e 20 pacientes sem esofagopatia com idades variando de 52 a 70 anos (média de 59,87 anos). No grupo de estudo, 29 pacientes eram do sexo masculino e 21 do feminino. Para amplificação do DNA viral pelo método de PCR utilizou-se *primers* específicos para detecção dos HPVs 16 e 18. A Medida do pH *in situ* foi realizada utilizando-se o pH meter digital SENTRON, com escala de 1 a 14. A pHmetria de 24h foi realizada com auxílio do DIGITRAPPER MKIII, sendo o sensor da sonda posicionado 5cm acima da região do esfíncter inferior do esôfago. **Resultados:** Dos 30 pacientes portadores de megaesôfago, detectou-se positividade para o HPV em 19 pacientes (63,34%) e dentre os 18 controles somente 3 (16,67%) foram positivos. Notou-se que em 15 casos, o subtipo prevalente foi o HPV16. A positividade do HPV foi significativa nos portadores de megaesôfago e apresentaram maior percentual de tempo de pH nas 24 horas entre 5 e 6. **Conclusões:** Notou-se que o líquido esofágico nos portadores de megaesôfago apresentou pH entre 4 e 6 e com maior positividade do HPV, sendo o subtipo 16 o mais encontrado.

Palavras-chaves: pHmetria de 24 horas; HPV; neoplasia de esôfago.

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