

ORIGINAL PAPER

HISTOLOGICAL FEATURES OF OESOPHAGUS MUCOUS MEMBRANE CHANGES IN PATIENTS WITH GASTROESOPHAGEAL REFLUX DISEASE AND TYPE 2 DIABETES MELLITUS

Iryna I. KARPENKO¹✉, Elina Yu. FROLOVA-ROMANIUK¹, Natalia M. ZHELEZNIKOVA¹

¹Department of Internal Medicine 1, Kharkiv National Medical University, Kharkiv, Ukraine

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ABSTRACT

Introduction. The high medical and social significance of type 2 diabetes mellitus (T2DM) is not only due to its prevalence, but also to serious complications that lead to a decrease in the quality of patients' life, early disability, and high mortality.

The objective of the study was to evaluate the histological and morphometric features of the oesophagus mucous membrane in patients with gastroesophageal reflux disease (GERD) and T2DM compared to patients with GERD without T2DM.

Material and methods. 67 patients with GERD and T2DM and 50 patients with GERD without T2DM, matched for age and sex, were examined. Comparative morphometry of histological changes of the oesophageal mucosa was performed.

Results. The histological investigation showed that in patients of the main study group (with GERD and T2DM), almost all the studied parameters had a significantly more severe course and exceeded similar indicators of the group with isolated GERD: the thickness of the basal layer (1.86 ± 0.04 points against 0.97 ± 0.02 points), papillae height (1.71 ± 0.06 points

RÉSUMÉ

Caractéristiques histologiques des changements de la membrane muqueuse de l'œsophage chez les patients avec de la maladie de reflux gastro-œsophagien en association avec le diabète sucré de type 2

Introduction. L'importance médicale et sociale accrue du diabète sucré de type 2 (DT2) n'est pas seulement due à sa prévalence, mais également à des complications graves qui conduisent à une diminution de la qualité de vie du patient, à une invalidité précoce et, par conséquent, à la cause de mortalité élevée.

L'objectif de l'étude a été d'évaluer les caractéristiques histologiques et morphométriques de la membrane muqueuse de l'œsophage chez les patients atteints de reflux gastro-œsophagien (RGO) en association avec DT2 et chez les patients atteints de RGO sans diabète sucré.

Matériel et méthodes. 67 patients atteints de RGO en association avec le DT2 et 50 patients avec RGO appariés par l'âge et le sexe ont été examinés. Une morphométrie comparative des changements histologiques de la muqueuse œsophagienne a été réalisée.

✉ Address for correspondence:

Iryna I. KARPENKO
Kharkiv National Medical University, Department of Internal Medicine 1,
Kharkiv, Ukraine
Address: Nauky Avenue no.4, Kharkiv, 61022, Ukraine
Emails: dep.inter.med1@gmail.com; zirina103@gmail.com

versus 0.72 ± 0.07 points), inflammatory epithelial infiltration (1.88 ± 0.03 points against 1.27 ± 0.07 points), width of intercellular spaces (2.04 ± 0.08 against 1.10 ± 0.07 points) ($p < 0.01$ in all cases).

Conclusions. The inflammatory process in the oesophageal mucosae in patients with GERD without T2DM has a more superficial character and is limited mainly to the epithelial layer, whereas hyperplastic changes in the mucous membrane of the oesophagus are more pronounced in patients with GERD and T2DM.

Keywords: gastroesophageal reflux disease, type 2 diabetes mellitus, oesophageal biopsy histological study.

Abbreviations

GERD – gastroesophageal reflux disease

T2DM – type 2 diabetes mellitus

MM – mucous membrane

HbA1c – glyated hemoglobin

INTRODUCTION

The high medical and social significance of type 2 diabetes mellitus (T2DM) is not only due to its prevalence, but also to serious complications that lead to a decrease in the quality of patient's life, early disability, and high mortality. Over the long course of T2DM, 10% of patients develop visual disturbances, 60% of patients are diagnosed with coronary artery disease, 40% of patients with nephropathy, and symptoms of gastrointestinal diseases are noted in 50-60% of patients¹. The real prevalence of gastrointestinal diseases in patients with T2DM is much higher, since diabetic gastropathies are often asymptomatic^{2,3}. With T2DM treatment for more than five years, the frequency of upper gastrointestinal tract diseases ranges from 60% to 80%, according to certain studies^{4,5}. Recently, there has been increased interest in this problem, but the results of different studies are contradictory and many questions remain without answer. Very often, diabetes is associated with gastroesophageal reflux disease (GERD)⁶⁻⁸. The development of neuropathy, along with metabolic and vascular disorders, play an important role in the appearance of this pathology⁹⁻¹¹. The inadequacy of anti-reflux mechanisms in diabetes can lead to both the development of GERD and the aggravation of the course of this disease. In turn, GERD's feature is the development of interconnected disorders of other organs and systems, which complicates the clinical

Résultats. L'enquête histologique montre que chez les patients du groupe principal (patient avec RGO et DT2) presque tous les paramètres étudiés avaient une évolution significativement plus sévère et dépassaient les indicateurs similaires du groupe avec RGO isolé: l'épaisseur de la couche basale ($1,86 \pm 0,04$ points contre $0,97 \pm 0,02$ points), la hauteur des papilles ($1,71 \pm 0,06$ points contre $0,72 \pm 0,07$ points), l'infiltration épithéliale inflammatoire ($1,88 \pm 0,03$ points contre $1,27 \pm 0,07$ points), la largeur des espaces intercellulaires ($2,04 \pm 0,08$ contre $1,10 \pm 0,07$ points) ($p < 0,01$ dans tous les cas).

Conclusions. Le processus inflammatoire dans les muqueuses œsophagiennes chez les patients atteints de RGO a un caractère plus superficiel et se limite principalement à la couche épithéliale, alors que les changements hyperplasiques de la muqueuse de l'œsophage sont plus prononcés chez les patients atteints de RGO avec T2DM.

Mots-clés: reflux gastro-œsophagien, diabète sucré de type 2, l'analyse histologique des biopsies œsophagiennes.

findings, as well as the diagnosis and treatment of both underlying pathologies.

Mutual diseases burden indicates the need to develop effective diagnostic methods and therapeutic interventions, considering concomitant pathology^{12,13}. However, there is little data on the pathogenesis and clinical findings when GERD is associated with T2DM.

Histological and morphometric examination of the oesophagus biopsy samples is a necessary condition for an individual approach in patients with GERD, which gives objective diagnostic criteria and complements the clinical picture¹⁴⁻¹⁷. Determination of the morpho-functional state of oesophageal mucous membrane (MM) in these diseases remains an important problem.

THE OBJECTIVE OF THE STUDY was to evaluate the histological and morphometric features of the oesophageal MM in patients with GERD and T2DM compared to patients with GERD without T2DM.

MATERIALS AND METHODS

The study was conducted at the Department of Internal Medicine no. 1 of Kharkiv National Medical University, Ukraine, between January 1st, 2015 and December 1st, 2018. The study was approved by the Ethics and Bioethics Committee of Kharkiv National Medical University. All the procedures and

experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

The inclusion criteria in the study were: patient's age between 35 – 55 years; verified diagnosis of GERD, erosive or non-erosive, with oesophageal and/ or non-oesophageal manifestations; for patients of the main group – presence of verified (documented) diagnosis of T2DM, for patients of the control group – absence of any carbohydrate metabolism disorders.

The exclusion criteria: type 1 diabetes mellitus; T2DM requiring insulin therapy; severe diabetes mellitus and complete decompensation ($HbA1c > 7.5\%$ in combination with fasting glucose > 7.5 mmol/L and postprandial glycemia > 10.0 mmol/L); Barrett's oesophagus; oesophageal adenocarcinoma and other malignant or benign gastrointestinal tumours; achalasia and oesophageal strictures; active peptic ulcers of the gastroduodenal zone, Zollinger-Ellison syndrome; absolute and relative contraindications to endoscopy; significant decrease of liver or/and kidney function; coronary artery disease; increased sensitivity to proton pump inhibitors; alcohol abuse, drug addiction; pregnancy and lactation in women; patient's refusal to participate in the study.

A study of 117 patients with GERD (72 women, 45 men) was performed. The mean age of the patients was 57.4 ± 7.6 years. The main group consisted of 67 patients with GERD and T2DM. 50 patients with GERD represented the control group. Written informed consent was obtained from all the participants.

GERD was diagnosed based on the complaints, the results of daily pH measuring using Gastroscan-24 and an endoscopic examination of the oesophagus with biopsy. The diagnosis of diabetes was done on the ground of anamnestic data, glycaemia levels, and studies of glycated hemoglobin (HbA1c).

The material for histological examination was obtained from the mucous membrane of the distal oesophagus, 3 cm above the conditional circular line connecting the stomach and oesophagus. In cases when erosion and ulcers were present, the material was additionally taken from the edge of the defect. Biopsies were carried out using a 10% formalin solution and hematoxylin-eosin staining. Samples were studied on a Micros microscope (Austria). In order to obtain a tissue photograph, a CAM 2800 digital video camera was used under light microscopy (x40 lens, x10 eyepiece). Ten images with clear boundaries were selected for the study. Morphometric determination of the epithelium thickness and height of the

papillae were obtained using the BioVision computer morphometric program.

A histological assessment of the activity of the inflammatory process in the oesophagus was carried out using a scale for assessing morphological changes in the oesophagus. The degree of basal cell hyperplasia and length of the papillae were evaluated on a scale, where 0 stands for no pathological changes, 1 – moderate changes, 2 – significant changes. The absence of basal cell hyperplasia was noted if the percentage of basal cell thickness did not exceed 15% of the total epithelium thickness. Moderate and significant basal cell hyperplasia was diagnosed when this indicator was exceeded by 16% and 66%, respectively. The length of the papillae was considered unchanged if it did not exceed 50% of the total epithelium thickness. In addition, an assessment of inflammatory epithelial infiltration was carried out (0 stands for lymphocytes only, 1 – slight infiltration with eosinophils and neutrophils, 2 – severe infiltration). When assessing the width of intercellular spaces, the following scale was used: 0 stands for no expansion, 1 – focal expansion, 2 – distinct sharply expanded intercellular spaces, 3 – diffuse expansion of intercellular spaces. Statistical processing was performed using „SPSS 13“ computer program, results were considered reliable if $p < 0.05$.

RESULTS

Patients with GERD and T2DM showed a high frequency of extra-oesophageal manifestations, namely in 2/3 of cases atypical manifestations of GERD were recorded, among which cardiac complaints and various ENT pathologies predominate. GERD typical manifestations (heartburn, regurgitation, acid burping) in patients with combined pathology were observed only in 30% of the cases. At the endoscopic examination of this group, the endoscopic signs of GERD are significantly more likely to occur: reflux esophagitis A in 25% of cases, B – 40.6% of cases and C – 6.25% of cases.

During the histological study of oesophageal biopsy materials, both in case of combined pathology and in isolated GERD, pronounced protein dystrophy of the epithelium and manifestations of parakeratosis are observed in most cases, there are signs of acanthosis, with severity depending on the chronicity of the process. In most biopsy materials, the perivascular connective tissue was fibrous, with signs of oedema and fibre homogenization. In the subepithelial space, oedema and vascular congestion were observed, as well as signs of fibrosis and sclerosis. In most cases, in the oedematous subepithelial space there was a lympho-histiocytic and leukocyte infiltration of different degree, with the transition of inflammatory

infiltrate to stratified squamous epithelium. In cases of erosive esophagitis, leukocyte infiltration of oesophageal MM reached its maximum, up to the formation of micro abscesses. Infiltrates were located both in the area of the papillae and in the deeper layers under the epithelium, provoking the stratification of collagen fibres and muscle cells. The cellular composition of the infiltrate was polymorphic, with a predominance of monocytes/macrophages, plasma cells, lymphocytes and fibroblasts. The epithelium basal membrane was unevenly thickened, homogeneous. The stratified squamous epithelium showed signs of impaired stratification of the layers and oedema. In most cases, basal hyperplasia was noted. The basal layer epithelial cells had enlarged, rounded, hyperchromic nuclei. Chromatin was placed in small lumps or a uniform mesh. In some areas, epithelial cells with wrinkled hyperchromic nucleus, which resembles a mulberry, were determined; this indicates the presence of apoptosis. The spinous layer was with shape polymorphism and different size of cells. Some of them are sharply increased in size, with signs of hydropic (ballooning) degeneration. The intercellular oedema of the spinous and basal layers of the epithelium manifested by the expansion of the intercellular space. In this case, the longitudinal axial orientation of the superficial cells was lost.

Significant differences between the groups ($p < 0.05$) were identified, with respect to the frequency of the following signs: epithelium swelling and stratification, epithelial layer hyperplasia and neutrophilic infiltration degree.

The morphometric study revealed a significant increase in epithelial layer thickness in patients with GERD and diabetes compared with isolated GERD ($p < 0.05$) (Table 1). Moreover, the differences identified are primarily due to hyperplasia of epithelium superficial layer. An increase in epithelial layer thickness may reflect an increase in the proliferation of its cells.

Connective tissue papillae length in some cases could reach 68% of the epithelial layer, and its growth is more likely to be determined by the release of pro-inflammatory mediators that stimulate the proliferation of fibroblasts, endothelium and smooth

muscle cells. No significant differences of this indicator in the studied groups were revealed.

The results of our study show that in patients with GERD and T2DM, T2DM is an important factor that influences not only the symptoms, but also the endoscopic and histological signs of GERD as well.

The comparative morphometry of histological changes of the oesophageal mucosa indicates that in patients with GERD and T2DM almost all the parameters studied had a significantly more severe course and exceeded similar indicators of patients with only GERD: thickness of the basal layer (1.86 ± 0.04 points against 0.97 ± 0.02 points), papillae height (1.71 ± 0.06 points versus 0.72 ± 0.07 points), inflammatory epithelial infiltration (1.88 ± 0.03 points against 1.27 ± 0.07 points), width of intercellular spaces (2.04 ± 0.08 against 1.10 ± 0.07 points) ($p < 0.01$ in all cases).

Patients with GERD and T2DM showed a positive correlation between postprandial glucose and intensity of basal hyperplasia ($\rho = 0.242$, $p < 0.05$), as well as between the width of intercellular spaces and BMI ($\rho = 0.305$, $p < 0.05$). A negative correlation between total epithelial thickness and fasting glucose was also recorded ($\rho = -0.291$, $p < 0.05$). In patients with GERD alone, none of the morphometry indices were dependent on external factors such as age, body mass index, and duration of the disease.

DISCUSSION

According to the global data, considerable progress has been achieved in the last years in the study of GERD pathogenesis, as well as optimization of its diagnoses and treatment. Differentiated treatment regimens for exacerbation of the disease and subsequent supportive therapy have been developed. Therefore, nowadays, the issues related to the course of GERD in combination with other chronic diseases and treatment of refractory, atypical forms of, are very important.

However, there are almost no papers devoted to the study of tissue resistance in the oesophagus, disorders of functional and structural integrity, and blood supply to the mucous oesophagus in patients with GERD and T2DM comorbidities.

Table 1. Morphometric assessment of distal oesophageal MM in patients with GERD and T2DM.

Groups	Morphometric indicators, μm					
	Epithelium total thickness		Epithelium basal layer thickness		Connective tissue papillae height	
	M \pm m		M \pm m	%	M \pm m	%
Only GERD	291.2 \pm 9.1		46.5 \pm 0.8	15.9	109.4 \pm 2.9	37.0
GERD + T2DM	349.3 \pm 13.5*		52.5 \pm 2.9	15.0	129.5 \pm 7.4	37.1

Note: * - significance of differences between groups ($p < 0.05$)

Comparing data on the prevalence of T2DM and GERD, the possibility of combining these nosologies becomes apparent, especially among middle-aged and elderly patients. Moreover, given that in diabetes there is an inability of antireflux mechanisms, then the presence of this pathology can both lead to the development of GERD and complicate its course^{5,7,9}.

Recent studies have shown that the symptoms of upper gastrointestinal tract disorders in the presence of diabetes are up to two times more frequent than in patients without it⁷⁻¹⁰.

There is no sufficient data in the modern literature regarding the prevalence of GERD and features of the clinical course when associated with T2DM. Some studies on the comorbidity of GERD and T2DM have examined the overall population of diabetic patients without identifying its type^{1,2,6}. The exact prevalence of GERD in patients with T2DM is unknown.

The incidence of GERD symptoms in T2DM is greater than in the general population^{3,11}. Thus, in a study by Wang et al, involving 150 patients with T2DM, the symptoms of GERD were observed in 40.7% of patients³. Japanese scientists Hirata et al demonstrated a prevalence of GERD symptoms in patients with T2DM of 23%¹¹. A similar analysis by Korean researchers showed the prevalence of reflux esophagitis in T2DM of 18.4%¹⁸. In 2014, Sun et al studied 775 T2DM patients in Shanghai and found out that the overall prevalence of typical GERD symptoms is 16%¹⁹.

Regarding the clinical manifestations of GERD in patients with T2DM, there is a tendency for an asymptomatic or atypical course^{20,22}. The prevalence of asymptomatic forms can be observed in a third of patients, frequency of acid symptoms, such as heartburn, acid regurgitation, dysphagia decreases, while incidence of dyspeptic symptoms such as nausea and hypersalivation increases²¹⁻²⁵.

The features of the GERD course in patients with T2DM can be explained in terms of pathogenesis mechanisms, e.g. chronic hyperglycemia leads to dysmotility and autonomic neuropathy disorders, which in turn lead to impaired visceral sensitivity²⁶⁻²⁸.

In an animal experiment, with the aim to study the esophagus morphological and biomechanical properties, some significant pathological changes have been revealed²⁹. The incidence of oesophageal dysmotility in patients with T2DM reached 63%, it was independent of the type of diabetes, sex, and had a strong correlation with retinopathy and T2DM persistency⁹. Despite the high incidence of motility disorders, classic GERD symptoms, such as heartburn and dysphagia, are present in a small number of patients^{9,15}.

With regard to histological features, there is evidence of a predominance of oesophagus dysplastic

changes in patients with GERD and T2DM⁵. There is no information available on the analysis concerning the morphological and morphometric indices of the oesophageal mucosa at these nosologies.

CONCLUSIONS

The inflammatory process in the oesophageal mucosae in patients with GERD without T2DM has a more superficial character and is limited mainly to the epithelial layer, whereas hyperplastic changes in the mucous membrane of the oesophagus are more pronounced in patients with GERD and T2DM. The revealed morphological and morphometric data must be considered in patients with combined pathology of GERD and T2DM when prescribing treatment, which should include not only PPI, but, possibly, drugs that affect the reparative properties of the oesophagus mucous membrane.

Author contributions:

Conceptualization, I.I.K., E.Yu.F. and N.M.Z.; methodology, E.Yu.F.; software, I.I.K. and N.M.Z.; validation, I.I.K. and E.Yu.F.; formal analysis, E.Yu.F. and N.M.Z.; investigation, I.I.K., E.Yu.F. and N.M.Z.; resources, I.I.K., E.Yu.F. and N.M.Z.; data curation, I.I.K. and E.Yu.F.; writing – original draft preparation, E.Yu.F. and N.M.Z.; writing – review and editing, I.I.K., E.Yu.F. and N.M.Z.; visualization, E.Yu.F. and N.M.Z.; supervision, I.I.K.; project administration, N.M.Z.. All the authors read and agreed with the final version of the article.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law.“

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