

Histomorphometric analysis with a proposed tissue lesion index in ischemia-reperfusion induced gastric mucosa damage

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Summary. Damage to the gastrointestinal mucosa caused by ischemia - reperfusion is a significant clinical problem associated with various physiopathological conditions. Our group has conducted various studies in patients in critical conditions and in animal models to identify early damage to the gastric mucosa under ischemia using impedance spectroscopy. It is important to perform a quantitative histopathological analysis which can be linked to changes in impedance of the gastric mucosa under conditions of ischemia and I/R.

Aim. To propose a tissue lesion index which considers pathological alterations inherent to the inflammatory process and cell damage which may be directly related to changes in impedance under conditions of ischemia and I/R.

Methods. The animals were randomly distributed into 4 groups: control, ischemia (30 min), and I/R (30 and 60 min). Qualitative histopathological analysis was performed; the vascular area, glandular lumen area, the number of damaged cells, and the depth of the erosion were also quantified to obtain a scale to propose a tissue lesion index (TLI).

Results. Under ischemic conditions, histopathological analysis showed edema and necrosis in epithelial cells, and vascular congestion. In I/R (30 and 60 min) conditions, areas of epithelial erosion were generated.

Damage was classified based on the TLI. A TLI threshold of 3 showed a predictive value of tissue lesion.

Conclusion. The proposed gastric lesion index allows us to objectively quantify and classify damage to the gastric mucosa produced by I/R.

Key words: Quantification, Index, Ischemia, Reperfusion, Gastric

Introduction

Damage to the gastrointestinal mucosa caused by ischemia - reperfusion (I/R) is a significant clinical problem associated with various physiopathological conditions, such as trauma, major surgery, anti-inflammatory drugs, hypovolemic shock, ischemic disease, or bleeding due to peptic ulcer (Brzozowski et al., 2000; Du et al., 2015). Transitory ischemic events in the gastric mucosa often occur in patients in critical condition with multiorgan dysfunction syndrome, with a 44 to 76% rate of mortality in such patients (Osterbur et al., 2014).

The pathogenesis of the lesion to the gastric mucosa caused by I/R is related to a series of events which involve the production of reactive forms of oxygen and nitrogen, and increased production of proinflammatory cytokines involved in the activation and migration of neutrophils toward the affected tissue (Konturek et al., 2000; Kobata et al., 2007). Under ischemic conditions, lethal cellular lesions are produced, characterized by cytoplasmic eosinophilia and nuclear condensation; also, characteristic signs of acute inflammatory process are

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observed, such as the presence of neutrophils, mainly in the lamina propria, and vascular dilation (Beltran et al., 2013). Under conditions of reperfusion, erosion areas in the surface epithelium can be identified, which may extend to the oxyntic portion of the gastric mucosa (Yi et al., 2012).

Our group has conducted various studies in patients in critical condition (Beltran et al., 2006; Beltran and Sacristan, 2015) and in animal models (Beltran et al., 2013) to identify early damage to the gastric mucosa under conditions of ischemia by means of impedance spectroscopy. This technique has been proposed to monitor and detect progression of damage in the gastric mucosa associated with tissue ischemia in patients in critical condition, for which reason it is important to conduct a quantitative analysis which can be linked to changes in impedance of the gastric mucosa under conditions of ischemia and I/R. In a preliminary study, a scale for quantification and a score for damage categorization were proposed arbitrarily (Pena-Mercado et al., 2016).

We identified 3 principal methods to quantify damage to the gastric mucosa induced by I/R. Magierowski et al. (2017) conducted a macroscopic analysis by computerized planimetrics, in which they reported the area of lesions to the gastric mucosa in mm². Another type of macroscopic analysis is that described by Hu et al. (2016), which was reported as an index of damage to the gastric mucosa based on assigning a score depending on the length of the erosion or hemorrhage points. Finally, Gezginci-Oktayoglu et al. (2016) examined microscopic damage to the mucosa using a quantification method which considered histopathological criteria, such as loss of epithelial cells, edema of the mucosa, bleeding, and presence of inflammatory cells, which were assigned a score of 0-3.

Due to the limitations found in the aforementioned methods of quantifying gastric lesions, to link to measurements of gastric impedance we proposed a tissue lesion index which considered pathological alterations inherent to the inflammatory process and cell damage which may be directly related to changes in impedance under conditions of ischemia and I/R. The proposed lesion index may be complementary, due to its quantitative contribution, to the semi-quantitative methods presently used to analyze damage to the gastric mucosa.

Material and methods

Animals

Male Wistar rats with body weight of 350-450 g from the biotherium at Universidad Autonoma Metropolitana - Iztapalapa were used for the experiment. The animals were housed in plastic boxes under light-dark cycles (12:12); they were given water and food *ad libitum* up to the time of surgery. The rats were distributed randomly in 4 experimental groups: control,

ischemia, I/R for 30 min, and I/R for 60 min (n=5 per group). The protocol was approved by the ethics committee at the National Center for Research in Medical Imaging and Instrumentation (CI3M), following Official Mexican Standard NOM-062-ZOO-1999.

Surgical procedure

The rats were anesthetized with 2 mL of an anesthetic cocktail consisting of 0.05 mL ketamine (2.5 mg/kg), 0.25 mL propionyl promazine (0.2 mg/kg), and 0.1 mL xylazine (1.1 mg/kg) given intraperitoneally. The gastric ischemia-reperfusion model was implemented using the method originally proposed by Wada et al. (1996). The abdominal cavity was opened (by sagittal incision), and for the ischemic group the stomach was exposed and the celiac artery was identified, and was clamped for 30 min blocking the blood flow. For the 30 and 60 min reperfusion groups, after ischemia was induced, the clamp was removed restoring the blood flow for 30 and 60 min, respectively. Then, a sagittal gastrotomy was performed at the level of the major curvature, lavage with saline solution was performed and incisional biopsies were taken from the glandular portion for histological analysis. The samples were fixed in formaldehyde 10% and stored at 4°C until processing. Blood samples were taken by cardiac puncture to perform a blood gas analysis (GEM Premier 3000; Instrumentation Laboratory, Lexington, MA) to measure pH and lactate levels.

Tissue lesion index (TLI) and classification of damage

Obtaining photomicrographs for the TLI

For quantitative histopathological analysis of damage to the gastric mucosa caused by I/R, a TLI was proposed which considers alterations inherent to cell damage (edematous, apoptotic, or necrotic cells) and acute inflammatory process (vascular area), as well as the area of the glandular lumen and the depth of erosion areas found in the gastric mucosa.

The biopsies were processed and embedded in paraffin (Paraplast Plus, Oxford Labware, St. Louis, Mo, USA) using the standard histological technique. Nine transverse and 9 longitudinal slices (5 µm) were taken from each of the biopsies, which were stained with hematoxylin and eosin. The observations were made with a clear field photon microscope (Axioscope II, Carl Zeiss Microscopy, Thornwood, NY, USA). The slices corresponding to the transverse plane of the gastric mucosa were observed with × 100 magnification (Fig. 1A1), 3 areas were identified in the field of vision. To promote randomness of measurements, the photomicrograph was divided in 9 quadrants (Fig. 1A2); in the first slice, fields 2, 4, and 9 were photographed; in the second, fields 1, 6, and 7 were taken; in the third slice, fields 3, 5, and 8 (magnification: ×400). For the remaining slices the same order was followed. Finally,

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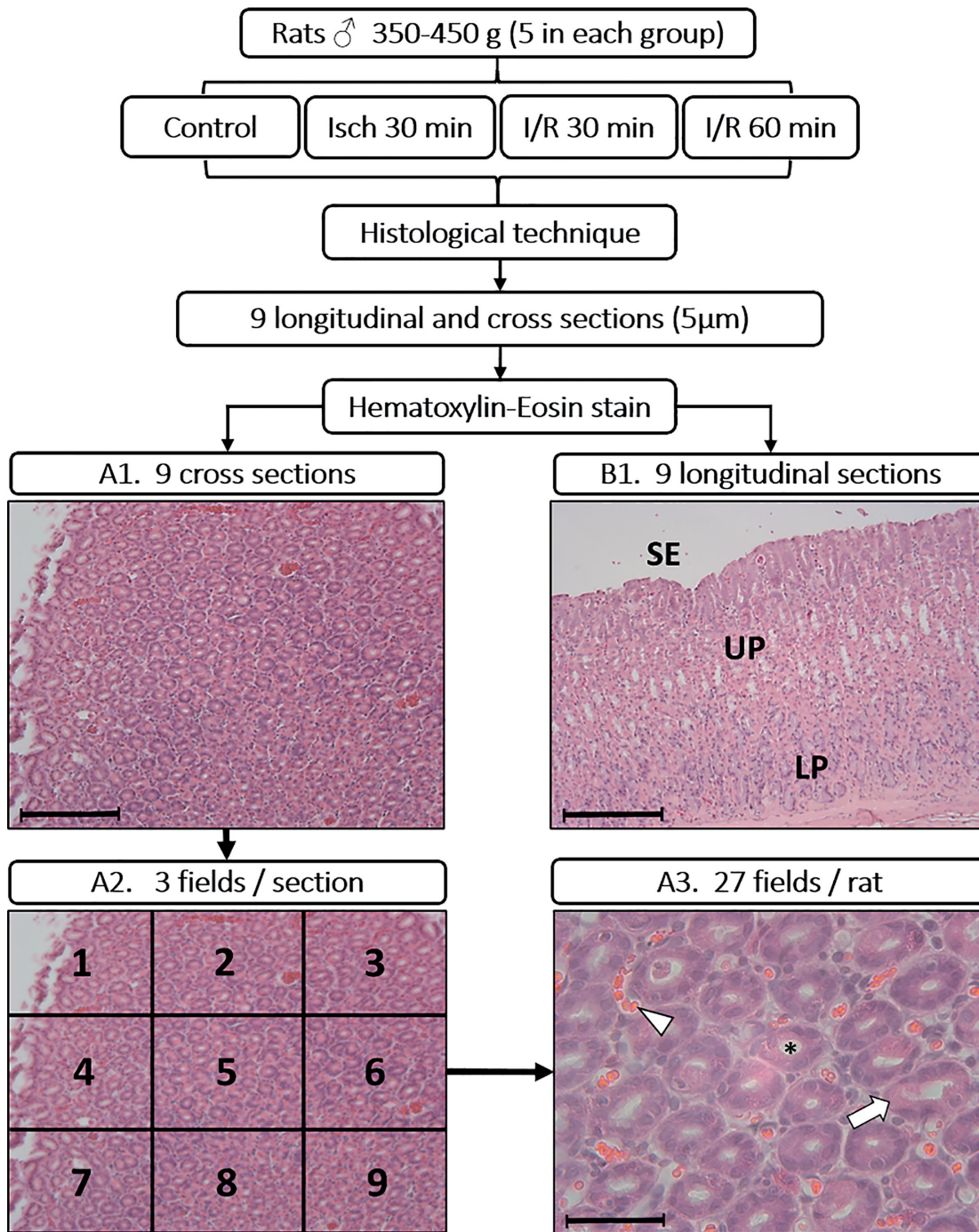


Fig. 1. Workflow to obtain the tissue lesion index. Photomicrographs of rat gastric mucosa. Longitudinal and transverse slides are presented. Identifiers: SE, superficial epithelium; UP, upper portion; LP, lower portion; *, glandular lumen; white arrow, damaged cell; arrowhead, blood vessels. Scale bars: A1, A2, B1, 200 μm ; A3, 100 μm .

27 photomicrographs were taken from each specimen (Fig. 1A3). The images (2584×1936 pixels, in TIF format) were digitalized with an AxioCamMRc5 camera (Carl Zeiss Light Microscopy, Göttingen, Germany). In each slice, variables were measured using AxioVision 4.9.1 software (Carl Zeiss Microscopy, Thornwood, NY, USA) with the following parameters: X and Y- scaling: 0.1418 $\mu\text{m}/\text{pixel}$. To identify areas of epithelial erosion, the 9 slices corresponding to the longitudinal plane of the gastric mucosa were observed with an original $\times 100$ magnification and 1 photomicrograph of each slice was taken, which allowed us to identify both erosion areas and their depth (Fig. 1B1).

Obtaining the scale to assign TLI scores

The variables and the score proposed to determine the severity of damage based on tissue alterations were as follows: vascular area (0-2); glandular lumen area (0-2); cellular injury (0-2), and epithelial erosion (0-2). To quantify the number of cells in the gastric mucosa which presented evident damage due to lesion, we considered the presence of indicators of cellular edema like increases in eosinophilia and cytoplasmic hydropic changes, as well as the presence of pyknotic or cariolic nuclei which indicate processes of apoptotic or necrotic cellular death, respectively. For the vascular area, the blood vessels were delimited, with the presence of erythrocytes and endothelial cells serving as reference. To analyze the glandular disruption area, the gland lumina (pits) of the gastric mucosa was delimited. Finally, to identify areas of epithelial erosion, the slices corresponding to the longitudinal plane were observed with $\times 100$ magnification and the depth of erosion areas in the gastric mucosa was identified. To obtain the grading scale for each of the variables, descriptive analysis of all the data was conducted to obtain the 25th, 50th, and 75th percentiles and the interquartile range (IQR). Then, a box-plot was made

and the grading scale was assigned based on the data distribution.

Classification of damage to the gastric mucosa

After establishing the grading scales, a Receiver Operating Characteristic (ROC) curve was made with all the data obtained based on the tissue lesion index to classify damage to the gastric mucosa as “without tissue lesion” and “tissue lesion.”

Validation of TLI

Finally, the TLI was validated in an I/R model, for which 20 Wistar rats were distributed randomly in 4 groups (n=5): Control, Sham, Ischemia 30 min, and I/R 60 min. The surgical procedure, processing of biopsies, and collection of TLI were carried out as described previously. In the sham group, the opening of the abdominal cavity was performed, the artery was touched and the stomach remained exposed for 90 min.

Statistical analysis

The data were analyzed with IBM SPSS 21 (IBM Corporation, NY, USA) and GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). Kruskal Wallis test was performed to compare pH and lactate levels between groups. The data are presented as mean $\pm$ SD.

Results

Bibliographic review of methods for quantifying damage to the gastric mucosa induced by I/R

To identify the most common quantification methods used by research groups interested in damage to the gastric mucosa induced by I/R, we conducted a

Table 1. Bibliographic references related to the quantification of damage to the gastric mucosa induced by I/R.

A. Macroscopic analysis (68%) (Measurement of the lesion area by computerized planimetry or stereomicroscope)
Wada et al., 1996; Smith et al., 1996; De La Lastra et al., 1997; Calatayud et al., 1998; Nakamoto et al., 1998; Ishii et al., 2000; Konturek et al., 2000; Tashima et al., 2000; Mojžiš et al., 2001; Pawlik et al., 2001; Iino et al., 2002; Kwiecien et al., 2002; Cabeza et al., 2003; Erkasap et al., 2003; Ichikawa et al., 2003; Kyoj et al., 2004; Kitano et al., 2005; Derin et al., 2006; Qiao et al., 2006; Kobata et al., 2007; Kotani et al., 2007; Yonezawa et al., 2007; Abdallah et al., 2008; Bülbül et al., 2008; Konturek et al., 2010; Nakagiri et al., 2010; Nakamori et al., 2010; Muthuraman et al., 2011; Naito et al., 2011; De Faria et al., 2012; Mogami et al., 2012; Cui et al., 2013; Gemici et al., 2015; Mard et al., 2015; Magierowski et al., 2017.
B. Macroscopic analysis (23%) (Scoring assignment dependent on the length of the injury)
Villegas et al., 2004; Zhang et al., 2008; Peskar et al., 2009; Feng et al., 2010; Gou et al., 2011; Wang et al., 2011; Yi et al., 2012; Zhu et al., 2012; Du et al., 2013; Liu et al., 2013; Gao et al., 2015; Hu et al., 2016.
C. Microscopic analysis (9%) (Evaluation of histopathological characteristics)
Michida et al., 1994; Hassan et al., 1997; Nakamoto et al., 1998; Hiratsuka et al., 2005; Gezginci-Oktayoglu et al., 2016.

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bibliographic review in the PubMed database between the years 1994 - 2017 with the key words “ischemia gastric reperfusion,” from which we reviewed 52 articles related to the study of damage to the gastric mucosa induced by I/R.

We found that 68 % of reports presented an analysis of damage measuring the total area (mm^2) or percentage (%) of erosion in the gastric mucosa, determining the extent of the lesion in the surface epithelium by planimetry or with a stereoscopic microscope. To measure the extent of a lesion, they reported a method to calculate the size of gastric ulcers caused by I/R establishing a lesion index which considered the length of ulcers and was expressed as the sum of the size of stomach ulcers in millimeters. Another similar lesion index, in the articles reviewed, considered the length (mm) and width (mm) of ulcers in the mucosa and was calculated using the formula: $\text{UI} (\text{mm}^2) = \text{length} (\text{mm}) \times \text{width} (\text{mm}) \times \pi/4$ (Table 1A).

In the bibliographic review, 23% of the investigators presented a lesion index for the gastric mucosa based on a scale of cumulative length, on which a limited individual lesion in the surface epithelium of the mucosa (including spot erosions, ulcers, and hemorrhages) was graded based on its length. The score used is described below: 1, lesion ≤ 1 mm; 2, lesion >1 and ≤ 2 mm; 3, lesion >2 and ≤ 3 mm. The sum of the scores represented the lesion index of the gastric mucosa. Based on this quantification model, some authors modified the score according to the variability in length of lesions. In addition, when lesions present a width > 1 mm, the score was doubled (Table 1B).

In the less used quantification method (9%), a

histological analysis was conducted and quantification was made using criteria of tissue damage such as: exfoliation of cells from the surface epithelium, depth of erosion, edema, vasocongestion, bleeding, and presence of inflammatory cells, which were assigned a score depending on the size of the lesion (Table 1C).

Analysis of pH and lactate under I/R conditions

Occlusion of the celiac artery produced a statistically significant drop in pH values ($p < 0.01$) with respect to control group; following ischemia (7.17 ± 0.03) a greater reduction was observed than the reperfusion (7.20 ± 0.04) and the control groups (7.28 ± 0.02). Under conditions of ischemia (13.73 ± 9.81 mg/dL) and I/R (18.5 ± 9.19 mg/dL), lactate increased significantly compared with the control (2.51 ± 1.12 mg/dL), indicating metabolic acidosis.

Obtaining the scale to assign TLI scores

Based on the results obtained by quantifying each variable for the 4 groups, a descriptive analysis of all the data was conducted using SPSS 21 software, to obtain the 25th, 50th, and 75th percentiles and the interquartile range (IQR) (Table 2).

Then, the intervals were delimited and a box-plot was made to identify the distribution of data and assign the following score: vascular area (μm^2), the score=0 ($\leq P_{75}$), was set for measurements of blood vessels which did not present apparent signs of lesion in the histological analysis; the score=1 ($>P_{75}$ to $\text{RIC} \times 1.5$), and the score=2 (outliers) correspond to dilated blood vessels

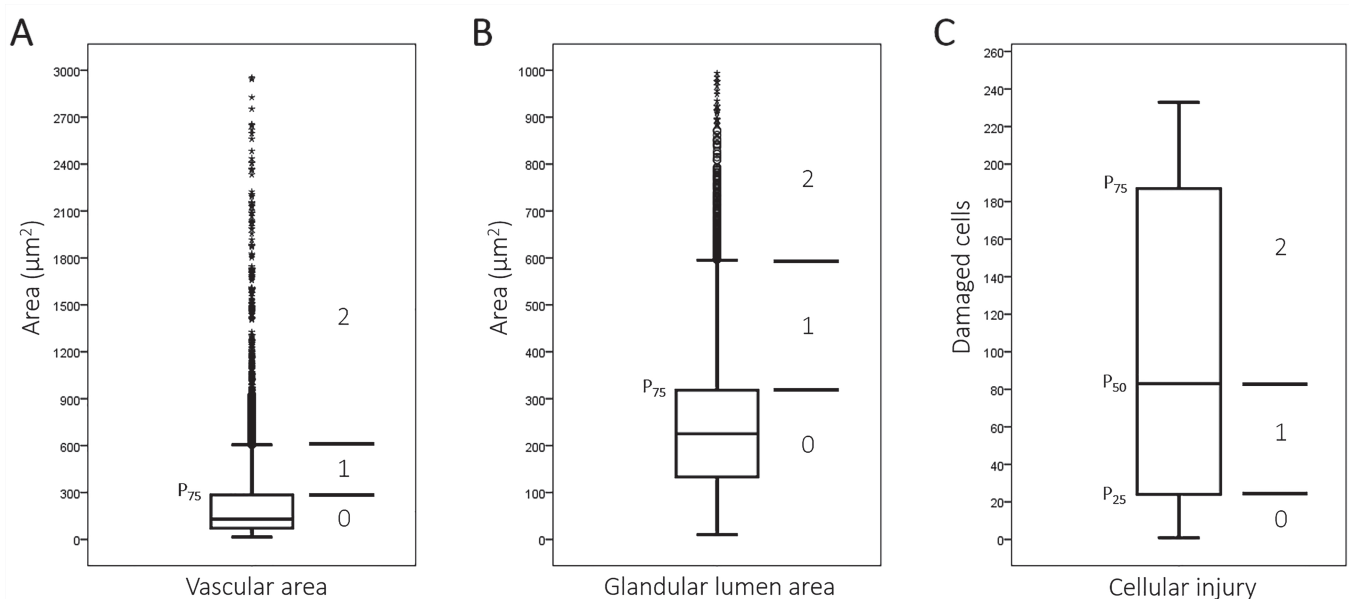


Fig. 2. Box plot graphs of the variables to assign the score for the tissue lesion index (0-2). **A.** Vascular area (μm^2). **B.** Glandular lumen area (μm^2). **C.** Cellular injury.

(Fig. 2A).

For the area of the gland lumina (μm^2), the score=0 ($\leq P_{75}$), was assigned to gland lumina measurements in which the epithelium was characteristically normal; the score=1 ($>P_{75}$ to $\text{RIC} \times 1.5$) was assigned to increase in the area of the gland lumina due to atrophy of the glandular epithelium; the score=2 (outliers), was assigned to areas with loss of glandular architecture, which produced erosion areas in the gastric mucosa (Fig. 2B).

For cellular damage, we considered the number of cells which presented edema, pyknosis, or karyolysis. The score=0, was assigned to the interval between the minimum value to P_{25} , the score=1 to the interval between P_{25} - P_{50} , the score=2 to the interval between P_{50} and maximum value (Fig. 2C).

The score for depth of erosion took into account whether the gastric mucosa presented erosion of the surface epithelium (1 point) or if the depth of the lesion extended to the oxyntic portion (2 points). Therefore, the score for each of the scales was assigned as shown in Table 3. Adding the scores for all the variables should produce a maximum of 8 points.

Classification of damage to the gastric mucosa

After the scales were established and scores were assigned, the lesion index was classified. A ROC curve was made with all the data obtained based on the tissue lesion index. A cut-off point was identified at TLI value of 3 with maximum sensitivity and specificity (0.96), and the area under the curve (AUC) was 0.993. With these results, the classification was established as “without tissue lesion” when the score was ≤ 3 and “tissue lesion” when the score was >3 (Fig. 3).

TLI Validation

The histological analysis under baseline conditions reported gastric glands, blood vessels, and lamina

propria with normal characteristics. The surface epithelium and the oxyntic and fundic regions presented a typically normal architecture (Fig. 4A,B). In sham conditions, vascular dilatation was identified (Fig. 4C,D). Under ischemic conditions, some cells of the glandular epithelium showed morphological changes associated with cellular edema, whereas areas of vascular congestion at the level of the lamina propria were identified, mainly in the superficial and oxyntic regions (Fig. 4E,F). Under conditions of reperfusion, the epithelial cells showed signs of substantial cellular damage (cellular edema, pyknotic or cariolytic nuclei) with loss of volume (cellular atrophy), which caused a significant increase in the luminal area. We identified erosion areas of the surface epithelium (Fig. 4G,H).

Quantitative analysis of vascular area, cellular damage, area of gland lumina, and erosion of surface epithelium was conducted for the 4 experimental groups. Statistical analysis of the vascular area (Fig. 5A) showed a gradual increase between experimental groups compared with the control, with the greatest vascular area found in the 60 min I/R group. The area of the

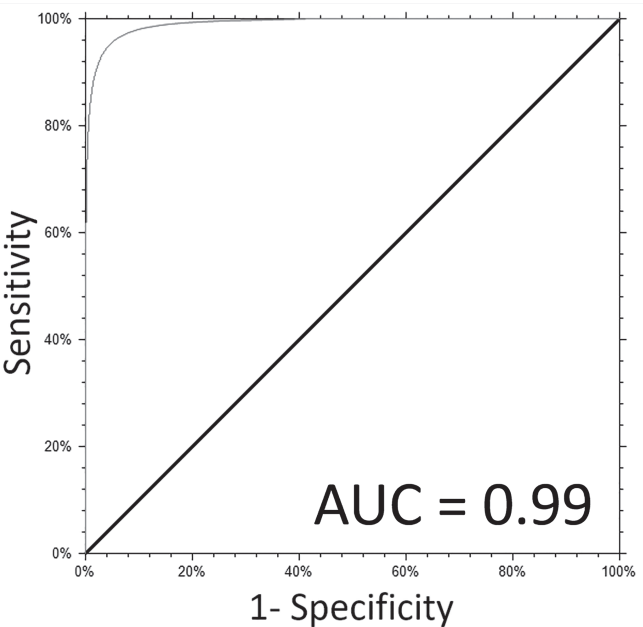


Fig. 3. ROC analysis to categorize the damage in the gastric mucosa comparing control and experimental groups.

Table 2. Percentiles 25th, 50th, 75th and interquartile range (IQR) of the quantified variables.

Variables	P ₂₅	P ₅₀	P ₇₅	IQR
Vascular area (μm^2)	67.46	127.7	268.0	200.54
Glandular lumen area (μm^2)	91.60	204.2	307.2	215.6
Cellular injury (number of damaged cells)	24	83	187	163

Table 3. Scale to indicate the score for each variable.

Score	0	1	2
Vascular area (μm^2)	100-300 μm^2	301-600 μm^2	≥ 601 μm^2
Glandular lumen area (μm^2)	50-300 μm^2	301-600 μm^2	≥ 601 μm^2
Cellular injury (number of cells with edema, pyknosis or karyolysis)	≤ 25	26-85	≥ 86
Epithelial erosion	Without erosion	Superficial epithelial erosion	Erosion to the oxyntic portion

Lesion index of gastric damage

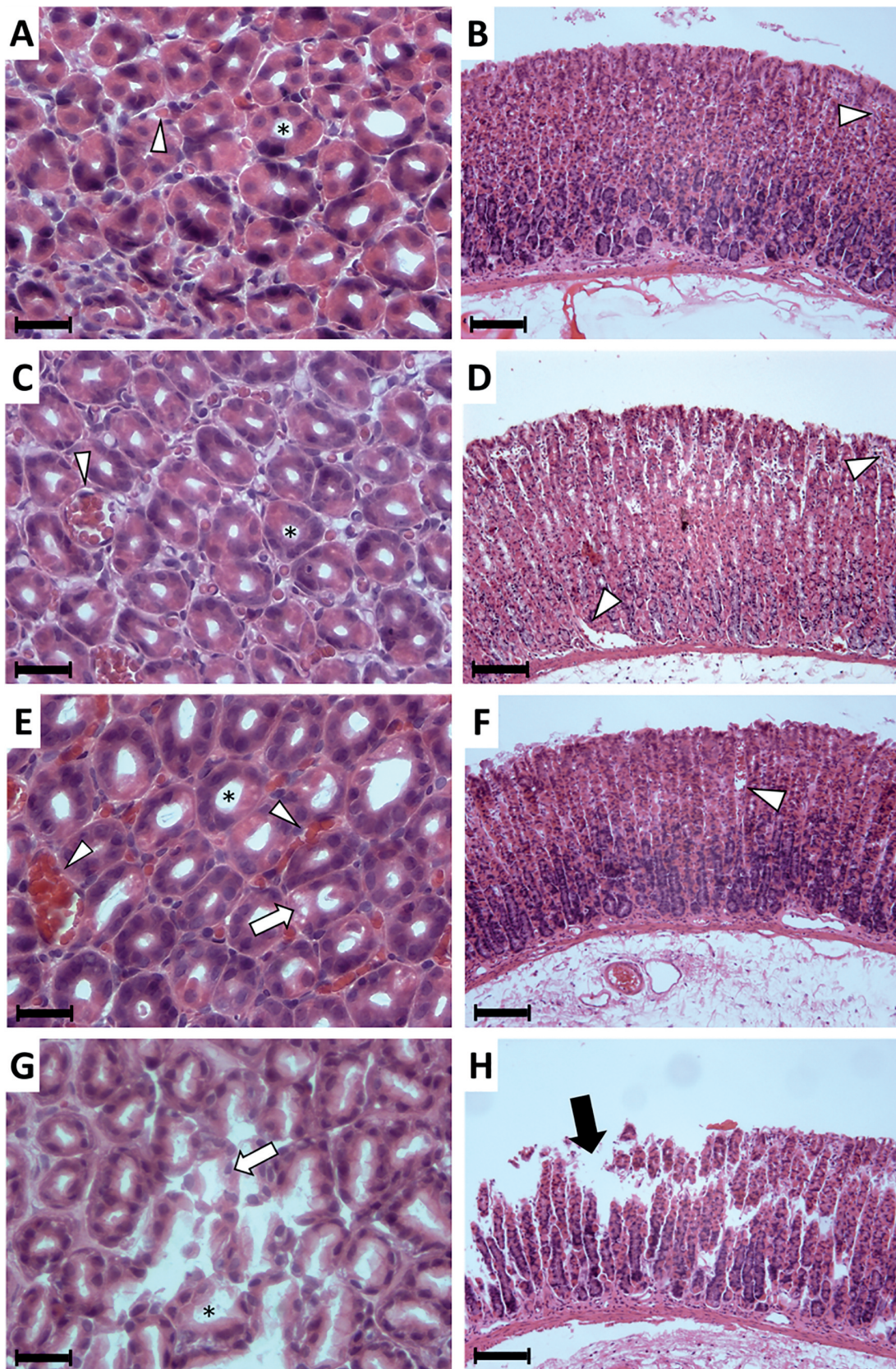


Fig. 4. Photomicrographs of rat gastric mucosa in control (**A, B**), sham (**C, D**), ischemia 30 min (**E, F**) and I/R 60 min (**G, H**) groups. Identifiers: *, glandular lumen; white arrow, damaged cell; arrowhead, blood vessels; black arrow, epithelial erosion. Note the vascular congestion in the ischemia group, besides the cellular damage and the loss of the epithelium integrity in the I/R 60 min group. H & E; Cross sections **A, C, E** and **G**; Scale bars: **A, C, E, G**, 50 μ m; **B, D, F, H**, 100 μ m.

gland lumina (Fig. 5B) presented an increase between the ischemia and I/R groups compared with the control and sham, with the largest increase in gland lumina area in the I/R group. The number of damaged cells (Fig. 5C) presented a gradual increase between the experimental groups compared with the control, with the largest number of cells in process of dying in the I/R group. Analysis of areas of epithelial erosion (Fig. 5D) found a gradual increase in depth of erosion in the 4 groups, with erosion to the oxyntic portion in the I/R group.

The sum of the scores assigned to each of the variables was calculated and the result was expressed as the TLI (Fig. 5E), which showed the highest scores in the I/R group.

Finally, damage was classified based on the TLI. In the sham and ischemia groups, the TLI score was <3 and no signs of apparent tissue lesion were found, although acute cellular damage and signs of inflammatory process were identified without compromising tissue integrity, whereas under conditions of reperfusion, the TLI score

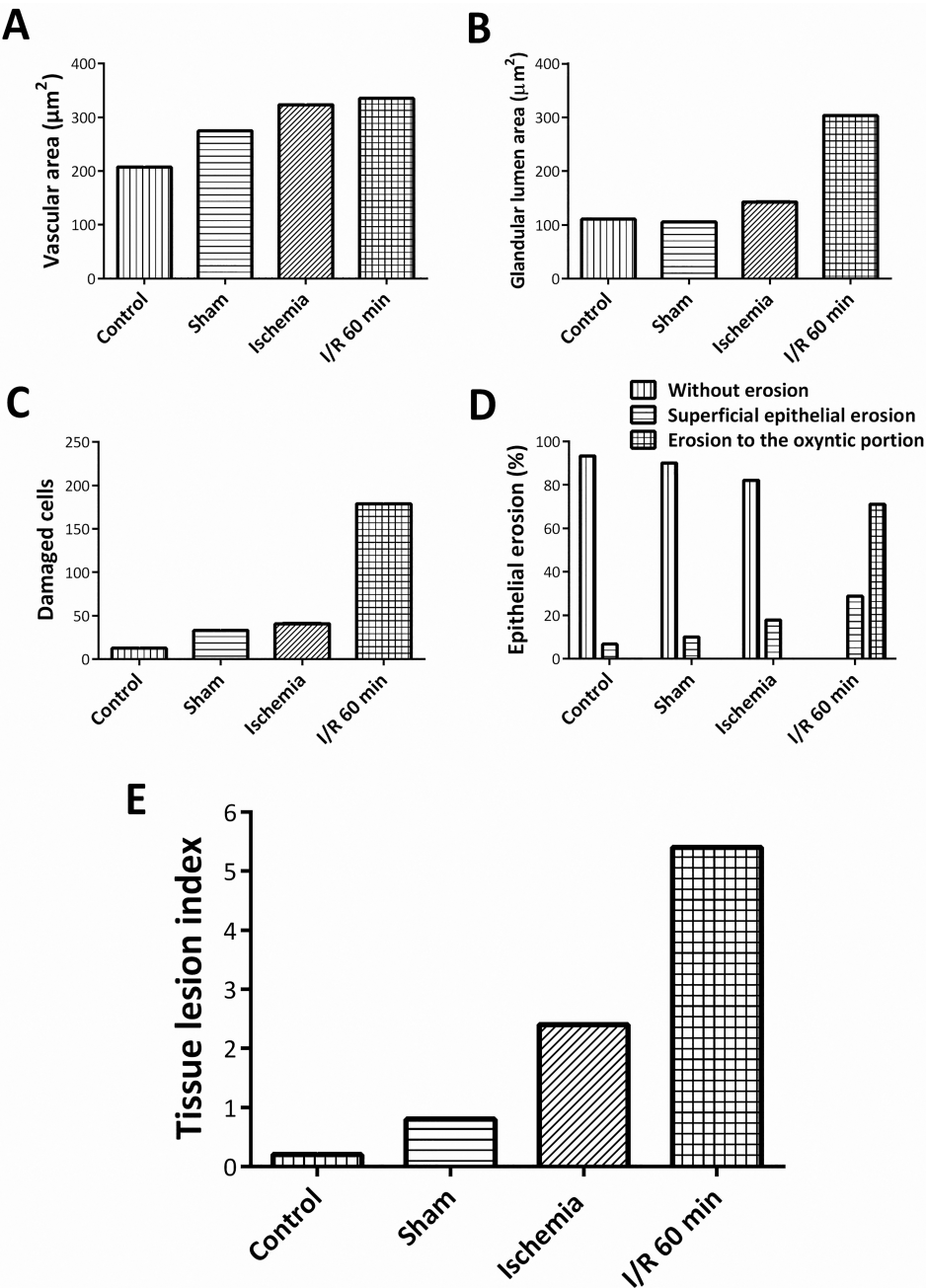


Fig. 5. Quantitative analysis of the variables considered in the histological analysis to obtain the index of tissue lesion (A, B, C, D). Score of the index of tissue lesion for the control and experimental groups (E): control (score: 0.2); sham (score: 0.8); ischemia (score: 2.4); I/R 60 min (score: 5.4).

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was >3 and based on the classification there was gastric tissue lesion, which is consistent with the histological analysis, because cellular death was observed, with compromise of the glandular architecture creating erosion areas in the epithelium of the gastric mucosa causing it to lose its barrier function.

Discussion

Under conditions of I/R in the gastric mucosa, oxygen deprivation to tissues alone causes cellular damage and eventually death. Also, reintroducing oxygen to the affected tissue increases the area of the lesion mainly due to production of ROS and nitrogen, produced primarily by neutrophils. The interaction of those molecules creates peroxynitrite, which damages the cell membranes by lipoperoxidation, increasing the size of the lesion in the gastric mucosa (Derin et al., 2006).

To evaluate the progression of tissue damage in the gastric mucosa under conditions of ischemia, impedance spectroscopy has been used, a technique which allows us to identify characteristic changes in impedance spectra in tissue, caused mainly by cellular edema, narrowing of intercellular bonds, and accumulation of metabolic products under conditions of ischemia (Casas et al., 1999). Studies have been conducted to characterize changes in impedance of the gastric mucosa in healthy adults and in patients under different degrees of splanchnic hypoperfusion during cardiovascular surgery. The greatest increase in resistance and reactance has been observed at low frequencies in ischemic patients with complications compared with healthy volunteers, but reactance is more consistent and is the electric parameter most sensitive to tissue damage. Changes at high frequencies occur later and are produced specifically in reactance (Beltran et al., 2006, 2010, 2013; Beltran and Sacristan, 2015).

To relate changes in gastric impedance associated with damage to the mucosa caused by ischemia and I/R, a TLI was proposed which considered pathological alterations associated with the inflammatory process and cellular damage which directly modified the parameters of gastric impedance; also, those variables may be affected by treatment which may inhibit or diminish damage to the gastric mucosa under conditions of ischemia or I/R.

Based on our review, the quantitative analysis most used by the investigators was macroscopic measurement of the size of the lesion, whether expressed as area (mm^2) (Kotani et al., 2007), percentage (%) of erosion (Calatayud et al., 1998; Qiao et al., 2006), or ulceration index (Abdallah et al., 2008; Mard et al., 2015). This kind of measurement allows us to determine only the area of lesion on the surface epithelium of the gastric mucosa. Such analysis may be affected by observer subjectivity and by the measuring techniques used, such as planimetry (Konturek et al., 2010; Cui et al., 2013; Magierowski et al., 2017) or use of a stereoscopic

microscope with a grid (Iino et al., 2002; Ichikawa et al., 2003; Nakamori et al., 2010) or a ruler (Derin et al., 2006; Gemici et al., 2015).

The macroscopic method to quantify damage to the gastric mucosa is based on a scale of cumulative length (length and width of the lesion), which is expressed as the “gastric mucosa damage index” (Zhang et al., 2008; Peskar et al., 2009; Hu et al., 2016); as in the previous method, measurements can be affected by observer subjectivity and measuring techniques; also, the score may be modified based on each investigator’s criteria, which may make it impossible to accurately compare the results with those of other investigators.

The least used method to quantify damage to the gastric mucosa is histological evaluation, which considers criteria of tissue damage; the drawback of this method is that, although the analysis is conducted by expert pathologists, this type of quantification may be subjective because it does not report an interval from which scores are assigned to each of the criteria, in order to objectively identify the possible degree of lesion present in the gastric mucosa. Also, investigators modify these criteria, some evaluate the depth of erosion in the mucosa (Nakamoto et al., 1998; Hiratsuka et al., 2005), while others focus on characteristics of cellular and tissue damage (Hassan et al., 1997; Gezginci-Oktayoglu et al., 2016).

The study of damage to the gastric mucosa is focused mainly on the search for treatments which can minimize the area of erosion of the gastric mucosa under conditions of I/R (Mard et al., 2015; Hu et al., 2016; Magierowski et al., 2017), for which the quantification methods described above may serve as a reference to identify whether the extent and depth of a lesion diminish with the treatments studied in I/R models. On the other hand, these quantification methods, because they are not objective, cannot be linked to changes in measurements of gastric impedance under conditions of I/R.

Unlike previous quantification methods, we proposed a TLI based on histopathological analysis of transverse and longitudinal slices to measure damage induced by I/R which considers variables of cellular damage (edema, pyknosis, and karyolysis), glandular area, depth of erosion, and early inflammatory process (vascular area), which were quantified and were statistically and graphically described to assign a score based on the distribution of data and the extent of damage reported in the histopathological analysis. Finally, a ROC curve was made to classify damage to the gastric mucosa as “without tissue damage” or “tissue damage.” These results allowed us to objectively conduct a quantitative analysis using statistically defined scales, which will help make the quantitative results obtained in other studies reproducible and comparable.

To validate the proposed TLI, we conducted a quantitative analysis of damage to the gastric mucosa induced by 30 min of ischemia and 60 min of I/R. The histological results observed in this study showed that,

under conditions of ischemia, there were signs of cellular damage which are identified as cytoplasmic eosinophilia and nuclear condensation or karyolysis. Also, we observed areas of vascular congestion which corresponds to an acute inflammatory response; these data coincide with the findings reported by Beltran et al. (2013). After 60 min of reperfusion, we observed primarily an increase in the area of the gland lumina, cellular damage, and erosion of the epithelium of the gastric mucosa. These results are consistent with the histological description presented by Liu et al. (2013), who implemented an I/R model (30 min/60 min), identifying damage to the gastric mucosa, primarily in the surface epithelium extending toward the glandular region. Gastric I/R models with greater reperfusion times (3-24 hours) described gastric lesions, such as multiple erosions, exfoliation, hemorrhage, necrosis of surface cells, and severe alterations in the glandular architecture, as well as infiltration of macrophages (Abdallah et al., 2008; Wang et al., 2011).

Based on the variables considered in the proposed TLI, we were able to identify under conditions of ischemia: acute cellular damage (cellular edema, pyknotic and cariolytic nuclei) and substantial vascular congestion without apparent signs of epithelial erosion compromising the barrier function, which was not the case under conditions of reperfusion, where we observed erosion of the epithelium of the gastric mucosa toward the oxyntic portion. Based on these results, we observe that the scale suggested in the TLI for classification of lesions is consistent with the macroscopic results reported previously. Under conditions of ischemia, Nakamori et al. (2010) found no visible macroscopic damage; however, under conditions of reperfusion for 60 min, multiple hemorrhagic lesions in the mucosa were observed. These results are consistent with those reported by Gou et al. (2011) in an I/R model similar to that used in this study.

The proposed gastric lesion index allows us to objectively quantify and classify damage to the gastric mucosa produced by I/R; also, the variables chosen may serve as references for treatments seeking to inhibit or diminish damage to the gastric mucosa.

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