Changes in Serum Concentration of VEGF Angiogenesis Factor and I-FABP Intestinal Barrier Biomarker in Different Outcomes of Treatment of Patients with Duodenal Injury

Authors’ Contribution:

A – Study design;  
B – Data collection;  
C – Statistical analysis;  
D – Data interpretation;  
E – Manuscript preparation;  
F – Literature search;  
G – Funds collection

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Received: 10.01.2020; Accepted: 24.02.2020; Published: 30.03.2020

Abstract
Abdominal injuries, due to a blunt and penetrating mechanism of origin, have a marked mortality rate from hemorrhage and sepsis. Diagnosis and treatment of damage to the duodenum is one of the complex problems of emergency abdominal surgery, although they are rare and account for 0.2-0.6% of all trauma patients and 1-4.7% of all abdominal injuries.

The aim of the study: to research the dynamics of the concentration in the blood of the factor of VEGF angiogenesis and a marker of damage to the digestive tract I-FABP in adults with various postoperative consequences of trauma to the duodenum.

Materials and Methods:
Studies were conducted in 60 patients (mean age 36 (18-73) years) with isolated and multiple traumatic injuries of duodenum ≥ grade II according to AAST criteria operated in the period 2006-2019.

Results: The level of I-FABP was higher in patients who had complications and needed reoperation and correlated with the severity of the patients’ condition, and according to Spearman’s criterion the comparisons were valid in all three periods of study (p=0.002, p=0.000, p=0.001).

Conclusions: Serum I-FABP level is a valuable, convenient, objectively early predictor of digestive injury in duodenal injury due to suppression of vascular proliferation, decrease in endothelial permeability, which is determined by the dynamics of VEGF changes in the treatment of this category of patients, and depends on their condition and severity, the presence of complications that require repeated surgery.

Keywords: duodenal injury, VEGF angiogenesis factor, I-FABP digestive tract damage markers, dynamics of changes

Conflict of interests: The authors declare that there is no conflict of interests

DOI and UDC: DOI 10.26697/ijes.2020.1.5; UDC 615.076:616.34:089:616.342

Academic research work (State registration number 0119U002909/15.07.2019)

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Peer review: Double-blind review

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Introduction
Abdominal injuries, due to a blunt and penetrating mechanism of origin, have a marked mortality rate from hemorrhage and sepsis (Mgguni, Muckart, & Madiba, 2012). Diagnosis and treatment of damage to the duodenum is one of the complex problems of emergency abdominal surgery, although they are rare and account for 0.2-0.6% of all trauma patients and 1-4.7% of all abdominal injuries (Rickard, Brohi, & Bautz, 2005; Siboni, Kwon, Benjamin, Inaba, & Demetriades, 2016). Related injuries in the trauma of the duodenum occur in 68-86.5% of patients, and major vascular injuries in 23-40% of cases. The presence and type of concomitant injuries significantly affect the outcome of treatment of patients due to the severity of the trauma itself and the complex pathophysiological mechanisms that lead to the development of local and systemic complications (Addison et al., 2016).

The processes of neovascularization in conditions of tissue damage and in wound healing are closely related to the molecular and cellular mechanisms of regulation of growth and remodeling of blood vessels (Folkman, 2007). Activation of angiogenesis is primarily associated with stimulation of endothelial cells under hypoxia under the action of hypoxia-inducible factor (HIF-1) and a number of angiogenic factors (Pugh & Ratcliff, 2003). Among the latter, vasculoendothelial growth factor – VEGF, which significantly influences vascular permeability, endothelial and monocyte migration, is an inducer of vasodilation via the NO synthase pathway in endothelial cells, is most important for vascular growth. It was shown (Mackavey & Hanks, 2013) that platelets release VEGF upon aggregation, and its high serum concentration correlates with an increased platelet count.

The processes of neovascularization in conditions of tissue damage and in wound healing are closely related to the molecular and cellular mechanisms of regulation of growth and remodeling of blood vessels (Folkman, 2007). Activation of angiogenesis is primarily associated with stimulation of endothelial cells under hypoxia under the action of hypoxia-inducible factor (HIF-1) and other angiogenic factors (Pugh & Ratcliff, 2003). Vascular endothelial growth factor (VEGF) is significantly influences vascular permeability, endothelial and monocyte migration. It is an inducer of vasodilation via the NO-synthase pathway in endothelial cells and it is most important for vascular growth. It was shown (Mackavey & Hanks, 2013) that platelets release VEGF upon aggregation, and its high serum concentration correlates with an increased platelet count.

In patients with severe digestive tract (DT) is quite vulnerable, the occurrence of its dysfunction plays a significant role in the development of complications of systemic complications, which is observed in other urgent conditions (Reintam, Farm, Kitus, Kern, & Starkopf, 2009). Dysfunction of DT is indicative of the severity of critical patients, as a number of publications have reported that nearly 50% of patients in ICUs have enterocyte damage at the initial stage of treatment (Piton et al., 2013), and among severely ill patients more often there are high mortality rates (Reintam et al., 2006). Acute dysfunction and lack of DT are increasingly being recognized in critically ill patients. The variety of definitions previously suggested has led to confusion and difficulty in comparing one study to another. The Working Group of Abdominal Problems (WGAP) of the European Society of Intensive Care Medicine (ESICM) identified acute injury of DT (AGI) as impaired in critical patients with acute illness, including surgery, and recommended classification for estimates of the severity of DT in 2012 (Blaser et al., 2012). It is believed that some biomarkers, such as protein binding fatty acids (I-FABP), D-lactate (D-lα) and lipopolysaccharide (LPS), can be used as possible markers for the determination of impaired barrier function DT and diagnosis of AGI (Grootjans et al., 2010).

For the last 20 years the diagnostic value of a new clinical-laboratory indicator, the fatty acid binding protein (FABP) which is one of the intracellular proteins and is involved in the transport and metabolism of long-chain fatty acids, has been studied. The FABP protein family has good diagnostic characteristics: 1) soluble in the cytoplasm; 2) have a high specificity for the tissue from which they originate; 3) are contained in the cell in high concentration; 4) have a low molecular weight (15 kDa). These characteristics allow the use of these markers in the peripheral blood to be sensitive and specific, indicating tissue damage (heart, liver, small intestine, etc.). It is known that intestinal FABP (I-FABP) is contained in the epithelial cells of the small intestine and the diagnostic value of increasing this biomarker in the blood in the heavy category of patients is being actively discussed in the literature (Krivoruchko et al., 2019; Pelsers, Hermens, & Glatz, 2005).

The aim of the study. To research the dynamics of the concentration of VEGF angiogenesis factor in the blood and the marker of I-FABP digestive tract damage with various postoperative effects of duodenal injury in adults.

Materials and Methods
A study was conducted in 60 patients with isolated and multiple traumatic injuries of duodenum ≥ II degree according to the criteria of the American Association of Injury Surgery (AAST) (Moore et al., 1990) who was operated at the Zaitsev Institute of General and Emergency Surgery NAMS of Ukraine (Kharkiv, Ukraine) and the Meshchaninov Clinical Hospital of Emergency and Emergency Medical Services for the period 2006-2018.

Patient selection and grouping
The study collected the following data: patient (gender, age), type and characteristics of duodenum injury, the presence of shock upon admission to the hospital (systolic blood pressure < 90 mm Hg), severity assessment on the scales of The Injury Severity Score (ISS) , The Revised Trauma Score (RTS) and
APACHE II (indicators were calculated online using a calculator on MDCalc), accompanying intra-abdominal injuries, local and systemic complications, nature of treatment (artificial lung ventilation, inotropic support), treatment outcomes (postoperative complications, 30-day mortality). All the patients were subjected to standard clinical and laboratory examination at the time of admission, the need to use instrumental examinations (radiographic, ultrasound, multispiral computed tomography, laparoscopy) was determined based on the estimated severity of the condition and the dominant area of injury. In the treatment period patients were initially stabilized in the intensive care unit or in the operating room with subsequent resuscitation laparotomy in 43 (71.7%) patients. Based on the clinical results of the analysis the following groups were formed: the first group: the patients who recovered with mild residual effects; the second group: the patients who had complications that required re-laparotomy. The examination was carried out in the following periods: 1st period (1-2 days after injury. It’s the period corresponding to the shock period and the early post-shock period); 2nd period (7-14 days. It’s the period of early manifestations of traumatic disease); 3rd period (14-30 days. It’s the period of late manifestations of traumatic disease) (Krivoruchko, Bojkó, Berezka, Teslenko, & Shevchenko, 2006).

Serum VEGF determination was performed on a STAT FAX-303 analyzer (USA) using the enzyme immunoassay and “Human VEGF 96 assay kit” (Invitrogen Corp., USA). Blood from the examined patients was sampled in the morning before meals from the cubital vein to obtain plasma without impurities and for 30 minutes after sampling, blood tubes were taken to the laboratory for further processing. The VEGF content in the serum of almost healthy people was very variable from 58 to 284 pg/ml which was taken as the reference interval for the content of this indicator. The level of I-FABP was determined using commercial kits “I-FABP, Human, ELISA kit” (The Netherlands) using the enzyme immunoassay. Blood samples were collected, were expected to coagulate at room temperature for 30 minutes, and then centrifuged at 3000 volumes within 10 minutes. The serum was collected in 2.0 ml Eppendorf tubes and sent to the laboratory for 30 minutes. The content of I-FABP in the serum of healthy subjects also turned out to be variable from 118 to 365 pg/ml which was also taken for the reference interval of the content of the indicator.

Surgical treatment

All patients were operated. Urgent operations included the following mandatory steps: hemostasis was performed, revision of the abdominal cavity and retroperitoneal space, and to exclude damage to the posterior wall of the intestine and portal vein was mandatory mobilization of the duodenum on Kocher-Clermon, and then its assessment of localization and in accordance with AAST criteria. Simple surgical operations such as drainage and primary repair were performed more often for duodenal injuries than more complex surgical operations, such as pyloric exclusion, duodenoejunosotomy and pancreaticoduodenectomy was used seldom. In the treatment of patients with multiple injuries with hemodynamic stability other damages was eliminated during operations and some of patients with hemodynamic instability performed tamponade of the bleeding source with surgical wipes, damage to the hollow organs were isolated with the help of cross-linking devices, and the abdominal cavity was not drained, but temporarily, quickly closed with surgical caps. Repeated surgery was performed 24-72 hours after stabilization of the patients to the principles of Damage Control Resuscitation (DCR) according to the principles of Damage Control Surgery (DCS). All patients received antibiotics before and during surgery and if this was due to antibiotic therapy continued in the postoperative period together with intensive treatment on the principles DCS.

Statistical analyses

Statistical data were processed using the STATISTICA 13.3 EN trial software. Using the Tukey criterion (Kobzar, 2012), the presence of emissions was checked and the normality of the distributions (Shapiro-Wilk criterion) of the selected indicators was evaluated. The correlation for interval indicators was estimated using the nonparametric Spearman correlation coefficient. The null hypotheses (H0) in the statistical tests were rejected at a significance level of p<0.05. The classification of the outcome of treatment using scales assessing the patient’s condition was performed using ROC analysis (Pepe, 2004). Traditionally, the predictive power of the scale and its discriminatory ability are based on the study of its sensitivity and specificity by calculating the area under the curve under the AUC (area under curve), taking into account its 95% confidence interval. The performance of the model was considered limited at AUC<0.70; good – at AUC=0.80; excellent – at AUC>0.90.

Results

Of the 60 patients analyzed, 16 (26.7%) received closed injuries, and 44 (73.3%) were penetrating injuries. 35 (79.5%) of them had stab wounds, 5 (11.4%) from firearms, 4 (9.1%) from mine and explosive. Men were 42 (70%), women – 18 (30%), the average age was 36 (18-73) years. RTS, ISS and APACHE II scores on admission were 6 (4-8), 14 (4-25) and 14 (8-32) respectively; 22 patients (36.7%) had shock (Table 1). Five scales were compared: APACHE II, ISS, AIS, RTS, and OIC, to determine the sensitivity of the model (the proportion of correctly predicted cases) to the specificity (the proportion of false predictions) to determine the optimal values of the threshold at which was the optimal sensitivity and specificity for this model. Only two scores turned out to be significant. These were APACHE II and RTS scores: the APACHE II score (AUC=0.912) was 21 points at a sensitivity of 0.909 and the specificity of 0.811, for the RTS score (AUC=0.876) the threshold was 6 points at a sensitivity of 0.803 and a specificity of 0.796 (Figure 1).
Table 1. Characteristics of patients with traumatic duodenum (n=60).

<table>
<thead>
<tr>
<th>Indicators</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), median (IQR)</td>
<td>36 (18-73)</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
</tr>
<tr>
<td>- male</td>
<td>42 (70%)</td>
</tr>
<tr>
<td>- female</td>
<td>18 (30%)</td>
</tr>
<tr>
<td>The mechanism of injury:</td>
<td></td>
</tr>
<tr>
<td>- penetrating</td>
<td>44 (73.3%)</td>
</tr>
<tr>
<td>- blunt</td>
<td>16 (26.7%)</td>
</tr>
<tr>
<td>The nature of duodenal injury:</td>
<td></td>
</tr>
<tr>
<td>- isolated</td>
<td>8 (13.3%)</td>
</tr>
<tr>
<td>- multiple</td>
<td>52 (86.7%)</td>
</tr>
<tr>
<td>OIS of duodenum:</td>
<td></td>
</tr>
<tr>
<td>II degree</td>
<td>31 (51.7%)</td>
</tr>
<tr>
<td>III degree</td>
<td>24 (40.0%)</td>
</tr>
<tr>
<td>IV degree</td>
<td>3 (5.0%)</td>
</tr>
<tr>
<td>V degree</td>
<td>2 (3.3%)</td>
</tr>
<tr>
<td>RTS score, median (IQR)</td>
<td>6 (4-8)</td>
</tr>
<tr>
<td>ISS score, median (IQR)</td>
<td>14 (4-25)</td>
</tr>
<tr>
<td>APACHE II score, median (IQR)</td>
<td>14 (8-32)</td>
</tr>
<tr>
<td>Shock</td>
<td>22 (36.7%)</td>
</tr>
<tr>
<td>Inotropic support</td>
<td>14 (23.3%)</td>
</tr>
<tr>
<td>Ventilation support</td>
<td>15 (25.0%)</td>
</tr>
<tr>
<td>Postoperative complications</td>
<td>48 (80.0%)</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>11 (18.3%)</td>
</tr>
</tbody>
</table>

Figure 1. ROC curves for rating scales: APACHE II Score – AUC=0.912, 95% 0.870-0.948; RTS Score – AUC=0.876, 95% CI 0.849-0.934; ISS Score – AUC=0.776, 95% CI 0.757-0.814; AIS Scale – AUC=0.588, 95% CI 0.415-0.761; OIS Score – AUC=0.516, 95% CI 0.356-0.675.

In the 1st study period VEGF levels were 22.1% lower on average in surviving patients (Figure 2) than in those who had complications after surgery (p<0.01). In all patients examined during this period the average level of this indicator was higher by 65.9% and 72.1% respectively than in the control group (p<0.01). Survivors in the 2nd and 3rd periods of the research were 75.9% and 109.9% higher than the baseline respectively (p<0.01). At the same time the VEGF level in patients with complications averaged 13.8% and 46.1% higher than the baseline data (p<0.01), but according to the Spearman criterion the comparisons were only reliable in 2nd study period (p=0.002). When analyzed in patients who survived, the mean level of I-FABP was on 46.2% lower (Figure 3) than in those who had complications after surgery (p<0.01). In all patients examined during this period the average level of this indicator was also higher by 207.7% and 349.9% than in the control group (p<0.01). In the 2nd and 3rd periods of the research the I-FABP level were...
lower on 31% and 55.8% than the baseline ($p<0.01$). The average level of I-FABP an average of 21.2% was higher in the 2nd period and on 15.5% was lower in the 3rd period compared to the initial data in patients with complications after surgery ($p<0.05$), and by Spearman’s criterion this indicator were reliable in all three research periods when comparing ($p=0.002$, $p=0.000$, $p=0.001$).

At the same time, when assessing the severity of the condition of patients with significant Spearman criteria were APACHE II score (respectively, $p=0.000$ and $p=0.002$) and RTS score (respectively, $p=0.01$ and $p=0.000$) in the second and third periods of the research, as well as the ISS score (respectively, $p=0.000$ and $p=0.026$) (Figures 4-6).

**Figure 2.** Dynamics of VEGF level changes in traumatic duodenum injuries.

**Figure 3.** Dynamics of changes in I-FABP levels in traumatic duodenum injuries.

**Figure 4.** Dynamics of APACHE II score changes in traumatic duodenum injuries.
Discussion

Changes in VEGF level in the serum of patients with different cases of injury to the duodenum differed in certain features: up to 2 days of the trauma in patients who was survived, this indicator was lower than in patients who had complications after surgery. A similar tendency was observed in the study in the 2nd and 3rd periods of traumatic illness, but according to the Spearman criterion the comparisons were only valid in the 2nd period of the research (p=0.002). Also, the average VEGF values were significantly higher at all research times compared to the control data. It is known that in traumas, especially accompanied by blood loss and shock, there is an activation of angiogenesis and the synthesis of VEGF due to hypoxia and enhanced release of VEGF from platelets due to their aggregation during blood clotting. It is likely that an increase in VEGF levels in trauma acts as a trigger for the activation of endothelial cell migration and proliferation processes, the most important manifestations of angiogenesis. The increasing migration of monocytes under the influence of VEGF may be directed to the “harvesting” of post-traumatic necrosis by these cells. In addition, monocytes are form elements expressing specific receptors for VEGF (Parfenova & Tkachuk, 2007). These processes of activation of angiogenesis cause the formation of primary vascular structures with high permeability, which are likely to contribute in one way or another to the improvement of oxygenation and, accordingly, facilitate the repair of damaged tissues. However, a relatively long period of maintaining high VEGF may be associated with uncontrolled distribution of endothelial cells (VEGF is a specific mitogen of endothelial cells) a significant and prolonged increase in vascular permeability, a persistent decrease in blood pressure (increased vascular permeability and vasodilation mediated through NO action) with the possible development of appropriate complications. Probably, the undulating nature of the change in VEGF content is associated with a long maturation process of the newly formed
vascular network: signs of suppression of proliferation, reduction of endothelial permeability, involvement of pericytes, establishment of an appropriate cellular matrix of interaction (Felcht et al., 2012; Saharinen & Alitalo, 2006). As a result of engaging angiogenesis and a gradual increase in the density of the capillary network, the volume and blood flow velocity of the damaged tissue should increase, which will improve oxygen delivery, plastic and energy substrates (Bender & Laughlin, 2015; Higashi, 2015).

There are many risk factors for mortality in duodenal injuries (blood loss, MODS, shock, vasopressors, surgical stress, development of abdominal sepsis, late MODS, others), and the bowel is known to be a “motor of MODS development”. The function of DT is very complex and many researchers have tried to develop different assessment systems to assess its severity in ICU. The AGI assessment proposed by the ESICM Working Group (Blaser, 2012), which includes abdominal signs and symptoms, assessment of intra-abdominal pressure, and organ function, is considered as an important indicator of the evaluation of DT function in patients who have been in ICU. This classification is now a classic and accepted by various medical societies. Understanding the pathophysiological basis of DT dysfunction in severe trauma in previous studies has shown that it can be the result of many factors that lead to enterocyte damage, and objective biomarkers of enterocyte damage, such as I-FABP, can be used to predict consequences of a number of critical conditions: acute decompensated heart failure and cardiac arrest (Kitai et al., 2017), septic shock and severe acute pancreatitis (Sekino et al., 2017), etc.

In trauma and hypoxia, changes in the duodenum are characterized by profound disorders of tissue metabolism, including local nutrient depletion, imbalance in tissue oxygen demand and generation of a large number of reactive nitrogen and oxygen intermediates (Taylor & Colgan, 2008). In this case, polymorphonuclear cells are activated by chemical signals such as interleukin 8, complement factor C5a, N-formulated peptides, platelet-activating factor, and leukotriene B4, which are formed at sites of active inflammation as part of the host's innate immune response and host micro-response (Pollard & Borisy, 2003). VEGF is one of the most important growth factors for regulating vascular development and angiogenesis and belongs to a family of homodimeric proteins consisting of at least 6 members: VEGF-A (VEGF), VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor – PI GF (Cross, Dixelius, Matsumoto, & Claesson-Welsh, 2003). It plays an important role in the proliferation, migration and activation of endothelial cells, as well as in enhancing vascular permeability. In this study, it was found that in patients with duodenum trauma there are wavy changes in the nature of VEGF content in the serum, which indicates the suppression of proliferation, reduced endothelial permeability, and the like.

Conclusions

Preliminary results of the study showed that serum I-FABP level is a valuable, convenient, objectively early predictor of TT injury in duodenal injury due to suppression of vascular proliferation, reduction of endothelial permeability, determined by the dynamics of VEGF changes in the treatment of patients, from the severity of condition and the presence of complications that require repeated surgery.

Funding source

This study was performed as part of the planned research work of the GA “V. T. Zaitsev Institute of General and Emergency Surgery of NAMS of Ukraine” and Kharkiv National Medical University (State registration number 0119U002909/15.07.2019).

Ethical approval

Permission for this study was obtained from the ethics committee of both institutions and informed consent was obtained from patients or their legal representatives.

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Angiogenesis: in organizing, organizing, organizing, organizing, organizing, organizing. 


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Литература


Зміни концентрації в сироватці крові фактора ангіогенезу VEGF і біомаркера кишкового бар’єру I-FABP при різних результатах лікування хворих на травми дванадцятипалої кишки

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Анотація

Вступ: Травми живота внаслідок тупого і проникаючого механізму виникнення мають виражений рівень смертності від крововиливів та сепсису. Діагностика та лікування пошкоджень дванадцятипалої кишки є однією із складних проблем екстреної хірургії живота, хоча вони рідкісні і становлять 0,2-0,6% всіх пацієнтів із травмою та 1-4,7% усіх травм живота.

Мета дослідження: Вивчити динаміку концентрацій в крові фактору ангіогенезу VEGF і маркера пошкодження травного тракту I-FABP у дорослих при різних післяоперативних наслідках травми дванадцятипалої кишки.

Матеріали і Методи: Проведено дослідження у 60 пацієнтів (середній вік 36 (18-73) років) з ізольованими і множинними травматичними ушкодженнями ДПК ≥ II ступеня згідно з критеріями AAST, оперованих за період 2006-2019 рр.

Результати: Середній рівень I-FABP також був вищим у хворих, що мали ускладнення та потребували повторного оперативного втручання та корегував з тяжкістю стану хворих, а за критерієм Спірмена порівняння були достовірними у всі трі періоди дослідження (p=0.002, р=0.000, р=0.001).

Висновки: Рівень сироваткового I-FABP є цінним, зручним, об’єктивним раннім підставником пошкодження травного при травмі дванадцятипалої кишки на тлі підвищення проліферації судин, зменшення проникності ендотелію, що визначається динамікою змін VEGF в процесі лікування даної категорії хворих, та вказує на тяжкість їх стану і наявності ускладнень, які вимагають виконання повторних оперативних втручань.

Ключові слова: такма дванадцятипалої кишки, фактор ангіогенезу VEGF, маркер пошкодження травного тракту I-FABP, динаміка змін.

Cite this article as:

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