

May Nesfatin-1 be a Biomarker in Acute Mesenteric Ischemia?

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ABSTRACT

Objective: To investigate the diagnostic value of nesfatin-1 in cases of intestinal ischemia and ischemia/reperfusion.

Study Design: An experimental study.

Place and Duration of Study: The Experimental Animals Laboratory of Bezmialem University, in June 2018.

Methodology: Twenty-one healthy male Sprague Dawley rats were randomly divided into three groups of 7 rats each. In group 1: 1-hour intestinal ischemia followed by 5-hour reperfusion was performed. In group 2: rats were subjected to 6-hour intestinal ischemia. In group 3: rats underwent laparotomy and closure without performing any further procedure. Changes in leukocyte count, amylase, blood sugar, LDH, SGOT, CRP, and nesfatin-1 levels were determined. For histopathological examination, a small intestinal sample was taken and preserved in 10% formaldehyde.

Results: Nesfatin-1 value in group 2 was significantly higher than that in group 1 and group 3 ($p=0.005$, and $p < 0.001$ respectively). Nesfatin-1 value in group 1 was significantly higher than that in group 3. A significant ($r = 0.864/p < 0.001$) positive correlation was observed between nesfatin-1 value and pathology score. The pathology score of group 2 was significantly higher than that of group 1 and group 3 ($p < 0.001$).

Conclusion: Serum nesfatin-1 can be a biomarker in acute mesenteric ischemia.

Key Words: Acute, Biomarker, Intestinal, Mesenteric Ischemia, Nesfatin-1, Reperfusion.

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INTRODUCTION

Acute mesenteric ischemia (AMI) is a clinical condition caused by sudden inadequate blood flow through any small intestinal segment accompanied by ischemia, cellular damage, and intestinal necrosis and can be life-threatening, if not treated. AMI is a rare cause of abdominal pain with a low overall incidence of approximately 0.09%-0.2% of patients admitted to the emergency department.¹

In AMI, there is severe abdominal pain that is non-proportional to the physical examination findings. In most cases, intestinal ischemia progresses transmurally and is diagnosed after peritonitis and sepsis develop. Certain imaging methods are also performed in case of clinical suspicion. X-ray imaging and abdominal ultrasonography have very limited diagnostic value, and abdominal computed tomography has low sensitivity and specificity. Contrast-enhanced CT may be used as the first-line imaging technique because of its excellent

reported sensitivity and specificity.² The American College of Gastroenterology guidelines define angiography as the gold standard in the diagnosis of mesenteric ischemia.³ However, the disadvantages of catheter angiography are: it is an invasive and time-consuming technique, and many hospitals are unable to perform it. Recent studies have reported that computed tomography angiography is a less invasive and less time-consuming method and suggested that it can be used as the gold standard for mesenteric ischemia diagnosis with its 96% sensitivity and 94% specificity.^{4,5} According to the guidelines of the World Society of Emergency Surgery, there are no laboratory studies that are sufficiently accurate to identify the presence or absence of ischemic or necrotic bowel, although elevated L-lactate and D-dimer levels may be indications (Recommendation 1B). Although biomarkers such as intestinal fatty acid-binding protein, serum alpha-glutathione S-transferase, and cobalt-albumin binding assay are reported as auxiliary tools for diagnosis, there are still no definite biomarkers.¹

Nesfatin-1, first discovered by Oh-I *et al.* in 2006, is an appetite-controlling peptide consisting of 82 amino acids and present in many nuclei of the hypothalamus, including the paraventricular nucleus.⁶ Metabolism that produces cytokines, such as IL-1 and tumor necrosis

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alpha, and adipose tissue, which is an important mediator of inflammation, also produce adipokines such as leptin, adiponectin, resistin, and visfatin.⁷ Nesfatin-1 is a recently discovered adipokine and is associated with obesity and metabolic syndrome. Studies have demonstrated the effects of nesfatin-1 on feeding behaviour, neuroendocrine regulation, autonomic control of visceral functions, development and differentiation of adipose tissue, inflammation, thermoregulation, pancreatic insulin secretion, glucose homeostasis in KC, sleep, attention, anxiety, and stress. In addition, it has also been reported to regulate gastric emptying, gastric acid secretion, gastric motility, and reproductive functions.⁸⁻¹¹

The objective of this study was to investigate the diagnostic value of nesfatin-1 in cases of intestinal ischemia and ischemia/reperfusion (I/R), which have not been previously studied in the literature.

METHODOLOGY

This experimental study was carried out at the Experimental Animals Laboratory of Bezmialem University, in June 2018 and approved by the Ethics Committee, Faculty of Medicine, Bezmialem University. In the study, 21 male Sprague Dawley rats weighing 370-480 g (standard pelleted diet) were randomly divided into three groups of 7 rats each.

In group 1, superior mesenteric artery (SMA) and superior mesenteric vein (SMV) were isolated by laparotomy, silk sutures were used for knotting, and the abdomen was closed. After 1 hour of ischemia, silk sutures on SMA and SMV were removed through relaparotomy under anesthesia, and 5 hours of reperfusion was allowed (Figure 1). In group 2, laparotomy was performed on the rats. Silk sutures were used for the knotting of SMA and SMV (exposed to 6-hour ischemia), and the laparotomy opening was closed (Figure 2). In group 3, laparotomy was performed, SMA and SMV were isolated, and the abdomen was closed without performing any further procedure (Figure 3).

After 6 hours, relaparotomy was performed under anesthesia in all animals, and pulsatility of SMA was assessed in the I/R group. Absence of pulsation was

considered as an exclusion criterion. Then, intracardiac blood samples were collected from all rats for biochemical examination; leukocyte count, amylase, blood sugar, LDH, SGOT, CRP, and nesfatin-1 levels were measured; then rats were killed. For histopathological examination, a small intestinal sample was taken and preserved in 10% formaldehyde.

Histopathological examination was performed by a pathologist who was blinded to the group assignments of the numbered samples. Microscopic examination was performed based on the scale developed by Chiu *et al.* using hematoxylin-eosin staining method (Table I).¹²

Table I: Histopathological examination.

Grade	Description
0	Normal mucosal villi.
1	Slight elevation of epithelium from lamina propria at the apex of villi.
2	Moderate elevation of epithelial layer from lamina propria.
3	Massive epithelial lifting down the sides of villi.
4	Denuded villi with lamina propria exposed and dilated capillaries.
5	Disintegration.

The SPSS 22.0 software was used for statistical analyses, and mean, standard deviation, median, minimum-maximum, frequency, and ratios were used in descriptive statistics. Distribution of the variables was tested using the Kolmogorov-Smirnov test. The One-Way ANOVA was used for the analysis of continuous independent variables and; if there was a statistical difference, post-hoc test was performed to find which group caused the difference. Also Kruskal-Wallis test used for ordinal independent variables. Spearman correlation test was used for the pathological and nesfatin value correlation and the statistical significance level was accepted as $p < 0.05$.

RESULTS

Mean pathology scores were 1.3 ± 1.3 in group 1, 4.4 ± 0.5 in group 2, and 0 ± 0 in group 3. Images of grades 0, 1, and 3 under 100× magnification with hematoxylin-eosin staining are shown in Figure 4. Mean nesfatin-1 values were 74.9 ± 30.3 in group 1, 137.1 ± 42.3 in group 2, and 35.7 ± 18.9 in group 3.



Figure 1: SMA and superior mesenteric vein (SMV) were isolated by laparotomy, silk sutures were used for knotting.

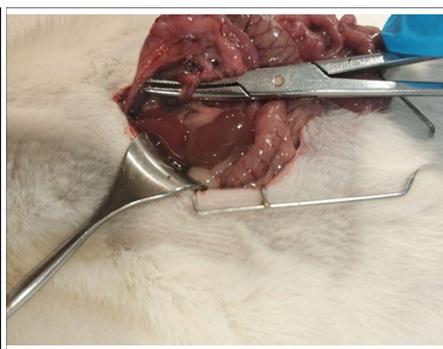


Figure 2: Isolation of SMA and superior mesenteric vein (SMV).

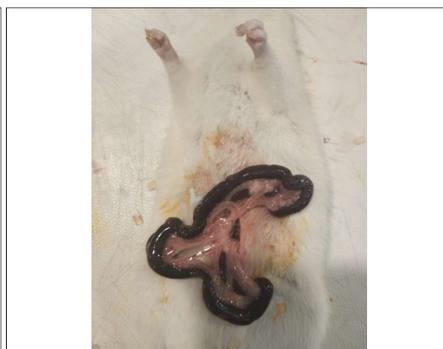


Figure 3: Ischemic intestines.

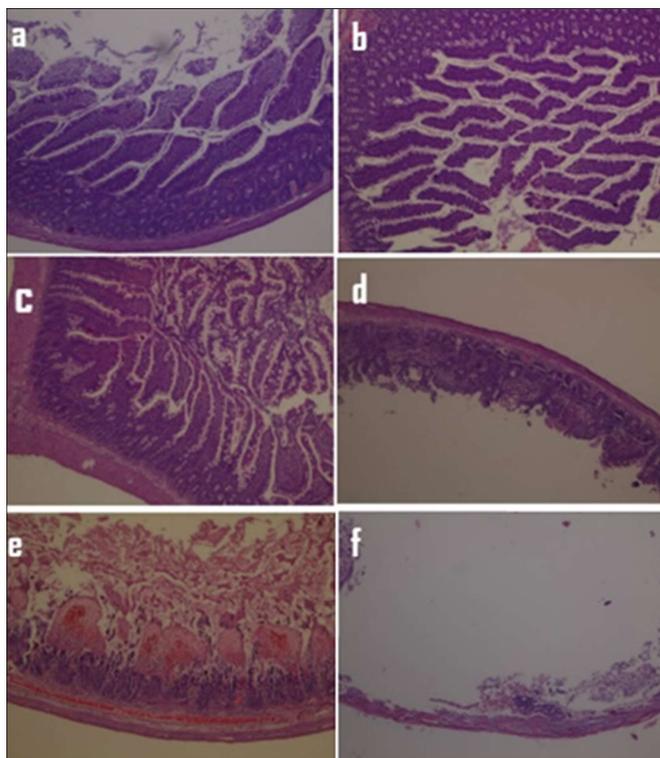


Figure 4: (a) Grade 0 (Hematoxylin-eosin, 100 x)
 (b) Grade 1 Hematoxylin-eosin, 100 x)
 (c) Grade 2 Hematoxylin-eosin, 100 x)
 (d) Grade 3 Hematoxylin-eosin, 100 x)
 (e) Grade 4 Hematoxylin-eosin, 100 x)
 (f) Grade 5 Hematoxylin-eosin, 100 x)

The pathology score of group 2 was significantly higher than that of group 1 and group 3 ($p < 0.001$).

Nesfatin-1 value in group 2 was significantly higher than that in group 1 and group 3 at the post-hoc test (respectively $p = 0.005$, $p < 0.001$). Nesfatin-1 value in group 1 was significantly higher than that in group 3 ($p = 0.026$).

A significant ($r = 0.864/p < 0.001$) positive correlation was observed between nesfatin-1 value and pathology score.

DISCUSSION

The mortality rates of AMI varying between 30% and 100% due to the absence of a specific diagnostic method for AMI.¹³⁻¹⁷ However, the absence of a biomarker for early diagnosis prevents the reduction of mortality rate.

Reperfusion is defined as the re-establishment of blood flow after ischemia, and this is necessary to prevent irreversible cell damage. In recent years, studies reporting nesfatin-1 as a protective peptide in cases of cardiac, renal, and intestinal ischemia have been published.¹⁸⁻²⁰ Dai *et al.* compared patients with acute myocardial infarct and patients with angina pectoris with a control group and found significantly lower nesfatin-1 plasma levels in the acute myocardial infarct group.²¹ The statistical analysis of our study revealed significantly

higher nesfatin-1 values in the ischemia group than in the other groups. In addition, the I/R group had significantly higher nesfatin-1 values than did the control group.

Intraperitoneal administration of nesfatin-1 was shown to have anti-inflammatory effect in acedid acid-induced gastritis model.²² Researchers have stated that nesfatin-1 had this effect through the inhibition of pro-inflammatory mediators as well as the establishment of a balance between oxidant and antioxidant systems. Ozturk *et al.* suggested in their ischemic colitis model that nesfatin-1 can exhibit an anti-inflammatory effect by preventing neutrophil infiltration into the tissue and by inhibiting free radical formation, and they added that it can have an antioxidant effect on oxytocin and ghrelin receptors and colitis.²³ Ayada *et al.* investigated the effects of chronic systemic nesfatin-1 administration on microcirculation effectors and oxidant-antioxidant states in an intestinal I/R model designed in rats.²⁰

In this study, nesfatin-1 balanced the oxidative state by decreasing the eNOS level and inhibiting the NO production.

Although this study revealed a positive correlation between nesfatin-1 values and pathology score, the causative mechanism could not be clearly understood. No definite data was obtained as to whether nesfatin-1 is produced as a result of an inflammation process or increased as a result of an anti-inflammatory response. In line with these findings, it can be interpreted that nesfatin-1 leads to an anti-inflammatory effect by inhibiting pro-inflammatory cytokines and establishes a balance in the oxidant-antioxidant system and can be a biomarker in acute mesenteric ischemia. Further studies are required on this aspect. Studies have suggested an association between nesfatin-1 and many diseases, and different mechanisms were addressed. The limitations of this study include its experimental design and limited number of rats.

CONCLUSION

In this study, nesfatin-1 levels were significantly higher in the AMI and I/R groups.

ETHICAL APPROVAL:

Ethical approval from the Ethics Committee of Bezmialem University was obtained prior to initiation of the study.

CONFLICT OF INTEREST:

Authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

CT: Concept, design, resource.
 FAA, UOI, AEN, SI, OA, CI, EB: Materials, data collection and/or processing, analysis and Interpretation, literature search, critical reviews.

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