

**FLUORESSEİN İLE INTRAOPERATİF BARSAK
CANLILIĞININ DEĞERLENDİRİLMESİ****ASSESSMENT OF INTRAOPERATIVE INTESTINAL
VIABILITY WITH FLUORESCEIN**

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ÖZET: Mezenter iskemisine bağlı barsak hastalığında demarkasyon hattının net olmadığı durumlarda intraoperatif barsak canlılığının değerlendirilmesi ve sınırlarının tespiti güçtür. Bu deneysel çalışmada, değişik mezenterik iske mi modelleri oluşturularak barsak iske mi derecesinin intraoperatif olarak değerlendirilmesinde yardımcı bir tanı yöntemi olan flouressein flouresansının yeri araştırıldı. Wistar - Albino erkek ratlarda strangülasyon, superior mezenterik arter ligasyonu ve kontrol grupları oluşturuldu. İki saat sonra intraoperatif gözlem bulguları ile barsakların canlılığı değerlendirildi. Kuyruk veninden 15 mg/kg flouressein intravenöz verildi. İki-üç dakika sonra karanlık odada 3600 A Wood ışığı altında flouressein flouresans derecesine göre barsak canlılığı değerlendirildi. Bütün gruplarda intraoperatif gözlem bulguları ve flouressein flouresans bulguları karşılaştırıldı. Tüm deneklerde, transmural nekroz varlığı düşünülen veya canlı olarak kabul edilen barsak segmentlerinden örnekler alınarak histopatolojik değerlendirme yapıldı. Flouressein flouresans yönteminin barsak iskemisini göstermede duyarlılığı %100, özgüllüğü %92 ve doğruluk oranı %96 olarak saptandı. Klinik değerlendirmede aynı değerler sırasıyla %76, %92, %83 olarak tespit edildi. Mezenter iskemisine yol açan emboli, tromboz, strangülasyon gibi barsak canlılığının şüpheli olduğu vakalarda ucuz ve pratik bir yöntem olan flouressein flouresansının kullanılması rezeksiyon sınırlarının tayininde ve gereksiz barsak rezeksiyonunun önlenmesinde faydalı olabilir.

Anahtar kelimeler: Mezenterik iske mi, barsak canlılığı, flouressein.

SUMMARY : The assessment of intraoperative intestinal viability and the determination of ischemic borders are difficult in situations where the demarcation line is not clear in intestinal disease due to mesenteric ischemia. In this experimental study, the value of fluorescein fluorescence, which is a helpful diagnostic procedure in the detection of the degree of damage in ischemic injuries of the intestine in the intraoperative period was sought by means of forming various mesenteric ischemia models. A strangulation group, a superior mesenteric artery ligation group and a control group were formed in Wistar-Albino male rats. Two hours later, intestinal viability was assessed via intraoperative observation findings. Fluorescein (Fluorescite Injection ampul 10%) was given intravenously with a dose of 15 mg/kg through the tail vein. Two-three minutes later, the intestinal viability was assessed in a dark room under wood light of 3600 A. In all groups, intraoperative observation findings and fluorescein fluorescence findings were compared. Histopathologic evaluation was performed in the samples taken from ischemic or normal intestines of all of the subjects. The accuracy of the methods was tried to be assessed by means of performing histopathologic evaluation. In the study, the sensitivity of the fluorescein fluorescence method in showing intestinal ischemia was found to be 100%, the specificity 92% and the accuracy 96%. In the clinical assessment, the same values were found to be 76%, 92%, 83%, respectively. In intestinal ischemic disorders such as embolism, thrombosis, and strangulation; if intestinal viability is questionable, fluorescein fluorescence which is a cheap and practical method can be useful in determining resection borders and preventing unwarranted intestinal resections.

Key words: Mesenteric ischemia, intestinal viability, fluorescein

In intestinal ischemia cases caused by various etiologic reasons, difficulties are encountered in the intraoperative prediction of the degree of intestinal ischemia and in the

surgical decision made as a consequence of this prediction. It was claimed that predicting intestinal viability solely based on clinical observations could lead to mistakes that would result in unnecessary intestinal resections (1,2). It was reported that investigative methods assessing viability such as doppler ultrasonography, intraluminal pH measurement, venous clearance, phosphocreatinin and ATP analysis, measurement of serosal oxygen pressure with surface oxymetry, and fluorescein injection would yield more accurate results in the intraoperative prediction

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Aksaray - İSTANBUL

(1,2,3,4,5,6) . The utilization of fluorescein in the prediction of intestinal viability is reported to be a practical, fast and cheap method with a high rate of accuracy (1,4,6).

The rate of cardiovascular diseases has risen in today's world because of alterations in nutrition and the increased consumption of alcohol and smoking. As a consequence of the improvement in therapy methods, those people, who could die of their primary disease, live long enough to have mesenteric ischemia (7). Resection for various reasons is performed on intestines which play an important role in digestion. When the short-bowel syndrome is taken into account, especially in recurrent resections and resections performed for ischemic disorders, the few centimeters of bowel which is protected will be crucial in patient nutrition. Although diagnostic methods such as fluorescein fluorescence have been utilized to assess intestinal viability intraoperatively aside from clinical findings, they have not been exercised commonly. In this experimental study in which we formed various ischemia models different from the previous ones, we aimed to evaluate the feasibility of the fluorescein method and the results of our study in light of the literature.

MATERIAL - METHOD

This study was conducted in DETAM. In the study, 30 Wistar-Albino male rats weighing 200-250 gr in average were used. The subjects were separated into three groups: The control group (Group I, n:10), the strangulation group (Group II, n:10), and the superior mesentery artery (SMA) ligation group (Group III, n:10). Under ether anesthesia, median laparotomy of 3 cm was performed on the subjects. In the control group, simple ligation with 2/0 silk was done 2 cm proximal to the caecum.

In the strangulation group, volvulus was applied to an intestinal segment of 8 cm beginning from 2 cm away from the caecum and it was tied with its mesentery without forming a tight knot which ensures sufficient pressure for strangulation by means of 2/0 silk (as performed in a strangulated hernia to form the hernia neck).

In the group undergoing superior mesenteric artery ligation (SMAL), the SMA was explored at the site where it

originates from the aorta and it was tied with a knot by means of 3/0 silk. The abdomen was closed as one layer with 3/0 atraumatic silk. Relaparotomy was performed 2 hours later and silk knots were untied. After 15 minutes, the viability of the intestines was noted by assessing it according to standard clinical criteria. Fluorescein (Fluorescite Injection ampul 10%) was given intravenously with a dose of 15 mg/kg through the tail vein. Two-three minutes later, the lights were switched off and the intestinal viability was assessed in a dark room under wood light of 3600 A according to table 1 which was formed by modifying the Bulkley (1) table. Under Wood light, the normally perfused tissues emit bright golden yellow-green fluorescence; the fluorescence of the tissues with reduced perfusion (ischemia) diminishes or disappears and these segments have a dark blue color. Tissue samples were taken from the segments, which were thought to be nonviable or viable for histopathologic investigation and they were treated with 10% formalin following cleansing with physiologic serum.

Intestinal segments in which no necrosis was detected or having mucosal necrosis histopathologically were evaluated as viable, segments with transmural necrosis were evaluated as nonviable.

Picture 1A



Picture 1 B



Table 1. Patterns of Fluorescein Fluorescence

Degree-Pattern	Intensity	Predicted Outcome
1-Hyperemic	Increased	Viable
2-Normal	Normal	Viable
3-Fine granular	Normal to Slightly Decreased	Viable
4-Patchy	Decreased	Nonviable
5-Perivascular or Hypofluorescent	Non	Nonviable

Table 2. The assessment of the fluorescence of the simple ligation group according to clinical criteria and histopathologic investigation. Negative result (-) shows viability and positive result (+) shows nonviability.

Subject No	Clinic assessment	Fluorescein Assessment	Fluorescence Pattern	Histopathologic evaluation	
				Necrosis	Result
1	-	-	2	absent	viable
2	-	-	2	absent	viable
3	-	-	2	absent	viable
4	-	-	2	absent	viable
5	-	-	3	mucosal	viable
6	-	-	2	absent	viable
7	-	-	2	absent	viable
8	-	-	1	absent	viable
9	-	-	2	absent	viable
10	-	-	2	absent	viable

Within the framework of histopathologic findings, the efficacy of clinical assessment and fluorescein fluorescence methods in determining intestinal necrosis was sought. With that purpose, the sensitivity, specificity and accuracy of the two methods were compared.

RESULTS

The results of clinical assessment and fluorescein method in the simple ligation group were found to be in concert with histopathologic findings and the intestines were evaluated as viable in all subjects (Table 2). In 1 subject, fine granular fluorescence (3.degree) was present and solely mucosal necrosis was detected histopathologically.

In all but one subjects in the strangulation group, transmural necrosis appearance was present clinically in the intestines (dilated, thin-walled, violet, no peristalsis) (Picture 1 A, Picture 1 B). One case was evaluated as clinically viable because of the detection of the

disappearance of the dark color, and resurgence of the peristalsis. However, this segment was evaluated as nonviable because of existence of patchy fluorescence (4.degree) and transmural necrosis was observed histopathologically in this case. Hypofluorescence was detected in 9 of the subjects in this group and patchy fluorescence was observed in one. The intestines were evaluated as nonviable (true positive) by means of fluorescein assessment and these results coincided with histopathologic findings (Table 3).

In the clinically assessment of the SMAL group, the intestine was evaluated as viable because of the presence of pulsation in the arteries closed to the intestinal wall following revascularization in three subjects; however, transmural necrosis was observed (false negative). In one case, the intestine was evaluated as nonviable; however, only mucosal necrosis was detected histopathologically, there was no transmural necrosis (false positive). Fluorescein assessment was also mistaken in this subject;

Table 3. The assessment of the fluorescence of the strangulated group according to clinical criteria and histopathologic investigation .

Subject No	Clinic assessment	Fluorescein Assessment	Fluorescence Pattern	Histopathologic evaluation	
				Necrosis	Result
1	+	+	5	transmural	nonviable
2	+	+	5	transmural	nonviable
3	+	+	5	transmural	nonviable
4	+	+	5	transmural	nonviable
5	-	+	4	transmural	nonviable
6	+	+	5	transmural	nonviable
7	+	+	5	transmural	nonviable
8	+	+	5	transmural	nonviable
9	+	+	5	transmural	nonviable
10	+	+	5	transmural	nonviable

Table 4. The assessment of the fluorescence of the AMLS group according to clinical criteria and histopathologic investigation

Subject No	Clinic assessment	Fluorescein Assessment	Fluorescence Pattern	Histopathologic evaluation	
				Necrosis	Result
1	-	-	3	mucosal	viable
2	+	-	3	mucosal	viable
3	-	+	5	transmural	nonviable
4	+	+	5	transmural	nonviable
5	+	+	5	transmural	nonviable
6	-	+	4	transmural	nonviable
7	+	+	5	transmural	nonviable
8	+	+	5	transmural	nonviable
9	-	+	5	transmural	nonviable
10	-	+	4	mucosal	viable

the intestine was evaluated as nonviable (false positive) because patchy fluorescence (4.degree) was detected (Table 4) (Picture 2 A, 2 B).

Comparing the standard clinical assessment and fluorescence results of all groups (30 subjects) with the histopathologic findings yielded the following outcomes: by means of clinical assessment, 12 true negative, 14 true positive, 4 false negative and 1 false positive results were obtained. By means of fluorescein assessment, 13 true negative, 16 true positive and 1 false positive results were obtained.

According to these results, sensitivity, specificity and accuracy rates of clinical assessment were found to be 76%, 92%, and 83%, respectively. By means of fluorescein assessment, these rates were found to be 100%, 92%, and 96%, respectively.

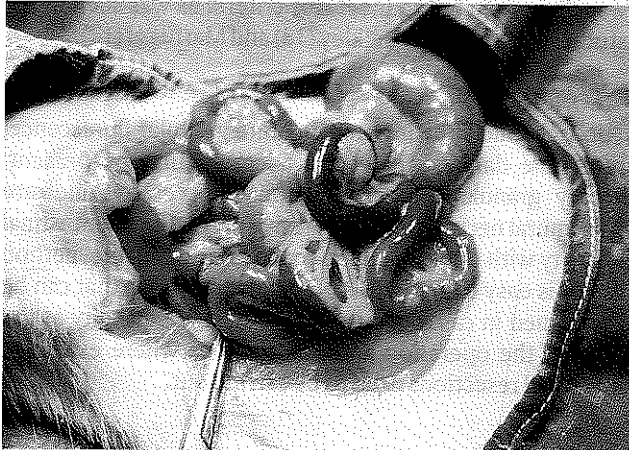
DISCUSSION

For a successful surgical therapy in mesenteric ischemia cases, the borders of the ischemic intestine must be determined accurately within the intraoperative period. Perforation and sepsis are inevitable unless all of the

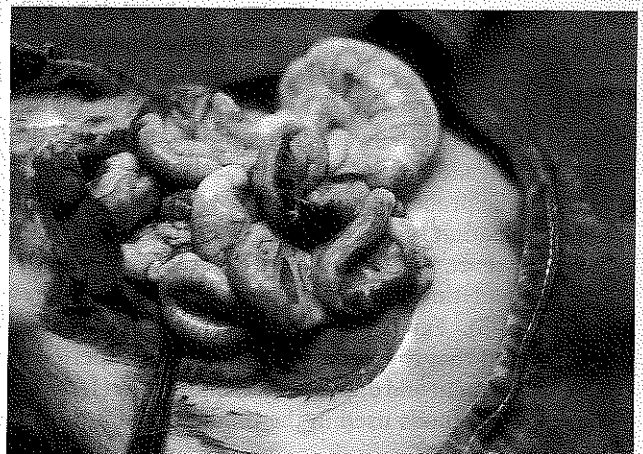
nonviable intestinal segments are resected. Intestine that is viable, but having an irreversible damage confined to a portion of the wall may later develop ischemic colitis, fibrosis or stricture (5,8). On the other hand, resection of long lengths of ischemic intestine which is able to survive may unnecessarily result in the short-bowel syndrome (5,7). Thus, preservation of even a few extra centimeters of small intestine may contribute to the quality of life of the patients. The difficulty of predicting intestinal viability intraoperatively in intestinal ischemia cases is known. This situation becomes more complicated in the border-line cases where intestinal necrosis does not develop totally, but there is doubt as to the viability of the intestine (5).

Surgeons, classically, intervene taking into account what the etiology is. After performing an operation for strangulation, volvulus, and vessel occlusion (embolism, thrombosis) which cause intestinal ischemia, they wrap the intestines having an ischemic appearance via warm pads. They assess the intestine after 10-20 minutes of observation. In this assessment, classical criteria for intestinal viability such as color, arterial pulsation, peristaltic activity, and the presence of active bleeding

Picture 2 A



Picture 2 B



from the edges after resection are used. This assessment might not always provide the accurate results. The detection of pulsation in the mesenteric arteries following revascularization of the intestinal segment does not necessarily mean that no necrosis will develop: Irreversible necrosis might have developed already (5). In recent years, nonocclusive mesenteric ischemia is being increasingly seen and constitutes approximately 20% of the disorders that cause mesenteric necrosis (4). In our study, transmural necrosis was detected in the intestines of three subjects, which were evaluated as viable because of the presence of pulsatile arteries. Hence, in the determination of intestinal viability, the presence of mesenteric artery pulsation may not be a reliable clinical criterium.

Peristaltism stops in the ischemic intestine and resurges following revascularisation. However, the anoxic spasms of the nonviable intestine may sometimes be confused with peristaltism and lead to mistakes (5). In our study, in 1 case of the strangulation group, the intestine was evaluated as viable because of the presence of the peristaltism, however, the histopathologic investigation revealed transmural necrosis.

Only in serious necroses (dilated intestine, thin-walled, dark color), nonviability can accurately be diagnosed by means of inspection of the intestine color. In our study, clinical assessment results of the strangulation group in which there was a serious degree of ischemia were found to be better than the ones of the SMAL group.

As a result of the intraoperative assessment, which was done by Bulkley et al. (1) using clinical criteria, it was reported that sensitivity was 78%, specificity was 91%, and unnecessary resections were done in 46% of the cases. Because clinical criteria are subjective and the results are not reliable, clinicians have needed objective diagnostic methods to be able to establish accurate diagnoses intraoperatively in mesenteric ischemia cases and created various methods (1,4,5). In 1942, Lange and Boyd (9) reported that when a vital dye was administered through a peripheral vein, the perfused tissues gave fluorescence under ultraviolet light and intestinal viability could be accurately predicted with sodium fluorescein fluorescence. Studies following this one have supported this conclusion (10,11,12). Stolar ve Randolph (13) described the characteristic images of this technique.

In doppler ultrasonography which is one of the intraoperative diagnostic methods intestinal viability can be evaluated through investigating the blood flow in the mesenteric arcade and in the small vessels in the intestinal wall via sterile probe, and the sensitivity of the method in the determination of the ischemic intestinal segments is 88% (6). However, its usage is limited because of being an expensive and time-consuming method which requires experience (5). Furthermore, in two studies, it was demonstrated that the fluorescein method is superior to clinical assessment and doppler ultrasonography (1,2).

The other methods used in the prediction of intestinal

viability intraoperatively comprise pH measurement and tonometry (14), photpletismography (6), measurement of serosal oxygen pressure with surface oxymetry (15), myoelectric analyses (16) and phosphocreatinin and ATP analysis (17). These methods are rather used for experimental purposes (17).

In the study conducted by Gorey et al. (4), it was reported that the rate of accuracy of fluorescein assessment was 95% and the specificity was 96%, however in venous occlusion cases, false positivity was reported in 17% of the cases. This connotes that there may be unwarented resections. In Akaydin's study (3), the sensitivity of the assessment done by means of fluorescein was reported to be 100%, specificity 88% and the rate of accuracy 93%.

Pearse et al. (6) found the sensitivity with fluorescein to be 88%, however reported the existence of irreversible ischemia (necrosis) histologically in the portion that was dyed normally with fluorescein in 2 of their cases. The same researchers have claimed that fluorescence formed late in these 2 cases and that the dye perfused into the domain where there was mucosal necrosis and hence assessment needed to be done in 2-3 minutes. The lack of false negative results in our study may be due to the shortness of this period.

Carter et al. (19) advocate that ischemic tissue damage develops faster and is more severe in strangulated obstructions than isolated arterial occlusions. Hence, they claim that the successful results obtained with fluorescein in the prediction of ischemic damage due to arterial occlusion can not be extrapolated to the other ischemia models. Because of this, in our study, a control group was formed as well as a SMAL group in which there was intermediate ischemia and a strangulation group with severe tissue damage due to ischemia. It was demonstrated in both groups that the fluorescein technique was superior to clinical assessment in the prediction of viability which is consisted with the other studies.

A method that will predict intestinal viability in the operating room should meet such basic criteria as being easily accesible, practical, cheap, harmless, reliable and easy to interpret (10). In our study, the fluorescein method was demonstrated to meet this criteria as was seen in the studies conducted by Bulkley (18) and Marfuggi (12).

CONCLUSION

In ischemic intestins emitting fine granular flurescence, reversible damage (mucaosal necrosis) may be present. Patchy granular fluorescence may not always indicate transmural necrosis. Intraoperative prediction of intestinal viability with fluorescein is a practical and reliable method which is easy to apply. It is one of the methods that can be performed in cases of suspected intestinal viability at the time of the operation and in situations where extended intestinal resections need to be carried out.

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