

The Effects of Kefir and Enteral Feeding Products on Colonic Anastomosis: Experimental Study

Hakan Yigitbas¹, Mustafa U Kalayci¹, Mehmet Abdussamet Bozkurt¹, Ahmet N Turhan¹, Selin Kapan¹, Halil Alis¹, Ersan Aygun¹, Hafize Uzun², Habibe D Genc²

¹Bakırköy Dr. Sadi Konuk Training and Research Hospital, General Surgery Clinics, İstanbul

²İstanbul University, Cerrahpaşa Medical Faculty, Laboratory of Biochemistry and Hematology, İstanbul

ÖZET

Kefir ve enteral beslenme ürünlerinin kolon anastomozu üzerine etkileri: Deneysel çalışma

Amaç: Kefir, tıbbi etkileri zengin ve etkili verilerle kanıtlanmış probiyotik beslenme ürünüdür. Kefirin antimikrobiyal, skatrizan ve anti-kanser etkileri ile ilgili pek çok çalışma olmasına rağmen intestinal anastomozların postoperatif iyileşme sürecindeki iyileştirme etkisi ve patlama basınçları ile ilgili çalışma yoktur. Çalışmamızda enteral beslenme ürünleri olan Kefir (Altinkılıç) ve Ensure'un (Abbott) kolonik anastomozlardaki iyileşmeye etkisi incelenmiştir.

Gereç ve yöntem: Çalışmada 40 adet dişi albino Wistar sıçan kullanıldı. Sıçanlar şam grup (grup A), anastomoz grubu (grup B), anastomoz sonrası kefir grubu (grup C), ve anastomoz sonrası ensure grubu (Grup D) olarak dörde ayrıldı.

Bulgular: Gruplar arasında hemoglobin, hematokrit, lökosit ve trombosit seviyelerinde anlamlı fark tespit edilmedi ($p>0.05$), ancak total protein seviyesinde gruplar arasında anlamlı fark tespit edildi ($p<0.05$). Kefir grubunda patlama basınçları şam gruptan ($p:0.003$, $p<0.01$) ve anastomoz grubundan ($p:0.001$, $p<0.01$) fazla bulundu. Ensure grubunda patlama basınçları şam grubundan ($p:0.035$, $p<0.05$) ve anastomoz grubundan ($p:0.035$, $p<0.05$) anlamlı olarak yüksek tespit edildi. Hidroksiprolin seviyeleri şam grupta diğer tüm gruplardan (kefir- $p:0.004$, $p<0.01$, ensure- $p:0.002$, $p<0.01$, anastomoz grubu- $p:0.001$, $p<0.01$) istatistiksel olarak anlamlı yüksek idi.

Sonuç: Kefirin akademik olarak kullanılmasında yeni çalışmalara ihtiyaç duyulmaktadır. Daha fazla klinik çalışma ile kefirin preoperatif kullanımının postoperatif mortalite ve morbidite oranlarını azaltacağı görüşündeyiz.

Anahtar kelimeler: Kolon anastomozu, enteral beslenme, kefir

ABSTRACT

The effects of kefir and enteral feeding products on colonic anastomosis: Experimental study

Objective: Kefir is rich and affective probiotic feeding material with evidence based medical effects. There are many studies about antimicrobial, scatrizant, anticancer effects of Kefir but there is not any study on anastomotic burst pressure and healing effects on intestinal wall around anastomosis in the postoperative period (8). In this study the efficacy of Kefir (Altinkılıç) and Ensure (Abbott) as enteral feeding products as colonic anastomotic healing has been investigated.

Material and Methods: In this study 40 Wistar-albino female rats were used. Rats were divided into 4 groups as sham group (Group A), anastomosis group (Group B), kefir after anastomosis group (Group C), and ensure after anastomosis group (Group D).

Results: There was no significant difference between the hemoglobin, hematocrit, leukocyte and thrombocyte levels of the groups ($p>0.05$) whereas there was statistically significant difference between the total protein levels of the groups ($p<0.05$). In the Kefir group bursting pressure was measured higher than in sham ($p:0.003$, $p<0.01$) and anastomosis group ($p:0.001$, $p<0.01$). In the Ensure group bursting pressure was measured statistically higher than in sham ($p:0.035$, $p<0.05$) and anastomosis ($p:0.035$, $p<0.05$) groups. In the sham group hydroxyproline levels were statistically higher than in anastomosis ($p:0.001$, $p<0.01$), Kefir ($p:0.004$, $p<0.01$) and Ensure ($p:0.002$, $p<0.01$) groups.

Conclusion: Kefir is currently being used for academically purposes as further clinical studies are needed. With more clinical studies preoperative use of Kefir would reduce postoperative mortality and morbidity rates.

Key words: Colonic anastomosis, enteral feeding, Kefir

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INTRODUCTION

There is not a consensus on timing and method of feeding after the abdominal operations with

Yazışma adresi / Address reprint requests to: Hakan Yiğitbaş
Bakırköy Dr. Sadi Konuk TRH, General Surgery Clinics, İstanbul

Telefon / Phone: +90-533-261-1310

Elektronik posta adresi / E-mail address: drhakanyigitbas@yahoo.com

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anastomosis of luminal organs. General concept suggests early enteral feeding in the postoperative period due to various physiological causes as to obtain better use of feeding products biologically, to prevent mucosal atrophy, to keep intestinal contents and immune response (1). It is suggested that early enteral feeding after gastrointestinal surgery reduces catabolic consequences of surgical stress (2,3). Comparison of enteral feeding with intravenous crystalloid or total parenteral nutrition in the care of trauma or critically ill

patients demonstrated that enteral feeding decreases risk of septic complications (4,5). Major causes of mortality and morbidity after colonic anastomoses are delay in healing of anastomosis and anastomotic leak. Anastomotic dehiscence with resulting pelviperitoneal sepsis is the most important and devastating complication in colorectal surgery (6). Appropriate feeding regimen seems to affect anastomotic healing in a good manner. Postoperative early enteral feeding increases anastomotic resistance and collagen synthesis significantly (7). Due to the structural integrity and physiological characteristics, prebiotics and probiotics are gaining acceptance in current feeding regimens. Kefir is rich and effective probiotic feeding material with evidence based medical effects. There are many studies about antimicrobial, scartzant, anticancer effects of Kefir but there is not any study on anastomotic burst pressure and healing effects on intestinal wall around anastomosis in the postoperative period (8). In this study the efficacy of Kefir (Altinkılıç) and Ensure (Abbott) as enteral feeding products as colonic anastomotic healing has been investigated.

MATERIAL AND METHODS

In this study 40 Wistar-albino female rats were used. Rats were taken into cages in groups consisting of 5 rats and arranged a life cycle of 12 hours of day following 12 hours of night. Rats were divided into 4 groups as sham group (Group A), anastomosis group (Group B), kefir after anastomosis group (Group C), and ensure after anastomosis group (Group D). All of the operations were performed by the same surgeon with a standard technique. Ketamine 10% 50 mg/kg and ksilazine 2% 10 mg/kg were used for anesthesia and analgesia. In group A, after median laparotomy abdominal wall and skin were closed with No: 0 continue silk sutures. In other groups after median laparotomy, a colotomy proximal to peritoneal reflection was anastomozed with a 5/0 synthetic absorbable polyglycolic acid interrupted suture. After irrigation of abdominal cavity with 2cc saline solution, abdominal wall and skin was sutured with no: 0 separately. After postoperative first day rats in group A and B were fed with 3 cc tap water through a no: 5 orogastric feeding tube, rats in group C were fed with 3 cc kefir and rats in group D were fed with 3 cc Ensure in the same manner. After a 7 day period of orogastric feeding

animals did not gain any weight, median thoracotomy and laparotomy under ether anesthesia was performed. Approximately 6 cc intracardiac blood was obtained and rats were sacrificed under deep ether anesthesia. After abdominal exploration rats in group B, C and D regions of anastomosis was found. In group A, a segment of 4 cm long, 3 cm above peritoneal reflection was resected. In other groups a colonic segment of 2 cm above and 2 cm below anastomosis was resected en-blok. No adhesiolysis performed and bursting pressure was measured with a silastic catheter tied up into both ends of colonic segment with 2/0 silk sutures in a tank of water performed ex-vivo. For group A rats, 1 cm colonic segment was resected and lumen was divided and separated for histopathological and biochemical studies. In the other groups, 1 cm anastomotic segment was resected and divided into two parts longitudinally. Tissues were fixed in 10 % formaldehyde solution for histopathological studies. Tissue samples were kept at -220C until tissue hydroxyproline level was measured.

Caloric intake for kefir in 100 ml was 58 calories and protein intake was 4 gr meanwhile caloric intake for ensure was 106 calories and protein intake was 4 gr in 100 ml. Although kefir had fewer calories than ensure, calories were equalized by adding equivalent amount of sucrose. Group A and B were fed with regular rat cow and tap water ad libitum. Group C and D were fed with Kefir and Ensure once a day through orogastric feeding tube in addition to regular rat cow and tap water ad libitum.

Blood samples were taken and hemoglobin, hematocrit, leukocyte, thrombocyte, total protein, serum albumin, C reactive protein (CRP), sedimentation levels were measured in Biochemistry and Hematology Laboratory. Tissues were histopathologically examined by Pathology Laboratory. Tissue hydroxyproline levels were measured by Bergmann and Loxley method at Biochemistry Laboratory. After weighting, tissues were homogenized in 5 ml HCL (6N); their absorbencies were evaluated by Shimadzu UV-120 spectrophotometer subsequently.

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) version 10.0 software package. Due to inadequate essential assumptions quantitative data comparing was made by Kruskal Wallis test and for the different group determination Mann Whitney U test was used. The difference was considered significant if $p < 0,05$.

RESULTS

This experimental study included 40 rats. Rats were divided into 4 groups as, Group A "Sham" (n=10), Group B "Anastomosis" (n=10), Group C "Kefir" (n=10), Group D "Ensure" (n=10). During the course of study one rat from sham group, 2 rats from anastomosis group, 1 rat from kefir group died. Statistical analyses were made with 9 rats of sham group, 8 rats of anastomosis group, 9 rats of kefir group, and 10 rats of ensure group. There was no significant difference between the hemoglobin, hematocrit, leukocyte and thrombocyte levels of the groups ($p>0.05$) whereas there was statistically significant difference between the total protein levels of the groups ($p<0.05$). Binary comparisons showed reduced total protein levels in anastomosis group than in sham group ($p:0.002$, $p<0.01$ respectively) and same as in ensure group than in kefir group ($p:0.04$, $p<0.05$ respectively). There was no significant difference between the total protein levels of the other groups ($p>0.05$). There was statistically significant difference between the serum albumin levels of the groups ($p<0.05$). Binary comparisons showed elevated serum albumin levels in sham group than in anastomosis group ($p:0.01$, $p<0.05$ respectively) and same as in sham group had highly elevated serum albumin levels than in kefir group ($p:0.006$, $p<0.01$ respectively). There was no significant difference between the albumin levels of the other groups ($p>0.05$). There was no significant difference between the CRP levels of the groups ($p>0.05$). There was significant difference between the bursting pressure of the groups ($p<0.01$). In the Kefir group bursting pressure was measured higher than in sham ($p:0.003$, $p<0.01$) and anastomosis group ($p:0.001$, $p<0.01$). In the Ensure group bursting pressure was measured statistically higher than in sham ($p:0.035$, $p<0.05$) and anastomosis ($p:0.035$, $p<0.05$) groups. There was no significant difference between the bursting pressures of the other groups ($p>0.05$). There was significant difference between the tissue hydroxyproline levels of the groups ($p<0.01$). In the sham group hydroxyproline levels were statistically higher than in anastomosis ($p:0.001$, $p<0.01$), Kefir ($p:0.004$, $p<0.01$) and Ensure ($p:0.002$, $p<0.01$) groups. In the anastomosis group hydroxyproline levels were measured less than in Ensure group but it was not statistically significant ($p:0.076$, $p>0.05$). There was no significant difference between the hydroxyproline

levels between the Kefir group and the anastomosis and Ensure groups ($p>0.05$).

Histopathological Examination

Before the histopathological examination tissue samples taken from the rats were fixed in 10 % formaldehyde solution embedded in paraffin blocks. Paraffin blocks were sectioned into 5 microns and stained with haematoxylin–eosin for evaluation. In the group which sham operation was performed, mucosal and submucosal dense mononuclear inflammation cell infiltration, lymphoid follicular bodies with evident germinal centers and mature lymphoid infiltration in mucosal and submucosal lymphatics were seen. After the anastomosis in the group which tap water was given orogastrically, blood and fibrin masses at the intestine surface, polymorphonuclear (PMN) leukocyte aggregates with active ulcer ground including cell debris, increased fibrous tissue at the intestine wall, capillary proliferation with evident endothelia, lymphoplasmocyte inflammation cell infiltration with granulation tissue including histiocyte aggregates were observed.

After the anastomosis in the group which Ensure was given orogastrically, exudate including PMN leukocyte aggregated at the intestine surface with fibrin masses in cell debris areas. Furthermore PMN leukocyte aggregates at the intestine surface, increased fibrous tissue at the intestine wall, capillary proliferation with evident endothelia, lymphoplasmocyte inflammation cell infiltration were seen.

After the anastomosis in the group which Kefir was given orogastrically, erosion in the intestine epithelium, PMN leukocyte aggregates in the intestine epithelium, increased fibrous tissue at the intestine wall, capillary proliferation with evident endothelia, PMN leukocyte aggregates with lymphoplasmocyte inflammation cell proliferation were seen.

DISCUSSION

Enteral feeding after elective colorectal surgery is tolerable and safe way for the most of the patients (9). Kefir is known in many countries and is cheap, easy to prepare and approved consumer good. Therefore it's much tolerable and much more preferable than fabric nutrients, as primary enteral feeding. Kefir is North Caucasian

originated milk product. It is known to be made from cow, lamb and goat milk to fresh up, but little is known about its origin. It's suggested that Kefir was made in the Elburus mountain piedmonts and kept secret until a "Kefir" book published in Russia was translated to German in 1884 and recognized in Europe (10). Antibacterial components of Kefir, like acetic acid, H₂O₂ and antibiotics have antibacterial effects to pathogen bacteria like E.Coli and Salmonella. Microorganisms that Kefir contains, reduces colon cancer risk by reducing fecal enzyme activity. This special feature is achieved by antimutagenic and immunomodulator effects together (11-14).

Colon lumen has more microorganisms than other parts of the gastrointestinal system, therefore, complications originating from separation of colon anastomosis are much more in addition to increased sepsis risk (15,16). Healing period is delayed by microorganisms. Shen et al. studied the effects of enteral feeding combined with probiotics and find out that epithelial tight junction areas and microvillies were more intact than in parenteral feeding (17). Bacterial translocation in blood, lymph nodes in the liver, lungs, mesenteric nodes and endotoxin levels were significantly lower in enteral feeding with probiotic group than in parenteral feeding group (17).

Acetic acid has an antibacterial effect on the bowel bacteria. The microorganisms inside the Kefir produce numerous bacteriocyne. A study by Morgan et al. evaluated that Kefir had an antiproliferative effect on *Listeria innocua* and *Escherichia coli* O157:H45 (18).

Previous studies reported that Kefir is effective on pathogen bacteria such as *Salmonella*, *Helicobacter*, *Shigella*, *Staphylococcus* and has some antiinflammatory activities. Rodrigues et al. had a research on Kefir and Kefir extract's antimicrobial and healing effect and showed that Kefir biofilms with polysaccharide components are good antimicrobial, antiinflammatory and scarring agents (8). In our study CRP, leukocyte and thrombocyte values showed that Kefir at least had no augmented inflammation effect.

As a matter of fact, pathologic evaluation showed no additive inflammation around the wound.






Cronin et al. demonstrated that by the postoperative third day bursting pressure measurements increased gradually, reached maximum levels on 7-10 days whereas hydroxyproline concentration at the anastomosis tissue decreased 40% and by 5th day reaches to normal levels, and by 10-14 days reaches over the normal levels (19). In our study bursting pressure measurements were made at the 7th postoperative day based on these facts. In our study there were statistically significant difference in bursting pressures between groups ($p < 0.01$). Kefir group's bursting pressures ($p: 0.003$; $p < 0.01$) were statistically higher than sham and anastomosis group ($p: 0.001$; $p < 0.01$). The bursting pressures of Ensure group ($p: 0.035$; $p < 0.05$) were statistically higher than sham and anastomosis group. There was no significant difference in bursting pressures between sham group with anastomosis group and Kefir group with Ensure group ($p > 0.05$).

In our study, evaluation of albumin, total protein and hydroxyproline results suggest that Kefir had enough protein sources for early healing. Additional rich nutrition like Kefir for early oral intake can have a positive effect on strength of anastomosis and less postoperative complications. Kefir can be appropriate nutrition for colonic mucosa. Kefir can be used for regulating gut microflora, nutritional nature and immunomodulation. In our study we did not experience any anastomotic leakage. Postmortem explorations showed that death occurred due to postaspiration asphyxia due to tracheal placement of orogastric feeding tube. Because of Kefir's low cost and high nutritional value, it can be used preoperatively for all patients. Kefir of 500 ml costs for 2 Turkish liras however 250 ml Ensure costs 4.5 Turkish liras.

In spite of these outstanding features, Kefir is currently being used for academical purposes as further clinical studies are needed. With more clinical studies preoperative use of Kefir would reduce postoperative mortality and morbidity rates.

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