

The effect of propolis and mesalazine on bacterial translocation in an experimental colitis model

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The etiology of inflammatory bowel disease (IBD), a term that comprises Crohn's disease and ulcerative colitis (UC), is not known clearly, but the occurrence of high antibodies to enteric bacteria supports the idea of bacterial origin in the pathogenesis.^{1,2} The presence of *Shigella* or *Shigella*-like toxin, *Salmonella*, and *Yersinia* has been investigated as a possible cause of UC, whereas *Clostridium difficile* toxin has been associated with disease exacerbation.³ The colonic mucosal barrier is a complex physicochemical structure that separates the internal milieu from a polluted luminal environment. Barrier function ultimately depends on the integrity of the mucosa and the reactivity of dynamic defensive factors, such as mucosal blood flow and epithelial secretions. Indeed, some bacteria might alter mucosal barrier function and be incriminated in this defect.⁴

Oral aminosalicylates (such as mesalazine) are used in the treatment of mild and moderate active UC. It has been shown that mesalazine had bacteriostatic effects on the sulphate producing and amino acid fermenting bacteria.^{1,2} Propolis (PP) is a vegetable resin collected by bees, which they used against fungus. Its composition differs with the geographical changes. There has been more than 150 kinds of PP detected. Usually PPs include different chemical components, such as polyphenols (flavonoid aglicons, phenolic acids and esters phenolic aldehydes alcohol, ketons terpenoids steroids, and amino acids).⁵ The antioxidant, antimicrobial, antiinflammatory, hepatoprotective, immune regulator and anticancer effect of PP has been shown by previous studies.⁵ In recent studies, it has been shown that PP had toxic effects at doses of 2000-7000 mg/kg, whereas it had antiinflammatory effects at a doses of 300-600 mg/kg.⁵ In our study, we investigated the effect of PP and mesalazine on bacterial translocation in experimental colitis.

Forty adult male Wistar Albino rats, weighing between 200 and 250 g were used. The animals were acclimatized for one week in our laboratory conditions prior to experimental manipulation. They had free access to standard laboratory chow and water *ad libitum*. The protocol of this study and animal experimental procedures were approved by the Ethical Committee of the Mustafa Kemal University School of Veterinary, Antakya-Hatay, Turkey.

A 5% propolis tincture was prepared by mixing 1900 ml of 70% ethanol and 100 g of propolis. They were kept in a container, sealed the top, and shaken twice daily for one week. It was filtered and kept in a clean, dark bottle at 4 °C until it was used. Distal colitis of rats was induced by intracolonic installation of 2 ml of 4% acetic acid (Merck Laboratories, Germany; pH=2.6). This model has been used extensively to investigate the pathogenesis of the acute phase of inflammation. After an overnight fast, each rat was lightly anesthetized with ether, and a 5F polypropylene catheter was inserted into the colon via the anus. The catheter was advanced so that the tip was 8 cm proximal to the anus, and at this point acetic acid was instilled. Finally, each rat received a 2 ml colonic wash containing saline. The animals were randomly assigned into 5 groups: group 1 (G1), control (n=8); group 2 (G2), colitis, received no treatment (n=8); group 3 (G3), colitis + mesalazine (Salofalk; Ali Raif, Istanbul, Turkey), 2 ml once a day via enema (n=8); group 4 (G4), colitis + PP, 600 mg/kg once a day via intragastric lavage (n=8); group 5 (G5), colitis + mesalazine + PP, 2 ml via enema and 600 mg/kg via intragastric lavage once a day (n=8), for one week. The control group received 2 ml of isotonic saline solution into the colonic lumen. Rats were sacrificed 7 days after the rectal installation of saline or acetic acid. On day 7, ether or ketamine anesthesia, or both, was performed after skin disinfection with betadine. A midline thoracoabdominal incision was performed and blood, mesentery lymph node (MLN), liver, and spleen samples were obtained. A blood sample of 1-2 ml was taken in blood culture tubes by cardiac function (Hemoline, Biomerieux, France). The bottles were incubated for 7 days. The samples taken from the tubes in which there was bacterial incubation were dyed with Gram. The samples of mesentery lymph nodes, liver, and spleen were incubated in tyogluconate for 24 hours. The samples were inoculated on Colombia agar with 5% sheep blood, and eosine methylene blue (EMB) agar (BioMerieux, Marcy l'Etoile, France), and incubated at 36°C for 18-24 hours. After homogenization, the samples were inoculated in EMB and 5% blood in aerob and anaerob conditions. The incubating bacteria were identified with standard conventional and automated methods (API system, Biomerieux, France). Bacterial identification was based on biochemical and automated methods (ID 32E API, BioMerieux, Marcy l'Etoile, France). Susceptibility testing was performed by the disk diffusion method using the National Committee for Clinical Laboratory Standards criteria. Susceptibility was tested on Mueller-Hinton agar at

37°C against amoxicillin, amoxicillin-clavulanic acid (20 mcg + 10 mcg), cephalotin, cefuroxime (30 mcg), cefixime, cefotaxime, ceftazidime, norfloxacin, ofloxacin, ciprofloxacin, cotrimoxazol, and gentamicin. The disk diffusion test was performed with antibiotic-containing disks on Mueller Hinton agar plates.

There is no bacterial incubation in the control group. There was incubation in 6 rats and 12 samples in G2 (Colitis group), in 3 rats and 5 samples in G3 (mesalazine group), in 4 rats and 6 samples in G4 (propolis group), and in 2 rats and 3 samples in G5 (propolis+mesalazine group). Fourteen (53.8%) *Escherichia coli* (*E.coli*), 6 (23%) *Staphylococcus species*, 4 (15.4%) *Enterobacter species (spp)*, one (3.9%) *Proteus spp*, and one (3.9%) *Clostridium spp* were isolated from the samples. There was incubation in 12 of blood samples, in 9 of MLN samples, in 3 of the liver samples, and in 2 of the spleen samples (Table 1).

Animal models of inflammatory bowel disease have provided an optimal setting in which to investigate host-bacteria interactions in the pathogenesis of intestinal inflammation. The concept that luminal bacteria are involved in mucosal inflammatory responses is supported by data from different experimental models.⁴ The intestine is in permanent contact with billions of bacteria belonging to the normal intestinal flora, food protein, and potentially pathogenic bacteria, and has to discriminate and define selective action towards non-pathogenic and pathogenic components. The commensal bacterial flora plays an important role in nutrition and immune functions and has metabolic activity such as detoxification.⁴ When the mucosal defense system fails to resist the body's own mucosal bacteria and luminal antigens, the inflammatory response begins and macrophage

activation and delayed-type hypersensitivity reactions occur. Furthermore, indigenous bacteria should migrate transmurally toward the extraintestinal sites in such situations and a bacterial translocation should occur.² In our study, we found that bacterial translocation occurred in an experimental colitis model.

As *E.coli* is the predominant aerobic Gram negative species of the normal intestinal flora, much more attention has been paid to a possible role of its subtypes. Besides commensal strains, certain clones possess virulent properties and cause disease in humans; the diarrheagenic subtypes of *E.coli* belong to this latter group, showing properties such as adherence to the gut mucosa, production of enterotoxins and cytotoxins, and tissue invasion.³ The presence of *E.coli* in patients with UC has been investigated, and it has been reported that *E.coli* could be detected only in a small proportion of tissue samples. Studies on mucosal adhesion of pathogenic bacteria in UC are controversial. A significantly enhanced adhesion of *E.coli* isolates from stool specimens and rectal biopsies from UC patients to buccal epithelial cells was found in comparison with patients with infectious diarrhea or normal controls. The adhesive properties were similar to those of pathogenic intestinal *E.coli*, suggesting that virulent *E.coli* strains might participate in the pathogenesis of UC.³

Anaerobes are the major organisms in the gut flora, and the studies on their role in an experimental colitis model is controversial. Yigitler et al² claimed that anaerobes rarely translocate because they do not have attachment sites, but on the other side Guarner and Malagadela⁴ reported that anaerobic bacteria caused deep colonic lesions, and could lead to a severe inflammatory response when the mucosal barrier is broken. In our study the determined rate of anaerob bacterial translocation was only 39%.

Table 1 - Translocated bacteria species isolated from blood, mesentery lymph node (MLN), liver, and spleen.

Group	Blood	n	MLN	n	Liver	n	Spleen	n	Total
G1		0		0		0		0	0
G2	<i>E.coli</i>	3	<i>E.coli</i>	2	<i>E.coli</i>	1	<i>E.coli</i>	1	12
	<i>Staph.spp</i>	1	<i>Enterobacter spp</i>	1			<i>Staph.spp</i>	1	
	<i>Enterobacter spp</i>	1	<i>Clostridium spp</i>	1					
G3	<i>E.coli</i>	1	<i>E.coli</i>	1	<i>E.coli</i>	1		0	5
	<i>Staph.spp</i>	1	<i>Proteus spp</i>	1					
G4	<i>E.coli</i>	1	<i>Staph.spp</i>	1	<i>E.coli</i>	1		0	6
	<i>Staph.spp</i>	1	<i>Enterobacter spp</i>	1					
	<i>Enterobacter spp</i>	1							
G5	<i>E.coli</i>	2	<i>Staph.spp</i>	1		0		0	3

G1 - control group, G2 - Colitis group, G3 - mesalazine group, G4 - propolis group, G5 - propolis + mesalazine group,
E.coli - *Escherichia coli*, *Staph.spp* - *Staphylococcus species*.

Yigitler et al² isolated *E.coli* (35%), *Enterobacter* (16%), and *E.fecalis* (15%) most commonly in their studies of bacterial translocation in experimental colitis. Similarly, we isolated *E.coli* (53.8%), *Staphylococcus spp.* (23%), and *Enterobacter spp.* (15.4%) most commonly in our study. However, they reported that mesalazine was not efficient on bacterial translocation, but in our study we determined that it had, although not significant statistically, important clinical effects on bacterial translocation. Topical treatment by suppositories or in enemas enhances the mucosal mesalazine concentrations obtained with oral treatment alone, by some 20-folds in the rectum, and its benefits are evident up to the splenic flexure. High mucosal mesalazine concentration could contribute to a more effective treatment of UC. Thus, we administered rectal mesalazine. The antibacterial effect of PP on gram positive and gram negative bacteria, *Helicobacter pylori*, protozoons, fungus, and virus has been shown *in vitro*.

We conclude in our study, that PP and mesalazine reduced bacterial translocation in experimental colitis. The most common bacteria causing translocation was the *E.coli*. Anaerobes are the major organisms of the colonic flora and rarely translocate because they do not have attachment sites to the epithelium, and they contribute colonies in the intestinal mucosa. It has been shown that there was significant bacterial translocation in experimental colitis, and that mesalazine and PP reduces translocation. The combination of the 2 agents was more effective on bacterial translocation.

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Peroneal tendon dislocation. A tendon protective and bone preserving technique of stabilization

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Peroneal tendon dislocation is rare. These lesions have a traumatic origin in 90% of cases, and can become a source of worry in athletes. The typical mechanism is a forced dorsiflexion-eversion of the ankle with rupture of the superior retinaculum, however, a history of ankle inversion sprain with consecutive chronic lateral instability is also frequent. Acute peroneal tendon dislocation is commonly misdiagnosed as severe ankle sprain. In fact the clinical findings may include external malleolar swelling and ecchymosis as the source of confusion.¹ In cases of acute lesions, a cast immobilization of the ankle in plantar flexed inversion for 4 - 6 weeks can be attempted. However, the success rate do not go over 15-56%. In a chronic situation, the diagnosis is facilitated by the patient's complaints that clearly describe a windlass mechanism, or can reproduce the dislocation by active dorsiflexion-eversion of the ankle. In most cases, a peroneal tendon snapping around the posterior margin of the lateral malleolus can be palpated, and even visualized by the examiner. Additional clinical investigations include testing the stability of the ankle, especially the lateral ligaments. A magnetic resonance imaging (MRI) is mandatory to evaluate the presence and extent of tendinous lesions, especially longitudinal tear of the peroneus brevis tendon, and to visualize accompanying tenosynovitis and ligamentous lesions. This imaging technique also provides information about the peroneal tendon aspect and its elongation, or its avulsion from the lateral malleolus, especially in cases where the liquid produced by the tenosynovitis acts as a contrast. If the fibular groove needs to be evaluated, a computed tomography scan is more accurate than the MRI. Surgical treatment is required for symptomatic chronic peroneal tendon dislocation, and includes reconstruction of the fibular groove and retinaculum.

Preoperative planning. A careful clinical examination is the first step of the preoperative assessment. Patients with chronic lesions often relate a history of a former ankle injury. Therefore, the clinical examination should be very specific and looking for a peroneal pathology once every other instability syndrome has been excluded. Palpation of the peroneal tendons in the retromalleolar area is painful and may be accompanied by a crepitus caused by the tenosynovitis. A passive circumduction can directly identify the dislocation; otherwise a dislocation can be

provoked by a passive mobilization of the foot starting in the neutral position, bringing the foot in plantar flexion-eversion and finishing in dorsiflexion internal rotation. The unique presence of pain or apprehension during this maneuver, without any accompanying or palpable dislocation, is not significant and should evoke other pathologies. A routine antero-posterior and lateral x-ray of the ankle should be performed to evaluate the osteoarticular integrity. Bony lesions are rare. However, an avulsion of the fibular periosteum (fleck sign) can be seen, thus indicating a grade III injury, according to Eckert and Davis classification. An MRI completes the investigations.

Technique. The patient is placed in a lateral decubitus position with a tourniquet applied on the thigh. The operation is made under general or loco-regional anesthesia. The incision is longitudinal curvilinear along the posterior aspect of the distal fibula, and extends 5 - 6 cm proximal to the fibular tip towards the upper margin of the calcaneus. The danger of this approach includes damage to the superficial peroneal nerve, especially in the proximal part of the incision, and also the sural nerve, although the latter is usually located more posteriorly. Full thickness skin flaps should be developed to avoid skin necrosis. The incision is deepened down to the fibular periosteal layer through a longitudinal incision of the peroneal sheath and superior peroneal retinaculum close to the posterolateral margin of the fibula. The peroneal tendons are inspected. The peroneus brevis frequently presents with a longitudinal lesion that we excise. Any surrounding synovitis is carefully removed. The periosteum is then incised in a linear fashion. The placement of this periosteal incision is one of the most critical parts of the procedure. It should be made close to the lip of the lateral aspect of the fibula to avoid an acute angle during periosteal elevation. If this periosteal incision is made too anteriorly on the fibula, it might cause a perforation of the backside of the sheath while elevating the periosteum resulting in direct contact of the tendon itself and possible exposed bone, being thus a potential source of tendon irritation later on. The periosteal flap is now meticulously elevated to avoid perforating the back of the sheath. This should not be very difficult since in the majority of cases the surface is relatively flat. The posterior surface of the lateral malleolus is now exposed. The deepening process of the fibular groove can be started. We use a high speed rotatory burr, with graduate drilling from the tip of the fibula proximally into the shaft. During this deepening process, it is important to keep sufficient thickness of the posterolateral fibular cortex on which transosseous sutures will be applied in the second part of the procedure. The burring process is completed by delicately impacting the cancellous bone, thus increasing the depth of the

groove. Increment of the depth of the groove is expected to reach 3 - 8 mm. Only then, the fibular periosteum is reapproximated by simple application into the groove. The tendons are reduced. The superior retinaculum and peroneal sheath are then advanced under the lateral wall of the fibular groove and secured transosseously using 1-0 resorbable sutures (**Figure 1**). A cuff of tissue should still remain on the fibular side, which is then sewn over in a "pants-over-vest" fashion. The free gliding of the peroneal tendons within their new tunnel is carefully checked. Hemostasis is controlled after tourniquet removal, and closure in 2 layers is performed.

Postoperative management. The ankle is placed into a short leg, removable walking splint in neutral position. After 24 hours of bed resting, partial weight bearing (20 kg) is initiated during 2 weeks, and then weight bearing as tolerated is allowed. At 4 weeks, the cast is replaced by an ankle brace, which is worn day and night during 3 additional weeks, and night only for 3 more weeks. The rehabilitation program includes smooth passive mobilization of the ankle in the sagittal plane from the 3rd postoperative week, free passive and active mobilization starting at the 6th week, strengthening and proprioceptive exercises beginning at 7 - 8 weeks after surgery. Patients usually return to their habitual sports activities 3 - 5 months after surgery.

Randomized control trials could contribute to determine the best surgical technique, but the relative rarity of this pathology associated to the large numbers of surgical techniques proposed, makes such a study difficult. Nevertheless, the existing reports allow to highlight some of the advantages and disadvantages of several techniques and to recommend a few principles. Porter and associates² emphasized the need to have a symmetrical deepened groove in the distal

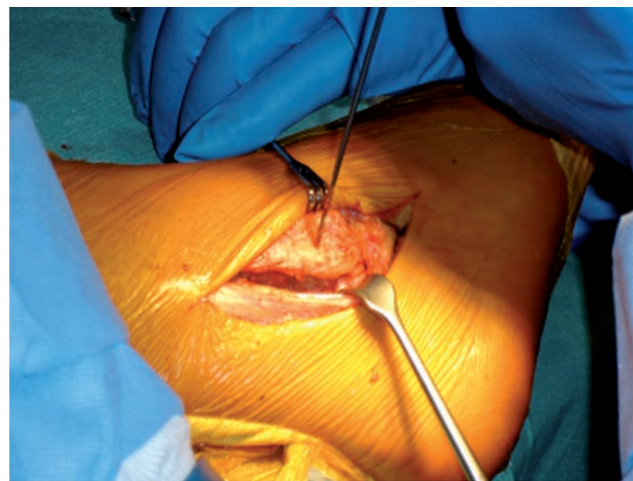


Figure 1 - New retromalleolar groove and preparation for transosseous sutures.

fibula. A bony flap resection prior to the grooving was carried out. No report of any complications from this, and related better results in the postoperative period since the inherent stability provided by this symmetrical groove allowed functional bracing rather than casting, and the patients were able to begin their exercises immediately after surgery.² Mendicino et al³ described an intramedullary drilling from the tip of the fibula backward, with particular attention paid to the 3-dimensional orientation of the drill, and the necessity to avoid disruption of the posterior cortex. Although it was described as an easy procedure, it remains a blind approach with a demanding learning curve.³ Maffulli et al⁴ insisted on the importance of using an anatomical approach to suture the superior peroneal retinaculum in order to get good results. Surgical treatment of peroneal tendon dislocation by deepening the fibular groove has also been reported with overall good results in the literature varying from 77 - 100%.⁵ A study by Hutchinson and Gustafson⁵ in 1994, reported in a series of 20 feet that had 12 good results, 5 fair related to concomitant peroneal tendon pathology discovered preoperatively, and 3 poor results. Porter et al,² in their study of 14 ankles, reported 4 excellent results where the patients had no pain, 9 fair with occasional pain but with no limitations in their activities.

The overall review of the literature concluded that a soft tissue procedure alone was not adequate in treating this pathology. The advantage of our technique is its simplicity combined with its efficiency, as it is a soft tissue-bone implicating procedure. Our low learning curve makes it attractive in this, nevertheless, rare pathology.

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A histopathological study of chronic granulomatous lymphadenitis

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Granulomatous inflammation is a distinctive pattern of chronic inflammatory reaction, characterized by focal accumulations of activated macrophages, which often develop an epithelial-like (epithelioid) appearance. Epithelioid granuloma formation is a common, and important histological finding in a lymph node. The possible etiology is diverse and wide. It is encountered in a number of immunologically-mediated, infectious and non-infectious conditions. Its genesis is firmly linked to immune reactions.^{1,2} *Mycobacterium tuberculosis* is the leading cause of the infectious granulomatous disease, especially if the granulomas show central caseous necrosis, but there are other etiological causes, such as *Brucellosis*, cat-scratch disease, lymphogranuloma inguinale, leprosy, syphilis, Whipple's disease, tularemia, and so forth. It is important to note that granulomas may be found in lymph nodes draining malignant tumors (such as carcinoma of the breast), or in association with primary lymphoid malignancy (Hodgkin and Non-Hodgkin lymphoma).² The use of conventional hematoxylin and eosin histopathological sections with other ancillary tests (microbiological, immunohistochemical, radiological, biochemical, and special staining techniques) are useful for obtaining a definitive diagnosis of the underlying possible etiology. The aim of this study was to determine the possible underlying cause for chronic granulomatous lymphadenitis in our locality by using different special histochemical stains in addition to the routine hematoxylin and eosin stain (H & E), and other laboratory tests.

We selected lymph node specimens from 63 patients with chronic lymphadenopathy that were filed in different public and private pathological laboratories in Baghdad, Iraq, from 1998 - 2001, and those who were diagnosed histopathologically as cases of chronic granulomatous lymphadenitis. The lymph nodes were received from different locations. Every lymph node was bisected and fixed for 24 hours in buffered formalin. On the following day, the samples were processed for paraffin embedding, and sections were made and stained for histopathological examination using the conventional H & E stain. Special stains were performed in each case in order to diagnose the possible underlying cause for granulomatous inflammation like Ziehl Neelsen stain for acid fast bacilli, periodic acid Schiff (PAS) and Grocott for fungus, Gram stain to

see Gram-positive or Gram-negative organisms, and Giemsa stain to see the parasites. When the results were inconclusive, the help of other laboratory methods were taken as micro-enzyme linked immunosorbent assay (ELISA) method to detect immunoglobulin G and immunoglobulin M, toxoplasma antibodies.

Forty (63.5%) patients were females and 23 (36.5%) were males. The age of patients ranged from 11 - 75 years, with mean age of 42 years. The maximum number of cases presented was at 21 - 30 years group (36.5%). Our study showed that the commonly involved sites were cervical (62%); other sites include inguinal and axillary (11.1% each), intra-abdominal (7.9%), and from paratracheal lymph nodes (3.2%). In 4.7%, the sites were not mentioned by the surgeon. The eventual diagnosis of granulomatous inflammation by histopathological diagnosis was subdivided into caseating and non-caseating granulomas. Caseating granulomas were present in 30 cases (18 females and 12 males) and non-caseating granulomas in 33 cases (20 females and 13 males). Depending on the results of conventional H & E stain, special histochemical stains, and other laboratory tests, the causative factors in the development of granulomatous lymphadenitis in our cases were summarized in **Table 1**. All neoplastic granulomatous lymphadenitis were of non-caseating type. Six lymph nodes were associated with primary lymphoid malignancy, Hodgkin lymphoma in 4 cases (3 lymphocyte predominant type, 1 mixed cellularity type), non-Hodgkin lymphoma in 2 cases, while 3 lymph nodes (2 axillary, 1 intra-abdominal) showed non caseating granulomas as a reaction to extra lymphoid malignancy (breast adenocarcinoma and colonic adenocarcinoma). The non-neoplastic causes for granulomatous lymphadenitis were mostly due to infections, predominantly tuberculosis (**Table 1**). Twenty seven lymph nodes (43%) showed acid fast bacilli, 20 were from caseating granulomas while 7 were non-caseating granulomatous lymphadenitis. Four lymph nodes were positive for Gram-negative bacilli; 2 of them were mesenteric lymph node proved by serological tests as *Brucellosis*. Two lymph nodes showed fungus confirmed both by PAS and Grocott special stains, and both were opportunistic infections; one of them were candida, having budding yeast and pseudohyphae (**Table 1**). Six lymph nodes with non caseating granulomas showed features of toxoplasmosis in histopathologic examination, and the results of Giemsa special stain were inconclusive but the diagnosis of toxoplasmosis was confirmed by serology. One lymph node showed *Leishmania donovani* bodies confirmed by Giemsa stain. In 14 lymph nodes (10 caseating and 4 non-caseating), the results of the special stains or the serologic tests or both, were inconclusive, where the

Table 1 - Final etiologies of granulomatous lymphadenitis in 63 patients.

Cause	n	Caseating granulomas	Non caseating granulomas
<i>Neoplastic (9)</i>			
Primary lymphoma	6	0	6
Secondary malignancy	3	0	3
<i>Infections (40)</i>			
Tuberculosis	27	20	7
Toxoplasmosis	6	0	6
Gram negative bacteria	4	0	4
Fungus	2	0	2
Leishmeniasis	1	0	1
Unknown cause	14	10	4
Total	63	30	33

underlying etiology for granulomatous inflammation remained unknown. In general, our study showed that the predominant cause for granulomatous lymphadenitis was non-neoplastic infectious etiology (63.5%) and among the infectious causes, tuberculosis was the most common cause of granulomatous lymphadenitis probably due to the emergence of antibiotic-resistant strains; while the emergence of AIDS has increased the rate of tuberculosis in the developed countries as well.³ Tuberculosis may be misdiagnosed at the cost of other etiological agents like toxoplasmosis, fungus, and the others, and if appropriate treatment is not given according to the etiological agent, the disease will not be eradicated. Different diagnostic methods are available for making a diagnosis but in our locality, we can at least do special stains to find out the causative agents. The acid-fast Ziehl Neelsen stain is the most rapid method for detection of mycobacterial infection in tissue sections but frequently presents negative results. In our study, 27 cases (42.8%) have positive results using the Ziehl Neelsen stain, (20 caseating granulomas), while in 10 with caseating granulomas, the Ziehl Neelsen stain was negative. The reason could be that the anti-mycobacterial therapy changes the capsule integrity that prevents acid fast staining, or because the bacilli is of small quantity. Immunohistochemical staining for *Mycobacterium* has shown higher sensitivity than acid fast staining, probably because of the detection of fragmented bacilli.⁴ Recently, the rapid diagnosis of tuberculosis by nucleic acid amplification using the polymerase chain reaction technique has become feasible in fresh material, and has also been used for formalin-fixed paraffin-embedded tissue,⁵ but both techniques are costly and not available for routine use in our locality. Our study showed that the presence of granulomata in a histological section may indicate the presence of a neoplastic process (9 cases), both primary (6 cases) and secondary (3 cases)

neoplastic disorders. Histologically, the granuloma is non-caseating, composed of epithelioid histiocytes with multinucleated giant cells, but these cannot distinguish granulomatous inflammation from other causes. A series by Khurana et al⁶ highlighted the difficulties encountered in making a definitive diagnosis of malignant neoplasm that mimics, or occurs in association with granulomata. The background cell population needs to be scrutinized if a malignant lymphoma is suspected. Granulomata may be encountered in both Hodgkin's disease and non-Hodgkin's lymphoma, particularly T-cell lymphoma. Hodgkin's lymphoma is characterized by the classical Reed-Sternberg cells in a background of sarcoid-like granuloma, reactive lymphoid cells, and occasional eosinophils.² Granulomatous inflammation found in lymph nodes draining carcinomas is a recognized phenomenon.² Such phenomenon are reported in different types of malignancies as breast carcinoma,⁷ colonic and gastric carcinoma,⁸ and the others. This has been suggested to be either a response to necrotic cell material or immunological T-cell mediated hypersensitivity reaction to cell surface antigens,^{7,8} however the precise mechanism is largely speculative, as the exact tumor or host factors that enable such a response remain unknown.

We conclude that a significant number of granulomatous lymphadenitis has an identifiable underlying causal pathology. Our experience suggests that infections, mostly tuberculosis, are the most common etiological factor. The conventional H & E histological examination combined with special stains, and the serological tests is useful as a first line investigation to detect the underlying infectious causative microorganism; however neoplastic disorders should be kept in mind as another possible predisposing factor for chronic granulomatous lymphadenitis.

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Endoscopic dacryocystorhinostomy for primary nasolacrimal duct obstruction

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Obstruction of the nasolacrimal drainage system is a common condition, and encountered in clinical practice both by ophthalmologists and otorhinolaryngologists. Dacryocystorhinostomy (DCR) is a standard procedure for the treatment of primary nasolacrimal duct obstruction. It can be performed through a cutaneous incision, traditionally referred to as external DCR, or via a transnasal approach under either microscopic or endoscopic guidance.¹ The published success rate of both procedures is 80 - 95%, with similar success for external and endoscopic approaches,^{2,3} however, the advantages of the endoscopic approach includes cosmetic advancement, minor traumatization, and preservation of lacrimal pump function and reduction of surgical time. Meanwhile, the minimal morbidity and the possibility to treat simultaneous sinonasal diseases urge the surgeons to advocate this technique.³ While these procedures are well documented in the developed countries, the paucity of published article from developing countries made us report our following series.

This prospective study was conducted in Besat and Imam Khomeini Hospitals, Hamedan University of Medical Sciences, Hamedan, Iran, during the years 2002 - 2005, with 67 patients (70 endoscopic DCR

cases) included in our study. All patients underwent a comprehensive ophthalmological examination, along with irrigation and regurgitation tests, and if needed, dacryoscintigraphy of the nasolacrimal drainage system, and an otorhinolaryngologic and endoscopic intranasal examination. Patients included were those with primary nasolacrimal duct obstruction and no other lacrimal disease. Patients were excluded if there was a history of trauma, previous lacrimal surgery, suspicion of malignancy, lower lid problems involving the canaliculi, secondary obstruction of the lacrimal duct (such as sarcoidosis), or a follow up period of less than one month after silicone stent removal. All surgeries were performed under general anesthesia. Endonasal endoscopic DCR uses sinus surgery instrumentation for the nasal mucosa and bone, with a sickle knife to open the lacrimal sac. After the mucosal elevation, without the usage of intracanalicular light pipe, an approximate 15 x 15 millimeter bony rhinotomy is created by protected-powered drilling on the lower and upper parts of the lacrimal sac. The lacrimal sac is incised vertically using a sickle knife, and the medial part of lacrimal sac is removed by Weil forceps. Finally, short silicone tubes are inserted and knotted in the nose. Outpatient review was at first to fourth weeks for endoscopic rhinostomy check, and removal of crusts as necessary. Silicone tube removal was planned 4 months after surgery, and each patient was reviewed 6 and 12 months after surgery. At the final visit, subjective and objective outcomes were assessed. Subjective success was based on the patient's symptoms of epiphora. This was recorded as asymptomatic, significantly improved (mild epiphora), moderate epiphora (intermittent epiphora but patient can endure it), or severe epiphora (persistent epiphora). The successful outcome was defined as asymptomatic or mild epiphora with a normal irrigation test. This definition is compatible with other well-known outcome measures for successful DCR, to emphasize the importance of subjective patient satisfaction after surgery. All postoperative complications were recorded by the surgeons. Complications includes post-operative periorbital ecchymosis, periorbital emphysema, severe pain (not relieved by oral non-steroidal anti-inflammatory drugs and needs parental analgesics), and severe epistaxis (needs hospital admission and nasal packing). Finally, all the data were extracted manually and displayed in a descriptive manner. Chi square test were used for non-parametric variables using the Statistical Package for Social Sciences version 13 software. This study was approved by the Ethics Committee of the Hamedan University of Medical Sciences, Hamedan, Iran.

Out of 67 patients, there were 48 (75.4%) females and 19 (24.6%) males. Age groups were 10 - 30 years (34.3%), 30 - 50 (55.2%), and 50 - 75 (10.4%). Only

2 cases were above 70 years and both had epiphora before surgery. From 70 operations, 28 cases were in the left, and 36 cases were in the right eyes, and 6 cases underwent bilateral operation. The mean operation time was 31.2 minutes (range from 25 - 41 minutes). After 6 months follow-up, 65 (92.5%) reported symptom free, 64 (91.4%) cases had mild epiphora or reported symptom free after 12 months. With irrigation test, passage of fluid failed in 6 patients after 6 months, and 8 patients after 12 months follow up. According to the defined criteria in this study, the success rate was 91.4% on 6 months follow up, and 88.5% on 12 month follow up. No difference between the success rates according to age, gender, and the other background variables ($p>0.05$). Postoperative complications involved 5 (7.1%) patients with periorbital ecchymosis, of which, 2 of them (2.8%) were accompanied by periorbital emphysema, treated by conservation. The postoperative complications are presented in **Table 1**. There were no smelling disturbances in our patients, and the silicone tubes were removed spontaneously in 2 patients.

The advantages of intranasal endoscopic DCR are better visualization; it avoids the external scar and damage to the angular vein; it preserves the normal function of the lacrimal pump; identification of the sac and correct placement of the opening between the sac and the nasal cavity; immediate correction of surgical mistakes such as immediate control of brisk epistaxis after anterior ethmoidal artery trauma by its direct cauterization; reduction of surgical time; and diagnosis and treatment of coexistent intranasal disturbances. The problems that are encountered in intranasal DCR has been previously reported, such as difficulties in recognition of intranasal structures and correct positioning of incisions, intra-operative bleeding, unsatisfactory bone removal because of thickness, cicatrization of lacrimal sacs, and complications of silicone tubing.³ The reported results of successful endonasal endoscopic DCR range from 63-99%, depending on the techniques used.^{2,3} We

Table 1 - Complications in patients who had endoscopic mechanical dacryocystorhinostomy.

Complications/time of occurrence	Free	Mild	Moderate
<i>Pain</i>	38 (54)	20 (28)	12 (18)
First 3 days	38 (54)	28 (40)	4 (6)
4-7 days	69 (98)	1 (2)	-
>7 day	53 (86)	15 (21)	2 (3)
<i>Epistaxis</i>	67 (96)	3 (4)	-
First 3 days	70 (100)	-	-
4-7 days			
>7 day			

analyzed our results based on the patient's symptoms of epiphora, and findings from irrigation test. The success rates of endoscopic DCR are as high as some studies with the same technique,^{3,4} that may be the result of the larger rhinostomy that is made by drilling. Meanwhile a report by the American Academy of Ophthalmology, reviewing series of reports on endoscopic DCR, stated that "it was still difficult to make definite evidence-based determinations about the relative efficacy of endonasal and external DCR".⁴ Lasers have been used for lacrimal surgery since 1990. The success rates using lasers are generally lower than the procedures in which, surgical instruments are used endonasally to create the rhinotomy.

Complications of endonasal DCR surgery can be divided into intraoperative and early or late postoperative. During surgery, the light pipe can make a false passage and cause canalicular obstruction or orbital fat prolapse with surgical emphysema. Instruments passed in and out of the nose can damage the nasal mucosa with resultant synechia. Early postoperative (up to one month) complications include hemorrhage, crusting, perirhinostomy granuloma, and transnasal synechia; 1 - 6 months side effects of surgery include surgical failure from impacted tubes, rhinostomy scarring, granuloma, and synechia. Most of these later complications occur between one and 3 months after surgery. In endonasal surgery, complications are greater with inexperienced surgeons. In our study, we were faced with few complications and the rate of complications was low (7.1%), which compares well with previous endonasal studies.⁵

We conclude that the success rates of mechanical endoscopic DCR in our series were 91.4% in 6 months, and 88.5% in 12 months follow-up period. Both patients and surgeons favor the lack of an associated facial scar, the reduction of surgical morbidity, and more rapid return to daily activities. The results support the routine usage of endoscopic DCR in Hamedan, Western Iran.

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Hepatosplenic abscess in brucellosis

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Brucellosis is endemic in the region of Sanliurfa, Turkey. Any individuals living in the endemic areas that have high fever and hepato-splenomegaly should undergo clinical and radiological investigations for salmonellosis, brucellosis, amebiasis, kala-azar, and tuberculosis.¹ Organomegaly and a moderate elevation in liver function enzymes can be observed in 50% of brucellosis complicated with granulomatous hepatitis. However, abscess formation is a rare entity, which is often undiagnosed.^{2,3} Abscess formation in bones and soft tissues (especially spinal and paraspinal areas) is very well defined in brucellosis, whereas the same is not true for hepatosplenic involvement.⁴ Brucellosis abscesses in liver and spleen are usually quiescent, and may improve with specific anti-brucellosis treatment.² Any abscess detected by either ultrasound (US) or computed tomography (CT) should be confirmed microbiologically.⁵ Through PubMed research, only 42 cases of hepatosplenic abscess was reported in the literature since 1904. The cases were diagnosed as brucellosis according to clinical manifestations, and conventional microbiological techniques (Rose Bengal slide agglutination, serum brucella antibodies $\geq 1/160$ in standard tube agglutination, and blood culture). Throughout 2004 - 2005, all brucellosis cases admitted to the Clinic of Infectious Diseases at Harran University, Hospital of Education and Practice, Sanliurfa, Turkey, underwent radiological assessment for hepatosplenic involvement prior to treatment. Abdominal and hepatic US (Toshiba SSA 370 A, Japan) was performed on all patients by 2 independent radiologists, before and after the treatment. Cases with hepatic involvement detected by US, underwent further evaluation using CT (Toshiba X Vision Spiral, Japan) images by the same radiologists.

Of the 80 cases, 3 female patients were defined as having hepatosplenic abscess related to brucellosis.

The first case was a 45-year-old female, who had complaints of high fever, malaise, and right flank pain. She had a history of brucellosis 3 years ago. She was hospitalized with a pre-diagnosis of acute hepatitis. The CT showed well circumscribed, homogeneous lesions at the right lobe of the liver, the largest being 5 x 4 x 1 cm in diameter, which had a weak enhancement in the wall following intravenous contrast material injection, had surrounding edema, and seemed to be hypodense when compared to the liver surrounding it (**Figure 1**). The very weak hyperdense areas in the center of the lesions were suggested to be calcifications. Abdominal US revealed multiple, ill defined, irregular lesions in the liver parenchyma that were hypoechoic when compared to liver tissue.

The second case was a 28-year-old female complaining of high fever, left flank pain, diarrhea and weight loss, and was diagnosed as having amebic dysentery, but both serum antibody and stool antigen for amebiasis were negative. The US and CT revealed multiple abscesses in her spleen. Abdominal US in the supine position revealed multiple, ill-defined, hypoechoic, irregular formations in the spleen, the largest measures 9 x 15 cm in diameter, and these were interpreted as splenic abscesses. The US showed scattered, point-like calcifications in liver parenchyma; the findings were confirmed by the CT.

The third case was a 65-year-old female, who had disseminated pain in her joints, mild fever, and bilateral



Figure 1 - Computed tomography without contrast enhancement revealed very weak hyperdense areas in the center of hypodense lesions in the left lobe of liver, the largest measuring 5 x 4 x 1 cm in diameter. After density measurement, the hyperdense areas around the smaller hypodense lesions were interpreted as calcifications. Loculated collections of fluid were observed around the lesions.

flank pain. Hepatomegaly, detected by the physical examination, was confirmed by the US. Abdominal US revealed multiple hypoechoic lesions, whereas CT revealed hypodense, heterogeneous pseudotumoral lesions with multiple calcium depositions in the center.

These lesions, exhibiting weak enhancement on their walls on CT with intravenous contrast material, had multiple loculated fluid collections. The radiological features of the patients were consistent with those in literature. All 3 patients recovered fully with anti-brucellosis treatment although none of them underwent surgical drainage. Both pathological and radiological findings completely disappeared after treatment.

Hepatic and splenic abscesses are caused either by direct or hematogenous spread of infectious agents. The liver is involved in 1.7% of brucellosis cases in the form of pseudotumoral necrotizing granuloma.^{3,4} Although hepatic brucellosis could be observed in the latent phases of chronic brucellosis, its incidence is rare.⁴ The reason may be latent brucella retained in calcified lesions. Ariza et al,¹ on their large-scale study reported that early phase treatment was effective in preventing hepatosplenic abscess formation in 50% of brucellosis. The US and CT features are characteristic in hepatosplenic abscesses. In brucellosis cases, hypoechoic, hypodense, heterogeneous, pseudotumoral lesions with central calcium precipitates were observed in the liver and the spleen.⁵ Lesions with contrast enhancement in the wall may contain one or more loculated fluid collection areas.² Magnetic resonance imaging findings are usually not superior to CT.^{4,5} The presence of calcium deposits in hepatosplenic abscesses is an invariable finding of chronic hepatosplenic brucellosis, and ascertains the chronic nature of the disease. Localized disease may be confused with hepatocellular carcinoma, hydatidosis, pyogenic, or amebic abscesses, or other granulomatous conditions including tuberculosis and histoplasmosis.^{1,3} The radiological finding of a hypoechoic mass that surrounds an area of calcification is interpreted as specific.⁵ Evaluation of hepatic radiograms of brucellosis cases revealed multiple calcifications in 5 cases, single calcification in 8, and no calcifications in 4.¹⁻⁵ Examination of 13 hepatic brucellosis cases resulted generally in a heterogeneous mass and calcification, whereas 9 cases exhibited a hypodense region, and 6 cases, a saccular collection of fluid.⁴ The typical radiological findings of hepatic brucellosis cases are calcium deposits surrounded by a hypodense region, neighboring image of snowflake, thin, lamellar calcifications, and halo effect.⁵ Splenic brucella abscess was reported as a wide, leaf-shaped lesion with thin borders.⁵ Histoplasmosis, tuberculosis, tularemia, malignancies (such as fibro lamellar carcinoma, mucin-secreting colonic and gastric cancers, and

cyst-adenocarcinomas), and cavernous hemangiomas may produce scattered, point-like calcifications in the spleen.¹ Hepatosplenic involvement may easily be misdiagnosed in some cases of brucellosis.² Although central calcium deposits are suggestive for brucella abscess, intrahepatic tumoral and pseudotumoral masses should be also kept in mind in the differential diagnosis. In conclusion, radio-diagnostic methods should be considered in brucellosis patients living in the endemic regions in order to define and treat abscess formation (brucellosis) successfully.

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