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Diagnosis of lymphogranuloma venereum from biopsy samples

The Health Protection Agency Centre for Infections launched an alert in October 2004 to improve the awareness, diagnosis, and control of lymphogranuloma venereum (LGV), a sexually transmitted chlamydial infection, following a series of outbreaks in Western Europe.¹ To date (9/3/2006), 334 cases of LGV have been diagnosed in 334 men. The case definition for a confirmed case of LGV is the presence of *C trachomatis* specific DNA, using two nucleic acid amplification tests (NAATs) with different primers, of serovars L1, L2, or L3, determined by genotyping (http://www.hpa.org.uk/infections/topics_az/hiv_and_sti/LGV/lgv.htm). All cases of LGV to date in the UK have been in men who have sex with men and typically present with proctitis and/or inguinal lymphadenopathy. Some of the men in the UK diagnosed with LGV reported long duration of symptoms presenting to gastroenterologists and having been wrongly diagnosed with inflammatory bowel disease before referral to genitourinary medicine (GUM).

Initially, the Sexually Transmitted Bacteria Reference Laboratory (STBRL) only accepted swabs, residual or unprocessed NAAT specimens, and enzyme immunoassay samples, which we had previously validated as being suitable for testing. In January 2005 we were approached about accepting a histology specimen from a male patient who in retrospect the clinician suspected as having LGV. Since this date we have processed seven histological specimens, which we now report.

Genomic DNA was extracted from formalin fixed paraffin embedded biopsies using a QIAamp DNA mini kit (Qiagen, Crawley, Sussex). The paraffin was removed by incubating at 56°C and then we proceeded with the manufacturer's recommended tissue protocol without any modifications. *C trachomatis*

Figure 1 Rectal biopsy of patient No 5. The lymph node architecture is preserved but severely distorted. There is a florid active and chronic inflammatory process within the node and extending into the surrounding soft tissues. This consists of epithelioid granulomata showing severe central necrosis which is geographic in outline. There is a severe acute inflammatory component with microabscess formation. There is also follicular hyperplasia.

was confirmed to be present in the extracted DNA by two different real time polymerase chain reaction methods, each having different gene targets. These targets are different to other commercially available NAATs. The genotype of the *C trachomatis* was then determined by the method of Lan and colleagues.² Since September 2005, all samples positive for *C trachomatis* were tested for LGV using the real time polymerase chain reaction method of Morre and colleagues.³

Seven biopsies were processed from six patients attending GUM clinics in the UK (table 1).

Of seven biopsy samples submitted to STBRL in the past nine months for LGV investigation, we have successfully been able to extract DNA from the biopsies and diagnose three cases of LGV in two patients (Nos 3, 4 and 5), two that were not LGV associated (Nos 1 and 7), and two samples were negative for *C trachomatis* (Nos 2 and 6) (table 1). A rectal biopsy from patient No 5 is shown in fig 1. It is interesting that two patients (Nos 1 and 7) presented with clinical symptoms characteristic of LGV that were determined to be infected with chlamydia, but of a non-LGV associated serovar. Further clinical research is indicated to consider whether these chlamydial infections were causing the severe inflammation of proctitis

and inguinal lymphadenopathy, or if these were incidental findings.

A diagnosis of LGV should be taken into consideration by gastroenterologists and histologists in patients presenting with proctitis or inguinal lymphadenopathy, particularly in men who have sex with men. Examination of biopsy tissue may be useful for diagnosing LGV in patients where cases are being re-examined due to the increased awareness of LGV in the UK, or where a swab for *C trachomatis* testing was not obtained. The algorithm for accepted samples has now been altered to include histological specimens.

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Table 1 Biopsy results from six patients attending genitourinary medicine clinics in the UK

Biopsy No	Site of biopsy	<i>C trachomatis</i> result	<i>C trachomatis</i> genotyping result	LGV specific real time result	Symptoms	Histological findings
1	Rectum	Positive	Not LGV associated	Not available	Proctitis, fibrotic stricture of rectum	Chronic inflammation, ulceration of mucosa
2	Rectum	Negative	–	Negative	Proctitis, bloody diarrhoea, weight loss,	Severe inflammation, goblet cell depletion, distortion crypt architecture
3, 4	Lymph nodes of left groin	Positive	L2 (LGV associated)	Positive	Inguinal lymphadenopathy	Lymph node necrosis, inflammatory infiltrate
5	Rectum	Positive	Not amplified	Positive	Colo-proctitis	Inflammatory infiltrate with early abscess formation
6	Left inguinal lymph node	Negative	–	Negative	Inguinal lymphadenopathy	Perivascular inflammation
7	Right groin	Positive	Not LGV associated	Not available	Inguinal lymphadenopathy	Suppurative granuloma reaction

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Efficacy of ecabet sodium enema on steroid resistant or steroid dependent ulcerative colitis

Ecabet sodium (ES), a 12-sulfodehydroabietic acid monosodium salt derived from an ingredient in pine resin, has been shown to topically enhance several gastric mucosal defensive factors.¹ ES is widely used for the treatment of gastritis and gastric ulcer in Japan. A recent open label study demonstrated that ES also has therapeutic effects in active ulcerative colitis (UC).² In that study, the authors demonstrated that six of seven UC patients with proctosigmoiditis achieved complete remission by treatment with ES enema. However, successive clinical trials of the efficacy of ES enema in UC patients have not been reported. Here we demonstrate the efficacy of ES enema in steroid resistant or steroid dependent UC patients.

Treatment with ES enema was performed after obtaining informed consent from steroid dependant, steroid resistant, or steroid contraindicated UC patients who had inflamed lesions mainly in the rectum and sigmoid colon except for one patient with ileal pouchitis after total proctocolectomy. This clinical trial was approved by the ethics committee of Akita University School of Medicine. Patient profiles are shown in table 1. ES enema was prepared with 1 g of ES and 20–50 ml of tepid water, and one or two enemas per day were instilled into the rectum. Disease activity was assessed before and after treatment using the ulcerative colitis-disease activity index (UC-DAI).³ In case No 1, the endoscopic point was not included in the score. Doses of drugs for UC, including sulfasalazine, 5-aminosalicylic acid, and prednisolone were not changed or increased during treatment with ES enema.

One patient (case No 6) was withdrawn because of a sense of abdominal fullness. Two of five patients (case Nos 1 and 2) significantly responded to the treatment and achieved almost clinical remission in three and seven weeks, respectively. Endoscopic findings in the rectum of case No 2 before and after treatment are shown in fig 1A and 1B. One patient with pouchitis (case No 4) dramatically responded to treatment after two weeks. Two patients (case Nos 3 and 5) with deep ulcers in the rectum and sigmoid colon also responded to the treatment but did not achieve clinical remission. Endoscopic findings of case No 3 before and after the treatment are shown in fig 1C and 1D. Mean disease activity index declined by 54.5% after ES enema treatment compared with that before treatment ($p = 0.0289$).

Our results suggest that ES enema may have therapeutic actions in some steroid resistant or steroid dependent UC patients. ES enema also had therapeutic effects in the patient with ileal pouchitis. However, the effect of ES enema in two cases with deep ulcer was slight and clinical remission was

Table 1 Patients profiles

Case	Sex	Age (y)	Extent of lesions	Clinical course	Intractability
1	F	45	Left sided colitis	Relapsing remitting	PS dependent
2	M	44	Left sided colitis	Relapsing remitting	Irritation to PS
3	F	41	Left sided colitis	Chronic continuous	PS resistant
4	F	33	Pouchitis	Relapsing remitting (post colectomy)	PS induced bone necrosis
5	M	23	Left sided colitis	Relapsing remitting	PS resistant
6	F	32	Left sided colitis	Relapsing remitting	PS resistant

PS, prednisolone.

not achieved. The detailed functional mechanism of ES enema for improvement in inflammation in colonic mucosa was not clearly elucidated but induction of several defensive factors and an antioxidative stress function⁴ are suggested as being involved. Further randomised case controlled trials with larger number are needed to assess the efficacy of ES enema in patients with UC.

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Figure 1 Endoscopic findings of the rectum in case Nos 2 case 3 before and after treatment with ecabet sodium (ES) enema. (A) Case No 2, before treatment; whitish coated shallow erosions are seen diffusely. (B) Case No 2, after treatment; lesions have improved dramatically. (C) Case No 3, before treatment; deep ulcers can be seen diffusely. (D) Case No 3, after treatment; deep ulcers are partially improved.