Practical Laboratory Medicine 27 (2021) e00255

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

A case report: Use of FT-IR analysis to improve Colovesical fistula diagnosis

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ARTICLE INFO

Keywords:
Colovesical fistula
Faecaluria
Fourier transform infrared spectroscopy

ABSTRACT

Colovesical fistula (CVF) is an abnormal connection between the colon and the urinary bladder. Faecaluria, reported in 40–70% of cases, is virtually pathognomonic for CVF. During the 5th day of recovery in an 84 years old subject, the passage of cloudy, malodorous urine with visible debris was observed. According to the pathognomonic character of faecaluria, the sample was signed to the laboratory for biochemical and microbiological investigation, able to define the type and origin of materials. Following clinical requirements, both biochemical pathways and instrumental procedures able to confirm or exclude the presence of faecal components in urine were considered. No biochemical compound or component addressing faecal compounds in urine results available between laboratory tests. The brown powder component of the pellet was identified as Keratin, with 90% overlapping with the reference spectrum of the compound. FT-IR analysis on urine pellet can be proposed as a simple, non-invasive, and fast method to improve the diagnostic course of CVF.

1. Introduction

Colovesical fistula (CVF) is an abnormal connection between the colon and the urinary bladder. CVFs are uncommon findings cause of significant morbidity that usually require surgical treatment. Its incidence is about 3 times higher in men than in women, probably due to the barrier formed by the uterus and broad ligaments between the sigmoid colon and the bladder [1,2]. Complications of diverticulitis, cancer, Crohn's disease result in the more representative cause of CVF. However, many other aetiologies with a low incidence, like abdominal trauma, complications from surgery, tuberculosis, and pelvic radiation therapy, are reported in the literature [1]. Diagnosis depends on a high index of clinical suspicion, as well as on the results of laboratory and radiological investigations (computerized tomography and magnetic resonance imaging) [3]. CVF's clinical manifestations, involving the lower urinary tract, frequently include pneumaturia (in 50–95% of cases), faecaluria (in 40–70% of cases), and suprapubic pain (in 30–90% of cases) [4]. Urine analysis and urinary cultures result in the most useful laboratory tests in CVF patients, whereas other routine blood tests are nonspecific [5]. Urine culture report pyuria and bacteriuria, often with the growth of a single specie (ranging from 43 to 71%), rather than a mixed flora [6]. The finding of faecaluria is virtually pathognomonic for CVF: the passage of cloudy, malodorous urine with

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https://doi.org/10.1016/j.plabm.2021.e00255
Received 30 July 2021; Accepted 31 August 2021
Available online 1 September 2021

visible debris is reported by some CVF patients [4]. Microscopic evaluation of urine can show sediments of vegetable matter, leukocytes, and undigested muscle fibers (rhabdomyocytes) [7].

2. Case description

A cloudy, malodorous urine sample with visible debris was collected, on the fifth day of hospitalization in the internal medicine department, by an 84 years old subject. According to the pathognomonic character of faecaluria, the sample was signed to the laboratory for the biochemical and microbiological investigation to define the type and origin of suspended materials. Following clinical requirements, both biochemical pathways and instrumental procedures to confirm or exclude the presence of a faecal component in urine were considered. Urine culture was performed on WASPLab (Copan, Brescia, Italy) system, while chemical-physical and microscopic urine analysis was performed on IRICELL system (Beckman Coulter, Pasadena. USA); particulalrly, the chemistry investigation were performed on iChem VELOCITY (Beckman Coulter, Milano, Italy) and the sediment analysis on Iris IQ 200 (Beckman Coulter, Milano, Italy), a digital flow Morphology investigation system supported by an Auto-Particle Recognition software. No biochemical compound or component able to characterize faecal presence in urine results from laboratory test: microscopic analysis of urinary sediment showed no clear and well-defined images. Thus, in order to characterize the urinary debris, the urinary pellet, obtained by centrifugation, was washed in 5 mL distilled water, dried in oven (60 °C for 2h), and analysed by Fourier Transform-Infrared spectroscopy coupled to Attenuated Total Reflectance (FTIR-ATR) (IRAffinity-1S, Shimadzu, Japan) in the range 4000 ÷ 400 cm −1, normally used for renal stone analysis [8]. ATR method allow to scan the reflected IR light beam with an acquisition depth between 0.5 and 2 μm on a window of 1.5 mm of radius, requiring a small amount of pulverized sample to perform the analysis. The resulting spectrum was compared with the instrument library to identify the debris' compound. Results of urine analysis and urine cultures at the emergency presentation and on the fifth day of hospitalization are reported in Table 1.

Shortly, no pathological findings were observed in the first sample. The presence of leucocytes, red blood cells, bacteria, and 100.000 UFC/mL bacteria of Escherichia coli in urine culture were observed the fourth day, instead. Moreover, in the analysis of urinary sediment, a high amount of dispersed pellet of light brown material, unclassifiable on optical microscopy and initially indicated as possible faecal origin, was observed in the background. ATR/FT-IR spectrum obtained from the light brown material was identified as Keratin, with 901/1000 overlapping with the reference spectrum of the compound present in Spectra Nicodom IR Library [9] (Fig. 1).

2.1. Patient follow-up

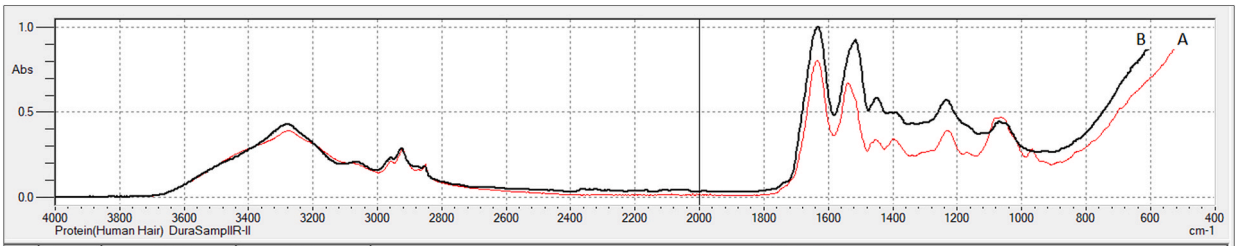
A computed tomography and a cystoscopy was suggested in order to investigate lesion evidences.

3. Discussion

Many imaging and laboratory techniques were proposed to confirm the diagnosis of an entero-urinary tract fistula [1,10]. Laboratory tests used to confirm the presence of a fistula are based on the recovery in the urine samples of different markers after oral or rectal administration. Bourne test, based on a barium enema x-ray examinations of the urine [11], and the 'poppy seed test' [12], based on the oral intake of 35–250 g of poppy seeds and the finding of seeds in the urine within 48 hours from consumption, are the most used

Table 1
Chemical-physical, microscopical analysis and urine culture results.

	Day 1	Day 5	Reference
Colour	light yellow	yellow	
Nitrite	Absent	absent	absent
SW	1.009	1.009	1.015–1.025
Leucocite Esterasi	Absent	500	absent
Aspect	Limpid	Very cloudy	Limpid
pH	5	5	5.0–6.5
Glucose	absent	absent	absent
Protein	10.0 mg/dL	50	0.0–10.0
Haemoglobin	0.03 mg/dL	>1	absent
Chetoacid	absents	absents	absents
Bilirubin	absent	absent	absent
Urobilinogen	absents	absents	0–1
Sediment*			
RBC	nothing to report	1609	/microL
WBC	nothing to report	13900	/microL
Bacteria	nothing to report	numerous	/microL
*Pellet: significant presence of brown fibrous material compatible with faecal origin			
Culture			
UFC/mL	n.d.**	100.000	absent
Species	–	E. Coli	absent
**n.d.: not detected			



	Score	Library	Name	Comment
1	901	21 - ATR-Polymer2	D_Protein1	Protein(Human Hair) DuraSamplIR-II
2	881	85 - Nierstenen met Golden G	STEEN 408 - HUM. SERUM1 A	STEEN 408 - HUM. SERUM1 A/E CHROMS.

Fig. 1. ATR/FT-IR spectra of urinary pellet (A) and Keratin (B) and Spectrum Score table.

and accurated tests, reporting a sensitivity close to 100% and 90%, respectively. However, oral administration is often discomfortable for the patients, especially barium preparations [13]. Many imaging and laboratory techniques were proposed to confirm the diagnosis of an entero-urinary tract fistula [1,10]. Based on the recovery in the urine samples of different markers after oral or rectal administration, the laboratory tests are used to confirm the presence of a fistula. Bourne test, based on a barium enema x-ray examinations of the urine [11], and the 'poppy seed test' [12], based on the oral intake of 35–250 g of poppy seeds and the finding of seeds in the urine within 48 hours from consumption, seems to be the most accurate tests, reporting a sensitivity close to 100% and 90%, respectively. However, oral administration is often discomfortable for the patients, especially barium preparations [13]. Radiological and imaging techniques like cystoscopy, cystography, computed tomography, nuclear medicine scans can provide, instead, more details about lesions' location and aetiology [3], and in some cases, they are better tolerated [13]. To our knowledge, this is the first case confirming the presence of CVF directly on the urinary pellet analysis on FT-IR spectroscopy. FT-IR instrument is used for renal calculi investigation, and it is frequently available in clinical laboratories [14]. In this case, the result obtained by analysing urinary pellet with FT-IR reported the presence of Keratin; this protein is reported as the main physiological component of faeces (as undigested fibers with cellulose). Therefore results of analysis unequivocally can confirm the presence of faecal material in the urine of the subject [15]. Thus, excluding faeces contamination of the urinary samples, faecaluria is pathognomonic for an entero-urinary tract fistula [16]. Deepening of the urine sediment on FT-IR has the same limits and, probably, a lower specificity of the Bourne or the 'poppy seed tests': the possibility of well-characterizing compounds in the urinary pellet with FT-IR depends on the amount of material collected after centrifugation. In fact, the possibility to carry out several analytical repetitions of the sample is crucial to obtain a significant matching with the spectra database. However, the FT-IR coupled with the Attenuated Total Reflection technique requires a very low quantity of dry samples, about 0.21 mm 3 [17]. Therefore, the FT-IR analysis on the urinary pellet, obtained from a sample of subjects with suspected CVF, can be proposed as a fast and cheap method able to improve diagnostic course avoiding any oral administration.

4. Conclusion

Urinary sediment could represent an easy to collect specimen and its non-invasive analysis by FT-IR, in presence on unclassifiable components, must be considered to guide and support clinical and diagnostic decisions, especially in patients with suspected CVF.

Credit author statement

All the authors included in this research contributed to the intellectual content of this paper and have met the following requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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