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Possible application of Raman microspectroscopy to verify the interstitial cystitis diagnosis after potassium sensitivity test: Phenylalanine or tryptophan as a biomarker

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Abstract. There is lack of a worldwide standard technique for clinical diagnosis of interstitial cystitis (IC). Raman spectroscopy with higher specificity and sensitivity has been extensively used to act as a non-destructive analytical technique without special sample preparation. In this preliminary study, possible use of Raman microspectroscopy as an IC diagnostic tool was attempted. Twenty-two participants were screened by clinical features, history, urodynamic evaluations and potassium sensitivity test (PST). The freeze-dried water samples voided from all the participants after PST were directly determined by using a confocal Raman microspectroscopy to search the biomarker. Participants with or without IC symptom were separated into control and clinical groups, according to the above screening. The participants in the clinical group were further divided into mild and severe subgroups by PST. The symptom of urinary pain and urgency was significant difference between the mild and severe subgroups ($p < 0.05$). A significant increase in urinary frequency but a marked reduction in bladder capacity, maximum cystometric capacity and maximum voiding flow rate were obtained for clinical group of IC participants, as compared with the result of control group ($p < 0.05$). By using Raman microspectroscopic determination, the band near 1003 or 1005 cm^{-1} assigned to phenylalanine was respectively detected from the freeze-dried water sample of control group or mild subgroup, but the band at 1010 cm^{-1} due to tryptophan was found in the freeze-dried water sample of severe subgroup. The result of this preliminary study first suggests a possible application of Raman microspectroscopy to strongly certify the results of PST for IC diagnosis. Phenylalanine or tryptophan might be acted as a biomarker to assist the diagnosis of IC after PST. Particularly, the appearance of tryptophan might be used to discriminate the severity of IC symptom.

Keywords: Interstitial cystitis, potassium sensitivity test, Raman microspectroscopy, phenylalanine, tryptophan

1. Introduction

Interstitial cystitis (IC) is a chronic bladder disorder occurring predominantly in women about 90 percent of all cases, which is characterized by urinary frequency, urgency, and pelvic/perineal pain at daytime and night-

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time. The onset of IC usually occurs from 30 to 70 years of age, but the prevalence of IC appears to be increasing among young and middle-aged women [1]. There are multiple theories regarding the cause of IC, but the reason has been not proven yet [2,3]. Because IC is a poorly defined entity of unknown etiology and pathophysiology, the clinical syndrome of IC is not uniform and the symptoms are variable in nature and severity. The difficulty in diagnosis for most IC patients is due to the lack of histological changes and absence of laboratory assays, and is also significant overlap between chronic pelvic pain syndromes of patients and IC [4]. Although several diagnoses for IC have been reported such as clinical assessment, urodynamic testing, cystoscopy, bladder biopsy, urinary markers and intravesical potassium sensitivity testing (PST), there is lack of standard diagnosis method for IC [4,5]. Until now a proper diagnostic tool for IC remains uncertain.

Although the PST has been introduced to help establish the diagnosis of IC by Parsons [6,7], the reliability of PST has been questioned due to the poor sensitivity, specificity, accuracy and controversial results [4,8,9]. Thus, the current PST is not a valid diagnostic tool. In addition, a major criticism of ambulatory urodynamic testing has been reported by its over-diagnosis [10]. In order to develop a uniform, definitive and worldwide standard technique with higher specificity, accuracy and sensitivity for the diagnosis of IC, spectroscopic determination has been considered. Raman spectroscopy is a powerful analytical technique capable of providing highly detailed chemical information for many biological samples [11,12].

Recently, Raman spectroscopy has been extensively reported to have potential for analysis and diagnosis of diseases, such as atherosclerosis, skin, cataractous lens, breast, cervix, and both the mineral and organic components of bone [13–17]. This technique not only has higher specificity and sensitivity but also acts as a non-destructive analysis without special sample preparation. For these reasons, we attempted to approach the possible use of Raman microspectroscopy as a clinical IC diagnostic tool via detecting the biomarker of freeze-dried water samples voided from all the participants after PST stimulation. The result of this preliminary study by using Raman microspectroscopic detection will be available for more certifying the result of PST.

2. Materials and methods

In this preliminary study, twenty-two female volunteers having 25 to 76 years old were presented to our outpatient department for their occasional painful sensation in the urinary tract. All the participants were fasted for at least 10 hrs before test to eliminate any possibility of the impact of dietary intake. All volunteers were undergone by cystoscopic screening, and glomerulations was essentially used to divide those volunteers with possible IC from the controlled participants. Two groups were divided by performing the IC screening (Table 1): the first one was a clinical group with IC syndromes ($n = 12$), the second one was a control group without IC or urethral syndromes ($n = 10$). The participants were examined by PST, according to the procedure of Parsons's report [5,6], but a modification of the test using 0.2 M KCl instead of 0.4 M KCl was made to reduce irritation [18]. All the participants were screened by a questionnaire (Table 1) after continual installing distilled water (solutions 1) and 0.2 M KCl solution (solution 2). The participants were asked which solution caused more urgency or more pain than the other, and then rated the degree of provocation with pain and urgency on a scale of 0 (no symptom) to 10 (maximal symptom). An urodynamic evaluation was also performed with a Menuet Compact Plus (Dantec, Denmark) in accordance with the guidelines established by the International Continence Society and Chuang's report [19,20].

After removing the 0.2 M KCl solution from bladder, 40 ml of distilled water was installed again for maintaining 5 min, and then collected 10 ml of voided water for further study. Aliquots (3.0 mL) were placed in a glass tube, then immersed in N_2 until frozen, and freeze-dried (Christ Gamma 1–20, Osterode am Harz, Germany; $p < 50 \times 10^{-3}$ mbar, condenser temperature $= -45°C$). Each freeze-dried sample was directly determined by using a confocal micro-Raman spectrophotometer (Ventuno, Jasco Co., Tokyo, Japan) equipped with a 30 mW green (532 nm) solid-state laser as standard via non-destructive analysis [21,22]. The pixel resolution was 1.3 cm^{-1}. A Teflon plate was used as a substrate for Raman determination. Each sample was determined three times and the averaged Raman spectra were obtained. No any degradation was found during several laser irradiations. This spectroscopic study was approved by the Institutional Review Board at the Taipei Veterans General Hospital, and all the procedures were adhered to the Declaration of Helsinki.

For comparisons of these variables, analysis of variance (ANOVA) was used. The p values <0.05 were considered significant.

Table 1

The results of clinical features, history, PST and urodynamic evaluations for participants with or without IC

Groups		IC Screening		History		PST		Urodynamic ecaluation				Raman result
		Glomerulation	Hematuria	Age (years)	Parity (No.)	Pain	Urgency	Frquency/ Day (times)	UD capacity (ml)	Maximum cystometric capacity (ml)	Maximum voiding flow rate (ml/s)	
Control	($n = 10$)	$-$	$-$	50.2 ± 7.3	2.6 ± 0.5	$-$	$-$	8.23 ± 2.6	444.3 ± 147.3	430 ± 252.9	25.7 ± 12.9	Phenylalanine
Clinical	Mild syndrome ($n = 7$)	$++$	$+$	37 ± 7.9 (*$p < 0.05$)	1.6 ± 1.6 (* $p > 0.05$)	2 ± 2.5	1.8 ± 2.4	20.9 ± 6.3 (* $p < 0.05$)	272.3 ± 130.8 (* $p > 0.05$)	237.9 ± 67.8 (* $p > 0.05$)	7.7 ± 3.3 (* $p < 0.05$)	Phenylalanine
	Severe syndrome ($n = 5$)			53 ± 17.3 (** $p > 0.05$) (†$p > 0.05$)	3.8 ± 2.1 (** $p > 0.05$) (†$p > 0.05$)	5.6 ± 1.8 (** $p > 0.05$)	5.8 ± 1.7 (** $p > 0.05$)	22.4 ± 6.1 (** $p > 0.05$) (†$p < 0.05$)	219 ± 63.9 (** $p > 0.05$) (†$p < 0.05$)	207 ± 77.4 (** $p > 0.05$)	9.7 ± 3.8 (** $p > 0.05$) (†$p < 0.05$)	Tryptophan

Note: $-$: not find; $+$: found; $++$: obviously found.
*: versus control group.
**: versus mild syndrome.
†: versus control group.

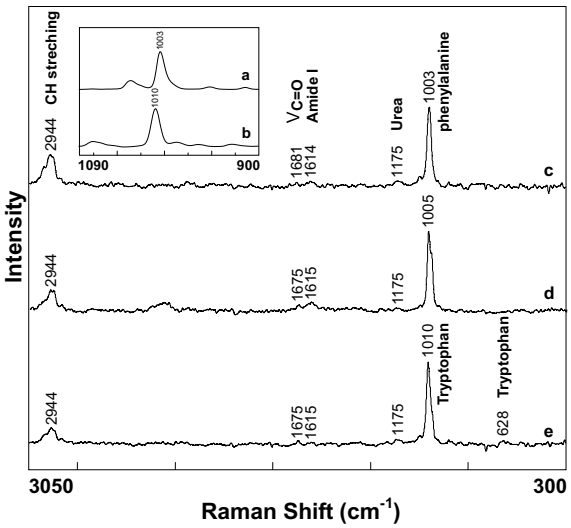

Fig. 1. Raman spectra of intact phenylalanine and tryptophan, as well as the representative freeze-dried water samples voided after PST. Key: a, intact phenylalanine; b, intact tryptophan; c, freeze-dried water sample of control groups; d, freeze-dried water sample of mild subgroups; e, freeze-dried water sample of severe subgroups.

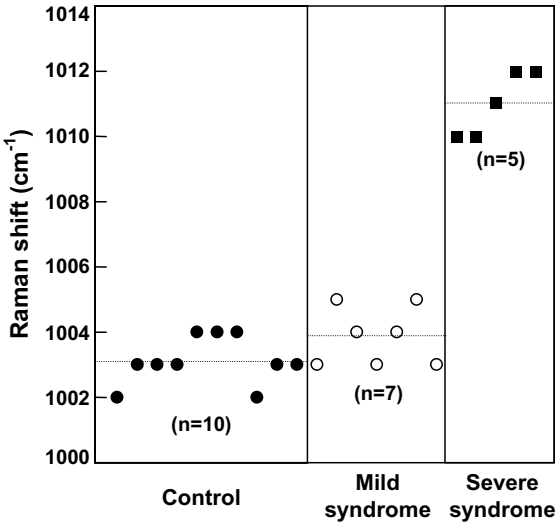

Fig. 2. Raman shift variability of samples in the same group or different groups after Raman microspectroscopic determinations. Key: dotted line indicates the mean value of Raman shift.

3. Results

The glomerulation and hematuria were first used to screen the participants with or without IC. According to the above examinations, participants with or without IC symptom were clearly separated into control and clinical groups, as shown in Table 1. Moreover, the participants in the clinical group were further divided into two subgroups after PST, one was a mild and the other was a severe. It is evident that the urinary pain and urgency after PST exhibited a significant difference between the mild and severe subgroups ($p < 0.05$). The data of urodynamic evaluations for clinical group of IC participants also showed a significant increase in urinary frequency but a marked reduction in bladder capacity, maximum cystometric capacity and maximum voiding flow rate, as compared with the result of control group ($p < 0.05$).

PST has been advocated as an office diagnostic screening method for IC, but it has been interrogated due to poor specificity and lacking sensitivity [4,8,9]. In order to further exactly confirm the results of PST for IC diagnosis, the freeze-dried water samples voided after PST has been once more examined by Raman microspectroscopy to detect the biomarker leaked from the lesions of the bladder. Figure 1 shows that Raman spectra of the intact phenylalanine and tryptophan, as well as the representative freeze-dried water samples voided after PST. The variability occurred between samples in the same group or different group is also displayed in Fig. 2. Obviously, all the data were so gathered up and the differences in variability between same groups were minimized. This indicates that the sample preparation and determination method were optimized. It clearly evidences that only a predominant band near 1003 or 1005 cm^{-1} due to phenylalanine was observed in the representative Raman spectra of freeze-dried water samples voided from control group ($n = 10$) or from mild subgroup ($n = 7$) of the clinical group (Fig. 1c and d), as compared with the Raman band at 1003 cm^{-1} of intact phenylalanine (Fig. 1a) [23,24]. From Fig. 2, however, the mean values of Raman shift for both control group and mild subgroup (1003.100 $\pm$ 0.738 for control group but 1003.857 $\pm$ 0.899 for mild subgroup) at this preliminary report did not show any significant difference ($p > 0.05$), due to a small sample size. Whereas only one domain band at 1010 cm^{-1} assigned to tryptophan (Fig. 1b) appeared in the Raman spectrum of freeze-dried water samples for severe subgroup ($n = 5$) of the clinical group (Fig. 1e). The result is also listed in Table 1. It is interesting to note that phenylalanine or tryptophan was uniquely detected by Raman microspectroscopy in each freeze-dried water sample voided after PST, suggesting that phenylalanine or tryptophan might be used as a biomarker to certify the results of PST.

4. Discussion

It is well known that the bladder epithelium of normal individuals is relatively impermeable. However, patients with IC symptom have a dysfunctional bladder epithelial membrane to improve the urothelial permeability of urinary compounds, especially potassium, resulting in urge sensation and consecutive stimulation [6, 7]. Although the current consensus concerning PST and urodynamics are not enough to diagnose the IC symptom, these techniques may still provide useful information on the differential diagnosis of painful voiding disorders and the symptoms of the overactive bladder [4,8,9]. In this preliminary study, the results of PST and urodynamic evaluations were the same as the other reports in which the sensory urgency and instability, reduced bladder capacities, and pain with bladder filling at low volumes were found for many IC patients [25, 26].

The result of this preliminary study proposes a new vibrational spectroscopic detection to further reinforce and certify the results of PST for IC diagnosis. Raman microspectroscopy is convenient and easy for spectroscopic detection of biosamples, particularly the freeze-dried water samples voided after PST were less complicated than that of the dried samples of common urine. A few bands were also observed in the Raman spectrum of each freeze-dried water sample voided after PST. The prominent Raman bands at 1003 (1005) or 1010 cm^{-1} might be assigned to the phenylalanine or tryptophan, respectively. The band at 825 cm^{-1} was also corresponded to the tryptophan. The weak bands within 1680–1610 cm^{-1} were ascribed to amide I vibration of protein, but the band at 1175 cm^{-1} was assigned to the urea [23,24]. In particular, the appearance of phenylalanine or tryptophan in the freeze-dried water samples voided after PST might be a possible biomarker to assist the IC diagnosis, though the leading mechanism of both amino acids is unknown. This preliminary study suggests that tryptophan might be used as a special marker to discriminate the severity of IC symptom, although phenylalanine could not yet separate the control group and mild subgroup by using less number of participants. Even if the absence of tryptophan in urine after dietary modification had been reported [27], the differences of patient's species and common food intake might be responsible for this reason. However, further study should be examined in more detailed to certify the possible use of Raman microspectroscopy for IC diagnosis.

5. Conclusions

The present study suggests that a non-destructive analytical technique of Raman microspectroscopy might possibly be used to exactly certify the results of PST for diagnosis of IC. Phenylalanine or tryptophan seemed to be a possible biomarker to assist the IC diagnosis after PST.

References

[1] J.F. Metts, Interstitial Cystitis: Urgency and Frequency Syndrome, *Am Fam Physician* **64** (2001), 1199–1206, 1212–1214.

[2] C.W. Butrick, Interstitial cystitis and chronic pelvic pain: new insights in neuropathology, diagnosis, and treatment, *Clin Obstet Gynecol* **46** (2003), 811–823.

[3] K. Bouchelouche and J. Nordling, Recent developments in the management of interstitial cystitis, *Curr Opin Urol* **13** (2003), 309–313.

[4] G.R. Sant and P.M. Hanno, Interstitial cystitis: current issues and controversies in diagnosis, *Urology* **57**(Suppl 1) (2001), 82–88.

[5] J. Nordling, Interstitial cystitis: how should we diagnose it and treat it in 2004? *Curr Opin Urol* **14** (2004), 323–327.

[6] C.L. Parsons, P. Zupkas and J.K. Parsons, Intravesical potassium sensitivity in patients with interstitial cystitis and urethral syndrome, *Urology* **57** (2001), 428–432.

[7] C.L. Parsons, M. Bullen, B.S. Kahn, E.J. Stanford and J.J. Willems, Gynecologic presentation of interstitial cystitis as detected by intravesical potassium sensitivity, *Obstet Gynecol* **98** (2001), 127–132.

[8] P. Hanno, Is the potassium sensitivity test a valid and useful test for the diagnosis of interstitial cystitis? *Int Urogynecol J* **16** (2005), 428–429.

[9] M. Gregoire, F. Liandier, A. Naud, L. Lacombe and Y. Fradet, Does the potassium stimulation test predict cystometric, cystoscopic outcome in interstitial cystitis? *J Urol* **168** (2002), 556–557.

[10] A.P. Cassidenti and D.R. Ostergard, Multichannel urodynamics: ambulatory versus standard urodynamics, *Curr Opin Obstet Gynecol* **11** (1999), 485–487.

[11] J.R. Baena and B. Lendl, Raman spectroscopy in chemical bioanalysis, *Curr Opin Chem Biol* **8** (2004), 534–539.

[12] R. Petry, M. Schmitt and J. Popp, Raman spectroscopy–a prospective tool in the life sciences, *Chemphyschem* **4** (2003), 14–30.

[13] E.B. Hanlon, R Manoharan, T.W. Koo, K.E. Shafer, J.T. Motz, M. Fitzmaurice, J.R. Kramer, I. Itzkan, R.R. Dasari and M.S. Feld, Prospects for *in vivo* Raman spectroscopy, *Phys Med Biol* **45** (2000), R1–R59.

[14] N.D. Sahar, S.I. Hong and D.H. Kohn, Micro- and nano-structural analyses of damage in bone, *Micron* **36** (2005), 617–629.

[15] J.T. Motz, S.J. Gandhi, O.R. Scepanovic, A.S. Haka, J.R. Kramer, R.R. Dasari and M.S. Feld, Real-time Raman system for in vivo disease diagnosis, *J Biomed Opt* **10** (2005), 031113–1∼031113-8.

[16] M.T. Liu, W.T. Cheng, M.J.Li, H.N. Liu, D.M. Yang and S.Y. Lin, Identification of chemical compositions of skin calcified deposit by vibrational microspectroscopies, *Arch Dermatol Res* **297** (2005), 231–234.

[17] S.W. Van de Poll, T.J. Romer, G.J. Puppels and A. Van der Laarse, Imaging of atherosclerosis: Raman spectroscopy of atherosclerosis, *J Cardiovasc Risk* **9** (2002), 255–261.
[18] L.K. Daha, C.R. Riedl, G. Hohlbrugger, M. Knoll, P.F. Engelhardt and H. Pfluger, Comparative assessment of maximal bladder capacity, 0.9% NaCl versus 0.2 M KCl, for the diagnosis of interstitial cystitis: a prospective controlled study, *J Urol* **170** (2003), 807–809.
[19] W. Schafer, P. Abrams, L. Liao, A. Mattiasson, F. Pesce, A. Spangberg, A.M. Sterling, N.R. Zinner and P. Van Kerrebroeck, International Continence Society. Good urodynamic practices: uroflowmetry, filling cystometry, and pressure-flow studies, *Neurourol Urodyn* **21** (2002), 261–274.
[20] T.Y. Chuang, K.J. Yu, I.W. Penn, Y.C. Chang, P.H. Lin and Y.A. Tsai, Neurourological changes before and after radical hysterectomy in patients with cervical cancer, *Acta Obstet Gynecol Scand* **82** (2003), 954–959.
[21] K.H. Chen, W.T. Cheng, M.J. Li, D.M. Yang and S.Y. Lin, Calcification of senile cataractous lens determined by Fourier transform infrared (FTIR) and Raman microspectroscopies, *J Microsc* **219** (2005), 36–41.
[22] S.Y. Lin, K.H. Chen, M.J. Li, W.T. Cheng and S.L. Wang, Evidence of octacalcium phosphate and Type-B carbonated apatites deposited on the surface of explanted acrylic hydrogel intraocular lens, *J Biomed Mater Res* **70B** (2004), 203–208.
[23] N. Stone, C. Kendall, J. Smith, P. Crow and H. Barr, Raman spectroscopy for identification of epithelial cancers, *Faraday Discuss* **126** (2004), 141–157.
[24] A.T. Tu, Peptide backbone conformation and microenvironment of protein side chains, in: *Spectroscopy of Biological Systems*, R.J.H. Clark and R.E. Hester, ed., Adv. in Spectroscopy, Vol. 13, John Wiley & Sons, Chichester, 1986, pp. 47–112.
[25] W.B. Steinkohl and G.E. Leach, Urodynamic findings in interstitial cystitis, *Urology* **34** (1989), 399–401.
[26] P. Toozs-Hobson, C. Gleeson and L. Cardozo, Interstitial cystitis–still an enigma after 80 years, *Br J Obstet Gynecol* **103** (1996), 621–624.
[27] L. Gillespie, Metabolic appraisal of the effects of dietary modification on hypersensitive bladder symptoms, *Br J Urol* **72** (1993), 293–297.