391

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QUANTITATIVE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY OESTRIOL SERUM LEVELS IN NEW AND CHRONIC USERS OF VAGINAL OESTRIOL CREAM

Hypothesis / aims of study

Vaginal oestriol (E_3) cream is frequently used by Urologists and Urogynaecologists to treat women with urinary incontinence, prolapse, and/ or recurrent cystitis. With increasing awareness of the association between oestrogen and breast cancer, patients often enquire about its safety. We have always considered it to be safe, based on (1) showing serum E_3 levels below the traditional menopausal cut off of 90 pmol/L in current cream users. However, these measurements were performed using radioimmunoassay (RIA), which has limited published validation data and is known to suffer from issues of both specificity and sensitivity, particularly at the very low levels of circulating oestrogens found during menopause.

With the advent of quantitative liquid chromatography tandem mass spectrometry (LC-MS/MS), the aim of this study is to measure changes in serum E_3 levels in vaginal E_3 cream users much more accurately than with the previously used RIA. We hypothesise that, although blood levels of patients using E_3 cream will be higher than controls, they will remain below 90 pmol/L. This is a novel study, to our knowledge it has not yet been performed.

Study design, materials and methods

The study comprised 2 groups of post-menopausal women with urogynaecological complaints. Group 1 (new users) tested at baseline and 12 weeks as follows: women not using E_3 cream or any other systemic or local oestrogen therapy had their oestrone (E_1), oestradiol (E_2) and E_3 levels determined prior to commencing E_3 therapy. These participants used 1mg/g E_3 cream, daily for the first 3 weeks, and then 3 times per week from weeks 4-12 as per written instructions. A compliance diary was kept. After 12 weeks of treatment, having applied E_3 cream the night before, a second sample was taken, thus patients acted as their own control. Group 2 (chronic users): women who had been using 1mg/g E_3 cream 3 times a week for more than 3 months, applied the cream at bedtime, then had serum levels between 9-11am the next morning. Target sample sizes are 30 new patients and 50 chronic users.

In Group 2, a small sub group of these chronic users agreed to have trough and peak levels done, as follows. After abstaining the cream for 2.5 days, venepuncture for trough levels was performed in clinic. They then applied the E_3 cream in clinic and subsequently had peak serum levels E_1, E_2 and E_3 measured 2 hours later (2). The lower limit of quantitation of the LC-MS/MS assay is 5 pmol/L.

Results

At present 30 new users and 32 chronic users have enrolled, blood levels for 10 new users and 9 chronic users are still awaited. Of the new users, 1 patient was excluded as she recommenced menstruating, 5 patients stopped using the E_3 cream.

Treatment compliance was good (range 98-100%). None of the new users had detectable serum E_3 at baseline (all <5 pmol/L, Table 1). The 12 week E_3 levels, which were measured in clinic at a mean of 11.8 hours (SD=1) following cream application the night before, were as follows: median 29.4 pmol/L (IQR 11.4; 84.1). Therefore, the results were generally well below the traditional 90 pmol/L cut off for menopausal serum levels. However 3 patients were quite elevated, relevant factors are being explored (max 188.0 pmol/L).

Of the chronic users (Table 2), 1 patient was excluded, as there was uncertainty about her menopausal status. The chronic users had been using E_3 cream for a median of 21.0 months (IQR 9.2; 38.4). Following a median of 12.4 hours after application of the cream (IQR 11.1; 13.6), serum E_3 levels were median 19.2 pmol/L (IQR 6.6; 35.3), ie. well below the menopausal level.

Peak and trough levels have been performed in 8 patients, however further recruits are required to attain meaningful results.

Interpretation of results: In general, aside from 3 outliers, serum oestriol results are well below menopausal levels. In contrast to previous authors we found that E_2 and E_1 were significantly decreased post E_3 cream application (3).

Concluding message: The authors were surprised to find firstly that the old literature using RIA was so scarce (n=8 papers) and secondly that this study appears to be the first report of E_3 data gathered using LC-MS/MS. These initial results are reassuring and we await the results of the remaining chronic users, our goal sample size (n=50) is still being recruited.

Table 1: New Users (n=19)

	median/mean	IQR/SD	range
Age (years)^	67	59.8 ; 73.5	47 – 79
Length of menopause (years)^	20	6.5 ; 24	1 – 38
Parity#	2.3	1.3	0 – 7
BMI#	29.6	5.1	21.2 – 39.5
E_3 serum levels pre oestriol cream in pmol/L^	<5*	<5 ; <5	<5 – <5
E_3 serum levels post oestriol cream in pmol/L^	29.4*	11.4 ; 84.1	4.0 – 188.0
E_2 serum levels pre oestriol cream in pmol/L^	18.3*	8.9 ; 26.3	3 – 113
E_2 serum levels post oestriol cream in pmol/L^	15.5*	5.1 ; 25.4	3 – 37.6
E_1 serum levels pre oestriol cream in pmol/L^	78.9*	51.5 ; 114.7	15.2 – 379.9
E_1 serum levels post oestriol cream in pmol/L^	57.8*	35.4 ; 111.9	16.1 – 135.6
Length of time oestriol cream used in weeks#	12.7	1.1	11.1 – 15.7
Period of time in hours between oestriol cream application and serum level sampling#	11.8	1	10.3 – 13.3

^median and IQR, #mean +/- SD (normally distributed), * Significant difference ($p<0.05$) between serum levels pre- and post oestriol cream

Table 2: Chronic Users (n=22)

	median	IQR	Range
Age (years)	74.5	65.8 ; 80.0	52 – 88
Length of menopause (years)	25	16 ; 33	2 – 41
Parity	2	2 ; 3	1 – 6
BMI	27.5	24.2 ; 30.1	22.6 – 44.9
E_3 serum levels in pmol/L	19.2	6.6 ; 35.3	<5 – 74.5
E_2 serum levels in pmol/L	8.4	3.0 ; 14.9	3.0 – 25.8
E_1 serum levels in pmol/L	63.7	44.7 ; 87.4	28.4 – 122.2
Period of time in hours between oestriol cream application and E3 serum level sampling	12.4	11.1 ; 13.6	8.8 – 14.7
Length of time oestriol cream used in months	21.0	9.2 ; 38.4	3.3 – 112.7

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Disclosures

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