Adequacy of hormone replacement therapy for osteoporosis prevention assessed by serum oestradiol measurement, and the degree of association with menopausal symptoms

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SUMMARY

Background. *Patients on hormone replacement therapy (HRT) for osteoporosis prevention rather than menopausal symptom control may be asymptomatic, despite inadequate replacement and low serum oestradiol (E_2) levels. In the primary health care setting, therapeutic monitoring of HRT is not carried out routinely so that patients with serum E_2 levels inadequate to protect bone may be missed.*

Aim. *To determine the proportion of women on transdermal E_2 preparations with serum E_2 levels insufficient to protect bone and to assess the value of a questionnaire-derived menopausal symptom score (MSS) for detecting these patients.*

Method. *A cross-sectional analysis of 45 patients aged 35–70 years using transdermal E_2 preparations obtained from a computer register of 14 500 patients in a suburban practice. One blood sample was obtained from each patient at the time the MSS questionnaire was completed. Serum E_2 concentration was measured using a fluoroimmuno-assay and compared with the MSS. Levels below 150 pmol/l were considered to be insufficient to protect bone. The diagnostic accuracy of the MSS in screening for levels below 150 pmol/l was determined using receiver operating characteristic (ROC) curve analysis.*

Results. *The median (95% CI) serum E_2 was 147 pmol/l (126–198 pmol/l) and levels were below 150 pmol/l in 24 out of 45 patients. There was no difference in the MSS (median, 95% CI) between those with serum E_2 < 150 pmol/l (8.5, 5.0–17) and 150 pmol/l (9.0, 5.0–14; P=0.477). The degree of association between the serum E_2 and the MSS, using the Spearman rank correlation coefficient, r_s (95% CI) was small and not significant (–0.04, –0.34 to 0.26; P=0.398). ROC curve analysis revealed an area under the curve (95% CI) of 0.51 (0.33–0.68).*

Conclusions. *More than half the women were inadequately replaced to protect against osteoporosis. Furthermore, the MSS was of no value in screening for those with low serum E_2 levels. Serum E_2 levels should be monitored in women on HRT for osteoporosis prevention and the E_2 dosage adjusted accordingly.*

Keywords: hormone replacement therapy; osteoporosis; menopause.

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Introduction

HORMONE replacement therapy (HRT) is widely used for the treatment of menopausal symptoms and osteoporosis prevention. Although the adequacy of menopausal symptom control can be assessed clinically, this is not the case in osteoporosis. In the absence of bone histology, the gold standard is bone mass measurement, but this requires a year or two between assessments to detect significant changes and hence the adequacy of treatment.[1,2] In the general practice setting, a surrogate marker for monitoring the adequacy of replacement is required.

In the course of HRT, average levels of serum oestradiol (E_2) in the early to mid-follicular phase range (60 pg/ml, 220 pmol/l) are adequate to normalize calcium excretion and protect bone in most women.[3] Furthermore, HRT causes gains in bone density that are usually correlated with serum oestrogen levels.[4] Thus, serum E_2 measurement may be of value as a marker for assessing the adequacy of replacement in the intervening period between bone mass measurements. This has not been used widely because of the poor specificity of serum E_2 assays. This problem is confined to oral HRT because of the increased circulating levels of oestrone and biologically inactive oestrogen conjugates that are associated with this mode of administration and that cross-react in the assay.[5-10] This may be overcome by including an extraction step to isolate unconjugated E_2, but this adds greatly to the duration and cost of the assay and is not generally available. Consequently, serum E_2 levels are not routinely measured in the general practice setting and patients may not be being adequately replaced.

The assay cross-reactivity encountered in patients on oral HRT is not a problem with parenteral administration.[7-10] Hence, serum E_2 levels were determined in patients on transdermal E_2 preparations. A serum E_2 of 150 pmol/l was defined as the minimum desirable level to protect against osteoporosis. The value of a questionnaire-derived menopausal symptom score (MSS)[11] for detecting patients with serum E_2 levels below 150 pmol/l was also evaluated to provide an instant assessment of replacement status, while reducing the need for blood testing.

Patients, materials and methods

Patients

The study was approved by Stockport Health Commission Ethics Committee and written informed consent was obtained from each patient taking part. The inclusion criteria were women on the practice register aged 35–70 years and on transdermal E_2 patches for at least one month. Exclusion criteria included oral HRT within the past month, subcutaneous E_2 implant within the last five years and a history of liver, kidney or bowel disease.

Of the 14 500 patients on the practice computer register, 7200 were women, of whom 503 were on HRT and 423 were on oral therapy. Of the 72 patients on transdermal preparations, 69 were in the age range 35–70 years and were sent a postal invitation

with a reply slip and a stamped addressed envelope. Fifty four agreed to take part, of whom 45 fulfilled the study criteria. Reasons for exclusion were non-responders (15), E_2 implant therapy within the past five years (7) and recent change in HRT (2).

The indications for HRT were menopausal symptoms (31) and osteoporosis (9). Patients on HRT following total abdominal hysterectomy and bilateral salpingo-oophorectomy (11) were considered to be on treatment for both menopausal symptom control and bone protection. The transdermal E_2 preparations used (number of patients) delivered doses of 25 μg/24 h (8), 50 μg/24 h (30), 75 μg/24 h (2), 80 μg/24 h (1) and 100 μg/24 h (4). Most patients were using reservoir (37) rather than matrix (8) patch formulations. The duration of patch application (median, 95% confidence intervals, CI) at the time of sampling was 24 h (20–36 h). The characteristics of the patients (median, 95% CI) were as follows: age 52.9 years (50.7–55.8 years), height 1.60 m (1.59–1.64 m) and weight 63.5 kg (61.0–70.0 kg).

Materials and methods

One blood sample was collected from each patient within 72 hours of patch application (except for three taken within 96 hours), allowed to clot, centrifuged within 2 hours of collection and the serum stored at -20ÞC until assay. The serum E_2 concentration was determined using a time-resolved fluoroimmunoassay system (DELFIA, Wallac Oy, Turku, Finland). The within-batch coefficient of variation (CV) was <12% over the range 50–1500 pmol/l and the between-batch CVs were 12.5%, 5.1%, 7.4% and 9.0% at 270, 472, 1763 and 3205 pmol/l respectively. The assay detection limit was 50 pmol/l and the bias was –18.9% [UK National External Quality Assessment Scheme (NEQAS) for steroid hormones, University of Wales, Cardiff].

Patients completed a questionnaire enquiring about 11 menopausal symptoms, based on the menopausal index reported by Kupperman *et al.*[11] The possible responses were absent, mild, moderate or severe, which scored zero, one, two or three respectively. Each symptom score was multiplied by a weighting factor: hot flushes/night sweats (x4); pins and needles, difficulty sleeping or nervousness/anxiety (x2); and the remaining symptoms (x1). The products were summed to give the MSS ranging from 0 to 51. Scores less than 16 were indicative of adequate menopausal symptom suppression.[11]

Data handling and statistical analysis

Non-parametric statistics were used because the data were not normally distributed. Results were presented as the median and 95% CI.[12] The degree of association between serum E_2 and the MSS, or the dose of E_2 per unit body mass and the serum E_2 was determined using Spearman's rank correlation coefficient, r_s, with a correction for ties.[13] The associated 95% CI was calculated as outlined in Gardner and Altman.[12] The significance of the difference between the median MSS values of the patients with serum E_2 < and ≥150 pmol/l was determined using the Wilcoxon-Mann–Whitney test for two independent samples with an adjustment for large samples (n>10) and ties.[13] The tests were one-tailed and values of P <0.05 were considered significant.

The diagnostic accuracy of the MSS in predicting whether the serum E_2 was low (<150 pmol/l) or sufficient (≥150 pmol/l) to protect bone was assessed by constructing a receiver operating characteristic (ROC) curve.[14-16] Diagnostic sensitivity (true-positive rate, TPR) and 1–specificity (false-positive rate, FPR) pairs were calculated for each possible MSS threshold value and the TPR was plotted against the FPR. The area under the curve (AUC) was an estimate of the probability of the MSS correctly classifying the serum E_2 as < or ≥150 pmol/l. The AUC and its 95% CI was calculated as described by Henderson.[15] An AUC value of 0.5 indicated a test of no discriminatory value, whereas values of 0.5–0.7, 0.7–0.9 and >0.9 indicated a test of low, moderate and high accuracy respectively.[17]

Results

The frequency distribution of serum E_2 levels among all 45 patients is shown in Figure 1. The median serum E_2 was 147 pmol/l (126–198 pmol/l) and 24 out of 45 patients had a serum E_2 concentration <150 pmol/l. The median E_2 dose received by patients with serum E_2 <150 pmol/l (0.71 μg/kg/24 h, 0.42–0.83 μg/kg/24 h) was significantly lower than those with serum E_2 ≥150 pmol/l (0.91 μg/kg/24 h, 0.79–1.03 μg/kg/24 h; P = 0.003). There was a significant correlation between the serum E_2 concentration and the E_2 dose received per unit body mass (r_s = 0.37, 0.09–0.60; P = 0.007; Figure 2). The age, height, weight, smoking, alcohol intake and duration of patch application did not differ significantly between the two groups.

The overall median MSS was 9 (6–12). The MSS was <16 in 36 out of 45 patients, of whom 17 had serum E_2 <150 pmol/l and

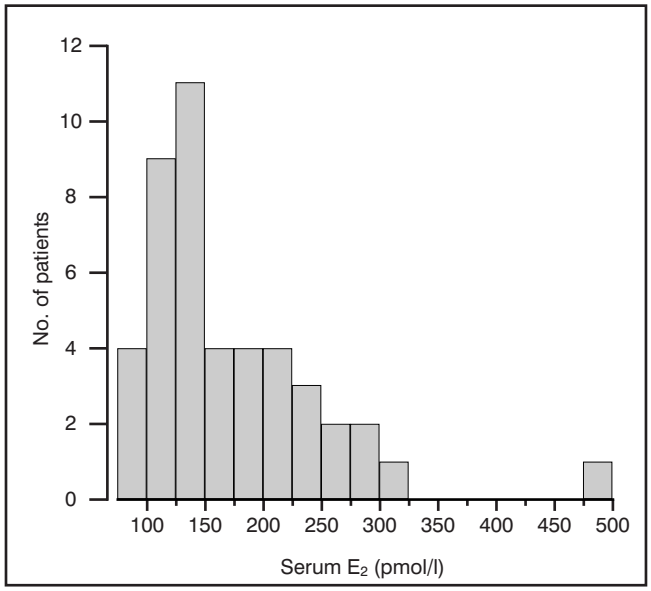

Figure 1. Frequency distribution of serum E_2 levels (pmol/l) in 45 patients on transdermal E_2 preparations.

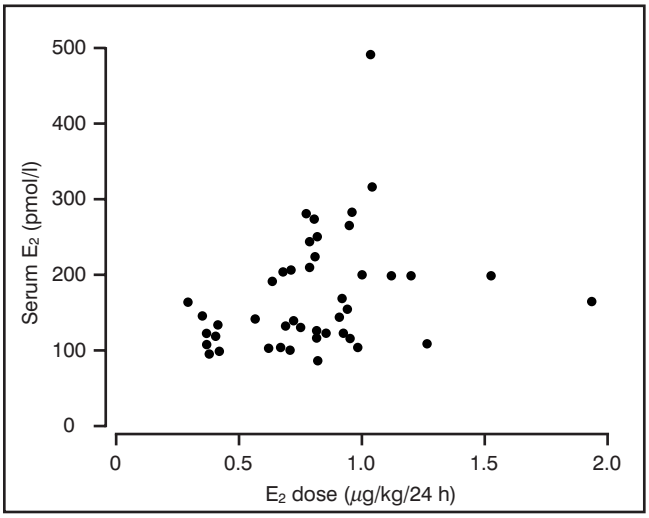

Figure 2. Scatter plot of serum E_2 levels (pmol/l) and the E_2 dose rate per unit body mass (μg/kg/24 h).

19 had serum E_2 ≥150 pmol/l. The MSS was ≥16 in 9 patients, of whom 7 had serum E_2 < 150 pmol/l and 2 had serum E_2 ≥150 pmol/l (Figure 3). There was no difference in the MSS between those with serum E_2 <150 pmol/l (8.5, 5.0–17) and ≥150 pmol/l (9.0, 5.0–14; P = 0.477). The degree of association between serum E_2 and MSS was small and not significant (r_s = –0.04, –0.33 to 0.26; P = 0.398). Furthermore, there was substantial overlap in the MSS values between the two groups (Figure 3). ROC curve analysis revealed an AUC of 0.51 (0.33– 0.68; Figure 4).

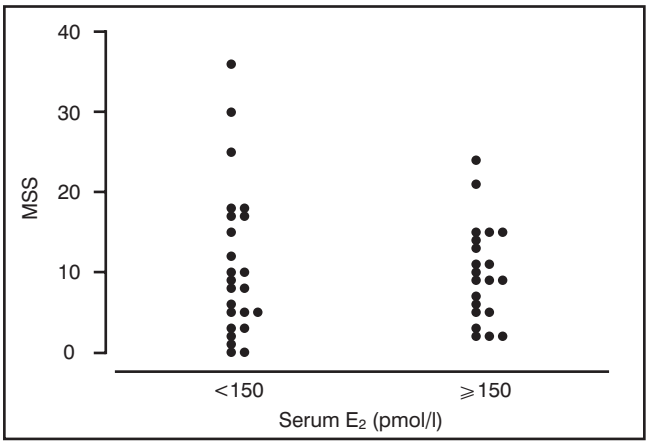

Figure 3. Dot-plot of menopausal symptom scores (MSS) according to serum E_2 group. This demonstrates the difficulty in finding an MSS threshold value that completely separates the two serum E_2 groups.

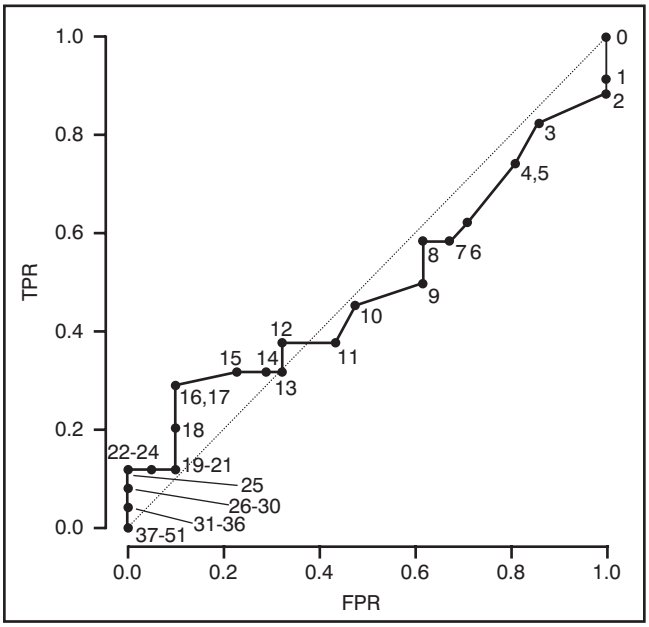

Figure 4. Receiver operating characteristic curve. Plot of true-positive rate (TPR) and false-positive rate (FPR) pairs calculated for each possible menopausal symptom score threshold value between 0 and 51 (figures in italics). The area under the curve (AUC) was 0.51 (95% CI 0.33–0.68). The dotted line represents the plot of a hypothetical screening test of no diagnostic value and AUC of 0.5. The plot of a perfect test describes a line commencing at coordinates (0,0), ascends perpendicularly to (0,1) then horizontally to (1,1) and has an AUC of unity.

Discussion

Adequate replacement is said to be achieved with conjugated equine oestrogens 0.625 mg daily or transdermal E_2 50 μg/24 h because these doses result in an overall rise in bone density relative to untreated groups.[18-21] However, although bone loss may be arrested overall, this may not be the case in an individual patient. For example, Evans and Davie[22] reported that transdermal E_2 of 25 μg/24 h or 50 μg/24 h prevented bone loss equally effectively. However, 3 out of 77 patients (3.9%) lost bone from the lumbar spine and 8 out of 77 patients (10.4%) lost bone from the femoral neck over a 3-year treatment period.

Serum E_2 levels are reported to correlate with gains in bone density[23,24] and are linearly related to the E_2 dose administered.[25,26] We found a significant correlation between the serum E_2 and the dose administered per unit body mass (r_s = 0.37, 0.09–0.60; P = 0.007). Furthermore, the median E_2 dose received by those with serum E_2 <150 pmol/l (0.71 μg/kg/24 h) was lower than those with levels 150 pmol/l (0.91 μg/kg/24 h; P = 0.003). Therefore, it would seem logical to use the serum E_2 level to judge the adequacy of replacement between bone mass measurements.

Serum E_2 levels between 50 and 100 pg/ml (184–368 pmol/l) are sufficient to reduce bone turnover[27] and prevent bone loss.[28] We chose 150 pmol/l to be the lowest desirable level based on these data, allowing for the E_2 assay bias of –18.9% (i.e. 184 x (1-0.189) = 150 pmol/l). The median serum E_2 in our patients was 147 pmol/l (126–198 pmol/l) and 24 out of 45 patients (53%) had levels below 150 pmol/l. Even when the 8 out of 45 patients on 25 μg/24 h patches were excluded, the median serum E_2 was only 164 pmol/l (134–204 pmol/l), with 17 out of 37 (46%) patients having serum E_2 levels below 150 pmol/l. Presumably, this was because those with low serum E_2 levels received a lower E_2 dose per unit body mass.

A contributory factor might have been poor compliance, particularly in asymptomatic patients on HRT for bone protection. Ryan *et al.*[29] reported almost 40% of patients started on HRT at an osteoporosis screening centre were not taking their medication 8 months later. However, a study of 348 patients in primary health care found three-quarters of women took their HRT regularly,[30] possibly because most were on treatment for menopausal symptom suppression.

These findings could have important implications for osteoporosis prevention. Altogether, 44% of our patients were on treatment to protect bone, and serum E_2 levels were insufficient in 53%. If these data were a true reflection of the situation nationally, up to 150 000 women on patches alone could be under-replaced (i.e. 0.53 x 72 x 56 m/14 500). There is an accelerated phase of bone loss in perimenopausal women[31] when many patients are started on HRT. Effective intervention at this time is likely to have the greatest impact on osteoporosis prevention. The opportunity may be lost because it could be two years before bone mass measurements detect the inadequacy of treatment.[1,2] However, serum E_2 measurement could permit timely adjustment of the E_2 dose or provide a check on compliance.

It was hoped that the MSS would detect patients who were under-replaced and required blood testing. Unfortunately, there was no correlation between the MSS and the serum E_2 (r_s = –0.04, –0.33 to 0.26; P = 0.398) even when those on treatment for menopausal symptoms alone were considered (r_s = –0.16, –0.48 to 0.20; P = 0.189). Indeed, the MSS values of those with serum E_2 <150 pmol/l completely overlapped those with serum E_2 ≥ 150 pmol/l. Furthermore, ROC curve analysis revealed the diagnostic accuracy of the MSS was no better than tossing a coin in predicting a patient's serum E_2 group (AUC = 0.51, 0.33–0.68).

The poor agreement between the MSS and serum E_2 could have resulted from a number of reasons. Padwick *et al*[32] have suggested that HRT should be continued for at least three months before the degree of menopausal symptom suppression is assessed. However, although our study inclusion criteria stipulated a minimum of just one month on treatment, only one patient was on HRT for less than three months, which would have been unlikely to have influenced the MSS overall.

Another factor might have been the variability in serum E_2 levels during the period of E_2 patch application. Powers *et al*[25] reported mean levels in 14 patients on 50 μg/24 h E_2 patches that varied between 22 and 57 pg/ml (81–209 pmol/l) over 18 days. However, when the sample timing was considered, the levels were 37–57 pg/ml (136–209 pmol/l) over 9–46 h, falling to 22–33 pg/ml (81–119 pmol/l) more than 59 h after application. Data with more frequent sampling confirm that levels are much less variable when sampling is restricted to 12–60 h following patch application.[33,34] In our study, the sampling time (median, 95% CI) was 24 h (20–36 h).

A further reason could be that menopausal symptoms may be related to changes in serum E_2 rather than the absolute level.[35-37] However, Steingold *et al*[26] reported a negative correlation between circulating E_2 and the frequency of hot flushes in women receiving transdermal E_2. Furthermore, in a study looking at untreated women with menopausal symptoms, not only was sweating frequency inversely related to serum E_2 levels, it was also superior to serum E_2 levels in predicting bone loss.[38] Nevertheless, even when vasomotor symptoms of flushing and sweating were considered alone, we found no correlation with serum E_2 (r_s = –0.04, –0.33 to 0.26; P = 0.403).

In conclusion, more than half our patients had serum E_2 levels inadequate to protect bone. The MSS was not a suitable screening test for low serum E_2 levels, or (as a result) for the adequacy of bone protection. Further research is needed to evaluate accurate, easily accessible and inexpensive methods of monitoring the response to therapy.[39] Certainly, the association between symptom assessment, serum E_2 and bone protection warrants further investigation. In the meantime, the adequacy of HRT for bone protection should be assessed using serum E_2 levels between bone mass measurements. The applicability of serum E_2 measurement to monitoring transdermal HRT[5-10] is a compelling reason for preferring this mode of administration to the oral route for bone protection.

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