Quantitative Immunological Detection of Total Estrogen Receptor (Cytosolic and Nuclear) in Term Decidua of Preeclampsia: a Preliminary Study, Sanaa Eissa,1* Mohamed M. Mostafa,2 Alan A.E.A. El-Gendy,3 and Ibrahim A. Senna3 [1 Oncology Diagnostic Unit, Biochem. Dept. (*address for correspondence: fax 202-285-9928), 2 Dept. of Gyn., Ain Shams Faculty of Med., Abbassia, Cairo, Egypt, and 3 Fayoum General Hospital, Egypt]

Although progesterone and estrogens are essential for maintaining human pregnancy after implantation, few reports have investigated the localization of their specific receptors in different uterine cell types throughout pregnancy [1, 2]. Wu et al. detected estrogen receptors (ER) in low concentrations in decidua in early pregnancy but not in term decidua [2]. ER concentrations in term decidua of pregnancies with complications (in particular, preeclampsia) have not been investigated.

Classical biochemical assay methods estimate ER either in cytosol fraction or in the high-salt extracted nuclei [3–5]. These assays need two subcellular fractionation steps and two ER assays in two fractions: cytosol and nuclear extract. To measure total ER (cytosolic and nuclear) in one fraction using one single assay in decidual samples, we modified the assay method by using a homogenization buffer containing KCl. The effective KCl concentration for maximum ER extraction without inhibition of the ER enzyme immunoassay (EIA) ranged from 0.4 – 0.8 mol/L KCl.

All steps of sample preparation in our laboratory were carried out at 4 °C, all reagents were purchased from Sigma Chemical Co. (St. Louis, MO), and all women included in the study gave informed consent. We used scrapped decidual samples from placental bed from women with uncomplicated pregnancies who underwent cesarean section for obstetrical causes (n = 10) and from women with preeclampsia (n = 20): mild (n = 4), moderate (n = 6), and severe (n = 10). Tissues were immediately washed in ice-cold saline and homogenized at 1 g/10 mL in ice-cold homogenization buffer (10 mmol/L Tris buffer, pH 7.5, containing 10 mmol/L K2EDTA, 100 mL/L glycerol, 5 mmol/L benzamidine, 10 mmol/L 2-mercaptoethanol, 0.39 mmol/L phenylmethylsulfonyl fluoride, and 5 mg/L aprotinin) with Ultraturax T-25 homogenizer for five bursts of 1 min each, separated by a 1-min pause. The homogenate was filtered and divided into two parts. The first part was centrifuged in a Beckman CS-6R centrifuge (Brea, CA) at 800 g for 15 min at 4 °C to obtain the crude nuclear pellet. The supernatant fluid was recentrifuged at 100 000 g for 1 h with a Beckman L7 ultracentrifuge to obtain the cytosol. The crude nuclear pellet was dissolved in 10 mmol/L ice-cold phosphate buffered saline (pH 7.2) by sonication for three 30-s bursts and recentrifuged at 800 g for 15 min at 4 °C. The washed nuclear pellet was incubated with 5 volumes of ice-cold high-salt extraction buffer (homogenization buffer containing 0.4 mol/L KCl) on ice for 30 min with vortex-mixing every 10 min. Then it was ultracentrifuged in a Beckman L7 ultracentrifuge at 100 000 g for 30 min at 4 °C. The nuclear extract (supernatant) was obtained.

The second part of the homogenate was incubated with 0.4 mol/L KCl on ice for 30 min with vortex-mixing every 10 min, then ultracentrifuged in a Beckman L7 ultracentrifuge at 100 000 g for 30 min at 4 °C. The supernatant was isolated. After quantifying the protein concentration in the cytosol, nuclear extract, and tissue extract by using

![Fig. 1](https://academic.oup.com/clinchem/article-abstract/43/2/405/5640693/423465648683?Expires=1607219367&OUperson=guest&Policy=academic)

Fig. 1. (A) Total ER in term decidua in normal pregnancy and mild, moderate, and severe preeclampsia; (B) cytosolic and nuclear ER in term decidua in severe preeclampsia. Dotted line represents the lower detection limit for immunological detection of ER.
Bradford’s method [6] with bovine serum albumin as the calibrator, we applied the samples directly to the ELISA plate for assay of ER with EIA kit from Abbott Laboratories (Chicago, IL) [7].

ER concentrations estimated by our modified method strongly correlated to those calculated as a sum of ER concentration in cytosol plus those in nuclear extract (y = −0.98 + 1.128x, r = 0.91, P = 0.005). Cytosolic, nuclear, and total ER were not detectable (lower detection limit, 1 fmol/mg protein) in term decidua from women with uncomplicated pregnancies or from women with mild or moderate preeclampsia, but were detected in term decidua from women with severe preeclampsia (Fig. 1) (cytosolic ER, 1.4–9 fmol/mg protein, mean 4.98 fmol/mg protein; nuclear ER, 1.5–11 fmol/mg protein, mean 4.68 fmol/mg protein; total ER 2.6–19.2 fmol/mg protein, mean 9.1 fmol/mg protein). This difference was statistically significant by Student’s t-test (P = <0.01).

Although our results suggest a significant association between ER detection in term decidua and severe preeclampsia, the cause–effect relationship cannot be determined in this study. Establishing a cause–effect relationship may improve understanding of the biological relationship and may have potential clinical implications through development of diagnostic or therapeutic markers for these lesions. We recommend the routine use of this modified assay for quantification of total ER in the clinical laboratory.

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References

Speciation of Arsenic in Serum, Urine, and Dialysate of Patients on Continuous Ambulatory Peritoneal Dialysis, Xinrong Zhang,1 Rita Cornelis,1,3* Jurgen De Kimpe,1 Louis Maes,1 and Norbert Lamere2 (1Lab. for Anal. Chem., Inst. for Nuclear Sci., Univ. of Gent, Pooeftuinstraat 86, Gent, Belgium; 2Renal Div., Dept. of Med., Univ. Hosp., De Pintelaan 185, B-9000 Gent, Belgium; *author for correspondence: fax 32 (0)9 2646699, e-mail Cornelis@inwchem.rug.ac.be)

Renal replacement therapy is currently achieved by hemodialysis (HD), hemofiltration, hemodiafiltration (HDF), continuous ambulatory peritoneal dialysis (CAPD), or renal transplantation. The concentration of trace elements in serum of patients on HD treatment has been reported [1, 2]. As concentrations higher than the reference value were also observed in serum of patients on HDF [3]. Although there are several studies describing the status of trace elements in serum, plasma, and dialysate in CAPD patients [4, 5], no data on As serum concentrations of CAPD patients are available. The aim of this work is to determine total As concentrations and to speciate As species in serum, urine, and dialysate of CAPD patients.

Fourteen CAPD patients were studied. Serum, urine, and dialysate samples were collected from the University Hospital. Patients gave their informed consent before blood sampling. To decrease the influence of As intake from the diet, patients were requested to refrain from ingesting seafood during the 3 days before blood and urine collection. The reagents and apparatus for the separation and measurement of As species and for the measurement of total As have been described [6]. Briefly, two types of HPLC columns were used for the separation of anionic and cationic As species: an anion exchange column (Supelcosil LC-SAX, 250 × 4.6 mm; Supelco, Bellefonte, PA) and a cation exchange column [Dionex (Sunnyvale, CA) Ionpac® CS 10, 250 × 4 mm]. A PerkinElmer (Norwalk, CT) 3030 atomic absorption spectrometer was used throughout for the detection of As signals. Analysis of serum creatinine was performed according to the Jaffe method [7].

The accuracy of the total As measurements was tested by simultaneously analyzing a Certified Freeze-Dried Reference Serum (CRM) of the University of Gent, Belgium. The accuracy of the As speciation measurements was tested by analyzing a BCR candidate Reference Material CRM 526 tuna tissue, as no serum reference material is yet available for the As speciation study. No significant differences were established between the analytical results and the certified values. The precision (CV) of the method for 10 replicate analyses of aqueous solution of As species at a concentration of 10.0 μg/L was always better than 5% for each form of As. The 3-day run-to-run precision of measurement of 10.0 μg/L As species added to serum was better than 10%.

The mean total As concentration in the serum of 14 CAPD patients is 4.67 ± 5.41 μg/L, significantly higher (P <0.001) than the reference values of 0.96 ± 1.52 μg/L in serum of healthy subjects previously obtained in our laboratory [8], indicative of an accumulation of As in serum of CAPD patients. The main As species in the serum of these patients are dimethylarsinic acid (DMA) and arsenobetaine (AsB), respectively carrying 15.2%