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ORIGINAL ARTICLE

THE USE OF ELECTRONIC SENSOR IN HORMONE ANALYSIS

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The development of antibody-based biosensor has grown steadily during recent years, and their use as a routine instrument in clinical application is not far from reality. This study has demonstrated the capability of conductometric sensor to quantitate human Follicle Stimulating Hormone (hFSH) from urine samples. The principles are adopted from Enzyme Linked Immunosorbent Assay (ELISA) technique. Self fabricated gold coated electrode was dipped in the microtiter well containing antibody-antigen complex. Substrate was added to the system to initiate a secondary reaction, which produced electroactive species and change the conductivity of the solution. The changes were proportional with the concentration of the hormone present. The results obtained correlate well with the conventional ELISA technique. Inter and intra assay variation (%CV) were under 6% and the lowest detection limit is 0.75 mIU/ml which was well under the physiological range of the hormone. This system offered advantages such as simplicity, reliability, minimal addition of reagents, freedom from turbidity and color problem, probability of miniaturizing the electrode thus minimizing the sample volume and the ability of on line data analysis. This study proved that Antigen-Antibody reaction via EIA could be detected electronically and it has a potential to be used as one of the measuring mode in clinical analysis.

***Keywords**: biosensor, immunossay, hFSH, antibody*

Introduction

Hormone analysis is a very useful tool in medical and biological sciences. It has been used as a diagnostic tool to confirm a disease and abnormalities or to be used as a tool to differentiate one physiological state from another. The method of hormone analysis has been changed throughout the years from bioanalysis to the more sophisticated techniques. However every technique has it own advantages and disadvantages and may be useful for different purposes. The best technique for measuring different hormones might also be different.

To date, three main techniques used for hormone analysis are bioassay, receptor assay and immunoassay. Bioassay is based on the observation of the specific physiological changes occurring upon the administration of the hormone. It is the first technique ever used for hormone analysis. It can be used either in vivo or in vitro. Currently it is not very popular for use in hormone level determination but it is more important for the study on etiology of the disease.

The receptor assay is a more specific technique, based on the interaction between a specific hormone and its receptor. It is more sensitive than the bioassay and will reflect the ability of the hormone to bind with its own receptor.

Another technique, which is routinely used especially for quantitative purpose, is the immunoassay. It is a very sensitive assay and is easy to perform. The progressive development in antibody technology has been a great advantage for this technique since we can customize our antibody. This enable us to produce antibody to almost any analyte of interest.

Enzyme Immunosorbent Assay (EIA), coupled with electrochemical detection, offers low detection limits but with high specificity. Electrochemical EIA is based on the conversion of an non-electroactive substrate to an electroactive product by the enzyme labeled.

In this study we developed a sensor to be used in hormone analysis based on its enzyme immunoassay principles. The substrate was added to the system and enzymatic reaction from the system was measured using a special electrode (1). The test showed a fast respond time, good sensitivity, reproducibility and has a great potential to be a leading technique in hormone analysis in the near future.

Materials and methods

The instruments used for this study were Sputter Coated Model SCD 005 from BAL-TEC (USA), conductometer Model LF 539 from Wissenschaftlich-Techniche (WTW) Germany and ELISA reader Model MR 5000 from Dynatec USA.

Chemicals used include bovine serum albumine (BSA), Tween 20, Urease type VII, urea, human Folicle Stimulating Hormone (hFSH) from Sigma (USA), hFSH ELISA kit from Veda Lab (USA) and polyclonal antibody to FSH from the Endocrine Unit, Universiti Kebangsaan Malaysia (UKM). Urine samples were from volunteers and patients in Gynaecology Clinic UKM.

Sample Collection

Human Follicle Stimulating Hormone (hFSH) from normal urine samples were collected from normal women, aged between 20-35 years who have menstrual cycles between 24-35 days. Early morning urine samples were collected every day from day seven to day 20 of the menstrual cycle. This time of the cycle was chosen because it contained the lowest and the highest level of hFSH in the body. For normal samples, six cycles from five individuals were used in this study.

Abnormal samples were obtained from patients in the Gynaecology Clinic, UKM. Most of the patients were menopausal and the younger patients suffered from polycystic ovary syndrome. They were collected randomly at the time the patients visited the clinic. All the samples were sent to the laboratory within 2 hours, aliquoted into small volumes and stored at -20^0C. If the samples could not be delivered to the laboratory within 30 minutes of collection, they were stored in a refrigerator at 4^0C. The difference in urine samples between normal and abnormal samples would not interfere with the study since we were looking into the correlation of each sample using different methods. The patient samples were used to ensure that the techniques can be used to analyze the higher range of hFSH level which usually presents in menopausal women and polycystic ovary patients.

Electrode Fabrication

An electrode was designed from double-sided Printed Circuit Board (PCB). The board was cut to 4.0mm x 100mm, cleaned with special sandpaper and rinsed under running tap water. Once dried the electrode was put in the chamber of sputter coater to be coated with a layer of gold. This was done to both surfaces of the electrode. A pair of cables were soldered to each side of electrode for conductometry meter (WTW model LF 359) connection. The electrode was then analyzed for its sensitivity and reproducibility (2). The electrode was used to analyze the conductivity of standard solution (potassium chloride (KCI). The readings were collected every day for one month. The results showed no changes in the sensitivity and reproducibility of the electrode for at least one month.

Hormone Analysis

A 96-microtiter plate was coated with 100μl capturing antibody at a concentration of 10μl/ml. It was sealed with parafilm and incubated at room temperature for 19 hours or for 2 hours at 37^0C. The plate was then stored at 4^0C until needed.

The prepared plate was then washed and incubated with 100μl of block solution containing 0.5% of bovine serum albumin (BSA) and 0.05% of Tween 20. After 30 minutes, the plate was washed and reincubated with 50μl of urine samples or hFSH standard. Washed process was repeated and the final incubation was done with 50μl of second antibody labeled with urease.

All the washing processes were done with deionized water and the incubation period was 30 minutes at room temperature. The plate was then washed for the last time and each well was filled with 200μl of deionized water. The plate was then ready for conductometry measurement.

The electrode was immersed in the well and the base line reading was recorded. The temperature in each well was measured with a thermometer. The value obtained was manually entered using the temperature button at the front panel of conductometry meter. 10μl substrate (1M urea) was added to the system and the conductometric change was recorded manually every 15 seconds starting from the time of substrate addition up to three minutes. The rate of conductometric change was calculated. The same sample was reanalyzed with ELISA hFSH Kit from Veda Lab and the results from both techniques were compared.

Results

The results for normal and abnormal samples were analyzed separately. Regression analysis was run for both groups to find the correlation between conductometry versus standard method. Figure 1 showed the result for the normal group and Figure 2 showed the results for the abnormal group.

Fig.1: Regression analysis for conductometric technique and ELISA, correlation coefficient r^2 is 0.703

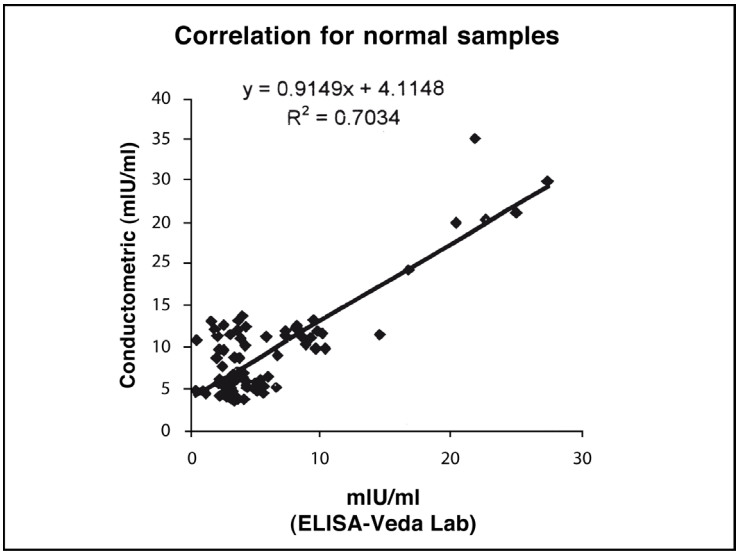
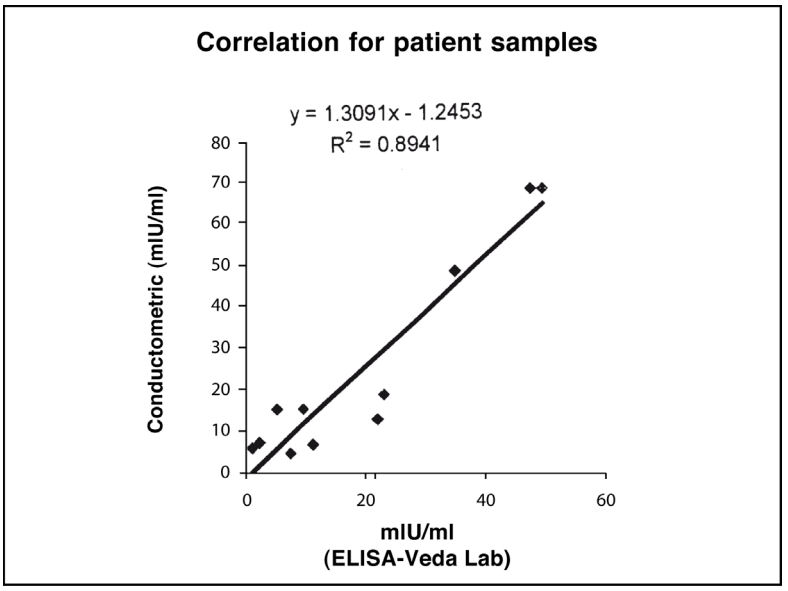


Fig.2: Regression analysis of patient samples for conductometric technique and ELISA, correlation coefficient r^2 is 0.894



From the results, we could see that the r^2-value for normal sample was 0.703 and for abnormal sample was 0.894. The abnormal sample showed a higher correlation due to the presence of a higher concentration of the hormone. The conductivity technique also showed a lower value of coefficient of variation percentage (%CV) at the higher concentration compared to the lower end. However, %CV for the overall reading was lower than 6% as shown in Figure 3. The lowest detection limit at 2 Standard deviation (3) was 0.75 mIU/ml. This value was much lower than the hormone level in the human physiological range.

Discussion

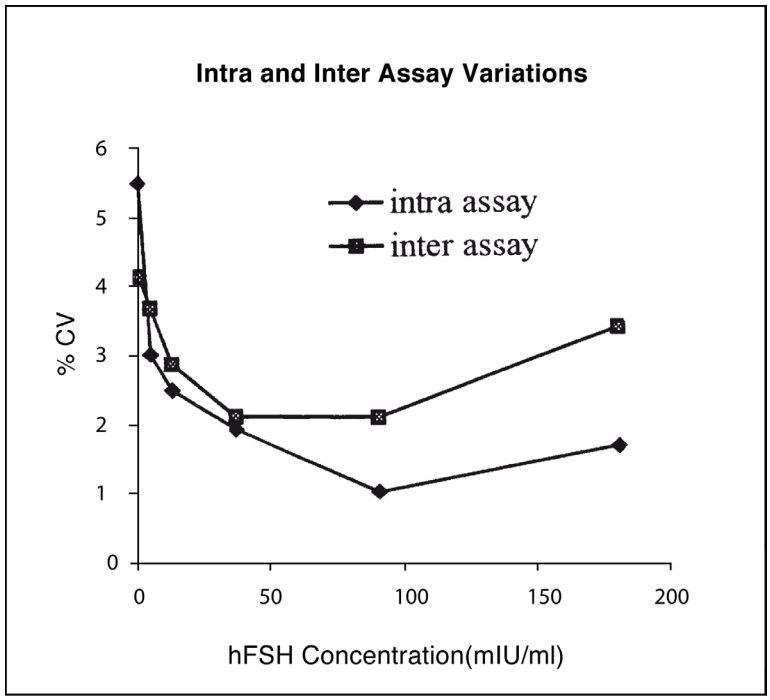
This study has shown the possibility of detecting hormonal assay electronically. Besides having the advantages of sensitivity and specificity due to its antibody characteristics, it also offers electronic capability such as fast response time and real time mode of analysis (4).

Conductometric technique was chosen as a detection mode because it offers the sensitivity needed and the easiness in electrode construction. The electrode was cheap, can be mass produced and durable. The lowest detection limit of the technique was 0.75mIU/ml. This value is much lower than physiological range of hFSH which is 2-12mIU/ml in follicular and luteal phase and 8-22mIU/ml during the mid-cycle peak. It showed a reproducible result up to 20 days with 200 samples analyzed (5). Utilization of inert material, like gold, made it more stable and did not react with the samples analyzed.

This study showed that the use of antibody as a biological recognition molecule in biosensor development has a great advantage. we also believed that the development for a *par excellent* biosensor should be derive from the utilization of antibody. However, more studies should be conducted to find the best detection mode so that the direct antibody-antigen reaction can be transformed to a meaningful signal. The proposed modes include optical, interferometry and acoustic detection modes utilizing devices such as piezoelectric oscillator or surface plasmon resonance membrane (6).

Even though this study has used the hFSH hormone as the model, the actual analyte can be anything from the environmental pollutants to a biological weapon for as long as an antibody to that analyte is available (7-9).

Fig. 3: Inter and intra assay variations for conductometric technique.



In conclusion, the study has demonstrated the ability of analyzing hFSH using the electrochemical detection method from untreated urine samples. However a lot of work need to be done before we can achieve a one step biosensor for hormone analysis.

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