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Comparison between ECL and ELISA for the detection of salivary cortisol and determination of the relationship between cortisol in saliva and serum measured by ECL

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ABSTRACT: Salivary cortisol has been increasingly used as a stress biomarker since saliva sampling induces less additional stress than blood sampling. Enzyme-linked immunosorbent assay (ELISA) has been commonly used to measure salivary cortisol in stress related research. Recently, electrochemiluminescence (ECL), a routine immunoassay analyser, has been suggested to measure salivary cortisol. Therefore, the aims of this study are: (1) to compare salivary cortisol level measured by ELISA and ECL and (2) to determine the relationship between salivary cortisol and serum cortisol measured by ECL. Both salivary and serum samples were collected from 83 volunteers for cortisol measurement by ECL analysis. The salivary cortisol value was 3% of that of the serum cortisol. For ECL, the positive correlation between salivary and serum cortisol levels was significant ($r = 0.84$; $p < 0.001$). The measurement by two different methods did not show any significant difference ($p = 0.5497$). The correlation of salivary cortisol values measured by both techniques was significant ($r = 0.81$; $p < 0.001$). The result suggests that ECL seems to be more practical and cheaper for salivary cortisol measurement.

KEYWORDS: stress, HPA axis, psychiatric illnesses

INTRODUCTION

Cortisol is measured from various samples including blood, saliva, and urine. Previously, serum cortisol has been determined in most studies. Salivary cortisol has been increasingly used as a physiological marker in endocrinology, psychobiology, and behavioural studies[1]. Salivary cortisol is in free form and directly diffuses along capillaries to tissues whereas most serum cortisol is in bound form[2]. Recently, the measurement of salivary cortisol has been developed since salivary sampling is simple, convenient, and painless, whereas blood sampling requires medical staff and is invasive[1]. Thus, salivary cortisol has been widely used as a stress biomarker in psychobiology studies[3–5].

Due to the low concentration of salivary cortisol, circa 3–5% of serum cortisol, the technique used to detect it must be effective, sensitive, and convenient. Enzyme-linked immunosorbent assay (ELISA) has been used in many research studies concerning the determination of salivary cortisol[6,7]. However, the limitations of ELISA include the cost of the kit, the contamination due to human error, and the duration of the procedure. Recently, electrochemiluminescence (ECL), measured by a routine automated immunoassay analyser, has been suggested to measure salivary cortisol since the technique is fast, reliable, and convenient[8]. However, there are no studies comparing the measurement of salivary cortisol by ELISA and ECL.

Therefore, the aims of the present study were to compare salivary cortisol level measured by ELISA and ECL, and to determine the relation between salivary and serum cortisol level measured by ECL.

MATERIALS AND METHODS

Subjects

The volunteers were 83 healthy students (40 males, 43 females) aged 18–25 years. The inclusion criteria were (1) not receiving any medication (2) not suffering from a psychiatric illness (3) not having a cortisol disorder including Addison's disease or Cushing's syndrome. Eligible volunteers consented to cooperate in the experiment according to the Human Ethics Committee of Srinakharinwirot University (SWUEC 19/2007) which is in compliance with the Interna-

tional Guiding Principles for Biomedical Research Involving Humans provided by the National Research Council of Thailand.

Saliva collections and measurements

The instructions were given to the volunteers. Two hours prior to salivary collections, the participants were told to avoid eating food and drinking beverages. Drinking an acidic beverage (like fruit juice) was absolutely prohibited since the low pH value would interfere with the immunoassay.

Saliva was collected during 09:00–11:00 AM to minimize the effect of the circadian rhythm on the hormone levels. The salivary samples were obtained by placing sterile cotton under the tongue without chewing for 3 min. All samples were centrifuged at 2400g for 5 min. Salivary supernatant was aspirated, divided into two sets (for measuring salivary cortisol by either ELISA or ECL) and then stored at $-20\,^{\circ}$C until analysis.

Salivary cortisol in the first set of salivary samples was determined by using an ELISA kit (Salimetrics, USA). Briefly, salivary samples were defrosted and centrifuged at 2400g for 10 min, which resulted in a clear supernatant of the low viscosity. Then, 25 µl of salivary samples, standards, or controls were transferred to antibody-coated plates and then 200 µl of diluted conjugate solution were added. The plate was mixed for 5 min and incubated at room temperature for 55 min and washed four times with wash buffer. Then, 200 µl of TMB (tetramethylbenzidine) substrate was added and incubated at room temperature for 25 min. Absorbance at 450 nm was determined after adding 50 µl of stop solution.

The second set of salivary samples was used to measure cortisol levels by using a cortisol Elecsys kit (Roche Diagnostics, Laval, Quebec). After thawing, salivary samples were centrifuged at 2400g for 10 min. Then 200 µl of salivary samples were transferred to a Elecsys sample cup and measured by a Elecsys model 1010 (Roche Diagnostics, Laval, Quebec).

Blood samples were drawn from the basilic vein of the arm. Clotted blood was centrifuged at 2000g for 5 min to obtain the serum which was then stored at $-20\,^{\circ}$C until analysis. Serum cortisol was measured using a 200 µl sample on the Elecsys machine.

Statistical Analysis

Paired t-test was used to compare the means of salivary cortisol values obtained from the ELISA and ECL measurements. For inter- and intra-assay variation, the %CV (coefficient of variation) was calculated. The relationship between salivary and serum cortisol was presented by the correlation and the linear regression analysis. The relationship between salivary cortisol measured by ELISA and ECL was also shown by correlation and linear regression analysis. Differences were considered statistically significant when $p < 0.05$.

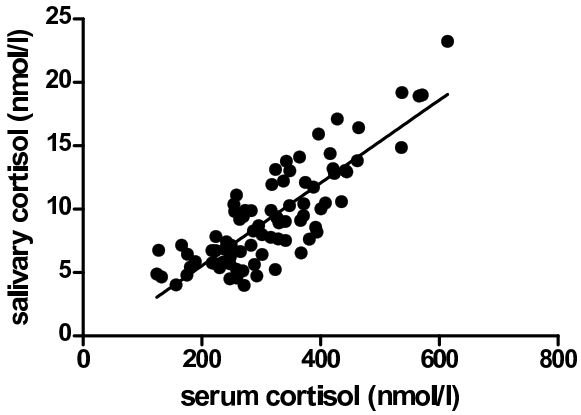

Fig. 1 Correlation between salivary and serum cortisol measured by electrochemiluminescence (ECL), $r = 0.84$ ($p < 0.001$).

RESULTS AND DISCUSSION

The relationship between cortisol in saliva and serum measured by ECL

The mean ($\pm$ SEM) concentration of salivary and serum cortisol measured by ECL were 9.39 ± 0.44 nmol/l and 318 ± 11 nmol/l, respectively. The salivary cortisol value was 3% of serum cortisol value. The relationship between salivary and serum cortisol measured by ECL is shown in Fig. 1. The positive correlation of salivary and serum cortisol levels was significant ($r = 0.84$; $p < 0.001$).

This finding is supported by previous studies showing a strongly positive correlation between salivary and serum cortisol levels measured by other methods[9,10]. Salivary cortisol reflects the biologically active free form of cortisol, thereby providing a more reliable measure than serum cortisol which is an inactive bound form[2]. Therefore, salivary cortisol measured by ECL can be used as a marker of HPA axis function.

Comparison of ECL and ELISA for the detection of salivary cortisol

The mean ($\pm$ SEM) concentrations of salivary cortisol measured by ECL and ELISA were 9.39 ± 0.44 nmol/l and 10.45 ± 0.47 nmol/l, respectively. Salivary corti-

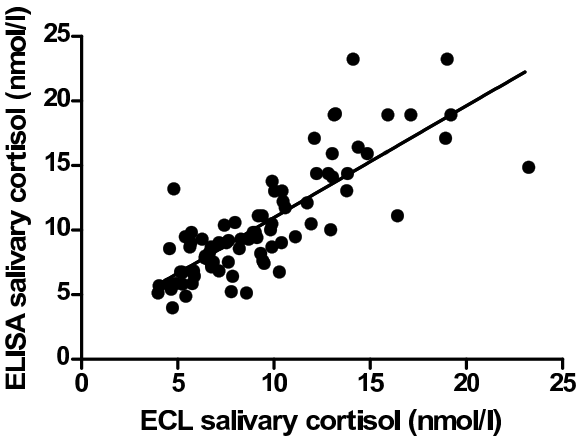

Fig. 2 Correlation between salivary cortisol measured by ECL and ELISA, $r = 0.81$ ($p < 0.001$).

sol concentration measured with ELISA did not differ from that measured by ECL ($p = 0.5497$). The correlation of salivary cortisol values measured by both assays was significant ($r = 0.81$; $p < 0.001$) (Fig. 2).

The present data showed for the first time that the mean concentration of salivary cortisol measured by ECL was not significantly different from that measured by ELISA. The correlation of salivary cortisol values measured with both assays was also highly significant. The price of the ELISA salivary cortisol kit was approximately twice that of ECL salivary cortisol kit. The other advantage of ECL technique was speed, as it took about 2 h to perform the assay (100 salivary samples) while the ELISA took about 5 h (80 salivary samples). The result showed that the intra-assay variations of ECL and ELISA were 2.35% and 6.86%, respectively. The inter-assay variations of ECL and ELISA were 6.48% and 14.36%, respectively. This suggests that the precision of ELISA technique is less than that of ECL probably due to human errors such as pipetting, timing, and washing.

Although the principles of both assays are different, the correlation value has been validated. However, there is a limitation of ECL because this method cannot detect concentrations of salivary cortisol lower than 0.5 nmol/l.

In conclusion, the present study confirmed that using the ECL method, the level of salivary cortisol was found to be highly correlated with serum cortisol. The correlation of salivary cortisol values measured by ELISA and ECL was highly significant, suggesting ECL might be an effective alternative technique to determine the level of salivary cortisol.

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