Biomedicine & Aging Pathology 4 (2014) 257–264

Available online at
ScienceDirect
www.sciencedirect.com

Elsevier Masson France
EM|consulte
www.em-consulte.com/en

Original article

Assisting the diagnosis of Cushing syndrome by pattern recognition methods, using a combination of eight routine tests and their multiple correlation with serum cortisol

Masataka Kudo[a], Fumitoshi Satoh[a], Sorama Aoki[c], Ryo Morimoto[a], Yoshitsugu Iwakura[a], Yoshikiyo Ono[a], Ken Matsuda[a], Akira Sugawara[b], Katsumi Yoshida[d], Kenichi Sato[c,*], Sadayoshi Ito[a]

[a] *Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryocyo, Aoba-ku, Sendai 980-8574, Japan*
[b] *Department of Molecular Endocrinology, Tohoku University Graduate School of Medicine, 1-1 Seiryocyo, Aoba-ku, Sendai 980-8574, Japan*
[c] *Medical and Pharmaceutical Information Science, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan*
[d] *Department of Health Supervision, Tohoku Kosai Hospital, 1-3-5 Oomachi, Aoba-ku, Sendai 980-0804, Japan*

ARTICLE INFO

Article history:
Received 31 March 2014
Accepted 18 April 2014
Available online 29 May 2014

Keywords:
Cushing syndrome
Cortisol
Screening
Routine tests
Pattern recognition methods

ABSTRACT

Cushing's syndrome (CS) is caused by excessive exposure to glucocorticoids, and the initial tests for diagnosis (urine free cortisol [UFC], late-night salivary cortisol, overnight low-dose dexamethasone suppression test [LDDST]) should be performed for patients. However, such test is burdensome and costly to patients, that is, not so easy examination. Therefore patients with CS can be underdiagnosed in general practice. If not diagnosed and treated appropriately, it can be lethal. The aim of our study was to establish a new method of assisting detection of CS patients using an appropriate set of routine clinical tests, similar to our successful previous works about screening patients with thyroid dysfunction. Thirty patients with CS and 49 healthy individuals were included in the present study. An optimal set of routine clinical tests (a new effective marker) to screen patients with CS was identified and the association of these clinical tests with serum cortisol levels was established by using pattern recognition methods. Serum cortisol was highly associated with a combination of 8 characteristics (γ-GTP, LDH, Na, K, and counts of neutrophils, lymphocytes, eosinophils and monocytes) in patients with CS. Their multiple correlation coefficients in the multivariate analysis of medical statistics were very high at 0.784. The analyses using pattern recognition methods of these 8 characteristics before endocrine workups were found to be useful to discriminate the patients with CS who needed to undergo surgery. These results indicate that we could screen the patients with CS using routine clinical test parameters, before performing the initial test for CS. In conclusion this efficient and versatile system is clinically very useful, and would improve patients' QOL.

1. Introduction

Cushing's syndrome (CS) is caused by excessive exposure to glucocorticoids, either from endogenous or exogenous sources [1]. CS is classified as "ACTH-dependent CS" in the setting of Cushing's disease (CD) and as ectopic ACTH or "ACTH-independent CS". CS occurs at a very low frequency and mostly in middle-aged or elderly females (female-to-male ratio, 4: 1) [2]. If not diagnosed and treated appropriately, it can be lethal [3–5]. Therefore, when CS is suspected, the state of cortisol production should be assessed as soon as possible. Potentially, CS can occur in patients with common diseases (metabolic syndrome, obesity, diabetes mellitus, hypertension, etc.) [6,7]. However, serum cortisol is usually not measured at the time of population health examinations to screen for different diseases. Both this and its very low frequency lead to patients with hidden CS being overlooked.

CS patients typically present striking characteristics, the so-called "Cushingoid" appearance, characterized by central obesity, hypertension, hyperglycemia and bone fragility. However, severe CS is rare, and patients with CS, particularly those with milder forms, do not always present the typical features, which makes it difficult for non-specialists to suspect this condition.

* Corresponding author. Tel.: +81227270206; fax: +81227270205.
E-mail address: ksato@tohoku-pharm.ac.jp (K. Sato).

http://dx.doi.org/10.1016/j.biomag.2014.04.001
2210-5220/

According to the guidelines for CS [8], the initial tests for diagnosis (urine free cortisol [UFC], late-night salivary cortisol, overnight low-dose dexamethasone suppression test (LDDST]) should be performed for patients suspected of having CS [9–12]. Moreover, it is recommended that patients with adrenal incidentaloma compatible with the diagnosis of adenoma be evaluated for CS [13,14]. There are many reports about the initial tests for diagnosing CS, but only a few deal with how to detect these patients efficiently, before performing the initial tests. Therefore, an assessment of the likelihood of CS from an early stage based on clinical findings and routine tests would be very useful. In Japan, activation of routine tests is easy since they are measured when people visit hospitals because of sickness or to have a health check-up (more than 7 million people participate in a general health check-up system referred to as "Ningen Dock").

It is known that the CS group has a tendency for lower serum potassium levels and suppressed eosinophilia, among others, compared with normal subjects, but such facts were used only qualitatively since some patients exhibit changes in part of these factors, while others exhibit different changes. To enable quantitative use of a combination of such routine tests, a sophisticated approach like informatics-based methods would be necessary to improve our diagnostic capability.

In previous studies [15–20], we analyzed the association of human thyroid hormone levels with the results of basic routine tests by adopting three types of pattern recognition method (PRM), in addition to medical statistics. Artificial neural networks, one of such methods, had already been used to identify myocardial infarction in emergency patients [21] and to evaluate cancer risk in patients with thyroid nodules [22].

Through data visualization, the Self-Organizing Map (SOM) enabled us to grasp the characteristic features of thyroid function from a map of well-clustered thyroid patients. These previous detailed analyses of routine test results showed that a set of three parameters (alkaline phosphatase [ALP], serum creatinine [S-Cr] and total cholesterol [TC]) allowed accurate detection of hidden Graves' hyperthyroidism [15–18], while a set of four parameters (lactate dehydrogenase [LDH], red blood cell count [RBC], serum creatinine [S-Cr] and total cholesterol [TC]) allowed accurate detection of hidden hypothyroidism [19]. Similar application of such a method for CS seems to be more difficult since cortisol also has a variety of pharmacological and physiological actions. However, it would be helpful to assist the diagnosis of CS based on a set of routine tests if we consider that PRMs have superior ability in classification than medical statistical analyses.

The principal aim of this study is thus to establish a new system for assisting the diagnosis of Cushing's syndrome using a set of routine clinical tests, not to give a protocol for differentiating CS from pseudo-Cushing's, which would then lead to a special test, for example, of abnormal ACTH and cortisol dynamics, that could provide a definitive diagnosis.

2. Subjects and methods

2.1. Subjects

2.1.1. Learning samples

To develop the new system for screening CS patients, 30 female patients with overt CS were selected among those receiving treatment at the Division of Nephrology, Endocrinology and Vascular Medicine, and at the Department of Internal Medicine of Tohoku University Hospital, from January 2001 to April 2009. Eight of them were diagnosed with ACTH-dependent CS caused by an ACTH-secreting pituitary adenoma, and the other 22 were diagnosed with ACTH-independent CS caused by an adrenocortical adenoma. Diagnosis of CS was made in accordance with several tests recommended by the Endocrine Society Clinical Practice Guidelines. All patients were operated on, and thereafter achieved complete remission. We pathologically confirmed that pituitary adenomas secreted only ACTH and that adrenocortical adenomas autonomously secreted cortisol; none of them showed signs of malignancy.

2.1.2. Test samples

To check the new system's screening ability to discover new CS patients, we used 749 female test samples, which differed from the ones used for learning, from subjects who visited the same division and hospital as mentioned above. The subjects were those for whom the diagnosis of various diseases had been established and who had undergone routine tests (more than 8 relevant items) together with cortisol measurement during May 2009 to June 2010.

2.1.3. Controls

Forty-nine healthy female volunteers were selected from those who underwent health examinations at JR Sendai Hospital. Two institutions, Tohoku University Hospital and JR Sendai Hospital, applied the same measurement method and reference values for all the routine tests that we used in this study. These women neither had a medical problem nor were taking any drugs. They were randomly selected and used as a gender- and age-matched control group.

Thirty patients with CS and 49 healthy individuals were included in the present study. The following 26 parameters were measured in samples collected at Tohoku University Hospital and JR Sendai Hospital from 2001 to 2009: alkaline phosphatase (ALP, IU/L), gamma glutamyl transferase (γ-GTP, IU/L), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, U/L), lactate dehydrogenase (LDH, IU/L), triglycerides (TG, mg/dL), total cholesterol (TC, mg/dL), high-density lipoprotein (HDL, mg/dL), low-density lipoprotein (LDL, mg/dL), blood urea nitrogen (BUN, mg/dL), serum creatinine (S-Cr, mg/dL), uric acid (UA, mg/dL), red blood cell count (RBC, $\times 10^8/\mu L$), hemoglobin (Hb, g/dL), hematocrit (HCT, %), platelet count (PLT, $\times 10^8/\mu L$), white blood cell count (WBC,/μL), differential white blood cell count (Neut%, eosinophils: Eosi%, basophils: Baso%, lymphocytes: Lymp%, monocytes: Mono%), serum sodium concentration (Na, mEq/L), serum potassium concentration (K, mEq/L), serum chloride concentration (Cl, mEq/L) and serum calcium concentration (Ca, mEq/L). Since basophils and lymphocytes were roughly measured, we did not use them in the present study.

Serum cortisol level (cortisol, μg/dL) was measured in CS patients using an ELISA kit (Abbott AxSYM TM Cortisol) and an automatic analyzer (ARCHITECT i2000). The biochemical markers and the blood cell counts were measured using an automatic analyzer (TBA-200FR NEO) and a cytometer (COULTER LH 750), respectively. In CS patients, we selected the values of these parameters examined at the initial visit, before any treatment.

The data from patients and healthy individuals were handled in a blinded manner.

2.2. Statistical analyses

The analytical approach consisted of descriptive statistics, comparative analyses and regression modeling. Data are expressed as mean ± SD or median (range). The characteristics between different groups were compared by Mann-Whitney's U test and Wilcoxon's rank-sum test. The statistical significance of the results was assumed at $P < 0.05$. SPSS software (Ver. 18; SPSS Inc., Chicago, IL) was used for statistical analyses.