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Original article

Thyroid-associated ophthalmopathy: linear regression analysis of single photon emission computed tomography

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Background: At present, there is still no significant progress in the diagnosis and treatment of thyroid-associated ophthalmopathy.
Objective: We explored the role of ^{99m}Tc-octreotide single photon emission computed tomography (SPECT) in determination of the clinical activity of thyroid-associated ophthalmopathy (TAO).
Methods: A prospective study including 36 cases of TAO and 8 controls was conducted by intravenous injection of ^{99m}Tc-octreotide, followed by SPECT. The radioactive counting ratio was calculated for the orbital/occipital regions of interest (ROI), and the Spearman rank correlation between the average ratio and the corresponding clinical activity score (CAS) mean of ADHOC (Recommendation of the Ad Hoc Committee of The American Thyroid Association in 1992) was analyzed. A curve regression equation for the CAS to orbital/occipital radio counting ratio was determined to calculate the cutoff for TAO activity.
Results: The Spearman rank correlation between orbital/occipital uptake ratio and CAS was calculated (coefficient $r = 0.972$, $p < 0.01$). The curve regression equation for all patients with TAO (including the active group and resting group) was $(Y) = -66.25 + 106.06\ X - 52.86\ X^2 + 8.94\ X^3$. The transformational equation of the cubical CAS of exophthalmos (dependent variable Y) to the square root of the orbital/occipital uptake ratio (independent variable X) was $(Y) = -752.67 + 676.74\ (X)$. A cutoff of 1.48 was used to identify the active phase of exophthalmos. The sensitivity was 96.0% with a standard error of 3.92%, and the specificity was 94.73 with a standard error of 5.13%.
Conclusion: ^{99m}Tc-octreotide SPECT can objectively and accurately measure TAO activity. An orbital/occipital uptake ratio $\geq$1.48 is the recommended cutoff to determine the active phase of TAO. This may guide clinical treatment of TAO.

Keywords: Clinical activity, SPECT, thyroid-associated ophthalmopathy, ^{99m}Tc-octreotide

Thyroid-associated ophthalmopathy (TAO), the most common orbit disease globally, accounts for approximately 20% of orbit disease in adults [1]. The symptoms associated with TAO include obvious photophobia, tears, tingling, and diplopia. Patients can thus display signs of soft tissue involvement such as exophthalmos, extraocular muscle involvement, corneal damage, optic nerve damage, and declining eyesight. Severe TAO can progress to blindness and can cause pain [2]. TAO can occur in patients with hyperthyroidism, hypothyroidism, and euthyroidism. Lesions may involve one or both eyes [3]. TAO may also be associated with organ-specific autoimmune diseases, a disease gene [4], altered activity of orbit fibroblasts, environmental factors, and smoking [5]. The early histology (inflammatory active period) presents massive lymphocyte infiltration in extraocular muscles interstitial and postocular connective tissue. The inflammation results in a deposition of collagen and glycosaminoglycans in the muscles, which leads to subsequent enlargement and fibrosis. The late stage (resting period) presents as postocular tissue fibrosis [6].

The severity of TAO is not directly related to the activity of postocular inflammatory lesions. Pamela et al. believed that the postocular radiotherapy or immunosuppressant therapy is effective in active TAO and ineffective in resting TAO [7]. Some patients have more severe TAO in the late stage (resting stage), when drug interventions are ineffective, because the

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postocular tissue has been overwhelmed by fibrosis. Some patients, without serious signs and symptoms, who are in the early stage (active inflammatory lesions), should start treatment as soon as possible to slow the progress of TAO. Therefore, evaluation of TAO inflammatory activity is important [8]. The exophthalmos clinical activity score (CAS) of the ADHOC (1992 Committee of The American Thyroid Association) has been used for years as a clinical guideline to determine the active stage of TAO, This ADHOC CAS is, however, mostly subjective and lacking in sensitivity.

Recently, there has been increasing interest in growth hormone and in statins or their analogues to treat TAO. In active TAO, massive lymphocytic infiltrations are seen in postocular tissues. Their activated lymphocyte surfaces are rich in somatostatin receptors (SSTRs). Radionuclide-labeled somatostatin analogues can bind to SSTRs at the lymphocyte surface and display the SSTR-positive region, resulting in high sensitivity. SSTR-mediated orbital imaging based on nuclear medicine tracer technology can visualize postocular inflammatory lesions in TAO [9-11]. Through surface imaging of lymphocytes and fibroblasts in active inflammatory TAO, postocular ROI (regions of interest) reveals the distribution and quantity of both lymphocyte infiltration and fibroblasts. These reflect the activity of local inflammatory lesions in TAO [12]. Octreotide is three times more effective in inhibiting growth hormone than natural somatostatin. It is very resistant to enzyme decomposition and has high affinity for SSTR2 and SSTR5. The drug is well tolerated by humans [13]. Currently, ^{99m}Tc-octretide is considered to be a cheap, convenient, sensitive imaging nuclide tracer that is expected to replace the first generation tracer ^{111}In-octretide [10, 14]. Galuska et al. (2005) attempted to improve or replace ADHOC with a ^{99m}Tc-octreotide single photon emission computed tomography (SPECT) score [10]. Sun et al. evaluated orbital ^{99m}Tc-hydrazionicotinamide-Tyr^3-octerotide (HYNIC-TOC) scintigraphy as a Graves' ophthalmopathy (GO) activity parameter in predicting retrobulbar irradiation response. The characteristic curve showed the best threshold for discriminating active and inactive disease and was 100% sensitive with a specificity of 83.3% [14].

However, limitations were cost, a small radiation burden, nonspecific examination for TAO, and finally, lack of evaluation of eye muscle swelling. This restricted the widespread use of this technique. It still remains to be seen if orbital octreotide scanning will become a widely available tool in the management of patients with TAO. Therefore, additional study of ^{99m}Tc-octreotide is necessary.

In this prospective study, ^{99m}Tc was used to label the somatostatin analogue octreotide. SPECT was conducted to objectively evaluate the activity of postocular inflammatory lesions in TAO. The technique was used to determine the cutoff of the active stage through linear regression analysis of TAO's CAS and thus be able to guide clinical management of TAO.

Materials and methods

Subjects

We interviewed 36 patients with TAO at our hospital from January 2007 to June 2010 (**Table 1**). We focused on the involved side of single eye cases and on the more severe eye of patients with bilateral disease. All patients provided informed consent. This study was approved by the Ethics Commission of the Second People's Hospital, China.

Diagnostic criteria

The diagnostic criteria were those from the American Thyroid Association (NOSPECS classification). NOSPECS is an acronym for "Only signs, no symptoms": soft tissue involvement, proptosis, extraocular muscle involvement, corneal involvement, and loss of sight. We made some minor modifications. Briefly, 0 grade: no signs or symptoms, I grade: only signs and no symptoms, II grade: soft-tissue involvement and appearance of signs and symptoms, III grade modified: proptosis degree >18 mm according to Chinese exophthalmos scoring criteria (>22 mm according to NOSPECS), IV grade: extraocular muscle involvement, V grade: corneal involvement, VI grade: declining eyesight and optic nerve involvement. Patients scored as III grade or above were diagnosed as having thyroid-associated exophthalmos.

Exophthalmos CAS criteria adhered to the ADHOC, e.g. (1) spontaneous postocular pain, (2) eye movement with pain, (3) eyelid red spot, (4) eyelid edema, (5) conjunctival congestion, (6) conjunctival chemosis, and (7) lacrimal caruncle swelling. Each of these factors was worth 1 point. Higher scores indicated higher activity; 4 points or more indicated active TAO; more than 4 points indicated higher activity of exophthalmos.

^{99m}Tc label octreotide

The labeling kit was provided by Nanjing Senke Pharmacal Co. (Nanjing, China). Octreotide (somatostatin analogue) powder was added to 925MBq $^{99m}TcO_4^-$ solution, followed by vortexing and immersion in boiling water (at 100 °C) for 30 minutes. ^{99m}Tc-octreotide was immediately cooled.

Treatment

Subjects took 400 mg of $KClO_4$ orally to block the thyroid, choroid plexus, and nasal mucosa. Forty-five minutes later, subjects underwent intravenous injection of ^{99m}Tc-octreotide 925MBq. Image acquisition was performed over the course of 2 hours by SPECT (GE Millennium VG, Hawkeye).

Layer selection for quantitative analysis

Using SPECT cross-sectional images of each layer, computed ROI technology allowed outlining of the orbit and occipital ROI. The layer of the highest average radiocount, where the orbital image should be clearest, was selected for quantitative analysis. The orbital/occipital average radioactive counting ratio was calculated. Two experienced clinicians performed image analysis independently.

Statistical methods

The SPSS 18.0 software package was used for statistical analysis. The orbital/occipital uptake ratio mean and the corresponding clinical activity score were analyzed using a Mann–Whitney rank sum test (two-sided) for the active group, resting group, and control group. For the active group and resting group, orbital/occipital uptake ratio mean and the corresponding clinical activity score were analyzed using a Spearman rank correlation test. A curve regression equation was constructed using Curve Expert 3.0 software, and the cutoff was obtained using the transformational equation to determine the active TAO.

Results

Demographics and characteristics

Our study included 36 patients, including 30 cases of thyroid-associated exophthalmos with hyperthyroidism (5/30 unilateral TAO), 4 cases of thyroid-associated exophthalmos with hypothyroidism, and 2 cases of thyroid-associated exophthalmos with euthyroidism. TAO was divided into active and resting cases in which lymph infiltration and uptake of ^{99m}Tc-octreotide by postocular tissue are different. Males and females have different TAO incidences. There were 25 (25/36) active TAO, 15 male and 10 female, aged 46.1±17.8 years with a course of illness lasting 8.8±3.9 months; 11 (11/36) had resting TAO, 8 male and 3 female, aged 39.7±14.0 years with courses of illness lasting 19.2±11.2 months. There were 8 normal controls with no thyroid or organic diseases of eyes (4 male and 4 female, aged 43.0±9.7 years) as shown in **Table 1**.

SPECT outcomes

Figure 1 shows orbital images of ^{99m}Tc-octreotide SPECT. ^{99m}Tc-octreotide was concentrated in the orbit to yield a clear image. The concentrated radiation is represented by red color in the posterior aspects of eyes. The concentration was in agreement with the corresponding ADHOC CAS.

Radiocounting and activity score

The orbital/occipital uptake ratio and CAS in patients with TAO and normal controls can be seen in **Table 2**. Normal controls are healthy undergraduates and signed informed consent forms. There were significant differences in orbital/occipital uptake ratios between the active group and the resting group ($p < 0.01$) as well as between the resting group and the normal controls ($p < 0.01$).

Table 1. Demographics and characteristics

	Age	Course of the illness	Overall no. of cases	No. of cases for each score							
				0	1	2	3	4	5	6	7
Active males	46.6±18.5	8.9±4.0	12					3	7	1	1
Active females	45.6±17.2	8.7±3.7	10					1	6	3	0
Resting males	39.1±13.3	19.3±11.3	7		2	2	3				
Resting females	40.1±14.4	20.1±12.1	7		3	1	3				
Control males	43.3±10.1		4	4							
Control females	42.7±9.3		4	4							

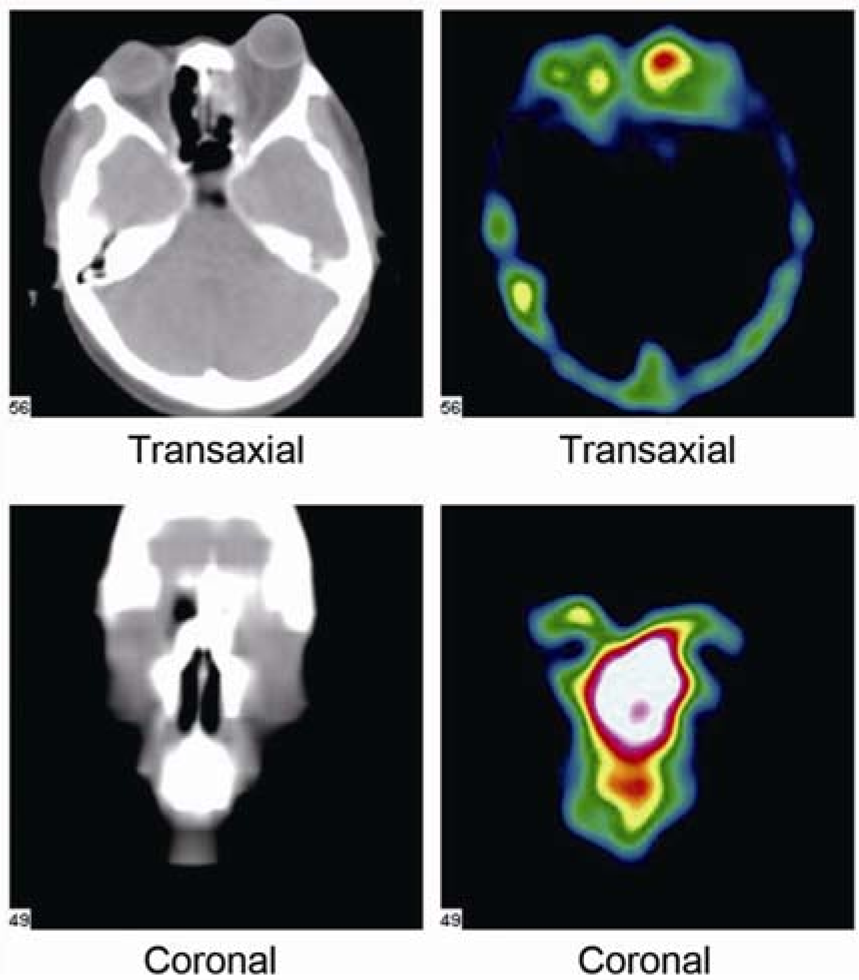

Figure 1. Orbital images from ^{99m}Tc-octreotide SPECT. A female patient aged 54 years suffered from left unilateral TAO of NOSPECS grade IV with an ADHOC CAS of 5. The two image frames were scanned with the same CT machine. The images in the second line on the left are ^{99m}Tc-octreotide SPECT. ^{99m}Tc-octreotide was denoted by a red color in the left postocular orbital region. The left occipital region and the right postocular orbital region presented reduced concentrations. The concentration was consistent with the corresponding ADHOC CAS.

Table 2. The orbital/occipital radiocount ratio and CAS in TAO patients and normal controls (mean±SD)

	No. of cases	Orbital/occipital ratio	CAS
Active	22	1.724±0.257	5.097±0.750
Resting	14	1.314±0.074	2.070±0.917
Control	8	1.000±0.071	0.000±0.000

There were significant differences between the active group and the resting group, in terms of both the orbital/occipital uptake ratio ($Z = -4.999$, $p < 0.01$) and clinical score ($Z = -5.146$, $p < 0.01$). There were significant differences between the resting group and the controls, with respect to both the orbital/occipital uptake ratio ($Z = -3.826$, $p < 0.01$) and clinical score ($Z = -3.986$, $p < 0.01$).

Figure 2 shows all patients with (active and resting) TAO. The positive Spearman rank correlation between the orbital/occipital uptake ratio and clinical activity scores was determined ($r = 0.972$, $p < 0.01$). The curve regression equation was (Y) = –66.25 + 106.06 X –52.86 X^2 + 8.94 X^3 (X: independent variable, orbital/occipital uptake ratio mean; Y: dependant variable, clinical activity score).

By data transformation and curve fitting, the cubic transformation of the clinical activity score of exophthalmos (dependant variable Y) and the square-root transformation of the orbital/occipital uptake ratio (independent variable X) were found to accord with the normal distribution. The resultant equation was (Y) = –752.67 + 676.74 (X). Based on the equation, a clinical activity score of 4 was used to determine the active phase that corresponded to the orbital/occipital uptake ratio of 1.48 (i.e., orbital/occipital uptake ratio of 1.48 or above determined the active phase of TAO). With use of 1.48 as a cutoff to mark the active phase of exophthalmos, the sensitivity was 96.0% with a standard error of 3.92%, and the specificity was 94.73 with a standard error of 5.13%.

Discussion

Galuska et al. applied the octreotide scanning technique to clinical diagnosis and management of patients with TAO. He then further investigated the correlation between ^{99m}Tc-DTPA, ^{99m}Tc-depreotide SPECT, MRI image scores and clinical activity scores [10]. The data demonstrated clear images by nuclide-octreotide SPECT, and the MRI activity scores correlated well with both ^{99m}Tc-DTPA and Tc-depreotide uptake activity. The approach described by us provides essential supplementary information to traditional clinical activity scores for assessing disease activity in Graves' orbitopathy. Kirsch et al. [15] believed that MRI is more suitable to discriminate between the active phase of TAO and the resting phase, and that CT is appropriate for evaluating need for orbital decompression because of its ability to produce high-resolution images.

In this study, ^{99m}Tc-octreotide SPECT produced clear images; the concentrations visualized accorded with the corresponding ADHOC CAS. Statistical analysis demonstrated that the orbital/occipital uptake ratio and the corresponding clinical activity score of exophthalmos had positive rank correlation in patients with active and resting TAO. Curve regression analysis demonstrated that the orbital/occipital uptake ratio $\geq$1.48 identified the active phase of TAO. These findings demonstrated that ^{99m}Tc-octreotide SPECT could determine the active phase of TAO with highly sensitive criteria.

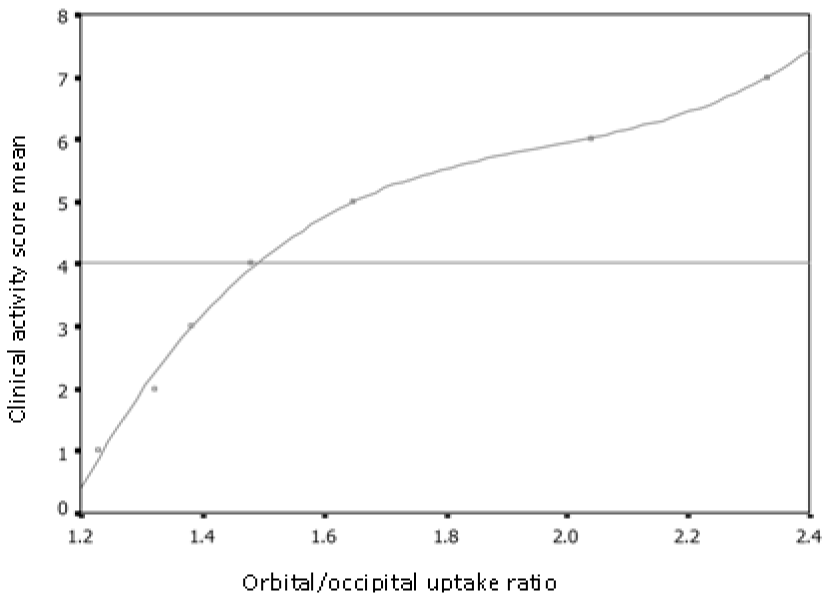

Figure 2. The regression curve of the clinical activity score to the orbital/occipital uptake ratio. Involving all patients with active and resting TAO, the regression curve was plotted and the curve regression equation was (Y) = –66.25 + 106.06X – 52.86 X^2 + 8.94X^3. X: independent variable, the orbital/occipital uptake ratio mean; Y: dependant variable, the clinical activity score mean.

Sun et al. studied ^{99m}Tc-HYNIC-TOC scintigraphy in evaluation of active Graves' ophthalmopathy (GO) [14] and reported outcomes similar to those reported here. They included 14 cases, 8 active and 6 resting, ^{99m}Tc-HYNIC-TOC scintigraphy at 4 hours (SPECT image) demonstrated that responders had a higher orbital/occipital uptake ratio than the nonresponders ($p = 0.001$). A significant correlation was observed between the orbital/occipital ratio and CAS ($p = 0.034$). The characteristic curve showed that the best threshold for identifying active and inactive disease was 1.40 (sensitivity, 100%; specificity, 83.3%).

In this study, a cutoff of 1.48 to mark active TAO was close to the value of 1.4 as reported by Sun et al. [14]. The sensitivity of 96.0% (+/- 3.92%) and the specificity of 94.7% (±5.13%) in this study are lower and higher respectively than 100% and 83.3% as reported by Sun et al. [14]. The differences in outcomes between both studies are most likely attributed to the different number of cases included. This study included 36 cases (25 active and 11 resting TAO), while Sun et al. investigated 14 cases (8 active and 6 resting TAO). Use of a similar cutoff (1.4) to determine TAO would result in greater error because of the limited number of cases. Accordingly, it is necessary to undertake more studies and include more cases to improve the accuracy of the cutoff in identifying active TAO. In addition, the CT time is also different. This study provides SPECT images obtained at 2 hours, while the evaluation by Sun et al. [14] provided SPECT images at 4 hours. In this study, the optimized cutoff to determine the active TAO was 1.48, which is similar to the value of 1.4 as reported by Sun et al. [14]. This outcome demonstrated that SPECT performed at 2 hours results that are consistent with those of other studies; therefore, 2 hours SPECT represents a reliable methodological approach. Clinical diagnosis time can therefore be shortened to improve efficiency.

In conclusion, ^{99m}Tc-octreotide SPECT was used to determine the objective quantitative cutoff (1.48) to identify exophthalmos cases in active TAO. The technique represents a more objective and accurate approach than using only traditional ADHOC CAS criteria and may improve clinical care.

The authors have no conflicts of interest to declare.

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