ความชุกและปัจจัยที่มีความสัมพันธ์กับ anti-neutrophil cytoplasm antibody ในโรคคอพอกตาโปนที่รักษาด้วยยาต้านไทรอยด์ โรงพยาบาลดำเนินสะดวก
Prevalence and Factors Associated with Positive Anti-neutrophil Cytoplasmic Antibody in Graves' Disease Patients Receiving Antithyroid Medications at Damnoen Saduak Hospital: A Prospective Cohort Study

นิพนธ์ต้นฉบับ **Original Article**

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บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาความชุกของการตรวจพบ anti-neutrophil cytoplasm antibody (ANCA) ในคนไข้โรคคอพอกตาชาวไทยก่อนและหลังการรักษาด้วยยาต้านไทรอยด์ และทดสอบความสัมพันธ์ระหว่าง ANCA และปัจจัยต่าง ๆ **วิธีการศึกษา:** เป็นการวิจัยเชิงวิเคราะห์ในผู้ป่วยโรคคอพอกตาโปนที่รับการรักษาที่โรงพยาบาลดำเนินสะดวก ตั้งแต่กรกฎาคม 2557 ถึง กรกฎาคม 2563 จำนวน 194 ราย มีผู้ได้รับยา methimazole 170 ราย และ propylthiouracil 24 ราย ตรวจ ANCA ก่อนและหลังเริ่มยาต้านไทรอยด์ หาความชุกด้วยร้อยละ เปรียบเทียบปัจจัยทางคลินิกที่อาจมีผลต่อการตรวจพบ ANCA ด้วย Fisher's exact test และ Mann–Whitney U test **ผลการศึกษา:** ความชุกของการตรวจพบ ANCA เท่ากับร้อยละ 3.61 มีผู้ป่วย 3 ราย (ร้อยละ 42.86) ตรวจพบ ANCA ก่อนการรักษา และ 4 ราย (ร้อยละ 57.14) พบหลังรักษาแล้ว 3 - 6 เดือน ทุกรายที่ผลตรวจพบ ANCA ไม่พบอาการของหลอดเลือดอักเสบ และพบว่าการตรวจพบ ANCA สัมพันธ์กับการตรวจพบ antinuclear antibodies (ANA) อย่างมีนัยสำคัญ (*P*-value = 0.047) แต่ไม่สัมพันธ์กับเพศ อายุ ชนิดของยาต้านไทรอยด์ ระดับ FT3, anti-thyroid peroxidase, anti-thyroglobulin และ Thyrotropin Receptor Antibodies (TRAb) **สรุป:** ความชุกของการตรวจพบ ANCA เป็นร้อยละ 3.61สามารถตรวจพบ ANCA ก่อนและหลังการรักษาในผู้ป่วยโรคคอพอกตาโปน การตรวจพบ ANCA สัมพันธ์กับการตรวจพบ ANA การตรวจพบ ANCA ก่อนการรักษาอาจเป็นผลจากภาวะภูมิคุ้มกันจากตัวโรคเองโดยไม่พบอาการและอาการแสดงของหลอดเลือดอักเสบ จึงไม่แนะนำให้ตรวจ ANCA ในผู้ป่วยทุกราย แต่ตรวจในรายที่สงสัยอาการหลอดเลือดอักเสบเท่านั้น

คำสำคัญ: anti-neutrophil cytoplasm antibody (ANCA), โรคคอพอกตาโปน, ยาต้านไทรอยด์

Abstract

Objective: To determine prevalence of anti-neutrophil cytoplasmic antibody (ANCA) positivity in Thai patients with Graves' disease at diagnosis and after treatment with methimazole (MMI) or propylthiouracil (PTU). **Method:** This prospective cohort study was conducted between July 2014 – July 2020. All 194 patients with GD and treated with PTU (n = 24) or MMI (n = 170) at Damnoen Saduak Hospital were included. Sera were screened for ANCA before and after the start of antithyroid drug treatment. Prevalence of ANCA positivity with percentage. Differences of clinical factors between patients with ANCA positive and negative status were compared with and Fisher's exact test and Mann–Whitney U test. **Results:** 3.61% of patients with GD were seropositive for ANCA. Three (42.86%) and 4 (57.14%) patients were ANCA positive before and 3 - 6 months after the start of the antithyroid drug treatment. ANCA positivity was significantly associated only with antinuclear antibodies (ANA) (*P*-value = 0.047), not with gender, age, type of antithyroid drugs, levels of FT3, anti-thyroid peroxidase, anti-thyroglobulin or Thyrotropin Receptor Antibodies (TRAb). **Conclusions:** ANCA positivity was 3.61% among GD patients, could be found before and after the initiation of drug therapy, and was associated with ANA positivity. ANCA positivity before drug treatment could point to the autoimmune disease nature of GD. Not all GD patients, but those with clinical syndromes might benefit from ANCA determination.

Keywords: anti-neutrophil cytoplasm antibody (ANCA), Grave's disease, antithyroid hormone drugs

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Introduction

Graves' disease is an autoimmune condition caused by circulating autoantibodies including thyroid stimulating hormone receptor antibody (TRAb) to stimulate thyroid gland function. Signs and symptoms of Graves' disease include enlargement of the thyroid gland or goiter, signs and symptoms of hyperthyroidism, and bulging eyes.[1] Most cases of Graves' disease are treated with antithyroid drugs such as propylthiouracil (PTU) and methimazole (MMI) which are common in Thailand.

Adverse effects of these antithyroid drugs could be non-serious and serious events. Non-serious adverse effects include rash or urticaria, and joint pain which could be found about 5% of the treated patients. In most cases, rash or urticaria could be resolved with or with antihistamine drugs; while some cases are too severe that antithyroid drugs need

to be discontinued.[2] Serious adverse effects of antithyroid drugs include agranulocytosis, hepatitis, and antineutrophil cytoplasmic antibody-positive vasculitis (ANCA–positive vasculitis). ANCA-positive vasculitis has been found 4 – 46% of the antithyroid drugs treated cases.[3] The majority of ANCA-positive vasculitis cases was perinuclear-ANCA (p-ANCA)[4] which is more common among patients treated with PTU than MMI.[5,6] The patient's younger age is a major factor of ANCA–positive vasculitis. Before the start of antithyroid drug treatment, prevalence of ANCA is between 0 – 13% and only 15% of the ANCA-positive patients will progress to vasculitis or nephritis.[7] One study revealed that 28% of the ANCA-positive patients developed vasculitis while the rest developed no adverse events.[8]

An international study showed that ANCA was positive in 6.7% and 64% of the patients before and after the initiation of antithyroid drugs, respectively.[9] In addition, ANCA was associated with anti-thyroglobulin antibody (anti-TG) but not with anti-thyroperoxidase antibody (anti-TPO).[9] For patients with signs of vasculitis including multiple-joint inflammation, fever, and purpura, discontinuation of antithyroid drugs is warranted if more severe signs, e.g., glomerulonephritis and pneumonitis, are developed.[10] Glucocorticoids with immunosuppressants could also be used.[10] These symptoms are usually resolved rapidly after antithyroid drugs discontinuation and most cases have good prognosis. However, some cases are life-threatening.[4] There have been cases of antithyroid drugs related vasculitis reported in Thailand and other countries especially Japan. However, prevalence of ANCA among Graves' disease patients in Thailand has not been known. To improve the care for Graves' disease patients regarding treatment safety, there was a need to know prevalence of ANCA, the associations of ANCA with signs and symptoms of vasculitis, and factors potentially associated with the ANCA presence. Specifically, the study aimed to determine 1) prevalence of ANCA in Thai Graves' patients receiving antithyroid drugs at Damnoen Saduak Hospital, 2) prevalence of vasculitis among ANCA-positive patients, and 3) associations of ANCA positive status with its potential factors. It was hypothesized that age, gender, type of antithyroid drugs, FT3 level, FT4 level, TSH level, TRAb level, anti-TPO level, anti-TG level and antinuclear antibody (ANA) positive status were associated with being ANCA positive.

Methods

In this prospective cohort analytic study, data collection took 6 years to complete. Each of all Graves' patient was tested for ANCA before and 3 - 6 months after treatment with antithyroid drugs. Data were collected using data collection form from patient interview and medical record including demographic and health status characteristics, signs and symptoms, and laboratory investigations at the start, at 3 – 6 months after the start, and at the end of the antithyroid drug treatment. All patients were treated with antithyroid drugs for 18 months and followed up for another 6 months resulting in a 2-year treatment period.

Definitions

1. Anti-neutrophil cytoplasmic antibody (ANCA) is an auto-antibody to antigen of neutrophil which is detected by indirect immunofluorescence assay (IIF). IIF is widely used to screen ANCA because of its high sensitivity. With IIF, 3 fluorescence stains including cytoplasmic (c-ANCA), perinuclear (p-ANCA) and atypical patterns could be identified. These ANCA types were associated with vasculitis.

2. In this study, vasculitis was defined as local skin signs alone or with vasculitis in internal organs such as glomerulonephritis and pneumonitis.

3.Thyroid receptor antibodies (TRAb) is an antibody to TSH receptor in Graves' disease patients. TRAb was determined by blood test and reported as IU/L.

4. Antinuclear Antibody (ANA) is an antibody to components within nucleus of cells of the patient. ANA could be identified in patients with diseases of tendon, muscle, joint fluid, and bone such as systemic lupus erythematosus (SLE). ANA was determined by blood test and qualitatively reported as positive or negative.

Population and sample

All patients diagnosed with Graves' disease and received antithyroid drugs at the Medicine Clinic of Damnoen Saduak Hospital were study population. Study population patients who were treated between July 2014 to July 2020 who met inclusion criteria were recruited as study sample. A sample size of 258 patients was recruited.

Inclusion and exclusion criteria

To be eligible, the patients had to be diagnosed with Graves' disease based on signs and symptoms and laboratory

findings including abnormally high FT3 or free thyroxine (FT4) levels, and abnormally low TSH level. They had to be 15 years old or older and treated with antithyroid drug for at least 2 years. They were Thai, able to communicate in Thai language, willing to participate in the study. Patients with the following status were excluded from the study. Patients who had not been under the medical care for 3 consecutive months were excluded. Patients with AIDS, systemic lupus erythematosus (SLE), cancer, cerebrovascular diseases, pulmonary tuberculosis, liver diseases or kidney disease with a glomerular filtration rate of < 30 ml/min/1.73m^2 were excluded. Those who were dead during the treatment, pregnant, or treated with hydralazine or prednisolone were also excluded.

Research instrument quality assurance

The study used an interview form to collect demographic characteristics, signs and symptoms, and laboratory investigation results of vasculitis. Content of this data collection form was examined for content validity (correctness, comprehensiveness, and clarity) by 3 experts. The data collection form was found to have an acceptable content validity with an index of congruence (IOC) of 0.92.

Participant protection

The study protocol was approved by the Ethics Committee for Human Study of Damnoen Saduak Hospital (approval number: 04/2557). The researcher approached and introduced himself to potential participants, provided details of study objectives and process, and voluntary nature of the study. The participant could participate or deny participation voluntarily, and could withdraw from the study at any time, without any consequences on their health care. No extra questions, interviews, examinations or laboratory investigations were made because all of these were conducted in the regular patient care. No additional blood draw was needed since blood sample collected regularly was kept for 15 days for any blood tests needed. The researcher asked the patients only for using their data for research analysis. The researcher informed the patients that their data were kept in secret and anonymous with no name or identity recorded. Research results were presented as summary statistics not individual patient data.

Data collection procedure

Once the study protocol was approved by the Ethics Committee for Human Study, data were collected from July 2014 to July 2020 on the patient's regular appointment date. The data of gender, age, signs and symptoms of Graves' disease, signs and symptoms of vasculitis, type of antithyroid drugs, laboratory investigation results including ANCA status, FT3 level (pg/mL), FT4 level (ng/dl), TSH level (μIU/mL), TRAb level (IU/L), anti-TPO level (IU/L), anti-TG level (IU/L) and ANA status were recorded.

Data analysis

Demographic and clinical characteristics were presented using descriptive statistics including frequency with percentage and mean with standard deviation. Prevalence values of ANCA positive status among all patients and vasculitis among ANCA-positive patients were present as percentage. Differences in clinical factors between patients receiving propylthiouracil and methimazole were tested using Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Differences in clinical factors between ANCA-positive and -negative patients were tested using Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Statistical significance was set at a type I error of 5 % (or *P*-value < 0.05). All statistical analyses were conducted using a statistical software program.

Results

From July 2014 to July 2020, there were 258 patients diagnosed with Graves' disease receiving care at Damnoen Saduak Hospital. Of the 258 patients, 64 were excluded because of missing the appointment, and having AIDS, SLE, cancer, cerebrovascular disease, pulmonary tuberculosis and death of other causes (52, 3, 2, 1, 1 and 2 patients, respectively.), resulting in 194 eligible participants.

Of the 194 participants, about three-quarters were women (74.2%) (Table 1). Most of them received MMI an antithyroid drug while the rest received PTU. The majority were 41 – 50 years old (23.7%), followed by 31 – 40 and 51 – 60 years old (21.6% for both). Almost two-thirds had no other chronic illnesses (61.3%). The most frequently found chronic illnesses were hypertension, hyperlipidemia, diabetes, and heart diseases, respectively (Table 1).

Table 1 Demographic and clinical characteristics of participants (N = 194).

Characteristics	N (%) by type of medications[#]		
	PTU (N = 24)	MMI (N = 170)	Total (N = 194)
Gender			
Men	5 (20.8)	45 (26.5)	50 (25.8)
Women	19 (79.2)	125 (73.5)	144 (74.2)
Age (years)			
< 20	1 (4.2)	5 (2.9)	6 (3.1)
21 – 30	5 (20.8)	25 (14.8)	30 (15.6)
31 - 40	8 (33.3)	34 (20.0)	42 (21.6)
41 – 50	6 (25.0)	40 (23.5)	46 (23.7)
51 – 60	3 (12.5)	39 (22.9)	42 (21.6)
> 60	1 (4.2)	27 (15.9)	28 (14.4)
Chronic illnesses			
No	17 (70.8)	102 (60.0)	119 (61.3)
Yes	7 (29.2)	68 (40.0)	75 (38.7)
Hypertension	4 (16.7)	38 (22.4)	42 (21.6)
Diabetes	4 (16.7)	10 (5.9)	14 (7.2)
Hyperlipidemia	6 (25.0)	26 (15.3)	32 (16.5)
Heart disease	0	8 (4.7)	8 (4.1)
Others	0	7 (4.1)	7 (3.6)

[#] PTU = propylthiouracil, MMI = methimazole.

In terms of signs and symptoms of Graves' disease, the most found symptom was lethargy (97.42%), followed by weight loss (81.96%), palpitation (59.79%), goiter (40.72%), fine tremor of hands (26.29%), increased perspiration (17.01%), and bulging eyes (11.34%) (Table 2). Symptoms found within 5 – 10% of the patients were increased appetite and fatigue.

For signs, the most found ones were tachycardia (68.04%), followed by tremor (54.64%), warm moist palms (45.88%), diffuse palpable goiter (41.24%), and periorbital edema (11.34%) (Table 2). Rare signs included muscle weakness and pretibial myxedema. Average heart rate was 107.56 ± 24.08 beats per minute. Additionally, atrial fibrillation (AF) was found in 11.34% of the patients (Table 2).

Table 2 Signs and symptoms of Graves' disease in participants (N = 194).

Symptoms	N	%	Signs	N	%
Lethargy	189	97.42	Tachycardia*	132	68.04
Weight loss	159	81.96	Tremor	106	54.64
Palpitation	116	59.79	Warm moist palms	89	45.88
Goiter	79	40.72	Diffuse palpable goiter	80	41.24
Fine tremor of hands	51	26.29	Periorbital edema	22	11.34
Increased perspiration	33	17.01	Atrial fibrillation	22	11.34
Bulging eyes	22	11.34	Edema	10	5.15
Increased appetite	20	10.31	Muscle weakness	3	1.55
Fatigue	13	6.7	Pretibial myxedema	2	1.03

* Heart rate = 107.56 ± 24.08 beats/min. by average.

In terms of clinical factors, duration since diagnosis of Graves' disease was 3.34 months by average (Table 3). Mean levels of laboratory tests at diagnosis were as follows: FT3 = 13.49 ± 6.05 pg/mL, FT4 = 4.60 ± 3.97 ng/dl, TSH = 0.02 ± 0.06 μIU/mL, anti-TPO = 160.86 ± 193.97 IU/liter, anti-TG = 328.4 ± 701.37 IU/liter, and TRAb = 5.98 ± 9.42 IU/liter. Differences of duration of diagnosis and each of most of these laboratory tests between patients receiving propylthiouracil and methimazole were not statistically significant, except for mean TRAb level where level in patients receiving propylthiouracil was significantly lower than that in those receiving methimazole (1.72 ± 1.57 and 6.58 ± 9.90 IU/liter, respectively, *P*-value = 0.005). In terms of remission at 2 years after treatment, proportion of patients with remission among those receiving propylthiouracil (58.33%) was slightly higher than those receiving methimazole (52.99%) but with no statistical significance (*P*-value = 0.620) (Table 3).

Of the 194 patients, 7 of them were ANCA-positive. Proportion of ANCA-positive patients in those receiving propylthiouracil (2 of 24 patients or 8.33%) was higher than that in those receiving methimazole (5 of 170 patients or 2.94%) but with no statistical significance (*P*-value = 0.209) (Table 3). Of the 7 ANCA-positive-positive patients, 6 patients had p-ANCA (85.71%) and 1 patient had c-ANCA (14.29%) (Table 3).

Table 3 Clinical factors in participants receiving propylthiouracil and methimazole (N = 194).

Clinical factors	Mean ± SD by type of medication[#]			*P*-value
	Total (N = 194)	PTU (N = 24)	MMI (N = 170)	
Duration since diagnosis (months)	3.34 ± 3.60	3.63 ± 2.97	3.30 ± 3.67	0.680[†]
Heart rate (bpm)	107.56 ± 24.08	108.33 ± 19.03	107.45 ± 24.75	0.866[†]
FT3 level (pg/mL)	13.49 ± 6.05	12.32 ± 6.53	13.66 ± 5.98	0.310[†]
FT4 level (ng/dl)	4.60 ± 3.97	3.34 ± 2.01	4.76 ± 4.13	0.210[†]
TSH level (μIU/mL)	0.02 ± 0.06	0.03 ± 0.07	0.02 ± 0.06	0.736[†]
anti-TPO level (IU/liter)	160.86 ± 193.97	152.68 ± 212.80	162.02 ± 191.81	0.227[†]
anti-TG level (IU/liter)	328.4 ± 701.37	216.05 ± 478.96	344.26 ± 726.97	0.089[†]
TRAb level (IU/liter)	5.98 ± 9.42	1.72 ± 1.57	6.58 ± 9.90	0.005[†]*
Remission at 2 years: N (%)	104 (53.60)	14 (58.33)	90 (52.94)	0.620[‡]
ANCA positive: N (%)	7 (3.61)	2 (8.33)	5 (2.94)	0.209[‡]
- p-ANCA: N (%)	6 (3.09)[a]	6 of 7 (85.71)[b]		
- c-ANCA: N (%)	1 (0.52)[a]	1 of 7 (14.29)[b]		

[#] PTU = propylthiouracil, MMI = methimazole.

* Significant (P-value < 0.05). [†] Mann-Whitney U test. [‡] Fisher's exact test.

[a] Compared with a total of 194 patients. [b] Compared with 7 ANCA-positive patients.

Of the 7 ANCA-positive patients, 5 of them were female (71.43%). Four of them had ANCA positive at 3 – 6 months after the start of the antithyroid drug treatment (57.14%) while

the rest were found positive before the start of the treatment. None of the 7 patients had vasculitis signs or symptoms. Only one patient had remission after the treatment (14.29%) (Tables 4 and 5). In terms of ANCA type, 6 of 7 ANCA-positive patients had p-ANCA (85.71%). The only 1 patient with c-ANCA type was a woman aged 54 years old who was found ANCA positive 3 – 6 months after starting propylthiouracil (Table 4).

Table 4 Characteristics of 7 ANCA-positive patients.

No.	Gender	Age (years)	Drug#	ANCA type	Time of identification	Presence of vasculitis	Treatment outcome
1	female	59	MMI	p-ANCA	Before treatment	no	Remission
2	female	27	PTU	p-ANCA	Before treatment	no	No remission
3	female	43	MMI	p-ANCA	3 – 6 months after treatment start	no	No remission
4	female	54	PTU	c-ANCA	3 – 6 months after treatment start	no	No remission
5	female	65	MMI	p-ANCA	3 – 6 months after treatment start	no	No remission
6	male	20	MMI	p-ANCA	3 – 6 months after treatment start	no	No remission
7	male	42	MMI	p-ANCA	Before treatment	no	No remission

PTU = propylthiouracil, MMI = methimazole.

Table 5 Factors associated with ANCA status (N = 194).

Factors	Mean ± SD by ANCA status		*P*-value
	ANCA positive (n = 7)	ANCA negative (n = 187)	
Age (years)	43.71 ± 16.18	44.35 ± 14.95	1.000†
Gender (n): male/female	2/5	48/139	0.578‡
TSH (mIU/liter)	0.017 ± 0.027	0.02 ± 0.06	0.915†
FT_3 (pg/mL)	12.61 ± 7.38	13.53 ± 6.01	0.626†
anti-TPO (IU/liter)	273.77 ± 229.54	156.63 ± 191.9	0.112†
anti-TG (IU/liter)	547.27 ± 532.87	320.21 ± 706.68	0.083†
TRAb (IU/liter)	4.16 ± 4.95	6.05 ± 9.54	0.923†
ANA (%)	85.71 (6/7)	45.99 (86/187)	0.047‡*
Titer 1/160	71.43	37.43	
Titer 1/320	0	4.28	
Titer 1/640	14.28	1.07	
Titer 1/1280	0	1.60	
Titer 1/5120	0	1.60	

PTU = propylthiouracil, MMI = methimazole.

* Significant (P-value < 0.05). † Mann-Whitney U test. ‡ Fisher's exact test.

Regarding differences of various factors between patients who were ANCA positive and ANCA negative, their mean age, TSH, and FT3 of the two groups were considerable comparable. Mean TRAb level of ANCA-positive patients was slightly lower than that of ANCA-negative patients (4.16 and 6.05 IU/L, respectively), with no statistical significance. Mean anti-TPO level (273.77 and 156.63 IU/L, respectively) and mean anti-TG level (547.27 and 320.21 IU/L, respectively) of ANCA-positive patients were higher than those of ANCA-negative patients, with no statistical significance. Proportion of ANA-positive patients in ANCA-positive patients (6 of 7, or 85.71%) was significantly higher than that in ANCA-negative patients (45.99%) (*P*-value = 0.047). Among the 7 ANA-positive patients, 5 of them had titer 1/160 (71.43%) and 1 of them had titer 1/640 (14.29%) (Table 5). All of these 7 cases were homogeneous and speckled type.

Discussions and Conclusion

This is the first prospective cohort study in Graves' disease patients in Thailand with ANCA identification with by immunofluorescence assay before and 3 – 6 months after the start of antithyroid drug treatment. The prevalence of ANCA-positive was prevalence of 3.61%. Of all 194 Graves' disease patients, proportions of ANCA-positive in patients receiving propylthiouracil and methimazole were comparable (8.33% and 2.94%) with no statistical significance (*P*-value = 0.209). Among 7 ANCA-positive patients, the most found type was p-ANCA (6 patients, or 85.71%) with only 1 with c-ANCA type. This finding was consistent with some studies which showed that 80 – 90 cases of antithyroid drug related vasculitis were found to be p-ANCA type.[4,11,12] Our finding of no difference of ANCA positive status between propylthiouracil and methimazole was different from the study of Ross and colleagues which found that ANCA–associated vasculitis was more common in patients receiving propylthiouracil tan methimazole.[4] In contrast, Gunton and colleagues reported that of the 27 patients with antithyroid drug related vasculitis, only one case was associated with methimazole.[13,14]

Most of the 7 cases with ANCA-positive were female (71.43%). Their ANCA status was identified 3 – 6 months after the start of the antithyroid drug treatment (57.14%) and before the treatment (42.86%). ANCA was found positive before the start of antithyroid drug is consistent with a study revealing that 6.7% of patients were ANCA positive before the start of propylthiouracil.[9] The studies of Huang and Visavachaipan showed that anti-thyroid drug-induced ANCA-associated vasculitis in patients who did not respond to antithyroid drugs usually had a high dose and long duration of drug treatment. They reported that propylthiouracil with a high dose of 5 tablets per day by average was needed and usually was given for as long as 25 – 60 months.[5,15] Some reports showed a duration of antithyroid use as long as 7.9 years till the ANCA positive status was found. These previous findings were different from ours which ANCA positive status was found at 3 – 6 months after the start of antithyroid drugs. In terms of

genetic disparity, ANCA positive status was found more prevalent among Japanese patients with comprised about half of the reported cases. However, no rationales to support such cases have not been found. In addition, more than 70% of the cases were female. When compared with cases from other countries, no difference of ANCA positive status regarding gender, age, type of antithyroid drugs, duration of drug treatment, dose, pathophysiologic status, treatment, and prognosis.[7] Our findings showed that gender, age, type of antithyroid drugs, FT3, TSH, anti-TPO, anti-TG and TRAb were not associated with ANCA status. This is different from the study of Sato and co-workers which found that p-ANCA but not anti-TPO was associated with ANCA status.[9] In our study, among ANCA-positive patients, 85.71% of them were ANA positive and had a titer of more than 1/160. This finding was consistent with the study of Gumà which ANA positive cases were 73% and anti-dsDNA positive cases were 10% of ANCA positive patients.[16]

All ANCA-positive patients showed no signs or symptoms of vasculitis. This finding was consistent with previous studies which found that only 15% of ANCA-positive cases had signs and symptoms of vasculitis or nephritis.[7,9,17] It is also consistent with the study of Zhao and colleagues which revealed that only 28% of ANCA-positive cases had symptoms of vasculitis and the rest 72% had no signs or symptoms.[8] However, since only 194 patients were examined in our study, a larger sample size is needed for future studies to prove the association between ANCA status and various factors with more statistical confidence.

Flu-like symptoms are common among patients receiving antithyroid drugs who experience vasculitis. Most cases are mild but certain cases could have other symptoms such as purpura, skin wound, interstitial pneumonia, pulmonary hemorrhage, anemia, nephritis and acute kidney failure.[7] The presence of any of these signs should prompt diagnostic investigations and antithyroid drug discontinuation.

Certain limitations were found in our study. In this cohort study, the practicing physician (the researcher) usually prescribed methimazole for Graves' disease patients. Based on previous reports, more ANCA-positive cases were found taking propylthiouracil than methimazole. Future studies should recruit more cases taking propylthiouracil. In previous studies, ANCA positive status occurred with patients taking antithyroid drugs for a duration that is longer a limited 2 years of our study. Future studies should have a longer follow-up time.

In conclusion, a prevalence of 3.61% of ANCA positive status among Thai Graves' disease receiving antithyroid drugs was found both before and after the drug treatment initiation. No cases showed signs or symptoms of vasculitis. ANCA-positive status was significantly associated only with ANA-positive status (P-value = 0.047) but not with gender, age, type of antithyroid drugs, FT3, TSH, anti-TPO, anti-TG or TRAb level. With a low prevalence of ANCA positive of 3.61% and no signs or symptoms of vasculitis in those who were positive, ANCA status determination is thus not recommended for all Graves' disease patients receiving antithyroid drugs but it might be beneficial for cases with vasculitis clinical syndromes.

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