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Autoimmune urticaria in Rumah Sakit Umum Pusat Dr. Moh. Hoesin Palembang

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ABSTRACT

M. Athuf Thaha - ***Autoimmune urticaria in Rumah Sakit Umum Pusat Dr. Moh. Hoesin Palembang***

Background: About 30% patients with chronic idiopathic urticaria (CIU) have circulating histamine-releasing autoantibodies against the α-subunit of high affinity IgE receptor (FcεRIα), or IgE. This subgroup of patients has a disorder commonly referred as autoimmune urticaria.
Objectives: This study was conducted to reveal the autoimmune urticaria cases in Indonesian patients
Methods: The autologous serum skin test (ASST) and histamine release assay (HRA) were conducted on 79 patients with CIU (53 females and 26 males). Patients with predominant physical urticaria and urticarial vasculitis were excluded from the study.
Results: Seventeen patients had both positive autologous serum skin test and histamine release assay confirmative of autoimmune urticaria.
Conclusion: Combined positive ASST and HRA were seen in 21.5% of CIU patients, indicating autoimmune urticaria.

Key words: chronic idiopathic urticaria - anti-FcεRIα histamine-releasing autoantibodies - autoimmune urticaria - autologous serum skin test, histamine release assay.

ABSTRAK

M. Athuf Thaha – ***Urtikaria autoimun di Rumah Sakit Umum Pusat Dr. Moh. Hoesin Palembang***

Latar Belakang: Kira-kira 30% pasien dengan urtikaria idiopatik kronis (CIU) mempunyai histamin beredar yang membebaskan antibodi beredar terhadap á-subunit dari reseptor IgE afinitas tinggi(FcεRIα), or IgE. Kelompok pasien ini mempunyai kelainan umum yang secara umum dipandang sebagai urtikaria autoimun.
Tujuan: Penelitian ini bertujuan untuk mengungkap urtikaria autoimun di Indonesia.
Metoda: Uji kulit serum autolog (ASST) dan pengukuran pengeluaran histamin (HRA) dilakukan pada 79 pasien dengan CIU (53 wanita dan 26 pria). Pasien dengan urtikaria yang fisis predominan dan vaskulitis urticarial dikeluarkan dari penelitian ini.
Hasil: Tujuh belas pasien menunjukkan positif uji kulit serum autolog and pengukuran pengeluran histamin yang konfirmatif untuk urtikaria autoimun.
Simpulan: ASST dan HRA ditemukan pada 21.5% pasien CIU, ini menunjukkan urtikaria autoimun.

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INTRODUCTION

Chronic idiopathic urticaria (CIU) is a skin disorder characterized by urticarial lesion with/ without recurrent angioedema, and takes place for 6 weeks or more. CIU consists of 2 subgroups. One subgroup is autoimmune urticaria (AU), caused by IgG autoantibody against the α-subunit of high affinity IgE receptor (anti-FcεRIα) or, against IgE (anti-IgE) at the surface of mast cells and basophilic cell. The mechanism stimulates the release of histamine and other mediators (eicosanoid, cytokines, and protease), resulting in urticaria and angioedema.[1] This subgroup covers 35-55% of CIU cases. Other subgroup, that covers 50% of chronic urticaria, has no clear etiology, and commonly referred as the real CIU.[1]

Functional autoantibody relationship with FcεR1α on dermal mast cell and basophilic cell was reported for the first time by Hide *et al.*[2,3] These autoantibodies were IgG1 and IgG3, isotypes that had roles in complement activation.[4] Histamine release from dermal mast cell by cross-linking between anti-FcεR1α and FcåR1α is reinforced by complement activation.

Urticaria case prevalence in the literature is estimated to be occurred in 15-23% population,[5] and it is estimated that 25% of them will suffer from chronic urticaria.[6] Epidemiological studies in Spain showed the prevalence of chronic urticaria was 0.6%.[7] The exact incidence and prevalence of AU is not known, but the incidence is estimated to be in the range of 0-3%.[8] In a study in India, AU was found in 12 of 45 patients (26.67%), consisting of 8 females and 4 males. The study was based its result on *in vivo* ASST.[9] Other study found AU in 33 of 107 patients (31%), based on HRA results[10], and Ferrer, Kinet, and Kaplan[11] found 31 of 68 patient sera (48%) released > 17% histamine in HRA test.

Based on gender, the prevalene of urticaria in women (0.48%) is higher than in men (0.12%), and the age group most often sufferred from urticaria is over 65 years (0.96%), followed by 45-64 year old age group (0.65%) and 25-44 year old age group (0.46%).[7]

Anti-FcεR1α and anti-IgE autoantibodies may be detected by histamine release from healthy donor basophilic cells that was induced with CIU patient serum. Gold standard of AU is *in vitro* histamine release assay (HRA). This test has been conducted in a few specialist laboratories but cannot be conducted by most clinicians. Histamine release is measured with enzyme immunoassay technique. Percentage of histamine release (%HR) from healthy donor basophilic cells incubated with 24 normal sera was used to determine the cut-off value (mean±SD) of histamine release in patients who had AU compared to patients who did not have autoantibody (non-AU). The value of %HR in urticarial patients was said to have autoantibodies if the %HR is ≥ (mean + 2SD).[12]

Autologous serum skin test (ASST) procedure is still the best *in vivo* clinical test to confirm the activity of histamine release from basophilic cell *in vitro*. In Western literature, positive ASST result was reported in 25-45% CIU patients, and ASST procedure most commonly used by researchers were ASST procedure with the method of Sabroe *et al.*[13] The sensitivity and specificity of the method is 65-71% and 78-81%. We have determined ASST parameter that gave optimal sensitivity and spesificity to detect CIU patients who had auto-antibody.

In this study the author conducted ASST and HRA tests on CIU patients to reveal AU cases in urticaria patients who came to Subdepartment of Allergy-Immunology in the Outpatient Unit of Department of Dermatovenereology, RSUP Moh. Hoesin, Palembang in 2007.

METHODS

It was a cross-sectional study on 79 patients with CIU (53 females, 26 males; 18-69 years old). All patients did not received treatment of steroid or cyclosporin at the time of this *in vivo* study, and did not take any antihistamine 3 days before the study. CIU patients with predominant physical urticaria, urticarial vasculitis, C1 esterase inhibitor deficiency, drug and alcohol users, pregnancy, and lactation were not included in this study.

ASST procedure was conducted with author technique and Sabroe technique on 79 patients, and HRA procedure was conducted on all CIU patients. The ethical clearance of this study was given by Bioethics and Humanity Unit, Faculty of Medicine, Sriwijaya University, Palembang.

ASST Procedure

All CIU were clinically active at blood sampling. ASST procedure with author's technique: 50 mL sterile fresh autologous serum, 50 mL histamine (10 mg/mL), and 50 mL 0.9% saline solution, separately injected intradermally (i.d.) in volar forearm (free of spontaneous urtica lesions for a minimum of 24 hours) with space of 5 cm inbetween.

Wheal and flare response were measured at minute 0 and 30. The difference between wheal caused by serum and wheal caused by saline (D) was assessed based on this formula:

$$D = \frac{(dser0 + dser30)}{2} - \frac{(dsal0 + dsal30)}{2}$$

Colour response of wheal caused by serum, saline, and histamine C were observed after 30 minutes: score 0 was given if the colour of the wheal was similar with the colour of the wheal caused by saline (skin coloured/pink); score 1 if the colour of the wheal caused by serum was pink, while the wheal caused by saline was skin colour; and score 2 if the colour of the wheal caused by serum was similar with the colour of the wheal caused by histamine (red). The result of ASST was positive if D ≥ 1.5 mm and C = 2.[14]

ASST with Sabroe technique: the determination of difference of diameter of the wheal caused by serum and wheal caused by saline was based on the reading at minute 30 (D_1), with this formula:

$$\frac{(dser30 - dsal30)}{2}$$

The result of Sabroe ASST was positive if D_1 ≥ 1.5 mm and C = 2.[13]

HRA Procedure

1. Separation of healthy donor leucocyte suspension
   Leucocyte suspension was obtained from healthy donor and contained $2x10^5$ leucocytes/mL. Leucocyte suspension was divided into 3 in duplo tubes, each contained 50 mL.
2. Incubation of leucocyte suspension
   Leucocyte suspension in tube 1 was incubated in 50 L CIU patient serum and non-urticaria for 40 minutes at 37°C, and then the reaction was stopped with chilling over ice. The suspension was centrifuged for 10 minutes at 3500 rpm, and the supernatant obtained was assessed with enzyme linked immunosorbent assay (ELISA) technique to measure histamin release caused by the stimulation of serum: stimulated HR (stiHR) of patients with CIU and non-urticaria; suspension of leucocytes in the tube 2 was added with 50 mL PBS, heated at 85°C for 40 minutes. The suspension was centrifuged at 3500 rpm for 10 minutes, and supernatant obtained was assessed with ELISA technique to measure the total histamine released by lytic cells (totalHR) of patients with CIU and non-urticaria; suspension of leucocytes in tube 3 was incubated in 50 mL PBS for 40 minutes at 37°C, and then chilled over ice. Suspension was centrifuged for 10 minutes at 3500 rpm, and supernatant obtained was assessed with ELISA technique to measure histamin release without the stimulation of serum: spontaneous HR (spoHR) of patients with CIU and non-urticaria.[14]
3. Measurement of histamine release with ELISA technique
   Competitive ELISA kit used was produced by Neogen's Corporation.[15] Measurement of histamin release followed the direction of the ELISA kit manufacturer. The calculation of %HR used the formula:
   [(stimulated HR – spontaneous HR) / total HR] x 100% (spontaneous histami < 5% total histamin).[16]

Production standard curve and linear equation followed the direction of Neogen's Corporation, 2004,[15] and cut-off values (mean±SD) of HRA (+) or (-) were obtained from 25 healthy donor serum samples. Patients were considered as having functional autoantibody if the sera produced %HR ≥ (mean + 2 SD) of the cut-off value.

History taking, physical examination, and laboratory examination were conducted in this study by the author and data were analyzed with SPSS version 12. *In vivo* ASST and *in vitro* HRA tests were conducted to find out the presence of autoimmune urticaria. ASST technique reliability was

determined with diagnostic test using Receiver Operating Characteristics (ROC) curve, with HRA as gold standard. The difference of significancy between the sensitivity (Sn) and specificity (Sp) of ASST by author's technique and Sabroe technique was analyzed with difference inproportion test using EpiCalc software.

RESULTS

The percentage of histamine release (%HR) of healthy donor and CIU patients with positive autoantibody

In this study, non-urticarial serum samples to determine the cut-off values of HRA +/- were obtained from 25 donors. Linear equation obtained was: $y = -641.4x + 1994.4$ ($R^2 = 0.8331$). Based on this equation, the %HR of healthy donor was 10.81±0.72. Patients with urticaria that having %HR ≥ (10.81 + 2 x 0.72) or ≥ 12.25% were considered as having functional autoantibody, or referred as HRA +.

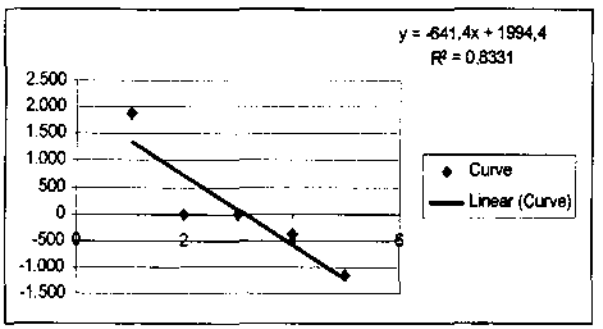

FIGURE 1. Standard curve and linear equation using Excel software

Note:
Linear equation obtained was: y = -641.4 x + 1994.4 (R^2 = 0.8331)
x: the level of stiHR/spoHR/totHR (ng/mL)
y: absorbance of stiHR/spoHR/totHR
%HR (mean±SD) of healthy donor = 10.81±0.72

HRA frequency

Based on total samples (79 patients, the youngest was 18 years old and the oldest was 79 years old, consisted of 53 females and 26 males), serum of CIU patients produced %HR ≥ 2.25% (HRA positive) was 17 patients (17/79 x 100% = 21.5%), mostly in 46-52 year old age group (8.86%), followed by 53-59 year old age group (6.33%), 60-69 year old age group, and 39-45 year old age group was 2.53%, and the remaining patients (62 patients) having %HR < 12.25% (HRA negative) was (62/79 x 100% = 78.5%) (TABLE 1.)

TABLE 1. The frequency of HRA positive based on age group

Age group	HRA		Total
	Positive	Negative	
18-25	0 (0.00%)	8	8
25-32	0 (0.00%)	2	2
32-39	1 (1.27%)	7	8
39-46	2 (2.53%)	11	13
46-53	7 (8.86%)	21	28
53-60	5 (6.33%)	11	16
60-69	2 (2.53%)	2	4
Total	17 (21.5%)	62 (78.%)	79

Frequency of HRA based on gender

There were 11 female CIU patients with HRA positive (13.92%), and 6 males with HRA positive (7.59% (TABLE 2.)

TABLE 2. The frequency of HRA positive based on gender

Gender	HRA		Total
	Positive	Negative	
Female	11 (13.92%)	42	53
Male	6 (7.59%)	20	26
Total	17	62	79

ASST procedure with author's technique, frequency of AST based on age group

There were 79 patients included in the study: 18 patients (22.8%) had ASST positive, mostly in 46-53 age group (7.5%), followed by 53-60 year old age group, 39-46 year old group (3.79%), and 18-25 year old group (3.79%), and the remaining 61 patients (77.2%) had ASST negative (TABLE 3.).

TABLE 3. The frequency of ASST based on age group

Age group	ASST with author technique		Total
	Positive	Negative	
18 – 25	3 (3.78%)	17	20
25 – 32	0 (0.00%)	2	2
32 – 39	1 (1.27%)	9	10
39 – 46	3 (3.79%)	9	12
46 – 53	6 (7.59%)	21	27
53 – 60	3 (3.79%)	1	4
60 – 69	2 (2.53%)	2	4
Total	18 (22.8%)	61 (77.2%)	79

Frequency of ASST based on gender

There were 11 patients who had ASST positive (13.92%), and 26 males with ASST positive (8.9%) (TABLE 4.)

TABLE 4. The frequency of ASST positive based on gender

Gender	ASST with author technique		Total
	Positive	Negative	
Female	11 (13.9%)	42	53
Male	7 (8.9%)	19	26
Total	18	61	79

Sensitivity and specificity of ASST

Sensitivity (Sn) and specificity (So) of ASST with author technique were 82.4% and 93.5%, respectively (AUC = 0.880, +PV: 77.9, -PV: 95.1, +LR: 12.76, dan –LR: 0.19); Sn and Sp of ASST with Sabroe technique were 70.6% and 71%, respectively (AUC = 0.708, +PV: 40.1, -PV: 89.8, +LR: 2.43, dan –LR: 0.41%) (TABLE 5).

TABLE 5. Sensititvity and specificity of ASST with author technique and ASST with Sabroe technique, with HRA as gold standard (SPSS and MedCalc)

ASST	Sn	Sp	AUC	S.E.	*p*	95% C.I.		LR		PV	
						Lower	Upper	(+)	(-)	(+)	(-)
Sabroe *et al.*[13]	70.6	71.0	0.708	0.072	0.009	0.566	0.850	2.43	0.41	40.1	89.8
Athuf [14]	82.4	93.5	0.880	0.057	0.000	0.767	0.992	12.76	0.19	77.9	95.1

Note:
AUC : area under the receiver operating character istic (ROC) curve
S.E. : standard error
C.I. : confidence interval
Sn : sensitivity
Sp : specificity
PV : predictive value
LR : likelihood ratio

Difference inproportion test

Difference inproportion test between Sn of ASST with author's technique and Sn of ASST with Sabroe technique, giving Z score of 1.56, p = 0.056, showed that the difference was insignificant (TABLE 6).

TABLE 6. Result of difference inproportion test between Sn of ASST with author technique and Sn of ASST with Sabroe technique (EpiCalc software)

Proportion of Sn and sample size	Z score	95% C.I	p-value (one-sided)	p-value (two-sided)
ASST with author technique (82.4%, 79) vs ASST with Sabroe technique (70.6%, 79)	1.56	11.8%	0.056	0.11

Difference inproportion test between Sp of ASST with author technique and Sp of ASST with Sabroe technique, giving Z score of 3.49, p < 0.000, showed that the difference was significant (TABLE 7).

TABLE 7. Result of difference inproportion test between Sp of ASST with author technique and Sp of ASST with Sabroe technique

Proportion of Sp and sample size	Z score	95% C.I.	p-value (one-sided)	p-value (two-sided)
ASST with author technique (93.5%, 79) vs ASST with Sabroe technique (71%, 79)	3.49	22.5%	0.000	0.000

DISCUSSION

Result based on in vitro HRA test showed that the proportion of females who had autoantobodies was higher than males (11 patients or 13.92% and 6 patients or 7.59%). The result was comparable with the result of the study by Gaig *et al.*[7] This proportion of gender was almost similar with the proportion of gender based on *in vivo* ASST test (11 patients or 13.9% and 7 patients or 8.9%).

Proportion of age group who had the highest autoantibody based on in vitro HRA test was in 46-53 year old age group (8.86%) and 53-60 year old age group (6.33%). This result was different with the result of Gaig *et al.*, where it was found mostly in the patients who were over 65 years old. This porportion was different with the proportion of age group based on *in vivo* ASST test, where the true difference was shown in 18-25 year old age group. The result of in vitro HRA test showed that there were no CIU patients with HRA positive in 18-25 year old age group, while in *in vivo* ASST test, there were 3 patients (3.78%) of the age group was ASST positive. The author suggested that the positive result of *in vivo* ASST test in the age group was caused by false positive.

HRA Procedure

HRA procedure with author's technique is a procedure using ELISA kit produced by Neogen's Corporation, America. The cut-off value for HRA+ in this study was ≥ 12.25%. This result was different with the result of a study by Ferrer *et al.*[11] (≥ 16.9%). This difference was caused by ELISA kit used. Nevertheless, the cut-off value obtained by the author was still in the range of 2-20%.

The combination of HRA+ and ASST+ in this study was 21.5%, lower than the result of Sabroe technique *et al.*[10] (31%) and Ferrer *et al.*[11] (48%). These results gave impression that the percentage of AU cases in this study (21.5%) was lower than in European studies (21-50%).

Based on the total visits to the Outpatient Unit of Department of Dermatovenereology of RSUP Moh. Hoesin Palembang in 2007 (9400 patients), the incidence of AU was 0.18%. This incidence was not different with the incidence reported by Greaves.[1] (0.1-3%).[15]

ASST Procedure

ASST diagnostic test by author's technique with HRA as gold standard resulted in sensitivity (82.4%) and specificity (93.5%) higher than Sn and Sp of Sabroe *et al.* technique (65-71% and 78-81%, respectively) and ASST result with Sabroe technique (70.6% and 71.0%, respectively). Sensitivity and specificity with Sabroe *et al.* technique[13] was almost similar with ASST with Sabroe technique.

Diagnostic value of ASST with author's technique and Sabroe technique showed that Sp of ASST with author's technique was far more specific than Sp of ASST with Sabroe technique, based on the result of analysis of difference in proportion ($Z = 3.49$, $p < 0.000$), but there was insignificant difference of the Sn between the two methods ($Z = 1.56$, $p = 0.05$).

The author suggested that the significant difference in specificity between ASST with author technique and ASST with Sabroe technique was caused by the method of measurement of diameter difference of the wheal caused by serum and the wheal caused by saline. Sabroe *et al.*[13] measured the difference only at 30 minutes, while author measured the difference at 0 and 30 minutes. At 0 minute, edema caused by saline and serum injection was not caused by immunological process, but by the effect of solution volume injected. At minute 30, due to the difference of saline and serum diameters, the absorption of solution volume at the location of serum and saline injection was not similar, and this might cause the difference in diameter between the two locations, not only resulted from immunological process but also from non-immunological process (by solution volume). The author was certain that the difference in diameters of the wheal caused by serum and wheal caused by saline at minute 0 may affect the difference in diameters of both wheals.

Besides, the reading at minute 0 was conducted based on author observation while conducting ASST procedure, where there were a different diameters of the serum wheal and saline wheal at minute 0, although the volume (mL) of serum and saline injected has been measured accurately (0.05 mL). It meant that there was no difference in diameters of wheal caused by serum and wheal caused by saline at minute 0, therefore, ASST procedure may be conducted with author's technique or Sabroe technique, because Sn and Sp of both methods were similar. The author cannot explain why the sensitivity of the two techniques was not significantly different.

CONCLUSION

Combined positive ASST and HRA were seen in 21.5% of CIU patients, indicating autoimmune urticaria.

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