SUBJECTIVE AND OBJECTIVE EVALUATIONS OF PERENNIAL ALLERGIC RHINITIS AFTER NASAL ALLERGEN CHALLENGE AND THE TREATMENT IN ACUTE PERIOD

"การประเมินผู้ป่วยโรคภูมิแพ้จมูกอักเสบโดยตัวผู้เองและการใช้มาตรวัดทางการแพทย์ หลังจากกระตุ้นโดยสารก่อภูมิแพ้เปรียบเทียบกับอาสาสมัครปกติและติดตามผลการรักษาด้วยยาต้านฮิสตามีนชนิดไม่ก่อการง่วงนอนหรือยาหลอกในระยะเฉียบพลันของโรค"

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INTRODUCTION

Allergic rhinitis is a prevalent disease in Thailand, particularly in urban districts. The diagnosis depends on history taking and skin prick testing. Rhinomanometry has been long used in several facets of research and clinical practice. Also, it helps physician diagnose allergic rhinitis when either skin-prick test does not establish the diagnosis or more specific testing is in need. Studies proposed positive cut-off level by this mean. Without report of cut-point value for patients in Thailand, this study performed nasal allergen (Der p, Der f) challenge of allergic rhinitis patients and healthy volunteers in order to record the nasal airway resistance. The data from both groups were used to construct ROC curve. The most accurate point for the rising resistance was then determined.

Orally non-sedating antihistamines have widely prescribed in Thailand for ages. Three commonly used of them are fexofenadine, cetirizine,and loratadine. The previous researches studied the efficacy of the drug in treating allergic rhinitis in western world where seasonal allergic rhinits is predominately found [1-3]. Although perennial allergic rhinitis was also studied in some countries, including Thailand [4;5]. There were two studies in Thailand regarding fexofenadine and astemizole but they were conducted in non-comparative manner and subjectively evaluated [1;6]. This study aims to do comprehensive investigation the efficacy, onset-of-action, and tolerability of these three agents by both subjective and objective methods in acute period after nasal challenge test in allergic rhinitis patients in Thailand.

PATIENTS AND METHODS

Written informed consent was obtained from all subjects prior to enrollment. For subjects under 18 years of age, a parent or guardian signed the informed consent document. The protocol and consent form for the study were reviewed and approved by Research Ethical Committee of the Chiang Mai University, Faculty of Medicine.

Subjects

The allergic rhinitis patients were *included* by these following criteria (1) Age between 15-50 years. (2) Diagnosis of allergic rhinitis was confirmed by history, physical examination as well as positive skin test to house dust mite (Der p, Der f). Positive wheals are those exceeding 3 mm in diameter greater than the negative control. The diameter evaluated was the average of the longest and the prependicular diameters. (3) No prior medications in limited period (i.e. one week for decongestant, two weeks for non-sedating antihistamine, and four weeks for topical or systemic steroid) was allowed before starting the study.

The *exclusion criteria* were the following : (1) history of severe asthmatic attack or anaphylaxis ; (2) Relevant septal deviation, polyps, or sinusitis remained in active. (3) The activity of allergic rhinitis was moderate and severe score 5 or more by criteria (Table 1). (4) The excesscively alcohol and coffee drinker. (5) History of antihistamine drug allergy. The child-bearing potential women must use an effective contraceptive controls. On the contrary, control subjects had no nasal symptoms and negative skin-prick tests with comparable other parameters. The vasomotor rhinitis patients, defined by patients who complained of nasal symptoms without positive skin testing, were classified as control subjects as well. The protocol and patient information guideline was given clearly.

Study Design

The study was a single center, randomized, double-blind. parallel, placebo-controlled trial. After initial screening visit where eligible subjects were enrolled. Demographic data was collected. Baseline total nasal symptoms score (TNSS) and total nasal airway resistance (NAR) were recorded by trained nurse. The nasal symptoms score was consisted of itching, stuffiness, sneezing count, and rhinorrhea.

The patients and healthy volunteers underwent nasal allergen challenge test by disc method. Firstly, the subjects challenged with diluent to prove the impurity of allergen solvents. The diluent was 0.4% phenol in 0.9% normal saline solution. In this study, the measurements at diluent challenge were regarded as baseline of those particular parameters. Mixture of D. pteronyssinus and D. farinae (Allertech, Thailand) was prepared to 50, 100, 500, 1,000, and 5,000 AU/ml and pipetted 20 microliters onto each dry filter paper disc (punched out of Whatman filter paper no.1, Whatman, England). This incremental doses of allergen were challenged at 10-min interval. The allergen discs were placed over inferior aspect of the inferior turbinate bilaterally. After 30 seconds, they were then removed. Ten minutes after diluent/allergen discs were placed, the NSS and NAR were assessed.

The nasal cavities were always inspected to confirm the absence of nasal secretions before measuring NAR. The NAR was measured bilaterally with anterior

rhinomanometry (Rhinomanometer, PC 200 ATMOS, Germany). Reading total NAR at 75 Pa of pressure gradient was based on previous study in Thais [7]. The value which was too high to be measured by rhinomanometer would be estimated by multiplying with the average of the increasing proportions of all measurable results in that subject.

Patients who had positive nasal challenge by allergen were enrolled to the next session of study. The positive nasal allergen challenge was arbitrarily chosen by symptoms score of 5 and over and the increment of NAR at least 2 times above the diluent baseline. The positive-challenged patients were randomly given a tablet either of cetirizine 10 mg (Zyrtec®, U.C.B., Thailand), loratadine 10 mg (Clarityne®, Schering-Plough/Zuellig, Thailand), fexofenadine 60 mg (Telfast®, Aventis/Zuellig, Thailand), or placebo (corn starch, Vidhyasom, Thailand). Patients with 150-ml water took an assigned drug without seeing (double-blind) fashion.

In the following 4-hour period, patients would have been challenged with single concentration of allergenic extract (5,000 AU/ml) every 30 minutes to elicit the symptoms. Ten minutes after each challenge, TNSS and NAR were evaluated. Also, at the baseline and every 30-minute milestone, special nurse conducted histamine-induced skin reaction and weighed nasal secretion by mandating patients to blow their noses. Tissue papers for blowing nose were weighed before. Besides, four adverse experiences (sleepiness, dryness, headache, fatigue) were graded hourly throughout four hours.

Wheal-and-flare area (W-F area) was elicited by histamine phosphate 1 mg/ml and 10 mg/ml (Allertech, Thailand) on the right and left volar surface of forearm respectively. A disposable hypodermic needle (26 gauge) was passed through a histamine drop and inserted into the epidermal surface at a low angle with the bevel facing up. The needle tip was then gently lifted upward to elevate a small portion of the epidermis without inducing bleeding. The needle is then withdrawn and the solution gently wiped away with a paper tissue approximately 1 minute later. A separate needle must have been used for each side to avoid mixing of solutions. Ten min interval needed to see the maximal responses by these histamine phosphate. The wheal and flare was traced and transferred to paper with transparent tape. Wheal-and-flare areas were measured by in-house developed software (by Thirasak Borisuthibandit, MD.). The W-F areas were filled with 16-bit gray color by the Proimage® programme. Then, pictures were saved in scale 300x300 dot per inch (DPI). Next, we entered these color-filled graphics to the particular software where they were replaced with alphabets (one pixel per one alphabet). Software would count exact number of alphabets in the appointed area. Eventually, according to known DPI, these pixel numbers were converted to the area in square millimeters (mm^2).

Endpoints

Owing to objectives of this study, the end points were widely divided into two categories. (1) Total NAR for ROC curve analysis; (2) Comparative efficacy and adverse effects of three non-sedating antihistamines and a placebo in patients with allergic rhinitis. However, the efficacy was further detailed in chiefly two ways, subjective and objective study. Subjective study denoted individual symptom score and total symptoms score. Objective way to evaluate the efficacy was attributed by W-F area, NAR, and secretion weight. To produce comprehensive, but systematic

study, the results at each time point would be analyzed in two-axis comparison (with placebo, and with other drugs).

There were three spots of baseline measurements in this study. $Baseline_0$ was the outcome prior to diluent challenge. $Baseline_1$ meant the outcome produced by diluent. $Baseline_2$ was the variable after challenge completed but before taking studied drug.

The studies involving symptoms score were stressed on the onset-of-action and in regard of "which drug does it improve the parameters better"? The latter would better expressed in terms of relative efficacy. It was defined as the number of time points at which 'no symptom' was achieved, divided by the total number of time points evaluated.

Treatment-emergent adverse events was the events noted during the double-blind treatment period but not during the baseline period). These adverse experiences were then handled to find the dissent among studied drugs and against placebo.

The secretion weights and W-F area were corrected by their individual $baseline_2$ (before taking medications) before analysis. The corrected values were presented in form of percent weight reduction.

Total NAR was similar to other parameters accounting for study the efficacy of drugs. Total NAR, as mentioned above, it was compared in two-axis design, as well. Notably, in stead of flow or unilateral NAR, the total NAR was engaged in actual analysis.

The input for ROC curve analysis was responsible by total NAR or total flow of the patients and controls resulting from each concentration challenged. Subsequently, investigators chose those NARs either of single concentration or multiple concentrations of allergen to create ways to approach ROC curve analysis. To ease the use of NAR in clinical practice, the NAR at each concentration challenged was divided by NAR produced by diluent (i.e., times over $baseline_1$). This final quotient was further applied to calculation.

Statistical analysis

Statistical analysis for comparative study

Basically, the symptoms score needed to use non-parametric statistical test. In case of comparison either two unrelated groups or three and more groups of treatment. the author would employ Kruskal-Wallis test. Whereas two related groups of treatment (suchas against its own baseline) would be calculated by Wilcoxon matched pairs signed rank test. The parametric statistical tests commonly used were student t-test (paired or unpaired) and oneway analysis of variance (ANOVA) with post *hoc* scheffe.

Statistical analysis for ROC curve analysis

The value for the *area under the ROC curve* interpreted as follows: an area of 0.84, for example, meant that a randomly selected individual from the positive group had a test value larger than that for a randomly chosen individual from the negative group in 84% of the time [8]. When the variable under study could not distinguish between the two groups, i.e. where there was no difference between the two distributions, the area would be equal to 0.5 (the ROC curve would coincide with the diagonal). When there was a perfect separation of the values of the two groups, i.e. no overlapping of the distributions, the area under the ROC curve was 1 (the ROC curve Reached the upper left corner of the plot). The *95% confidence interval* for the area could be used to test the hypothesis that the theoretical area was 0.5. If the confidence

interval did not include the 0.5 value, then there was evidence that the laboratory test was able to distinguish between the two groups [8;9].

Statistical analysis for association study

Wheal induced by 1 and 10 mg/ml (W1and W10 respectively) was separately calculated throughout this analysis. Time of onset for parameters i.e., TNSS, individual symptoms score, NAR, and secretion weight were examined in individual subjects. They all were appointed to be the dependent variables, and onset of wheal suppression were an explanatory variable. They were then tested in pairwise for the correlation.

Efficacy of test drugs was generally tied up with percent of wheal suppression as well as percent suppression of secretion weight and nasal airway resistance. Whilst symptom scores were not converted to percent change alike (they were treated as ordinal scale), but subtracted with baseline score to produce differential score. Then, percent of wheal suppression was matched with each parameter-of-interest to calculate simple correlation coefficient. When parameter-of-interest belonged to ordinal scale (i.e., symptoms score), Spearman's rank order coefficient was applied. Subgroup analysis was looking for correlation between percent wheal suppression and target parameters within group of similar treatment. Furthermore, variable effects from both time course after treatment and active treatments were tested with multiple regression.

The statistical software used for these analysis was intercooled STATA 5.0 for Windows 95 (STATA corporation, Texas, USA). The software to perform ROC curve analysis was MedCalc version 6.14 for Windows (MedCalc Software, Mariakerke, Belgium). Statistical significance was defined for all test at $P< 0.05$. All comparisons were based on two-sided tests.

RESULT

Patients

Among 30 normal volunteers, there were 9 subjects (30.0%) whose the history resembled to the non-allergic rhinitis. These subjects were also counted as control subjects. Of 46 atopic patients screened, one of them administered prednisolone for a month before testing. As a result, the total 45 patients who had been challenged were included as patients in ROC curve analysis. Of them, 31 (68.8%) met the criteria for positive nasal allergen challenge testing, fourteen (31.2%) allocated to negative challenge. Negative challenge patients remained to be followed (intention-to-treat analysis) for ROC curve analysis. Their data (N=14) were pooled towards atopic group. The patient and control demographics were presented in table 2. Among those who had positive nasal challenge were randomly given a single dose of placebo (n=7). 10-mg loratadine (n=8), 10-mg cetirizine (n=8), or 60-mg fexofenadine (n=8). They all continued till the end of study and none dropped out or violated the protocol. Demographic data for these 31 patients were shown in table R1.

ROC curve analysis for NAR (cut-off level for the NAR)

Congestion score, total NSS, and total NAR of the patients shown no significant difference before and during diluent challenge (Table 2). This possibly confirmed the purity of the diluent. Although, TNSS at first of the patients were slightly higher than controls but did not reach statistically significance (0.8 ± 1.1 vs 0.52 ± 1.2). The congestion score was comparable in the two groups during $baseline_1$ period. Later, the allergic rhinitis patients had significantly higher congestion score after completed. For itching score even at $baseline_0$, patients were significantly more itchy than controls (1.81 ± 0.83 vs 0.5 ± 0.81, $p < 0.05$). Sneezing count score was absolutely zero for both groups at $baseline_1$ and patients finally had higher score after challenge (0.03 ± 0.19, vs 3.5 ± 3.69 $p < 0.05$). Secretion score at $baseline_1$ found insignificant in both patients and controls, but it was increased by nasal challenge in the former (1.94+0.92 vs 0.42+0.80, $p < 0.05$).

Like TNSS and congestion score, Total NAR at $baseline_2$ could distinguish most patients from normal subjects. Total NAR in patients mounted from 0.27+0.36 to 1.4+2.95 Pa/ml/sec. Yet, almost unchanged NAR was noted in controls (0.31+0.22 to 0.31+0.17 Pa/ml/sec).

The impurity of diluent was negligible by considering TNSS changes before and during diluent challenge. In control group, TNSS hardly changed (0.70 ± 1.1 vs 0.70 ± 1.4). TNSS increased from 0.8 ± 1.1 to 1.4 ± 2.3 in patient group ($P>0.05$). The evidence by total NAR and congestion score supported the same trend.

ROC curve report

To find the most proper cut-off value of NAR in practical application, the investigators carefully included the concentration in analysis the ROC curve (Table R4). By the way, the way to approach this analysis always realized the sequential placement of five-concentration allergenic extract in the noses.

The maximum diagnostic value at the discriminator position on ROC curve was 1.57 (84.4% sensitivity, 73.3% specificity) with 1,000 AU/ml dose. At that dose,

the NAR cut-off value was >1.1923 times (round to 1.2 times) over baseline produced by diluent. The maximum diagnostic value at the discriminator position on ROC curve was 1.68 (68.9% sensitivity, 100% specificity) with 5,000 AU/ml dose. At that dose, the NAR cut-off value was >1.5556 times (round to 1.5 times) over baseline prooduced by diluent. Combine of many concentrations did not yield higher accuracy at all. Of these, 1,000 accompanied with 5,000AU/ml represented the summit of the rest with 84.4% sensitivity, 73.5% specificity under cut-off level >1.2 times over baseline. It was noteworthy that inclusion entire of concentrations to analyze provided quite low sensitivity, specificity of the test, and despite low AUCROC.

In addition to sensitivity, specificity, and AUCROC, the likelihood ratio was used to judge as well. The criteria >1.5 times at 5,000 AU/ml dose presented with negative LR was 0.33 and positive LR was 23.2. As the criteria elicited at 1,000 AU/ml had postive LR=3.18 and negative LR=0.22.

There were a number of patients presented with complete obstruction resulting in unmeasurable data as follows : 2 subjects at 100 AU/ml, 1 subject at 500 AU/ml, 8 subjects at 1,000 AU/ml, and 7 subjects at 5,000 AU/ml. Nonetheless, extrapolation method caused these data applicable.

The rising pattern of NAR in patient group fitted to the dose-dependent fashion (Fig. R1). One patient had exaggerated NAR at concentration 1,000 and 5,000 AU/ml because of above-mentioned extrapolation. At 1,000AU/ml, for example, his NAR rose from 4.04 to 14.28 Pa/ml/sec. Absolutely, this enormously affected the curve prediction. Other spots of extrapolation were also tested to prevent falsely fitted curvature. But no significant impact was found. Merely his data at these concentrations were thereby excluded from plotting the curve. The NARs of the patients from 500 to 5,000AU/ml statistically differed from the controls (Table R2).

There was only one non-diseased subject (3.3%) had shown escalating NAR up to 1.5 times of baseline. None of them had NAR higher than the proposed cut-off level at 5,000 AU/ml.

Flow analysis

Contradictorily, there was a significant difference of sum of the flow (left and right) before (378.4±162.9 ml/min) and after (346.0±141.5 ml/min) the diluent challenge underlying the flow analysis in patient group (P =0.023). Result in control group could not witness this finding. The figure R2 shown the flow ran downhill at the concentration of 50, 100, 500, 1,000, and especially 5,000AU/ml. The statistical differences between patients and controls were noticed at the concentration 1,000 and 5,000AU/ml (P<0.05).

Cut-off level for the symptoms score and flow

The symptoms score during 5-concentration allergen challenge displayed remarkably silence in non-allergic cases. ROC curve analysis for all studies were best predicted at 5,000-AU/ml concentration (Table R6 to R10). The cut-off level for sneezing was > 0 sneeze at 5,000-AU/ml concentration (sensitivity = 68.9% , specificity = 96.3% ,+LR =18.60, -LR=0.32). The cut-off level for itching score was > 0 at 5,000-AU/ml concentration (sensitivity = 97.8%, specificity = 70.4%, +LR =3.30, -LR=0.03). The cut-off value for rhinorrhea score was > 0 at 5,000-AU/ml concentration (sensitivity = 93.3% sensitivity, specificity = 74.1%, +LR =3.60, -LR=0.09). And the congestion score >0 complained by subjects at 5,000-AU/ml allergen challenge gave 93.3% sensitivity, and 70.4% specificity, 3.15 positive LR and 0.09 negative LR. In case of total symptoms score (TNSS), the cut-off level for

positivity of the nasal allergen challenge was >4 (sensitivity = 82.6%, specificity = 85.7%, +LR = 5.78, -LR = 0.2). The AUCROC of the symptoms score with SE and 95% confidence interval were present in table R6 to R10.

ROC curve analysis for the flow was best predicted at 5,000-AU/ml concentration as previously. The most suitable criteria for the reduction of sum of the flow (left and right) was ≤140 ml/min (46.7% sensitivity, 92.9% specificity, 6.53 positive LR, 0.57 negative LR). At this level, the AUCROC appeared to be 0.704 (SE=0.065, 95%CI=0.586-0.805).

Sensitivity Analysis of ROC curve for NAR

Under investigation in depth, there were fourteen patients (31.2%) who did not give rise to the NAR as much as >1.5 times over baseline (at 5,000-AU/ml single point). They all were incorporated to analysis compared to the 31 remainders. Three parameters of interest presented with *p*-value in table R5. In low-sensitive polarity, they had lower TNSS, but it did not reach the statistical significance. Whereas the skin prick reaction was not obviously insignificant. The patients whose nasal allergen challenge with helpless rhinomanometry had shorter average diameter. The high-sensitive group significantly preferred 5,000:1,000 AU/ml NAR ratio 1 or more to the other. The scattergram in figure R5 clearly depicted that most of insensitive members resided below the line of 1. Besides, the author searched for possible parameters, such as asymmetrical baseline and rising pattern of 14 individuals. There was no further discrepancy in these two groups. Patients with high $baseline_1$ was found 5 (35.7%) and 13 (41.9%) in insensitive and sensitive group respectively (P>0.05). Also, the former had 8 patients (57.1%) whose NAR unilaterally elevated. The latter had 13 (41.9%) whose NAR increased in unilateral manner (P>0.05).

Efficacy of non-sedating antihistamines in clinical suppression.

There was no significant difference of demographic parameters among patients who took placebo, loratadine, fexofenadine, and cetirizine (Table R1). After challenge done, the record shown equal $baseline_2$ again both NAR and TNSS. The secretion weight and wheal-and-flare area at the time just before taking medication had no statistical difference either. The secretion weight at $baseline_2$ was 1.49±1.5, 2.42±1.8, 2.28±2.1, 2.07±1.1 gm for placebo, loratadine, fexofenadine, and cetirizine respectively. The wheal area was 7.83±6.1, 5.90±2.2, 5.12±1.7, 4.70±1.9 mm^2 for placebo, loratadine, fexofenadine, and cetirizine respectively. The flare area was 47.7±49.9, 64.6±31.1, 60.3±43.5, 48.7±41.1 mm^2 for placebo, loratadine, fexofenadine, and cetirizine respectively.

Impact of the agents on individual score was present in table 3.1 to 3.4 which were emphasized on minutes of statistical significance. Whilst, effect on TNSS was illustrated in table 4. The patterns of TNSS response of every drug was shown in figure 1.1 to 1.4.

- For sneezing score, loratadine group was noted different from placebo group at 240 min, so was cetirizine group. Fexofenadine group started to differ from placebo group since 180 min.
- Statistical difference from their baseline was seen in patients with loratadine since 60 min, followed by since 90 min in patients with fexofenadine and cetirizine.
- The statistical difference on sneezing score was not observed when the studied agents were compared to one another.

- Compared with placebo, fexofenadine group showed improvement on itching score since 150 min. Loratadine group achieved it since 180 min, and followed by cetirizine group since 210 min.
- Regard to their baseline, loratadine- and cetirizine-treated group showed significantly improvement since 120 min, and in fexofenadine-treated patients exerted that since 150 min.
- The statistical difference on itching score was not observed when the studied agents were compared to one another.

- For secretion score, patients with cetirizine were seen different from placebo group since 120 min. Fexofenadine group started to differ from placebo group since 210 min. The loratadine-treated group attained lately at 240 min.
- Statistical difference from their baseline was noticed in patients with cetirizine since 120 min. It was pursued by patients with fexofenadine which showed that since 150 min; patients with loratadine did not produce an effect before 240 min.
- Placebo-treated group also had secretion score reduced significantly since 210 min.
- The statistical difference on secretion score was observed when the studied agents were compared to one another. Cetirizine group was superior to loratadine group at 120-150 min, and to fexofenadine group at 120-180 min.

- Compared with placebo, cetirizine group showed improvement on congestion score only single time point at 150 min, while fexofenadine demonstrated that improvement only at 180 min.
- In relation to their baseline, congestion score decreased significantly in cetirizine-treated group since 120 min, in fexofenadine-treated group since 150 min, and lastly in loratadine-treated group at 240 min.
- Placebo-treated group also displayed markedly improvement since 210 min.
- The statistical difference on congestion score was observed among studied agents. Cetirizine group was superior to loratadine group at 150 min.

- In grand picture of TNSS, compared with placebo, cetirizine group showed improvement since 150 min, while fexofenadine demonstrated that improvement since 150 min. Thirty minutes later, loratadine suppressed TNSS over placebo group.
- In relation to their baseline, TNSS decreased significantly in cetirizine- and fexofenadine-treated group since 90 min (probably 30 min in the former), and lately in loratadine-treated group at 120 min.
- Placebo-treated group also displayed markedly improvement since 240 min.
- The statistical difference on TNSS was observed among studied agents. Cetirizine group was superior to loratadine group at 120-150 min, and fexofenadine to loratadine group at 90 min.

The relative efficacy for sneezing score was 31.1±4.1%, 45.2±3.3%, 73.2±1.2%, and 42.2±4.3% for placebo, loratadine, fexofenadine, and cetirizine respectively (Table 5). The relative efficacy for itching score was 11.2±2.2%, 23±2.3%, 43±3.5%, and 34±3.3% for placebo, loratadine, fexofenadine, and cetirizine respectively (Table 5). The relative efficacy for secretion score was 11±2.3%,

17±2.4%, 31±3.6%, and 34±4.4% for placebo, loratadine, fexofenadine, and cetirizine respectively (Table 5). The relative efficacy for TNSS was 11.5±13.8%, 16.7±10.2%, 36.9±19.7%, 37.1±23.5% for placebo, loratadine, fexofenadine, and cetirizine respectively (Table 5).

The fexofenadine exhibited higher relative efficacy in aspect of sneezing and itching score. Whereas cetirizine superiorly provided improvement in secretion score. All active treatment groups evidenced greater relative efficacy on TNSS than placebo group. No statistical difference in relative efficacy of TNSS was observed among studied agents.

Efficacy of non-sedating antihistamines on secretion weight

Percent weight reduction was found no significant difference among patients treated with medications, including placebo at any time pionts (Fig. 3). Respect to their $baseline_2$, all medications and even placebo showed statistical differences at certain time points (Table 6). The effect of loratadine on secretion weight reduction appeared to be delayed around 240 min. Fexofenadine could lighten secretion weight by beginning at 60 min. Cetirizine had dramatically secretion weight reduced since 30 min with the gap at 60 min and then became positively reduced again. The weight was also lessened at 60- and 180-min time point for placebo.

Efficacy of non-sedating antihistamines on congestion

The NARs prior to taking medications demonstrated equaled baseline. They revealed no significance when oneway ANOVA was applied to test discrepancy among various antihistamines against placebo. This was true in despite of individual baseline were brought to adjust before calculation. Total NAR was also tested versus the $baseline_2$, and all agents exhibited null effect on NAR (Fig. 4).

Efficacy of nonsedating antihistamines on suppression Wheal-and-flare area

Placebo was able to suppress W-F area incited by histamine of any neither minutes nor concentrations (Table 8 to 11)

Loratadine was noted to suppress W-F area produced by both concentrations at later minutes of evaluation (at 240 min for the most). The percent wheal reduction was invisible in W10 (against placebo and $baseline_2$). The inconsistency of suppression pattern was clearly seen in loratadine-treated group.

Fexofenadine and cetirizine, on the other hand, had relatively early onset of W-F suppression. They obviously shown undulating pattern of prediction either. Also, there were gaps of statistical insignificance (and/or almost significance) existing among those significant time-points. Therefore, the exact time of onset was difficult to define. Although, 90-min time point was seemingly preferred by both agents. As early as 30 min, the suppression could be seen. Even though, the time of onset was occasionally as late as 120 min.

Table 12 illustrated the percentage of individuals who experienced greater than 95% inhibition of wheal (12A) and flare (12B) surface areas at some time point during the day of observation. Seven and six out of 8 subjects had almost complete suppression of W1 and W10 respectively after administration of cetirizine. Also, they were significantly different from loratadine and placebo group. The responses to other antihistamines were quite less, with just 6 (for W1) and 3 (for W10) of the subjects having 95% suppession of wheal area after fexofenadine therapy. Only 95% suppession of W1 in fexofenadine group was superior to loratadine and placebo group. The loratadine-treated patients had one out of 8 (for W1) and zero out of 8 (for

W10) subjects having 95% suppression of wheal area. None in the placebo group reached 95% wheal suppression.

Inhibition of the flare response to histamine challenge was somewhat greater than that of whealing for most treatments except in cetirizine group. However, all subjects by F10 had more than 95% inhibition at one time after cetirizine. This response was followed by a gradual decline in numbers experiencing near complete suppression of flare in those who took fexofenadine and loratadine. None in the placebo group had 95% flare suppression.

Adverse experiences of non-sedating antihistamines

Study medications were well tolerated. No patient stopped treatment because of side effects or intercurrent illness. Treatment-emergent adverse events (i.e., events noted during the double-blind treatment period but not during the baseline period), were reported in table 13. The most common side effects in each group were somnolence for loratadine (70%) and fexofenadine (70%), somnolence (41.7%) and fatigue (41.7%) for cetirizine, and headache (42.9%), somnolence (42.9%), and dizziness (42.9%) for placebo. No statistical difference, however, were detected among groups in this study.

The incidence of treatment-related side effects was similar among groups, with headache reported most commonly in each group. Headache occurred in 27% of patients receiving cetirizine, 33% of patients receiving loratadine, and 28% of patients receiving placebo. Fatigue occurred in 3% of patients receiving cetirizine, 1.5% of patients receiving loratadine, and 0% of patients receiving placebo. Other events occurring in 2% or more of patients included abdominal pain in 4% of patients receiving placebo; back pain, dyspepsia, and migraine, each occurring in 3% of the placebo group; and chest pain and fever, each occurring in 3% of the loratadine group.

Association the time of onset of parameters and wheal suppression

According to TNSS, three active agents caused statitiscal significance from placebo group at 120, 150, 180 min for cetirizine, fexofenadine, and loratadine respectively. Three agents suppressed W1 more effective than placebo since 90 min for both cetirizine and fexofenadine, and since 120 min for loratadine. Three agents suppressed W10 more effective than placebo since 90, 120, 180 min for cetirizine, fexofenadine, and loratadine respectively. The correlation coefficient for W1 was 0.86 ($p > 0.05$). The correlation coefficient for W10 was 0.98 ($p > 0.05$).

The median time of onset for W1 was 60 (range 30-150), and for W10 was 30 (range 30-210). There was no correlation between independent and any dependent variables. This was seen in both concentrations of histamine (W1 and W10).

Associations of efficacy of wheal suppression and parameters

By both W1 and W10, the results failed to discover significantly correlation between wheal suppression and parameters-of-interest. (Table C1) Moreover, subgroup analysis showed no significant correlation in all parameters.

By multiple regression revealed that time course of treatment and any given drugs had no effect on prediction of wheal (W1 and W10) suppression towards TNSS reduction (R=0.30, F-ratio=0.66, P=0.62 for W1 , R=0.39, F-ratio=1.17, P=0.34 for W10). This was similarly found when statistic tool was engaged in analysis other parameters-of-interest. Hourly W1 and W10 percent suppression was negligibly correlated with any particular parameters. Under multiple regression analysis regarding arms of treatment, multiple correlation coefficient (R) between W1 and

TNSS for various groups of intervention was 0.16 (F-ratio=0.19, p=0.94). Of those W10 and TNSS was 0.16 (F-ratio=0.19, p=0.94). When wheal (W1 and W10) suppression underwent testing with other target parameters, there was also no statistical significant *R* among them.

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Discussion for ROC curve analysis

The diagnostic value of measurement of changes in nasal airway resistance in nasal provocation tests has been reported. [1] Although, the total NAR before and after diluent challenge did not change importantly. The investigators remained grasp the total NAR produced by diluent as an actual baseline. Normally, the rhinomanometric researches must have discarded certain data due to zero denominator. NAR obtained by pressure gradient divided by flow [2]. When complete obstruction encountered, the NAR was ensuingly unmeasurable. Even though extrapolation seemed to give the highly exaggerated and irrelevant results. This was shown by presence of wide standard deviation of total NAR when dose antigen challenge was increased. But the outcome of treatment (challenge) would not be eliminated or given way to 'uninterpretable data'. Besides, an irrelevance of extrapolation was thoroughly realized during construct the dose-response plot. As reported by other sources, the nasal response to allergen challenge in allergic rhinitis patients corresponded with dose-dependent manner.[3;4]

It was difficult to precisely compare our cut-off level for positivity with other rhinomanometric works. Hytonen recommended in his study an increase of > 50% (1.5 times) in NAR to regard the result as positive in provocation test.[5] Measured with passive anterior rhinomanometry after nasal challenge, atopic patients appeared to have an 100% (2 times) increase in NAR in 94% of patient group.[6] Shusterman challenged seasonal allergic rhinitis with chlorine; he proposed an increase of 21% (1.2 times) in NAR.[7] Patients suffering from perennial allergic rhinitis were provoked with histamine noticed an 3-fold increase in NAR as the most suitable criterion.[8] Only fifty-five percent of 106 Der p-allergic children had doubled total NAR over baseline following dust mite challenge. [9] 10 perennial allergic rhinitis children who underwent consecutively histamine and Der p challenge had doubled NAR in 80% of histamine- and 90% of Der p- challenged subjects. [10] A study in out-of-season seasonal allergic rhinitis patients under well-designed for ROC curve analysis pointed to 2.7 times over baseline as a cut-off level. [4]

The cut-off level for positivity in present analysis (>1.2 times at 1,000 AU/ml and >1.5 times at 5,000 AU/ml) appeared to be distinct from some of above-mentioned references. This was caused in part by (1) Specific antigen to challenge, rather than non-specific stimuli like histamine. (2) Different concentrations to challenge. (3) Innate structural narrowing of nasal fossa in orientals. [11;12] (4) Perennial nature of patients (priming effect). The seasonal allergic rhinitis was able to be tested out of season. (5) Age group. (6) Design and methodology. For instance, most of referable studies did not employ ROC curve for analysis.

It was known that not every single patient with allergic rhinitis would have elevated NAR after artificial challenge. In this study, there were 14 patients (31.1%) defined as non-responsive by the chosen cut-off point. Their inactive response brought about low sensitivity of the test. Two variables of interest in the sensitivity analysis were distinct, skin prick reaction and 5,000/1,000 AU/ml ratio of total NAR. The association of skin reaction and nasal reactivity was well described in one study [13] Patients with poor skin reactivity inclined to have less nasal reactivity, either. There was also an evidence indicated that nasal responsiveness correlated well with specific IgE level. [3] Five of non-responders showed decline in magnitude of response at 5,000 AU/ml in relation to 1,000 AU/ml. The mast cell degranulation might be exhausted by researcher's unremittent challenge. Histologically, the mast cell number progressively increased during 2-12 hours after challenge, but histamine

content paradoxically decreased at 2-4 hours after challenge.[14] Final TNSS and other parameters of interest in responders and non-responders were comparable by statistical viewpoint.

While 5,000 AU/ml exerted very high specificity, its low sensitivity of the test (68.2%) led to doubt in screening purpose. A-thousand allergen dose was probably more suitable than five-thousand one in this respect. In addition, total NAR of patient group was detected significantly different from the control since 500 AU/ml. This was correspondent with a study which only few patients required 1,000 AU/ml dose of house dust mite; it was extremely true for whose skin reactivity was strong. [13]. Also, less irritation and less time consuming were its adjunctive advantages. When the high specificity of the test was required, however, test could be extendable. Amidst divers diagnostic tools, the place of nasal allergen challenge with rhinomanometry remained to be defined. An comparative multi-ROC curve analysis was subject to accomplish this quest. [15]

Flow analysis showed trivial arguments onto NAR analysis. These included (i) clearly reduction of the flow by diluent in patient group, and (ii) 1,000 rather than 500 AU/ml which could discriminate patients from controls. As stated above, equation of NAR, the resistance was derived from pressure gradient divided by flow. This rationale was far more prudential in this case. Pressure gradient proximal and distal to obstructed zone was included to consider. Since, even in an individual, the effort to breath was sometimes not steady.

A drawback of our research was lacking of nasal cycle study prerequisite to challenge. Hence, fluctuation of NAR might give us falsely positive result. However, nasal cycle study had to take plenty of time (3-4 hrs) which was unbearable to succeed in genuine situation. Secretion weight concomitantly kept during challenge could possibly be an alternative.[16;17]

Conclusion

Nasal provocation test together with active anterior rhinomanometry under well-designed for ROC curve analysis showed that an increase of total NAR >1.2 times over baseline after challenge with dust mite dose 1,000 AU/ml appeared to be more sensitive (81.2%). On the other hand, an elevation of NAR >1.5 times over baseline following challenge with 5,000 AU/ml antigen dose provided 100 % specificity of the test. It was supposed to be an adjunct diagnostic tool for allergic rhinitis other than specific-IgE-dependent test.

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House dust mites are the most important aeroallergen sensitizers among allergic rhinitis patients in Thailand. [1] First- and second-generation antihistamines were widely dispensed over-the-counter, and commonly prescribed by physicians. Although, there were studies described the efficacy of some antihistamines in Thai patients. [2;3] There was no study which extensively compared the efficacy and tolerability of commonly prescribed agents by both subjective and objective measures under allergen challenge model among Thai patients. To create well-calibrated mileau, all patients were subject to nasal allergen challenge. Unfortunately, the effects of a series of nasal challenge subsided within 15 minutes. Hence, the continuous challenge was necessary to carry on throughout the study.

Time point which active drugs reduced TNSS to a greater extent than placebo was counted as time-of-onset. In present study, trend of onset among drugs was closed to recent reports in despite of not exactly similar in minutes. [4-6] The rank of onset appeared to be cetirizine(120 min), fexofenadine(150 min), and loratadine(180 min). This was contradicted by Horak who found that cetirizine and fexofenadine had a comparable onset of action in alleviating the nasal symptoms. [7]

Generally, tested antihistamines possessed distinctive efficacy on individual nasal symptoms score and TNSS over placebo within 240 minutes. Cetirizine was superior to the rest of studied antihistamines in aspect of secretion score (to both), congestion score (to loratadine alone), and TNSS (to loratadine alone). [4-6] Notably, loratadine group did not significantly affect congestion score regarding to placebo group. A paper with continuous challenge model discovered the similar result for loratadine. [8] Fexofenadine was able to suppress TNSS more than loratadine. None reported comparative efficacy of loratadine versus fexofenadine in an acute phase of allergen challenge. On the other hand, van Steekelenburg did not see any differences in efficacy among these antihistamines at 4 hours after intake. [9]

Although all antihistamines verified a benefit at certain time points, the relative efficacy (definite relief ratio) of these antihistamines on individual nasal symptom score was variably detected. Fexofenadine had greater relative efficacy than placebo on sneezine and itching score (i.e., symptoms of nerve endings irritation). The relative efficacy of cetirizine on secretion score was more excellent than placebo. Nonetheless, all agents contained higher relative efficacy on total nasal symptoms score than placebo. On the contrary, this study did not reveal statistical differences of relative efficacy among treated groups as stated in recent alike model. [4]

Incidentally, an elevation of NAR in first 30 min after challenge (in fexofenadine group) was occasionally seen. [10] There were an injection of NAR and TNSS in each group of cetirizine and loratadine during 150 to 240 min. This maybe caused by late-phase nasal responses. It would better discuss this agenda along with the effect of study drugs on NAR. Although all three agents showed significantly reduced inflammatory cells and mediators, including ICAM-1 expression. [11;12] The importance of this antiallergic activity of the H_1-receptor antagonists in contributing to their overall clinical efficacy has not known. [13] Effects of second-generation antihistamines on NAR was mixing and published few negative reports.

- Frossard noted significantly NAR reduction: cetirizine > loratadine > placebo after histamine challenge. [14]

- No differences of allergen concentration to induce nasal obstruction among various antihistamines, but they all could shift reaction threshold induced by pollen compared with placebo. [9]

- Under continuous challenge, Horak did not see loratadine have an affect on NAR. [8]

- Frossard also observed no changes in NAR at 24 hours after a single dose of loratadine-treated group, but of cetirizine-treated patients. [15]

- Fexofenadine subjectively reduced nasal congestion in chronic therapy, but this study was not under nasal challenge trial. [16]

- Wang revealed cetirizine significantly attenuated the histamine-induced increase in nasal airway resistance by nearly 50% . [17]

Our study found a null effect of different antihistamines on NAR. Apparently, this was not true with congestion score. Since patients themselves might sense decongestant effect from either the result of antihistamines on secretion or the placebo effect.

The finding that loratadine lacked significant symptomatic effects compared with placebo differed from many other studies of this agent in patients with seasonal allergic rhinitis. [18-21] Most of these studies extended over a period of many weeks, and it was possible that the full effects of loratadine had not yet emerged in the 4-hour study period.

Astonishingly, percent change in secretion weight was not significantly different among treatments in present study. The trendline shown in figure.... was somewhat steady in placebo group. Trendline in active treatments showed separately reduction at 4-hour time point. but statistical significance did not appear. Persistent follow-up beyond four hours might be required to uncover the significance. This was also observed in a study. [9] Baroody did not find secretion weight different among loratadine versus terfenadine. But there was no placebo control in that study. [22]

Adverse events found in our experiment was exclusively high. especially somnolence (over 40% in all groups). Other study reported fewer than 5%. [4] Some other reported higher but not beyond 33% with predominately headache side effect. [5;6] Somnolence and fatigue which greatly experienced in this study was likely resulted of continuous challenge with excessively high in allergen concentration.

There were certain limitations in our study model worth of mentioning. This continuous model of challenge with high allergen doses was actually not tolerable by patients in their real life. They are supposed to escape from that site. In this experiment model, patients were informally asked by investigator concerning placing allergen discs into their noses. Most of them complained of hugely stinging and burning sensation. In addition, it must be emphasized that this is the study of a single-dose antihistamine in an acute phase of nasal reactivity.

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Discussion for W-F suppression

The author avoided comparing an exact time of onset with other studies. Since concentration of histamine and procedure (such as intradermal or prick test) were variable. Time point which active drug first produced a greater extent of suppression than placebo was regarded as an onset. From this study, cetirizine and fexofenadine had an onset at 90 min. Loratadine exhibited an onset at 120 min (based on histamine 1 mg/ml) and at 180 min (based on histamine 10 mg/ml). Loratadine was able to suppress wheal and flare responses earlier.

It was ubiquitously mentioned the onset-of-action and the efficacy of W-F suppression by following rank: cetirizine→fexofenadine→loratadine→placebo. [1-5]

Unfortunately, only wheal suppression in present study obeyed those reports. Flare response yielded a little different result. Flare was resulted from vasodilatation. The margin in colored-skin patients was difficult to define precisely. Although most studies showed that flare inhibition supported the same trend of wheal suppression. Some of them excluded flare response from analysis.

Moreover, in this study, fexofenadine had equal onset of suppression with cetirizine. This was noticed in both concentrations of pricked histamine. This probably caused by short period of observation. Purohit followed this throughout 24 hours, there was no statistical difference between the two for first 4 hours. Four to 24 hours postdose was labeled as maximal inhibitory phase for both cetirizine and fexofenadine at which cetirizine showed distinctively more powerful. [6]

Loratadine had more delayed onset of action and lower potency of suppression than the other two in order that the statistical difference was detected during 120 to 240 min of wheal responses incited by 10 mg/ml histamine. The delayed onset might explain why loratadine differed from cetirizine and fexofenadine according to flare responses incited by 10 mg/ml histamine during 120-150 min. Absolutely, it did not explain why significant difference was seen throughout 120-240 min of inhibiting wheal responses incited by 10 mg/ml histamine. Of that interval, loratadine should have reached an onset regard to placebo. Thereby, low potency of loratadine was supposed to be an underlying reason. Our observations were also closed to findings of many researchers [3;5;7;8] Possibly, another controversy originated from Simons's finding that loratadine had exhibited a peak inhibitory effect lately (4 hours) compared with other agents (1-2 hours). [4] This study covered only four-hour period.

Moreover, the extent of suppression was analyzed in detail. Percent of greater than 95% wheal suppression was exceptionally low in loratadine recipients during 4-hour period. It was consistent with Grant's report, although his research ran over 24 hours. That study, however, did not attach clearly statistic results within. [2] Paradoxically, a recent paper indicated that no significant difference on wheal suppression was found among various non-sedating antihistamines, including loratadine. [9]

Loratadine was frequently guarded as the least potent inhibitor and slowest action. [2;10] Thus, newer antihistamines was commonly and logically compared the efficacy and onset with 10-mg loratadine.

Rihoux reported occasional failure of 10-mg loratadine in inhibiting histamine-induced cutaneous reaction. It was stated that cetirizine, 2.5 mg, was as potent as loratadine, 10 mg in inhibiting the histamine skin reactivity. [11]

By an interesting model, Ramboer studied ED50 for wheal suppression of both loratadine and cetirizine. ED50 for loratadine ranged from 9.1 to 40 mg, depending on

time after dose. On the other hand, cetirizine required only 1.9-4.7 mg to make a result. [12] Again, this paper reminded us a peak effect of antihistamines at different time points after dose.

Many published papers also reported dose-dependent efficacy of loratadine, and a well tolerability of higher doses loratadine. [13;14] Due to lower potency, the recommended daily dosage of loratadine (i.e., 10 mg qd) should be reappraised.

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Discussion for association and prediction of w-f suppression

Several studies of comparative antihistamines on allergic rhinitis often mentioned time of onset and efficacy of test drugs interchangeably between cutaneous reaction and nasal reaction. This may be justified. Since certain works showed interactions between these two sites [1-4] Indeed, this finding has been long known, and it has likely brought about skin prick testing in allergy field these days.

Kanthawatana reported that markedly increased nasal reactivity to the allergen was observed among those with 4+ skin test positivity. [5] Recently, Simola indicated that a decline in histamine nasal reactivity was associated with a concomitant change in skin and nasal mucosa to specific allergen. Although the correlation of the sizes of weals elicited by the histamine control in skin prick test and nasal response to histamine was poor. In addition, the study demonstrated that milder nasal responses to histamine was associated with lack of reactivity in skin prick tests. [6]

Our data collected between challenge patients versus control subjects to define reaction threshold for nasal airway resistance also supported previously papers (unpublished data).

If it proved true, this will be very helpful in future research. Particularly, prediction between variables can be established. The research of future antihistamines might be based largely on a simple skin test method rather than nasal challenge procedure. Contrarily, it appeared to be false, researchers and readers should be aware of limitation of citing both endpoints interchangeably regarding time-of-onset and efficacy among test drugs.

This study aims to audit associations and predictions, if any, of skin reactivity and nasal responses in sole subjects during comparative efficacy of antihistamines study.

It appeared that this study showed negative findings regarding association of suppression the reaction histamine-induced skin reaction and antigen-challenged nasal reactivity. No association between suppression of independent (i.e., parameter-of-interest, in this study) and dependent (wheal) variables was detected. This included both time-of-onset and efficacy-of-suppression aspects.

One thing, earlier studies which introduced skin test to allergy practice had emphasized on pricking by allergen (not histamine). [1-4] Recently, Simola in his follow-up study found that correlation of the sizes of wheals elicited by the histamine (as positive control in skin prick test) and nasal response to histamine which were done in same subjects of allergic rhinitis was poor. Whereas a decline in histamine nasal reactivity was associated with a concomitant change in skin and nasal mucosa to specific allergen. [6] Therefore, the interaction remained attractive by means of specific antigen approach. However, this study aimed to examine the model which frequently used in evaluating antihistamine previously. It was beyond the scope to discuss more details.

There were an excellent review which enlightened us the validity of histamine-induced skin reaction in antihistamines study.[7] After administration of the antihistamine astemizole for 8 weeks, the histamine-induced wheal and flare reaction was strongly suppressed. [8] With the same pretreatment, however, astemizole was less effective in inhibiting allergen-induced wheal and flare reactions. One interpretation of this finding is that the response after the epicutaneous injection of the usual pharmacologic doses of histamine used in these tests is not fully analogous to the activation of cutaneous IgE-loaded mast cells by an allergen.

Antihistamines can suppress the histamine-induced wheal and flare by blocking the binding of histamine to its receptors on nerves, vascular smooth muscle, endothelium, and mast cells, but they cannot fully suppress the cutaneous responses to the epicutaneous injection of other important mediators, such as eicosanoids, substance P, and calcitonin gene-related peptide (CGRP). A clinical study in which loratadine and cetirizine exhibited the same therapeutic effect after nasal challenge with grass pollens but reacted differently after skin tests with allergen or histamine.[9]

Symptoms score is rather subjective and emotional scale. On the other hand, wheal diameter is objective measurement. The data (was not present) showed that patients treated with placebo had even TNSS improvement along the 4-hour time course, whereas placebo was unable to reduce wheal diameter. This resulted in correlation between them is hard to appear.

Conclusion

There was no correlation between any symptom scores of allergic rhinitis and wheal diameter during suppression with antihistamines. Also, no correlation between objective measurements (NAR & secretion weight) and wheal diameter was seen during treatment with antihistamines. The conclusion covered both time-of-onset and efficacy aspects. It was invalid to link improvement in histamine-induced wheal with relief of nasal symptoms in allergic rhinitis sufferers. Interpretation an effect of any antihistamines on wheal diameter should be separate from nasal symptoms suppression.

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Table 1 Explanation of the nasal symptoms score

Score	Description	Definition
0	Absent	No symptom
1	Mild	Present symptom but not annoying
2	Moderate	Symptom annoying but not interfere normal activity
3	Severe	Symptom interfering normal activity

Sneezing count was 0 for no sneeze, 1 for 1-5 sneezes, 2 for 6-10 sneezes, 3 for 11 and more sneezes {Pipkorn 1982 20 /id}.

Table 2 Demographic data of controls and allergic rhinitis patients[a]

Variables	Controls (n=30)	Patients (n=45)
Mean age in year (range)	28.5 (21-42)	30.2 (22-45)
Male : female	10 : 20	16 : 29
Total NSS $Baseline_0$	0.70±1.4	0.8±1.1
Total NSS $Baseline_1$	0.70±1.1	1.4±2.3
Total NSS $baseline_2$	3.2±0.5	7.1±2.7[b]
Congestion score $baseline_1$	0.41±0.65	0.38±0.73
Congestion score $baseline_2$	0.57±0.94	1.86±0.90[b]
Total NAR before $baseline_0$ (Pa/ml/sec)	0.29±0.13	0.24±0.14
Total NAR $baseline_1$ (Pa/ml/sec)	0.31±0.22	0.27±0.36
Total NAR $baseline_2$ (Pa/ml/sec)	0.31±0.17	1.40±2.95[b]

[a] Figures were expressed in mean±SD except age and gender data.

[b] $p < 0.05$ compared to controls

Table 3 Nasal symptoms score after taking medications

Sneezing Score (3.1)

Agents	Comparison with	Significantly different at (min)	Prevailed by
Loratadine	Placebo	240	Loratadine
Fexofenadine	Placebo	180, 240	Fexofenadine
Cetirizine	Placebo	240	Cetirizine
Loratadine	Fexofenadine	NS	-
Loratadine	Cetirizine	NS	-
Fexofenadine	Cetirizine	NS	-

* NS = not significant

Itching Score (3.2)

Agents	Comparison with	Significantly different at (min)	Prevailed by
Loratadine	Placebo	180,240	L
Fexofenadine	Placebo	150,180,210,240	F
Cetirizine	Placebo	210,240	C
Loratadine	Fexofenadine	NS	-
Loratadine	Cetirizine	NS	-
Fexofenadine	Cetirizine	NS	-

* NS = Not significant

Secretion score (3.3)

Agents	Comparison with	Significantly different at (min)	Prevailed by
Loratadine	Placebo	240	Loratadine
Fexofenadine	Placebo	210,240	Fexofenadine
Cetirizine	Placebo	120,150,180,210,240	Cetirizine
Loratadine	Fexofenadine	NS	-
Loratadine	Cetirizine	120,150	Cetirizine
Fexofenadine	Cetirizine	120,150,180	Cetirizine

* NS = Not significant

Congestion score (3.4)

Agents	Comparison with	Significantly different at (min)	Prevailed by
Loratadine	Placebo	NS	-
Fexofenadine	Placebo	180	Fexofenadine
Cetirizine	Placebo	150	Cetirizine
Loratadine	Fexofenadine	NS	-
Loratadine	Cetirizine	150	Cetirizine
Fexofenadine	Cetirizine	NS	-

* NS = Not significant

Table 4 Total nasal symptoms score

Agents	Comparison with	Significantly different at (min)	Prevailed by
Loratadine	Placebo	180-240	Loratadine
Fexofenadine	Placebo	120-240	Fexofenadine
Cetirizine	Placebo	120-240	Cetirizine
Loratadine	Fexofenadine	90	Fexofenadine
Loratadine	Cetirizine	120-150	Cetirizine
Fexofenadine	Cetirizine	NS	-

Table 5 Relative efficacy

Relative efficacy for sneezing score (5.1)

Agents	Comparison with	*p*-value	Prevailed by
Loratadine	Placebo	0.422	-
Fexofenadine	Placebo	0.009	Fexofenadine
Cetirizine	Placebo	0.552	-
Loratadine	Fexofenadine	0.029	Fexofenadine
Loratadine	Cetirizine	0.885	-
Fexofenadine	Cetirizine	0.066	-

Relative efficacy for itching score (5.2)

Agents	Comparison with	*p*-value	Prevailed by
Loratadine	Placebo	0.280	-
Fexofenadine	Placebo	0.018	Fexofenadine
Cetirizine	Placebo	0.102	-
Loratadine	Fexofenadine	0.141	-
Loratadine	Cetirizine	0.448	-
Fexofenadine	Cetirizine	0.534	-

Relative efficacy for secretion score (5.3)

Agents	Comparison with	*p*-value	Prevailed by
Loratadine	Placebo	0.200	-
Fexofenadine	Placebo	0.088	-
Cetirizine	Placebo	0.045	Cetirizine
Loratadine	Fexofenadine	0.577	-
Loratadine	Cetirizine	0.072	-
Fexofenadine	Cetirizine	0.110	-

Relative efficacy for congestion score (5.4)

Agents	Comparison with	*p*-value	Prevailed by
Loratadine	Placebo	0.561	-
Fexofenadine	Placebo	0.125	-
Cetirizine	Placebo	0.125	-
Loratadine	Fexofenadine	0.294	-
Loratadine	Cetirizine	0.259	-
Fexofenadine	Cetirizine	0.853	-

Relative efficacy for TNSS (5.5)

Agents	Comparison with	*p*-value	Prevailed by
Loratadine	Placebo	0.474	-
Fexofenadine	Placebo	0.026	Fexofenadine
Cetirizine	Placebo	0.039	Cetirizine
Loratadine	Fexofenadine	0.040	Fexofenadine
Loratadine	Cetirizine	0.076	-
Fexofenadine	Cetirizine	0.999	-

Table 6 Secretion weight (gm) after treatment

Drug	0	30	60	90	120	150	180	210	240
Placebo	1.44±1.6	0.55±0.8	0.52±0.9	0.21±0.2	0.47±0.5	0.26±0.3	0.45±1.0	0.59±1.1	0.61±0.7
Loratadine	1.95±1.9	1.45±1.1	1.05±0.9	0.96±0.2	0.54±0.5	0.53±0.3	0.38±1.0	0.41±1.1	0.07±0.7[a]
Fexofenadine	2.28±2.1	1.53±1.6	1.01±1.6[a]	0.95±1.5[a]	0.73±1.1[a]	0.31±0.5[a]	0.46±0.5[a]	0.24±0.4[a]	0.56±1.1[a]
Cetirizine	2.08±1.1	1.24±1.2[a]	1.20±1.7	0.94±1.0[a]	0.96±1.4[a]	0.47±0.8[a]	0.24±0.3[a]	0.37±0.4[a]	0.12±0.2[a]

[a]$p < 0.05$ compared to baseline$_2$ [af](0 Min)

Table 7 Mean±SD of total NAR after taking medications (Pa/ml/sec)

Drug	0	30	60	90	120	150	180	210	240
Placebo	1.96±2.6	0.72±0.7	0.61±0.7	0.42±0.3	0.41±0.3	0.38±0.3	0.63±0.9	0.33±0.2	0.33±0.2
Loratadine	1.16±1.5	0.58±0.6	0.57±0.5	0.47±0.4	0.48±0.3	0.40±0.3	0.34±0.3	0.71±1.0	0.39±.0.2
Fexofenadine	0.81±0.5	1.59±1.6	0.63±0.3	0.48±0.1	0.45±0.1	0.37±0.1	0.38±0.1	0.40±0.2	0.37±0.2
Cetirizine	2.29±3.0	0.70±0.5	0.50±0.3	0.64±0.5	0.57±0.4	0.96±1.5	0.78±1.2	0.62±0.6	0.65±0.5

Table 8 Wheal 1 mg/ml

Drug	0	30	60	90	120	150	180	210	240
Placebo	7.83±6.1	9.27±7.2	10.62±6.3	9.21±5.0	12.09±8.5	10.32±7.3	8.99±5.4	8.13±4.8	9.28±4.4
Loratadine	5.90±2.2	6.89±4.0	5.42±3.1	5.27±3.6	4.32±4.0	$3.33\pm1.4^{a,b}$	$2.92\pm2.3^{a,b}$	$2.80\pm1.9^{a,b}$	$1.95\pm1.2^{a,b}$
Fexofenadine	5.12±2.7	4.18±3.0	4.1 ± 2.7^{a}	$2.30\pm0.9^{a,b}$	$2.30\pm1.1^{a,b}$	$1.82\pm2.4^{a,b}$	$1.34\pm1.4^{a,b}$	$0.83\pm1.6^{a,b}$	$0.46\pm1.1^{a,b}$
Cetirizine	4.70±1.9	4.74±2.2	3.07 ± 1.7^{a}	$1.88\pm1.7^{a,b}$	$1.32\pm1.4^{a,b}$	$1.07\pm1.3^{a,b}$	$0.68\pm1.1^{a,b}$	$0.87\pm2.0^{a,b}$	$0.33\pm0.9^{a,b}$

[a] $p< 0.05$ compared to placebo

[b] $p< 0.05$ compared to baseline (0 Min)

Table 9 Flare 1 mg/ml

Drug	0	30	60	90	120	150	180	210	240
Placebo	47.72±49.9	64.19±50.4	50.24±32.2	53.42±24.3	72.83±39.8	71.30±44.6	55.95±37.5	51.90±28.9	60.89±41.6
Loratadine	64.60±31.1	73.38±41.6	43.14±33.1	42.78±56.7	39.34±45.5	25.86±28.9[b]	31.39±38.3[b]	14.47±22.4[b]	13.92±19.2[a,b]
Fexofenadin	60.35±43.5	38.39±42.4[b]	37.76±26.4[b]	12.08±14.3[a,b]	10.30±12.0[b]	4.24±4.6[a,b]	5.67±7.5[b]	4.03±3.7[b]	2.13±2.0[b]
Cetirizine	48.74±41.1	34.35±26.9	16.95±13.4	5.58±7.3[a,b]	3.02±2.0[b]	3.05±2.2[a,b]	2.51±1.3[b]	2.50±1.6[b]	1.87±1.1[a,b]

[a] $p < 0.05$ compared to placebo

[b] $p < 0.05$ compared to baseline (0 Min)

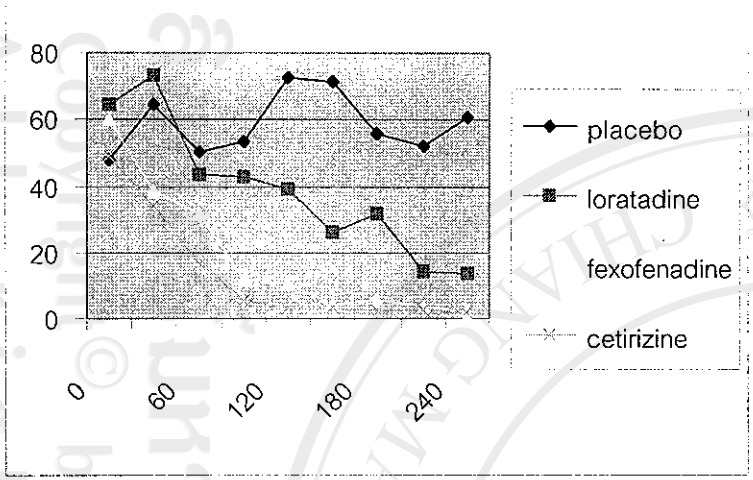


Table 10 Wheal 10 mg/ml

Drug	0	30	60	90	120	150	180	210	240
Placebo	15.08±8.9	19.64±14.0	19.29±11.1	15.74±9.2	17.37±10.6	15.06±7.9	14.51±6.7	15.68±8.3	13.34±6.8
Loratadine	9.34±2.9	10.28±5.7	11.98±8.9	10.21±8.7	11.56±8.4	8.56±5.1	8.95±6.7	6.32±3.9[a]	6.10±4.3[a]
Fexofenadine	11.78±5.2	8.23±4.0[a,b]	9.07±4.3[a,b]	7.33±3.7[a,b]	4.98±2.6[a,b]	3.64±2.7[a,b]	3.07±3.3[a,b]	3.80±5.1[a,b]	2.30±2.4[a,b]
Cetirizine	10.16±2.4	8.56±2.7[a,b]	7.38±3.4[a,b]	4.56±2.2[a,b]	2.12±2.2[a,b]	1.56±1.8[a,b]	0.98±1.4[a,b]	0.51±0.7[a,b]	0.76±1.6[a,b]

[a] $p < 0.05$ compared to placebo

[b] $p < 0.05$ compared to baseline (0 Min)

Table 11 Flare 10 mg/ml

Drug	0	30	60	90	120	150	180	210	240
Placebo	105.50±71.5	123.97±92.5	133.02±70.9	100.84±47.5	115.16±47.44	88.02±49.76	118.63±71.55	106.01±62.6	113.22±39.1
Loratadine	129.13±84.8	143.01±84.0	131.25±87.7	126.53±117.7	132.48±82.9	83.74±54.0	73.53±53.6	55.94±52.9	48.79±49.8[a,b]
Fexofenadine	112.20±67.3	74.71±52.2	78.23±76.7	50.61±41.7[b]	22.68±24.5[a,b]	19.43±19.1[a,b]	17.78±22.8[a,b]	8.45±7.7[a,b]	6.46±4.0[a,b]
Cetirizine	153.70±47.2	101.25±51.6	90.88±44.7[b]	66.08±49.2[a,b]	15.90±16.5[a,b]	5.87±5.7[a,b]	5.25±3.2[a,b]	5.10±2.0[a,b]	3.81±2.1[a,b]

[a] $p < 0.05$ compared to placebo

[b] $p < 0.05$ compared to baseline (0 Min)

Table 12A Percentage of subjects who had more than 95% wheal suppression

Drugs	% of subjects who had >95% wheal suppression	
	W1	W10
Placebo	0	0
Loratadine	12.5	0
Fexofenadine	75.0[a,b]	37.5
Cetirizine	87.5[a,b]	75.0[a,b]

[a] = p< 0.05 compared to placebo

[b]= p< 0.05 compared to loratadine

Table 12B Percentage of subjects who had more than 95% flare suppression

Drugs	% of subjects who had >95% flare suppression	
	F1	F10
Placebo	0	0
Loratadine	62.5[a]	37.5
Fexofenadine	75.0[a]	50.0
Cetirizine	50.0	100.0[a,b]

[a] = p< 0.05 compared to placebo

[b] = p< 0.05 compared to loratadine

Table 13 Treatment-emergent adverse events for each treatment group

Drugs	n	Somnolence	headache	dry mouth	dizziness	fatigue	nausea
Placebo	7	3 (42.9%)	3 (42.9%)	2 (28.6%)	3 (42.9%)	4 (57.1%)	0 (0.0%)
Loratadine	10	7 (70.0%)	3 (30.0%)	2 (20.0%)	2 (20.0%)	3 (30.0%)	0 (0.0%)
Fexofenadine	10	7 (70.0%)	2 (20.0%)	5 (50.0%)	2 (20.0%)	5 (50.0%)	0 (0.0%)
Cetirizine	12	5 (41.7%)	3 (25.0%)	1 (8.3%)	3 (25.0%)	5 (41.7%)	0 (0.0%)

Table R 1 Demographic data of patients taking antihistamines and placebo[a]

Parameters	Placebo (n=7)	Loratadine (n=8)	Fexofenadine (n=8)	Cetirizine (n=8)
Age (yr)	30.2±11.6	28.6±12.3	28.7±16.2	31.5±13.5
Wt (kg)	52.6±3.5	50.9±3.2	51.4±5.5	52.3±4.8
M : F	3 : 4	5 : 3	3 : 5	4 : 4
Mod-to-severe persistent	4	4	5	5
Mild persistent	3	4	3	3
TNSS at $baseline_0$	0.28±0.48	0.87±1.45	1.25±1.90	0.50±1.07
TNSS at $baseline_2$	10.3±3.9	12.5±5.37	12.6±4.9	10.7±3.4
NAR at $baseline_0$ (Pa/ml/sec)	0.34±0.27	0.23±0.15	0.23±0.12	0.23±0.12
NAR at $baseline_2$ (Pa/ml/sec)	1.96±2.57	2.75±5.9	1.08±0.94	2.28±2.96
Secretion weight at $baseline_2$(gm)	1.49±1.5	2.42±1.8	2.28±2.1	2.07±1.1

[a]There was no statistical significance among groups concerning parameters at baseline study.

Table R2 Total NAR (Pa/ml/sec) at various allergen concentrations in controls and patients

Concentration	Controls (n = 30)	Patients (n = 45)
$Baseline_0$	0.29±0.13	0.24±0.14
$Baseline_1$	0.33±0.17	0.27±0.22
50 AU/ml	0.34±0.36	0.36±0.24
100 AU/ml	0.35±0.27	0.44±0.62
500 AU/ml	0.32±0.67	0.54±0.18[a]
1,000 AU/ml	0.33±1.08	0.75±0.24[a]
5,000 AU/ml	0.32±1.17	0.85±0.17[a]

[a]P<0.05 compared to controls

Table R3 Nasal flow (ml/min) during challenge

Group	baseline	diluent	50	100	500	1000	5000
patient	378.41±162.9	349.07± 141.5	301.36±143.3	272.87±136.6	239.10±131.2	224.79±147.9	95.70±92.6
control	303.71±139.4	301.14±143.3	289.29±140.8	285.72±132.3	290.43±125.2	288.37±132.7	284.57±129.7

Table R4 The ROC curve report stratified by variably chosen of concentrations.

Conc. Included[a]	Proposed cut-off [b]	Sensitivity	Specificity	Pos LR	Neg LR	AUCROC[c] ±SE	95% CI of AUC
All	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
50	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
100	>1.2	60.0 (44.3- 74.3)	73.3 (54.1- 87.7)	2.25	0.55	0.65±0.06	0.53-0.75
500	>1.06667	80.0 (65.4- 90.4)	66.7 (47.2- 82.7)	2.40	0.30	0.78±0.05	0.67-0.87
1000	>1.1714[g]	82.2 (67.9- 92.0)	73.3 (54.1- 87.7)	3.08	0.24	0.81±0.04	0.70-0.89
5000	>1.5556[f]	68.9 (53.3- 81.8)	100.0(100.0-100.0)	-	0.31	0.82±0.04	0.72-0.90
50+100	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
50+100+500	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
50+100+500+1000	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
100+500	>1.2	60.0 (44.3- 74.3)	73.3 (54.1- 87.7)	2.25	0.55	0.65±0.06	0.53-0.75
500+1000	>1.0667	80.0 (65.4- 90.4)	66.7 (47.2- 82.7)	2.40	0.30	0.78±0.05	0.67-0.87
1000+5000	>1.1714[g]	82.2 (67.9- 92.0)	73.3 (54.1- 87.7)	3.08	0.24	0.81±0.04	0.70-0.89
50+500	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
50+1000	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
50+5000	>1.0541	75.6 (60.5- 87.1)	53.3 (34.3- 71.6)	1.62	0.46	0.67±0.06	0.55-0.77
500+5000	>1.0667	80.0 (65.4- 90.4)	66.7 (47.2- 82.7)	2.40	0.30	0.78±0.05	0.67-0.87
100+1000	>1.2	60.0 (44.3- 74.3)	73.3 (54.1- 87.7)	2.25	0.55	0.65±0.06	0.53-0.75
100+5000	>1.2	60.0 (44.3- 74.3)	73.3 (54.1- 87.7)	2.25	0.55	0.65±0.06	0.53-0.75

[a] Data from various concentrations of allergenic extract given to the patients which were included in analysis(AU/ml).

[b]Time above baseline of the total NAR which was proposed by software as the most suitable criterion for cutting positive off negative cases. The value corresponded with the highest accuracy (maximum sensitivity and specificity).

[c]Area under the curve of ROC curve at concentrations included.

[d] Pos LR = positive likelihood ration (+LR)

[e] Neg LR = negative likelihood ratio (-LR)

[f] Highest AUCROC and specificity

[g] Highest sensitivity and pos LR

Table R5 Sensitivity analysis

Parameters	Patients with NAR <1.55 times of baseline$_1$ (n=14)	Patients with NAR > 1.55 times of baseline$_1$ (n=31)	*p*-value
TNSS at the end of challenge	5.92±2.9	7.76±2.4	0.071
Skin prick reaction: average of the two diagonal diameters[a] (mm)	Der p 3.82±0.7 Der f 3.64±0.5	Der p 5.48±1.9 Der f 4.91±1.9	0.002 0.004
Ratio of NAR at 5000 and 1000-AU/ml concentration[b] ≥ 1.0[b]	5	22	0.027

[a]The maximal diameter of the wheal reaction by Der p or Der f were averaged with its diagonal diameter.

[b]The individual total NAR produced by 5000-AU/ml allergen was divided by the one produced by 1000-AU/ml extract.

Table R6 ROC analysis for sneezing score

Conc Included	Proposed Cutoff	Sensitivity	Spec	posLR	negLR	AUROC
Whole	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
50	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
100	>0*	33.3(20.0-49.0)	100.0(100.0-100.0)	-	0.67	0.667
500	>1*	37.8(23.8-53.5)	100.0(100.0-100.0)	-	0.62	0.699
1000	>0*	66.7(51.0-80.0)	100.0(100.0-100.0)	-	0.33	0.833
5000	>0*	68.9(53.3-81.8)	96.3(81.0-99.4)	18.60	0.32	0.837
0+100	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
0+100+500	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
0+100+500+1000	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
00+500	>0*	33.3(20.0-49.0)	100.0(100.0-100.0)	-	0.67	0.667
00+1000	>0*	33.3(20.0-49.0)	100.0(100.0-100.0)	-	0.67	0.667
000+5000	>0*	66.7(51.0-80.0)	100.0(100.0-100.0)	-	0.33	0.833
0+500	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
0+1000	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
0+5000	>0*	17.8(8.0-32.1)	96.3(81.0-99.4)	4.80	0.85	0.572
00+5000	>1*	37.8(23.8-53.5)	100.0(100.0-100.0)	-	0.62	0.699
00+1000	>0*	33.3(20.0-49.0)	100.0(100.0-100.0)	-	0.67	0.667
00+5000	>0*	33.3(20.0-49.0)	100.0(100.0-100.0)	-	0.67	0.667

Table R7 ROC analysis for itching score

Conc Included	Proposed Cutoff	Sensitivity	Spec	posLR	negLR	AUROC
Whole	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
50	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
100	>0*	84.4(70.5-93.5)	70.4(49.8-86.2)	2.85	0.22	0.775
500	>0*	91.1(78.8-97.5)	66.7(46.0-83.4)	2.73	0.13	0.82
1000	>0*	95.6(84.8-99.3)	66.7(46.0-83.4)	2.87	0.07	0.853
5000	>0*	97.8(88.2-99.6)	70.4(49.8-86.2)	3.30	0.03	0.868
0+100	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
0+100+500	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
0+100+500+1000	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
00+500	>0*	84.4(70.5-93.5)	70.4(49.8-86.2)	2.85	0.22	0.775
00+1000	>0*	91.1(78.8-97.5)	66.7(46.0-83.4)	2.73	0.13	0.82
000+5000	>0*	95.6(84.8-99.3)	66.7(46.0-83.4)	2.87	0.07	0.853
0+500	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
0+1000	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
0+5000	>0*	80.0(65.4-90.4)	70.4(49.8-86.2)	2.70	0.28	0.761
00+5000	>0*	91.1(78.8-97.5)	66.7(46.0-83.4)	2.73	0.13	0.82
00+1000	>0*	84.4(70.5-93.5)	70.4(49.8-86.2)	2.85	0.22	0.775
00+5000	>0*	84.4(70.5-93.5)	70.4(49.8-86.2)	2.85	0.22	0.775

Table R8 ROC analysis for rhinorrhea

Conc Included	Proposed Cutoff	Sensitivity	Spec	posLR	negLR	AUROC
Whole	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
50	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
100	>0*	73.3(58.1-85.4)	81.5(61.9-93.6)	3.96	0.33	0.788
500	>0*	75.6(60.5-87.1)	77.8(57.7-91.3)	3.40	0.31	0.785
1000	>0*	91.1(78.8-97.5)	77.8(57.7-91.3)	4.10	0.11	0.87
5000	>0*	93.3(81.7-98.5)	74.1(53.7-88.8)	3.60	0.09	0.889
50+100	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
50+100+500	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
50+100+500+1000	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
00+500	>0*	73.3(58.1-85.4)	81.5(61.9-93.6)	3.96	0.33	0.788
00+1000	>0*	75.6(60.5-87.1)	77.8(57.7-91.3)	3.40	0.31	0.785
000+5000	>0*	91.1(78.8-97.5)	77.8(57.7-91.3)	4.10	0.11	0.87
0+500	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
0+1000	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
0+5000	>1*	20.0(9.6-34.6)	100.0(100.0-100.0)		0.80	0.611
00+5000	>0*	75.6(60.5-87.1)	77.8(57.7-91.3)	3.40	0.31	0.785
00+1000	>0*	73.3(58.1-85.4)	81.5(61.9-93.6)	3.96	0.33	0.788
00+5000	>0*	73.3(58.1-85.4)	81.5(61.9-93.6)	3.96	0.33	0.788

'able R9 ROC analysis for congestion score

Conc Included	Proposed Cutoff	Sensitivity	Spec	posLR	negLR	AUROC
/hole	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
50	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
100	>0*	75.6(60.5-87.1)	70.4(49.8-86.2)	2.55	0.35	0.698
500	>0*	77.8(62.9-88.8)	74.1(53.7-88.8)	3	0.30	0.732
1000	>0*	93.3(81.7-98.5)	66.7(46.0-83.4)	2.8	0.10	0.801
5000	>0*	93.3(81.7-98.5)	70.4(49.8-86.2)	3.15	0.09	0.834
0+100	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
0+100+500	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
0+100+500+1000	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
00+500	>0*	75.6(60.5-87.1)	70.4(49.8-86.2)	2.55	0.35	0.698
00+1000	>0*	77.8(62.9-88.8)	74.1(53.7-88.8)	3.00	0.30	0.732
000+5000	>0*	93.3(81.7-98.5)	66.7(46.0-83.4)	2.80	0.10	0.801
0+500	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
0+1000	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
0+5000	>0*	55.6(40.0-70.3)	70.4(49.8-86.2)	1.88	0.63	0.6
00+5000	>0*	77.8(62.9-88.8)	74.1(53.7-88.8)	3.00	0.30	0.732
00+1000	>0*	75.6(60.5-87.1)	70.4(49.8-86.2)	2.55	0.35	0.698
00+5000	>0*	75.6(60.5-87.1)	70.4(49.8-86.2)	2.55	0.35	0.698

Table R10 ROC analysis for total nasal symptoms score

Conc Included	Proposed Cutoff	Sensitivity	Spec	posLR	negLR	AUROC
Whole	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
50	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
100	>0*	95.7(85.1-99.3)	67.9(47.7-84.1)	2.98	0.06	0.844
500	>1*	87.0(73.7-95.0)	71.4(51.3-86.7)	3.04	0.18	0.848
1000	>2*	93.5(82.1-98.6)	78.6(59.0-91.7)	4.36	0.08	0.906
5000	>4*	82.6(68.6-92.2)	85.7(67.3-95.9)	5.78	0.20	0.924
50+100	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
50+100+500	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
50+100+500+1000	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
100+500	>0*	95.7(85.1-99.3)	67.9(47.7-84.1)	2.98	0.06	0.844
500+1000	>1*	87.0(73.7-95.0)	71.4(51.3-86.7)	3.04	0.18	0.848
1000+5000	>2*	93.5(82.1-98.6)	78.6(59.0-91.7)	4.36	0.08	0.906
50+500	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
50+1000	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
50+5000	>0*	91.3(79.2-97.5)	57.1(37.2-75.5)	2.13	0.15	0.75
500+5000	>1*	87.0(73.7-95.0)	71.4(51.3-86.7)	3.04	0.18	0.848
100+1000	>0*	95.7(85.1-99.3)	67.9(47.7-84.1)	2.98	0.06	0.844
100+5000	>0*	95.7(85.1-99.3)	67.9(47.7-84.1)	2.98	0.06	0.844

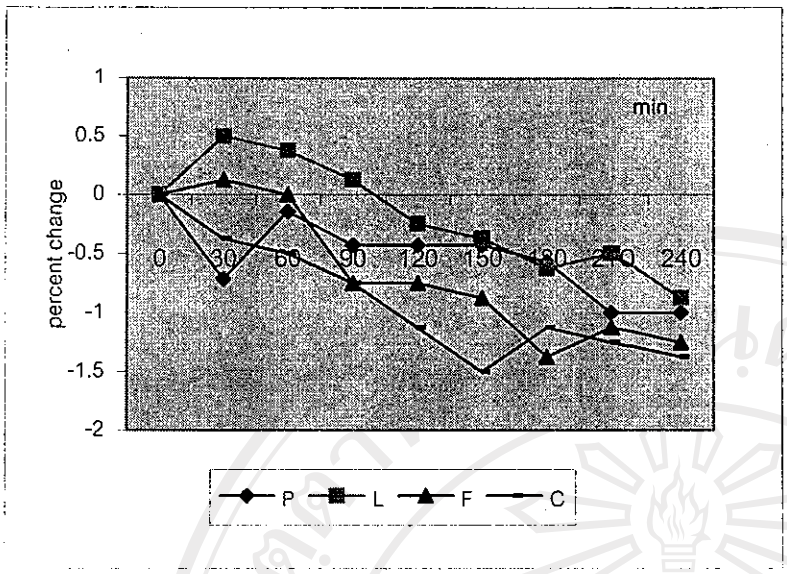


Fig 1.1 Congestion score

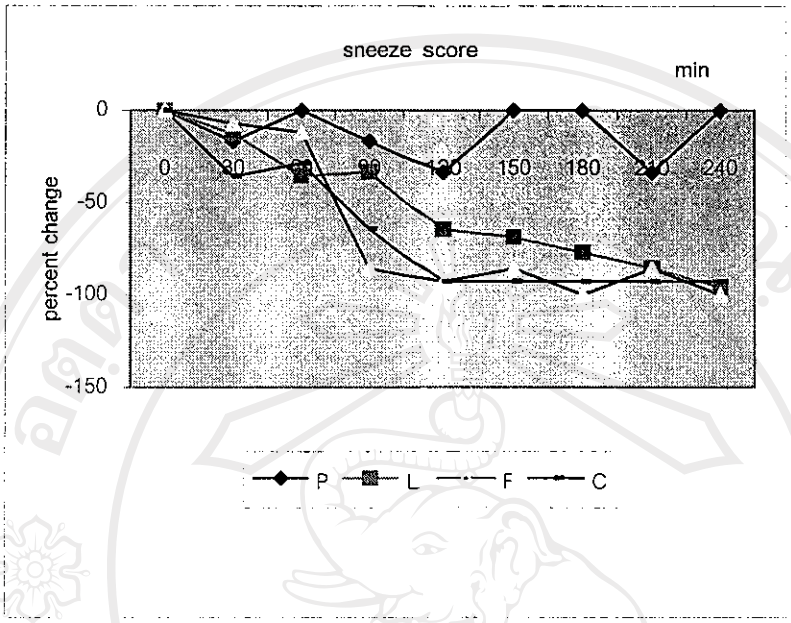


Fig 1.2 Sneezing score

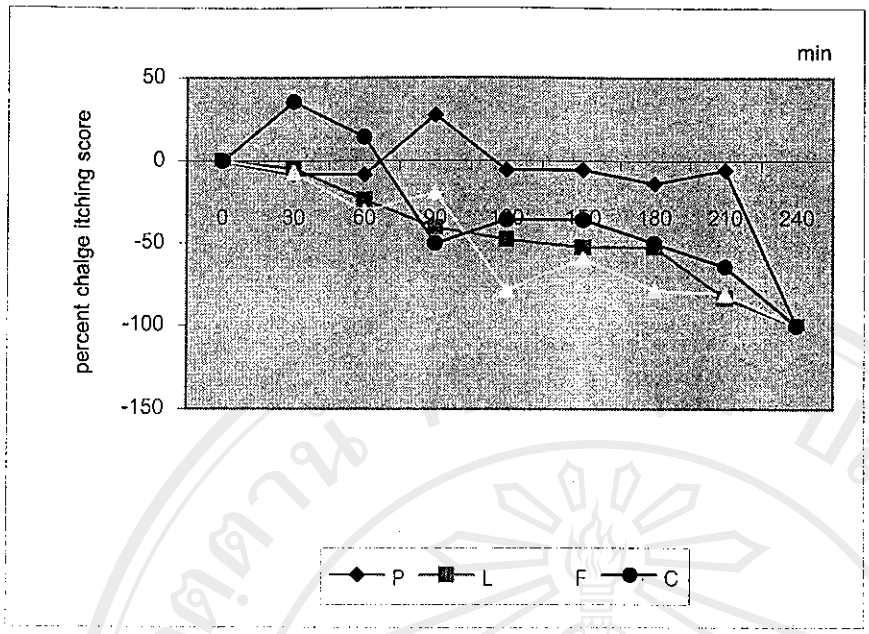


Fig. 1.3 Itching Score

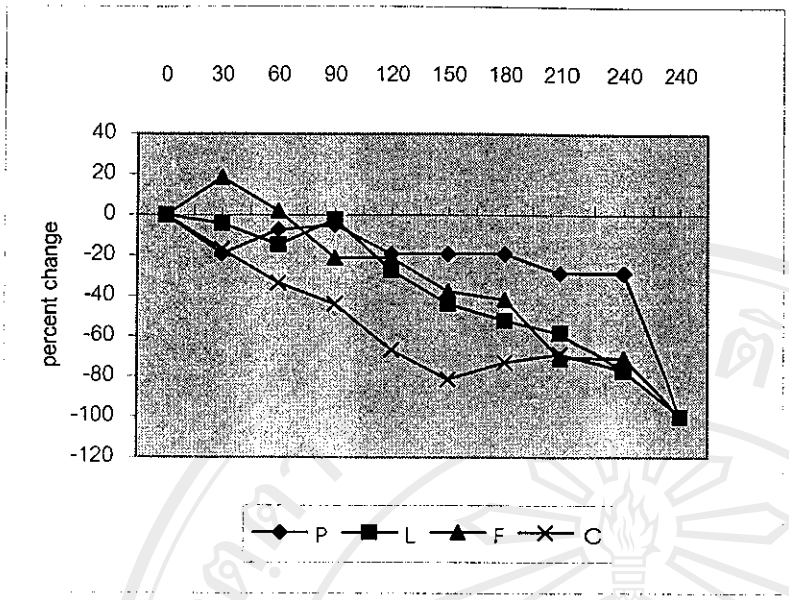


Fig 1.4 Secretion score

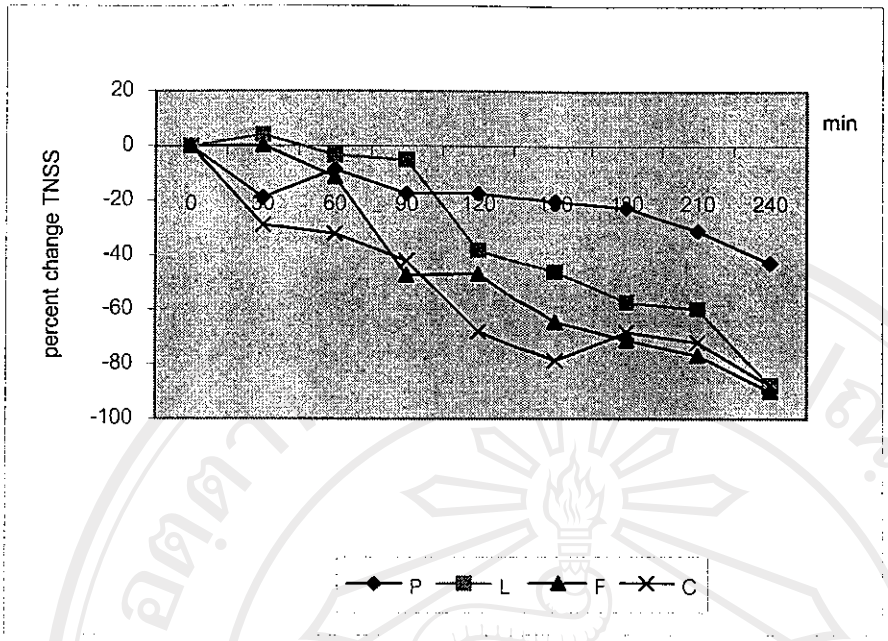


Fig. 2 Total nasal symptoms score

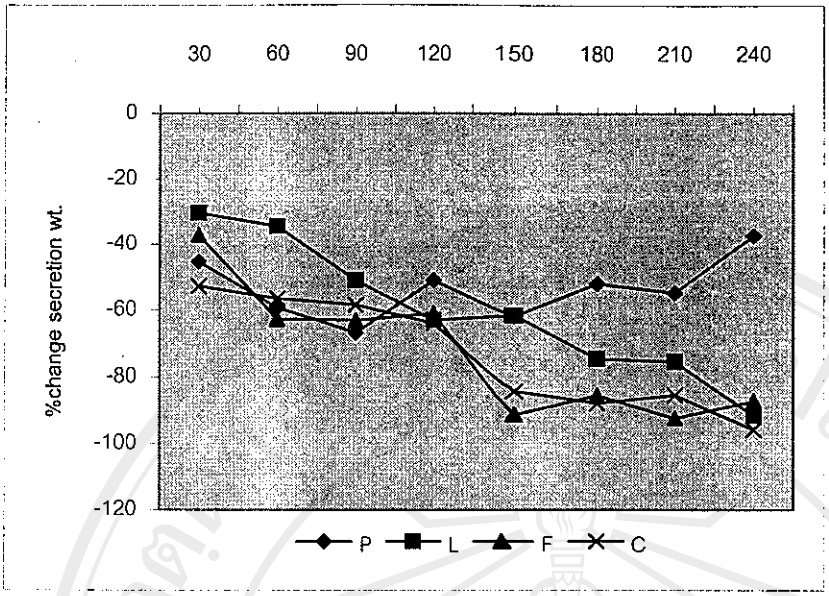


Fig. 3 Secretion weight

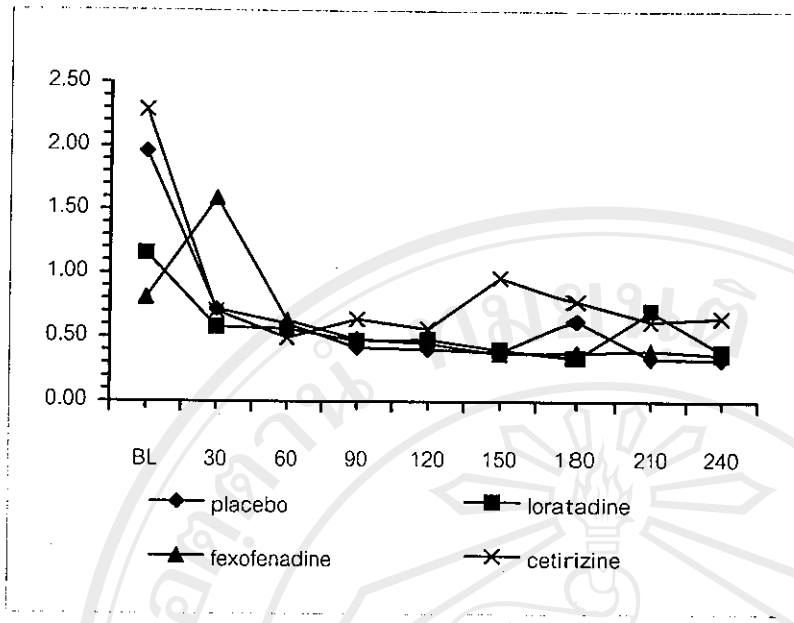


Fig.4 Mean of total nasal airway resistance

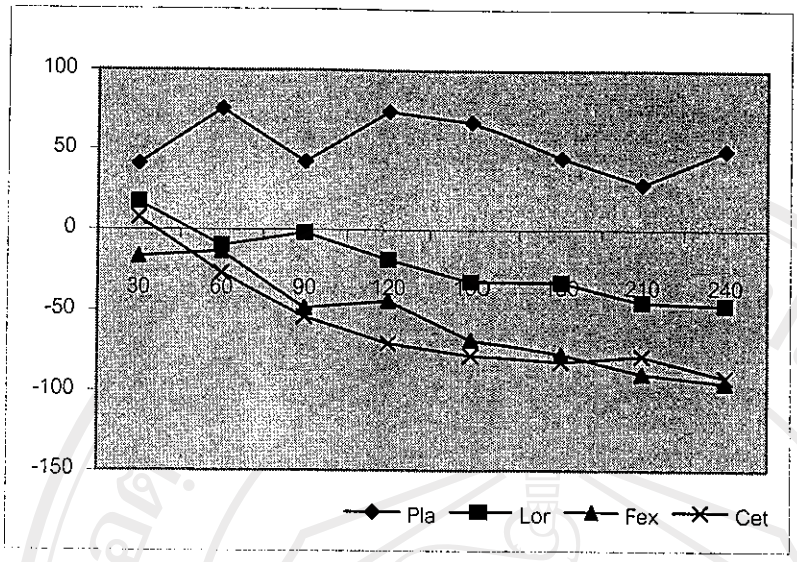


Fig.5 Percent wheal (1mg/ml) suppresion

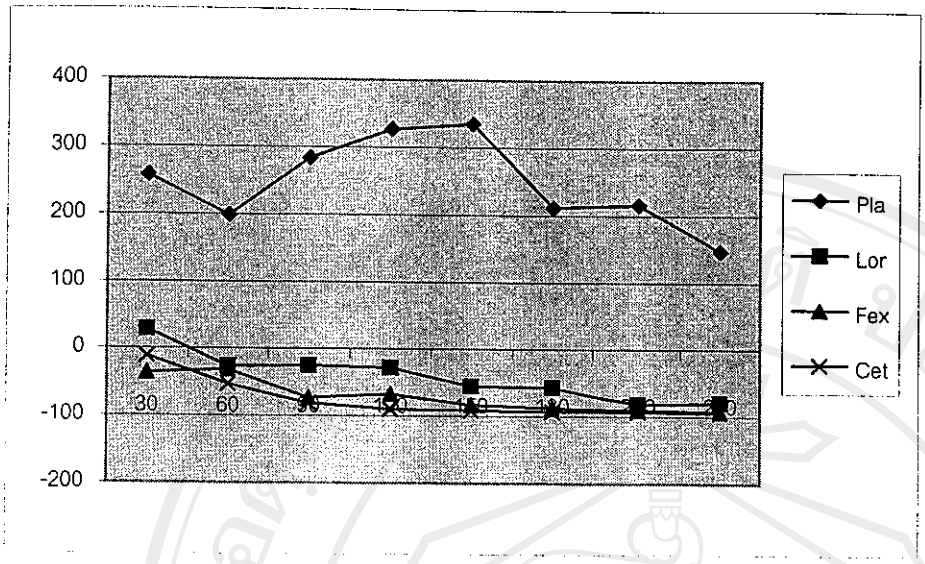


Fig.6 Percent flare (1mg/ml) suppression

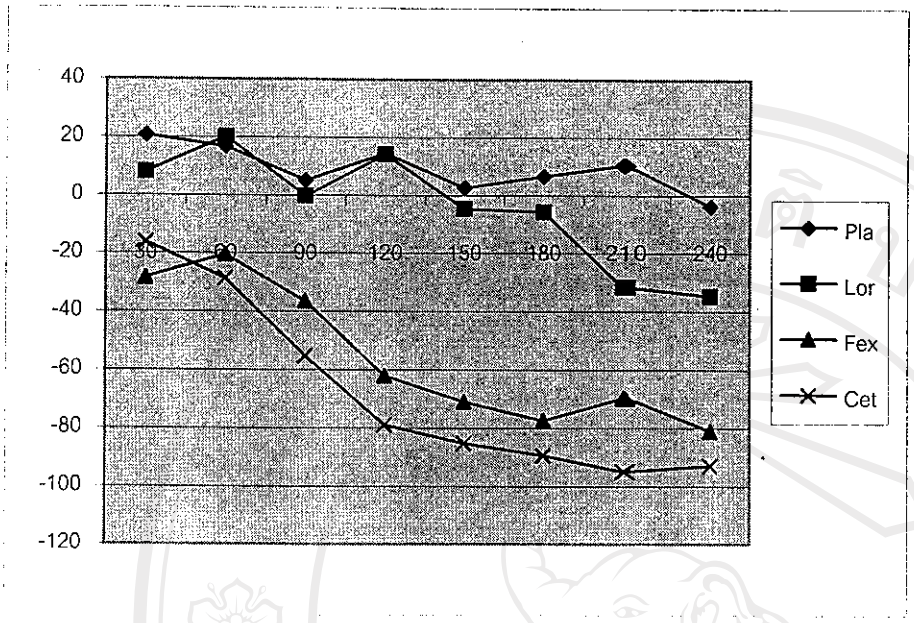


Fig.7 Percent wheal (10mg/ml) suppression

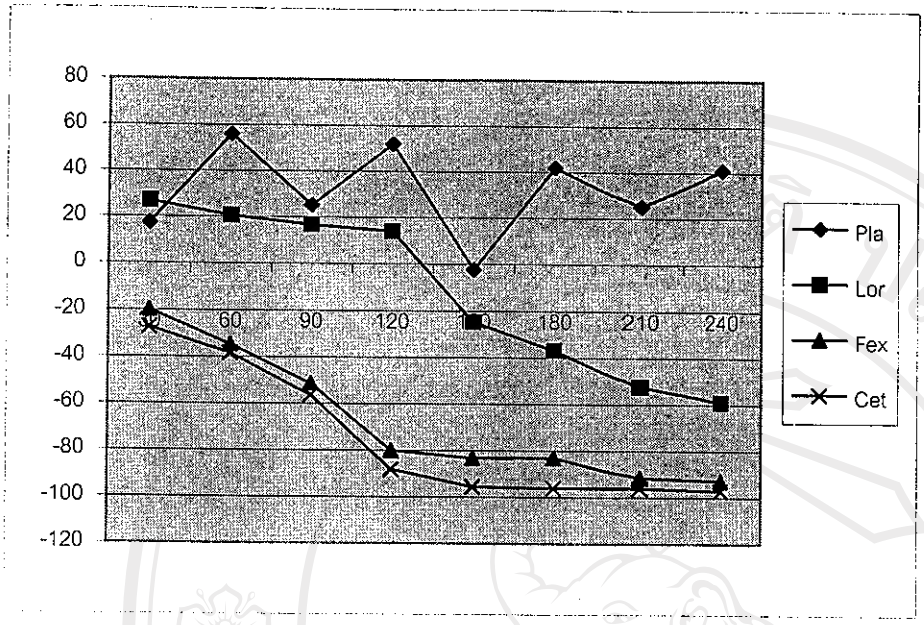


Fig.8 Percent flare (10mg/ml) suppression

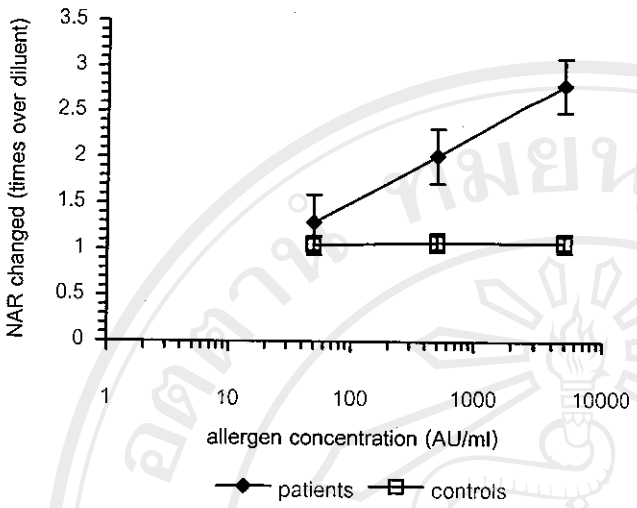


Fig. R1 NAR during allergen challenge

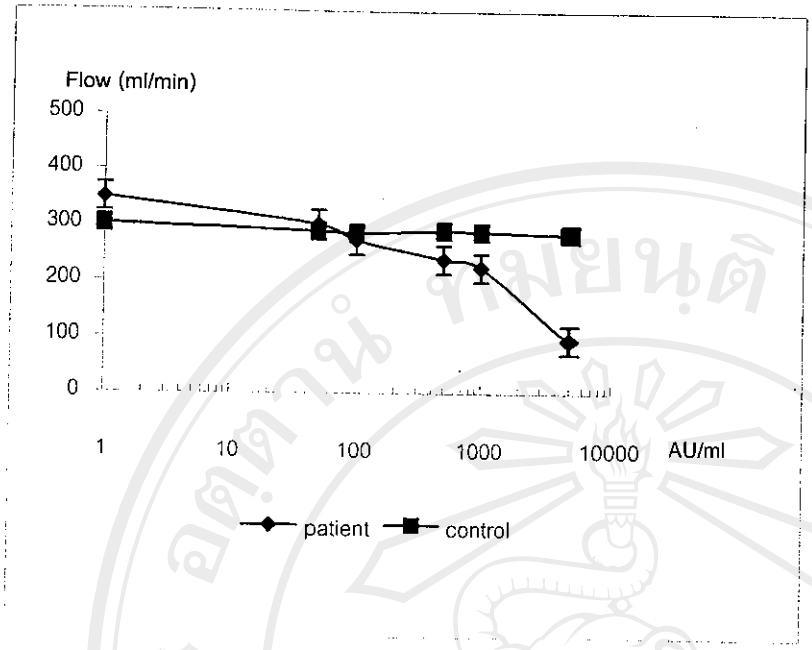


Fig. R2 Nasal Flow during challenge (ml/min)

Fig. R3 ROC curve report for 1,000 AU/ml allergen dose

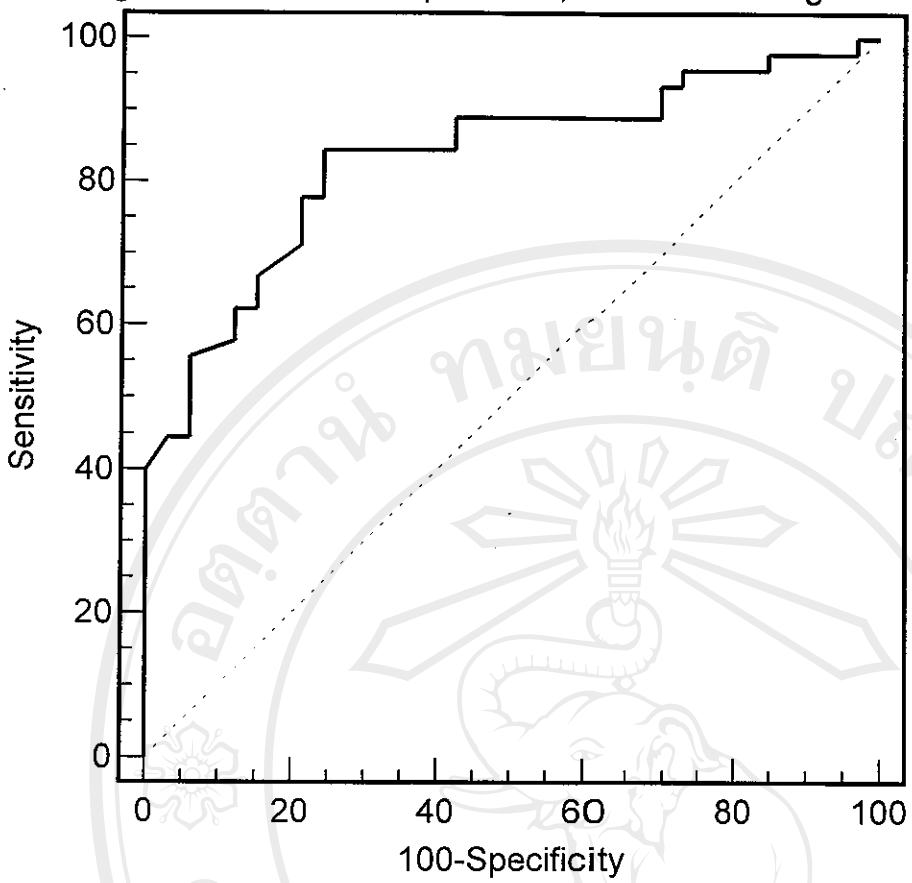
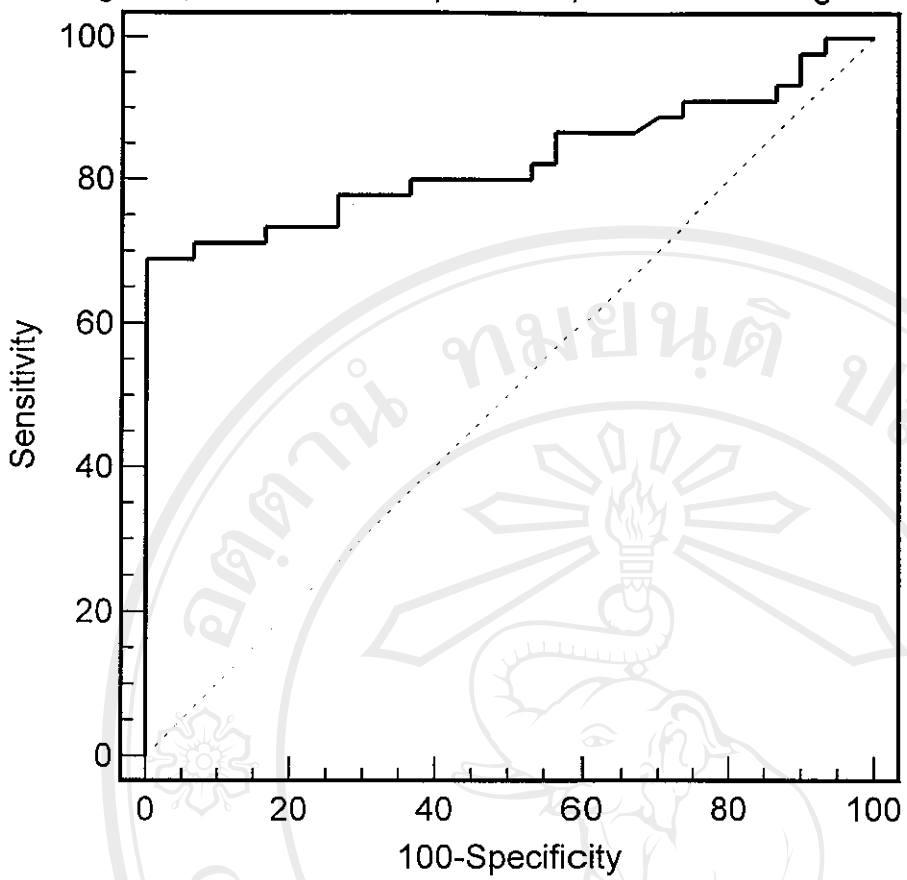


Fig. R4 ROC curve report for 5,000 AU/ml allergen dose



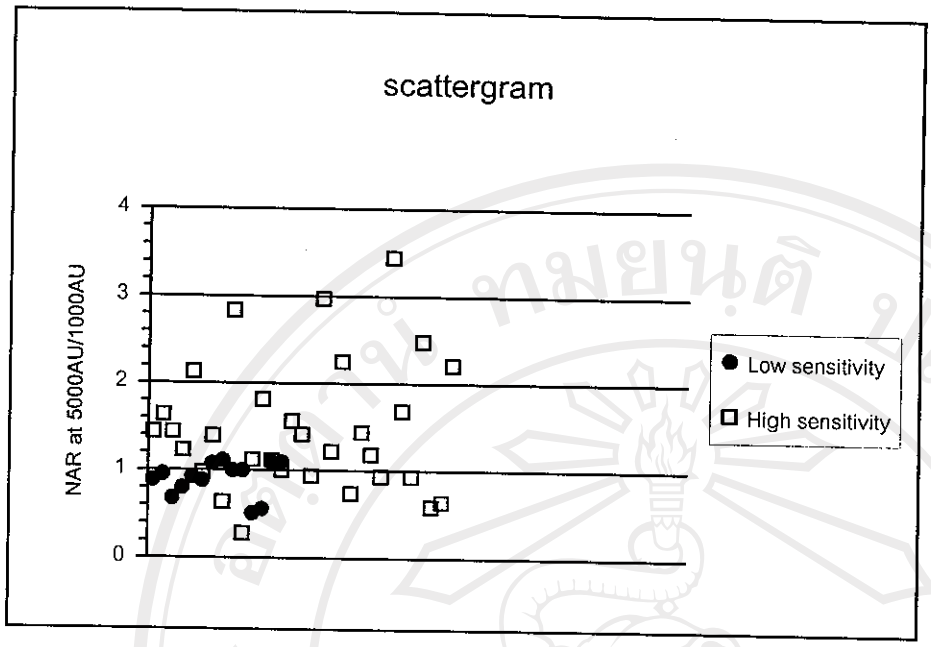


Fig. R5 Scattergram for sensitivity analysis