A Clinical Checklist for Fragile X Syndrome : Screening of Thai Boys with Developmental Delay of Unknown Cause

PORNPROT LIMPRASERT, M.D., Ph.D.*,
PUNNEE VASIKNANONTE, M.D.***,
SOMCHIT JARURATANASIRIKUL, M.D. ***,
HUTCHA SRIPLUNG, M.D.*
NICHARA RUANGDARAGANON, M.D.**,
THANYACHAI SURA, M.D.**** ,
NOPPAWON SRIWONGPANICH, M.D.*****,

Abstract

The aim of this study was to determine a cost-effective clinical checklist for fragile X syndrome (FXS) screening in a Thai male pediatric population with developmental delay of unknown cause. We studied 179 non-FXS male patients and 27 FXS patients from 18 families (age ≤ 15 years). A six-item clinical checklist was used including family history (FH), long and narrow face (F), prominent and large ears (E), attention deficit/hyperactivity (AH), autistic-like behavior (AT) and testicular volume (T). These were scored as 0 if absent, 1 if borderline, and 2 if present. All patients were tested by using PCR and/or southern blot for the *FMR1* gene. We used a logistic regression model from a computer program to analyze the data (Stata, version 5.0). We used logistic regression with cluster in the same family (average score) to eliminate bias from the related FXS cases. We found that a five-item checklist, 2FH + F + 0.5E + 2AH + T = total score, was the best model. When we used this clinical checklist with a threshold of total score of 4, 78.7 per cent of the screened cases with total scores ≤ 4 could be eliminated as negative cases. In addition, all positive FXS cases had total scores > 4. We propose this five-item model for FXS screening in clinical pediatric practice, particularly from Asian population settings.

Key word : Clinical Checklist, Fragile X, Mental Retardation, *FMR1* Gene

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* Department of Pathology, Faculty of Medicine, Prince of Songkla University,
** Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital,
*** Department of Pediatrics, Faculty of Medicine, Prince of Songkla University, Hat Yai, Songkhla 90110,
**** Department of Internal Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400,
***** Rachanukul Hospital, Bangkok 10400, Thailand

Fragile X syndrome (FXS) is an X-linked dominant genetic disease. It is the most common cause of inherited mental retardation with a prevalence of approximately 1:4,000 males[1-3]. Both males and females can be affected, but affected females are generally less severe[4]. The FXS gene, *FMR1*, contains polymorphic CGG repeats. The patients almost always have CGG repeats of more than 200 copies accompanied by methylation of the adjacent CpG island causing absence of the FMR1 protein[5,6]. In our experience, approximately 7 per cent of samples referred for FXS testing, regardless of clinical status, showed positive results on molecular analysis[7]. Variability of FXS clinical expression showed overlap with other disorders, but some clinical features were commonly found in FXS [8-10]. This has emphasized the importance of a clinical checklist for screening purposes. All clinical checklists for FXS so far have only been reported in Caucasian populations[11-16]. Prior to our studies, no standardized FXS clinical checklist for Asian populations had been reported.

We report a FXS clinical checklist in Thai boys with unknown etiology for developmental delay. We used logistic regression model and found that a five-item checklist was the most efficient. Our checklist is the first FXS screening model to assign different weights to each item.

METHOD

Subjects

Two hundred and eighty eight selected Thai patients, age ≤ 15 years, with developmental delay (DD) of unknown cause were studied. The patients attended two major medical centers in Songkhla and Bangkok which are located in southern and central Thailand, respectively. The project was approved by the faculty ethics committee. The patients were divided into 2 groups. Group A consisted of 92 cases who were tested between June 1991- December 1996 by cytogenetic methods and were now re-tested by molecular methods. Group B consisted of 196 cases prospectively screened who were tested between January 1997- October 1999 using molecular methods.

Clinical checklist

We used a six-item clinical checklist modified from the report of Giangreco et al[14] as shown in Table 1. These included family history (FH), long and narrow face (F), prominent and large ears (E), attention deficit/hyperactivity (AH), autistic like behavior (AT), and testicular volume (T). Family history included learning difficulties, developmental delay and mental retardation. A narrow and long face was based on clinical impression of long jaw and high forehead. Prominent ears were considered to be present when the angle of the ear and face was approximately 90 degrees. The longest axis of the ears were measured and compared to the standard scale using the 95 percentile as the threshold[17]. Attention deficit and hyperactivity were scored according to DSM-IV criteria[18]. Autistic-like behaviors were scored as positive when one of the following behaviors was present: tactile defensiveness, hand flapping, hand biting (excluding nail biting), delayed or perseverative speech and poor eye contact[11]. Testicular volume was measured with an orchidometer as milliliters and compared to a modified standard scale [17] (age ≤ 8 years, 1-2 ml: score = 0, 3 ml: score = 1, > 3 ml: score = 2; age > 8 years, 95 percentile - 2 ml: score = 0, 95 percentile ± 1 ml: score = 1, 95 percentile + 2 ml: score = 2). All DD patients were physi-

Table 1. Six-item checklist for FXS screening modified from Giangreco et al[14].

Clinical items	Score		
	0	1	2
Family history*	None	Unidentified	X-linked
Narrow/long face	None	Borderline	Present
Prominent/large ears	None	Either	Both
Attention deficit/Hyperactivity	None	Either	Both
Autistic-like behaviors	None	1 behavior	> 1 behavior
Macro-orchidism	None	Borderline	Present

* Mental retardation, developmental delay and learning difficulty

cally examined by pediatricians before the report of laboratory tests. In addition, the laboratory personnel did not see the checklist results. Due to some missing data (i.e. orphans or non-cooperative physical examinations) or unavailable information from retrospective cases, we used the subjects' data only when at least five items of the checklist were available.

DNA testing

A modified non-radioactive method[19] was used to PCR amplify the CGG repeat region of the *FMR1* gene in all patients. Repeat sizes were determined by comparison with known size markers. EcoRI/EagI double enzyme digestion, southern blot analysis and hybridization of the StB12.3 probe were tested in affected FXS samples and suspected positive PCR results. The StB12.3 probe was labeled with Fluorescein and detected using the Gene Images CDP-Star protocol from Amersham. StB12.3 was kindly supplied by Dr. J. L. Mandel.

Statistics analysis

We used a logistic regression model to analyze the data. Logistic regression has been commonly used to describe the probability of developing some diseases over a specified period as a function of certain risk factors[20]. We adopted this model for our study since it was based on a similar concept. Likewise, we used six items of the clinical checklist as a predictor of a DD child being a FXS case. The logistic regression module of the Stata version 5.0 program[21], logit command, was used for data analysis to determine the weight to assign to each item and to test the significance of each item in the model.

RESULTS

We analyzed 206 cases, from total 288 tested cases (~72%), that had completed at least 5 items of the clinical checklist. Of 65 cases from group A, 52 cases were unrelated non-FXS cases and 13 cases were FXS cases from 7 families. Of 141 cases from group B, 127 cases were unrelated non-FXS cases and 14 cases were FXS cases from 11 families. Fig. 1 showed schematic of the cases studied. There were 28 missing item-records described as the following: 2 age, 1 FH and 23 T in the non-FXS group, 1 FH and 1 T in the FXS group. The mean age of the FXS positive group was 7.9 years (N = 27, range 8 months to 13.6 years). The mean age of the non-FXS group was 6.5 years (N = 177, range 8 months to 14.8 years). There was no statistical difference between the ages of the FXS and non-FXS groups (*t*-test, equal variance, $P = 0.07$). We used logit command with cluster analysis in the FXS group to reduce bias from related FXS cases (average scores from the same

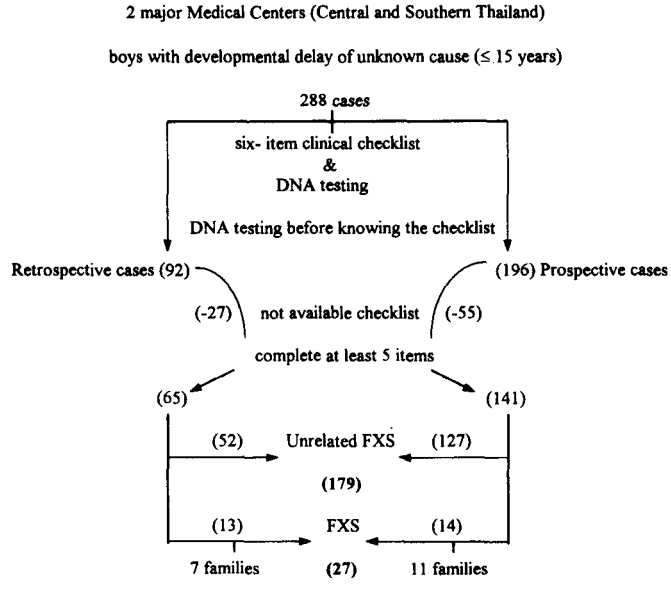

Fig. 1. Schematic of the study.

families were analyzed by the Stata program). We tested each item as an univariate. We found that AT was not statistically significant (P = 0.5). We analyzed the remaining five items using logit command with cluster and found the coefficient to be 1.59FH, 0.93F, 0.69E, 1.41AH and 1.20T. We first used a simulated model without weight, FH + F + E + AH + T= total score. We found that with a threshold total score of 2, 68.4 per cent (specificity) of the non-FXS cases could be eliminated (total score ≤ 2). In addition all FXS cases would have been detected (sensitivity = 100%, total score > 2). When we used the coefficient as a multiplicative weight in the model, the specificity was improved. All five-item checklist models had 100 per cent sensitivity at different threshold scores. The model, 2FH + F + 0.5E + 2AH + T = total score, showed the best specificity (78.7%) with a threshold score of 4 from a total score of 13. Fig. 2 shows a graph comparing non-FXS and FXS groups using the best weight model. We observed that the unavailable data was usually the testicular volume. Therefore, we also tried to use two four-item models (FH + F + E + AH and 2FH + F + E + 2AH). The summary of the models is shown in Table 2.

DISCUSSION

Although FXS testing has been recommended for both males and females with mental retardation of unknown etiology[22], this might not apply to many developing countries with limited facilities. Therefore, a clinical checklist for FXS screening is still applicable for many clinical settings. Table 3 is a comparison of clinical checklists studies for FXS. Our six-item checklist was a modification of the FXS checklist by Giangreco et al[14]. Three FXS clinical checklist reports were based on cytogenetic methods[11-13]. However, our study and the other three reports[14-16] were based on molecular methods. Molecular methods replaced cytogenetic methods since the identification of the *FMR1* mutation. We prospectively and retrospectively studied male cases with developmental delay or mental retardation of unknown cause, but the reports of Giangreco et al[14] and Hecimovic et al[16] retrospectively studied both male and female cases with or without mental retardation. All reported clinical checklists, except for the report of Arvivo et al[15], studied in pediatric subjects. We compared non-

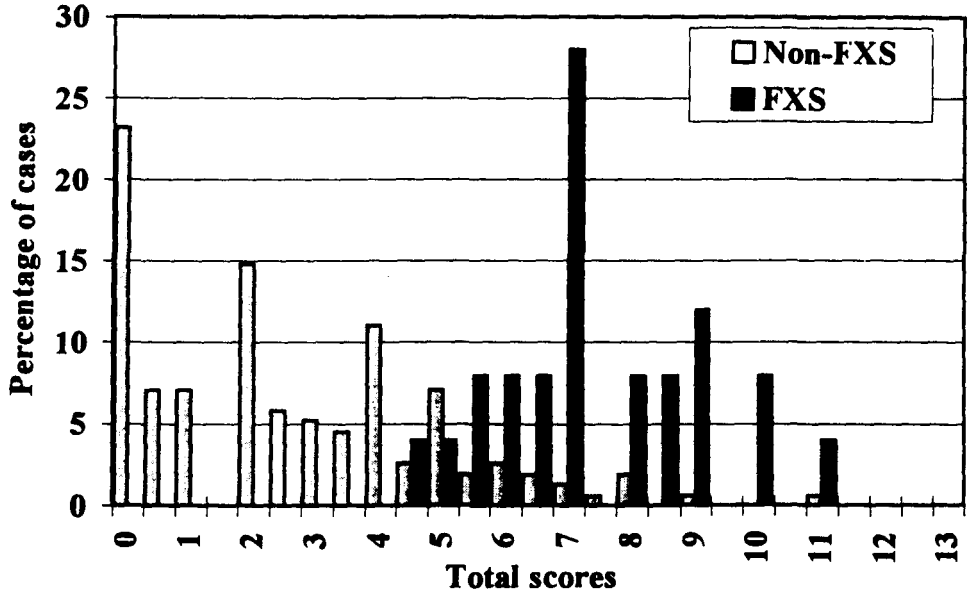

Fig. 2. Comparison of non-FXS and FXS groups using model, 2FH + F + 0.5E + 2AH + T = total score. All FXS patients had total scores of more than 4. Approximately 79 per cent of the non-FXS group had total scores of 4 or less. The total score of 4 is the threshold score for FXS screening in this model.

Table 2. Summary of simulated models for FXS screening.

Models	Total scores	Threshold	Sensitivity (%)	Specificity (%)
FH + F + E + AH	8	> 1	96.15	74.72
2 FH + F + E + 2 AH	12	> 2	100	65.73
FH + F + E + AH + T	10	> 2	100	68.39
1.5 FH + F + E + 1.5 AH +T	12	> 3.5	100	76.13
2 FH + F + E + 2 AH +T	14	> 4	100	76.77
2 FH + F + 0.5 E + 2 AH +T	13	> 4	100	78.71

Table 3. Comparison among clinical checklists for fragile X syndrome.

Studies (population, country)	Screening methods	Number of items (total score)	Scores (sensitivity, specificity)
Hagerman et al (1991) [11] (males, USA)	Cytogenetics	13 (26)	> 15 (86.7%, 84.8%)*
Laing et al (1991) [12] (males and females, Australia)	Cytogenetics	5 (10)	8-10 (67%, NA)
Butler et al (1991) [13] (males, USA)	Cytogenetics	15 (30)	> 7 (100%, 43.2%)*
Giangreco et al (1996) [14] (males and females, USA)	Molecular	6 (12)	> 4 (100%, 60%)
Arvivo et al (1997) [15] (males age > 16 years, Finland)	Molecular	17 (NA)	> 5 (100%, NA)
Hecimovic et al (1997) [16] (males and females, Croatia)	Molecular	6 (12)	> 4 (96%, 57%)*
This study (males age ≤ 15 years, Thailand)	Molecular	5 (13)	> 4 (100%, 78.7%)

* calculated from data in the reports, NA = not available

FXS and FXS males because only positive FXS cases from index males were found (data on screened females not shown). However, we recommended that FXS testing should be done in female patients with a family history of mental retardation or suspected clinical features.

Autistic-like behavior was not a significant item as reported in a previous study[14]. Therefore, it was discarded from the model. Our checklist retained five clinical items. The standard curves of ear length and testicular volume have not been studied in the normal Thai population. For this reason, we used standardized normal curves from the report of Butler et al[17]. Although these standardized curves came from Caucasian subjects, we found that these two items had statistical significance between non-FXS and FXS groups. These findings revealed that FXS patients tended to have much longer ear length and much larger testicular volume than normal children[17]. We suggest that using the Caucasian data may assist a population with lack of these standardized curves.

For the purpose of screening, we need to have a checklist with 100 per cent sensitivity and the highest possible specificity. We proposed different models as shown in Table 2 because these might be beneficial for a similar study. Our study is the first report on a FXS clinical checklist in Asians. In addition, this study is the first report on a FXS clinical checklist with multiplicative weight assigned to each item. We propose this clinical checklist for male FXS, particularly from Asian population settings. However, we suggest that a clinical checklist may not be applicable to all clinical settings, but individual settings may need to be modified according to their experiences.

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REFERENCES

1. Turner G, Webb T, Wake S, Robinson H. Prevalence of fragile X syndrome. Am J Med Genet 1996; 64:196-7.
2. Morton JE, Bundey S, Webb TP, MacDonald F, Rindl PM, Bullock S. Fragile X syndrome is less common than previously estimated. J Med Genet 1997;34:1-5.
3. de Vries BBA, van den Ouweland AMW, Mohkamsing S, et al. Screening and diagnosis for the fragile X syndrome among the mentally retarded: An epidemiological and psychological survey. Am J Hum Genet 1997;61:660-7.
4. Hagerman RJ. Physical and behavioral phenotype. In: Hagerman RJ, Cronister A, eds. Fragile X syndrome: diagnosis, treatment and research. 2nd ed. Baltimore: Johns Hopkins University Press, 1996:3-87.
5. Fu YH, Kuhl DPA, Pizzuti A, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. Cell 1991;67:1047-58.
6. Pieretti M, Zhang F, Fu YH, et al. Absence of expression of the *FMR-1* gene in fragile X syndrome. Cell 1991;66:817-22.
7. Limprasert P, Ruangdaraganon N, Sura T, Vasiknanonte P, Jinorose U. Molecular screening for fragile X sndrome in Thailand. Southeast Asian J Trop Med Pub Health 1999;30 (suppl 2) : 172-5.
8. Thake A, Todd J, Bundey S, Webb T. Is it possible to make a clinical diagnosis of the fragile X syndrome in a boy? Arch Dis Child 1985;60:1001-7.
9. Simko A, Hornstein L, Soukup S, Bagamery N. Fragile X syndrome: recognition in young children. Pediatrics 1989;83:547-52.
10. Nolin SL, Snider DA, Jenkins EC, et al. Fragile X screening program in New York State. Am J Med Genet 1991;38:251-5.
11. Hagerman RJ, Amiri K, Cronister A. Fragile X checklist. Am J Med Genet 1991;38:283-7.
12. Laing S, Partington M, Robinson H, Turner G. Clinical screening score for the fragile X (Martin Bell) syndrome. Am J Med Genet 1991;38:256-9.
13. Bulter MG, Mangrum T, Gupta R, Singh DN. A 15-item checklist for screening mentally retarded males for the fragile X syndrome. Clin Genet 1991;39:347-54.
14. Giangreco CA, Steele MW, Aston CE, Cummins JH, Wenger SL. A simplified six-item checklist for screening for fragile X syndrome in the pediatric population. J Pediatr 1996;129: 611-4.
15. Arvio M, Peippo M, Simola KOJ. Applicability of a checklist for clinical screening of the fragile X syndrome. Clin Genet 1997;52:211-5.
16. Hecimovic S, Barisic I, Pavelic K. DNA analysis of the fragile X syndrome in an at risk pediatric population in Croatia: simple clinical preselection criteria can considerably improve the cost-effectiveness of fragile X screening studies. Hum Hered 1998;48:256-65.
17. Butler MG, Brunschwig A, Miller LK, Hagerman RJ. Standards for selected anthropometric measurements in males with the fragile X syndrome. Pediatrics 1992;89:1059-62.
18. American Psychiatric Association. Diagnostic statistical manual disorders. 4th ed . Washington DC: American Association Press, 1994.
19. Brown WT. Molecular analysis of fragile X syndrome. In: Dracopoli NC, Haines JL, Korf BR, Moir DT, Morton CC, Seidman CE, Seidman JG, Smith DR, eds. Current protocols in human genetics. New York: John Wiley and Sons, 1994; 9.5.1-9.5.14.
20. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other multivariable methods. Boston: PWS-KENT, 1988;512-4.
21. StataCrop: Stata statistical software. release 5.0. College Station, Texas: Stata Corporation, 1997.
22. Curry CJ, Stevenson RE, Aughton D, et al. Evaluation of mental retardation: recommendations of consensus conference. Am J Med Genet 1997; 72:468-77.

แบบประเมินลักษณะทางคลินิกสำหรับการตรวจกรองกลุ่มอาการโครโมโซมเอ็กซ์เปราะในเด็กชายไทยที่มีพัฒนาการช้าโดยไม่ทราบสาเหตุ

พรพรต ลิ้มประเสริฐ, พ.บ., Ph.D., นิชรา เรืองดารกานนท์, พ.บ.**, พรรณี วาสิกนานนท์, พ.บ.***, ธันยชัย สุระ, พ.บ.****, สมจิตร์ จารุรัตนศิริกุล, พ.บ.***, นพวรรณ ศรีวงศ์พานิช, พ.บ.*****, หัชชา ศรีปลั่ง, พ.บ.**

การศึกษานี้เพื่อหาลักษณะทางคลินิกที่สำคัญสำหรับใช้ในการตรวจกรองกลุ่มอาการโครโมโซมเอ็กซ์เปราะในเด็กชายไทยที่มีพัฒนาการช้าโดยไม่ทราบสาเหตุก่อนส่งตรวจทางห้องปฏิบัติการเพื่อลดการส่งตรวจที่ไม่จำเป็น ผู้วิจัยศึกษาเด็กชายที่มีพัฒนาการช้าโดยไม่ทราบสาเหตุที่ไม่ใช่กลุ่มอาการโครโมโซมเอ็กซ์เปราะจำนวน 179 คนและผู้ป่วยกลุ่มอาการโครโมโซมเอ็กซ์เปราะจำนวน 27 คนจาก 18 ครอบครัว (ผู้ป่วยทุกคนอายุน้อยกว่าหรือเท่ากับ 15 ปี) โดยใช้แบบประเมินลักษณะทางคลินิก 6 ข้อ คือ ประวัติครอบครัว (FH), ใบหน้ายาวแคบ (F), หูกางและ/หรือใหญ่ (E), สมาธิสั้นและ/ หรือซนอยู่ไม่นิ่ง (AH), พฤติกรรมแบบออทิสติก (AT) และขนาดของอัณฑะ (T) การให้คะแนนมี 3 ระดับคือ 0 คะแนนเมื่อไม่พบ, 1 คะแนนเมื่อไม่ชัดเจนหรือพบลักษณะแบบใดแบบหนึ่งในข้อประเมินนั้น และ 2 คะแนนเมื่อชัดเจน โดยตรวจดีเอ็นเอของยีนในกลุ่มอาการโครโมโซมเอ็กซ์เปราะทุกราย วิเคราะห์ผลการศึกษาจากข้อมูลผู้ป่วยทั้งสองกลุ่มโดยใช้ Logistic regression จากโปรแกรมคอมพิวเตอร์ Stata version 5.0 ในผู้ป่วยกลุ่มอาการโครโมโซมเอ็กซ์เปราะใช้ Cluster จากโปรแกรมหาค่าเฉลี่ยในครอบครัวเดียวกันเพื่อขจัดความลำเอียงจากลักษณะคล้ายคลึงกันในครอบครัว ผลการวิเคราะห์ได้แบบประเมินที่สำคัญ 5 ข้อคือ 2FH + F + 0.5E + 2AH + T = total score เป็นโมเดลที่ดีที่สุด ค่าของคะแนนรวมที่เท่ากับ 4 เป็นจุดวิกฤตของการตัดสินใจว่าจะส่งตรวจดีเอ็นเอในกลุ่มอาการโครโมโซมเอ็กซ์เปราะหรือไม่ ในกลุ่มที่ไม่ใช่กลุ่มอาการโครโมโซมเอ็กซ์เปราะมีคะแนนน้อยกว่าหรือเท่ากับ 4 คือมีความจำเพาะ (specificity) ร้อยละ 78.7 แต่กลุ่มอาการโครโมโซมเอ็กซ์เปราะทุกรายมีคะแนนรวมมากกว่า 4 คือมีความไว (sensitivity) ร้อยละ 100 ผู้รายงานเสนอว่าควรนำแบบประเมินลักษณะทางคลินิก 5 ข้อนี้ไปใช้ในการตรวจกรองกลุ่มอาการโครโมโซมเอ็กซ์เปราะในผู้ป่วยเด็กโดยเฉพาะจากคนเอเชีย

คำสำคัญ : แบบประเมินลักษณะทางคลินิก, โครโมโซมเอ็กซ์เปราะ, ภาวะปัญญาอ่อน, ยีน FMR1

พรพรต ลิ้มประเสริฐ, นิชรา เรืองดารกานนท์, พรรณี วาสิกนานนท์, และคณะ
จดหมายเหตุทางแพทย์ ฯ 2543; 83: 1260–1266

* ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์,
** ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี,
*** ภาควิชากุมารเวชศาสตร์, คณะแพทยศาสตร์ มหาวิทยาลัยสงขลานครินทร์, จ.สงขลา 90110
**** ภาควิชาอายุรศาสตร์, คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10400
***** โรงพยาบาลราชานุกูล, กรุงเทพ ฯ 10400