

การทดสอบความเปราะต่อแรงดันออสโมติกของ
เม็ดเลือดแดงที่ได้รับการปรับปรุงพัฒนา :
ทางเลือกสำหรับการตรวจคัดภาวะฮีโมโกลบินผิดปกติ



นายอสมัน สเนปาร์

ศูนย์วิทยทรัพยากร

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาการพัฒนาสุขภาพ

บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

พ.ศ. 2538

ISBN 974-631-974-4

ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

A MODIFIED OSMOTIC FRAGILITY TEST (O.F.T.) : AN ALTERNATIVE
FOR SCREENING FOR HEMOGLOBINOPATHIES



OSMAN SIANIPAR, M.D., DMM.

A THESIS IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE
HEALTH DEVELOPMENT PROGRAMME
GRADUATE SCHOOL
CHULALONGKORN UNIVERSITY

1995

ISBN 974-631-974-4



Title : A Modified Osmotic Fragility Test (O.F.T.) : an
Alternative for Screening for Hemoglobinopathies
By : Osman Sianipar, M.D., DMM.
Program : Health Development
Advisor : Assoc. Prof. Kamjorn Tatiyakavee, M.D.
Co-advisor : Prof. Pirom Kamol-ratanakul, M.D., M.Sc.

Accepted by the Graduate School, Chulalongkorn
University in Partial Fulfillment of the Requirements for
Master's Degree

Santi Thoongsuwan
.....Dean of graduate school
(Assoc. Prof. Santi Thoongsuwan, Ph.D.)

The committee

Somjai Wangsuphachart
.....Chairman
(Assoc. Prof. Somjai Wangsuphachart, M.D., M.Sc.)

Kamjorn Tatiyakavee
..... Thesis advisor
(Assoc. Prof. Kamjorn Tatiyakavee, M.D.)

P. Kamolratanakul
..... Member
(Prof. Pirom Kamol-ratanakul, M.D., M.Sc.)

Tanin Intragumtornchai
..... Member
(Assoc. Prof. Tanin Intragumtornchai, M.D.)



: MAJOR HEALTH RESEARCH
KEY WORD: SCREENING/HEMOGLOBINOPATHY/OSMOTIC FRAGILITY TEST

OSMAN SIANIPAR, M.D.,DMM. : A MODIFIED OSMOTIC FRAGILITY TEST
(O.F.T.) : AN ALTERNATIVE FOR SCREENING FOR HEMOGLOBINOPATHIES.
ADVISOR : ASSOC. PROF. KAMJORN TATIYAKAVEE, M.D.; CO-ADVISOR :
PROF. PIROM KAMOL-RATANAKUL, M.D., M.SC. 111 PP. ISBN 974-631-974-4

Objectives : (1) to prove that the modified O.F.T. visualized by the button formation of unlysed erythrocytes has a good diagnostic performance to detect hemoglobinopathies (a thalassemia trait, β thalassemia trait and hemoglobin E trait) ; (2) to compare diagnostic performance of the modified and the previous O.F.T.; (3) to investigate factors that may influence test performance of the modified O.F.T.

Study design : descriptive, cross-sectional study.

Methodology : 468 volunteers were involved in this study. Each volunteer was tested by both the modified and the previous O.F.T. by different laboratory technician. Each test was validated blindly and separately by a standard screening procedure. Then, diagnostic performance of each test as a result from computation by using 2 x 2 table were compared to each other. Total bilirubin level that may influence test performance was also observed.

Results : It was found 51 cases of hemoglobinopathy, consisted of 12 cases of β thalassemia trait and 39 cases of hemoglobin E trait. The other (417 volunteers) were classified into non-hemoglobinopathy group. Diagnostic performance of the modified O.F.T. is as follows : sensitivity is 86.3 % ; specificity is 75.3 % ; positive and negative predictive values are respectively 29.9 % and 97.8 % as well as accuracy is 76.5 % . In term of detection rate both the modified O.F.T. and the previous O.F.T. are significantly different ($p < 0.05$). The previous O.F.T. has a higher sensitivity i.e. 88.2 % . However, the other test characteristics show that the modified test is better. Total bilirubin level up to 3.3 mg/dl. does not show any different compared with its normal level to influence the test result ($p > 0.05$).

ภาควิชา.....ศูนย์อภิชยาการวิจัยแพทยศาสตร
สาขาวิชา.....การพัฒนาระบบสุขภาพ
ปีการศึกษา.....๒๕๖๔

ลายมือชื่อนิสิต.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....Assoc. Prof. Kamjorn Tatiyakavee
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....Prof. Pirom Kamolratanakul



ACKNOWLEDGEMENT

I would like to express my gratitude to the following respectable persons;

My advisor Assoc. Prof. Kamjorn Tatiyakavee, M.D. and my co-advisor Prof. Pirom Kamol-ratanakul, M.D., M.Sc. for their valuable advice and guidance when I joined the course and conducted this study.

The Thai CERTC Consortium under the director of Prof. Chitr Sitthi-amorn which gave me the opportunity to join this Clinical Epidemiology Course, and also gave an additional allowance during my wife stayed with me in Bangkok for three months. Very deep appreciation is addressed to all of preceptors under The Thai CERTC Consortium, Mrs. Herminia (Tati) Mekanandha and other supporting staffs of the Thai CERTC and CEU who were very helpful during a period of the course.

The INCLEN Inc. under director of Dr. Arturo Morrilo which gave a start-up grant in this study.

The CEU sponsor, Soenarto Sastrowijoto, M.D., Ph.D. and also Tony Sadjimin, M.D., Ph.D., DSAK. who always supported me during the course undertaken. Thank to Clinical and Biostatistics Unit Dr. Sardjito Hospital, Yogyakarta which provided me some facilities.

Soeharjanto, M.D., DSPK. the head department of Clinical Pathology, Gadjah Mada University/Dr. Sardjito Hospital who gave a financial support and permitted to work in this laboratory, and also all of my colleagues in this department for their encouragement.

Laboratory technicians in Clinical Pathology Department, Dr. Sardjito Hospital who helped me to conduct this study. Those who participated in this study as volunteers for their cooperation and blood donation. My classmates for their cooperation and warm friendship during the course period.

Finally my wife, Exsyupransia Mursyanti, my parents as well as all of my brothers and sisters for their encouragement, love and care.



CONTENTS

| | Page |
|--|------|
| ABSTRACT (THAI) | iv |
| ABSTRACT (ENGLISH) | v |
| ACKNOWLEDGEMENT | vi |
| CONTENT OF TABLE | x |
| CONTENT OF FIGURE | xii |
| | |
| CHAPTER I. INTRODUCTION | 1 |
| Background and rationale | 1 |
| Operational definition of button formation | 3 |
| Conceptual framework | 4 |
| Research questions | 7 |
| Primary research question | 7 |
| Secondary research question | 7 |
| Research objectives | 7 |
| | |
| CHAPTER II. REVIEW OF RELATED LITERATURE | 8 |
| Definition and clinical manifestation of β thalassemia | 8 |
| Epidemiology | 9 |
| Problem of thalassemia in Indonesia | 10 |
| Methods for screening for β thalassemia trait .. | 11 |
| Osmotic Fragility Test (O.F.T.) | 12 |

| | |
|--|--------|
| Characteristic test for screening | 13 |
| β Thalassemia trait | 15 |
| α Thalassemia | 16 |
| α thalassemia trait | 17 |
| Silent carrier | 17 |
| Hemoglobin E disease | 18 |
| CHAPTER III. METHODOLOGY | 20 |
| Research design | 20 |
| Population | 20 |
| Observation and measurement | 21 |
| Sample size calculation | 22 |
| The modified O.F.T. | 22 |
| The previous O.F.T. | 24 |
| Gold Standard | 26 |
| 1. MCV and/or MCH values | 27 |
| 2. Hemoglobin electrophoresis on CAM | 27 |
| 3. Hemoglobin A ₂ determination | 29 |
| 4. Hemoglobin F determination | 31 |
| 5. Plasma ferritin level determination ... | 33 |
| Other variables to be measured | 33 |
| Diagnostic criteria of hemoglobinopathies | 34 |
| Data collection | 34 |
| Data analysis | 35 |
| Ethical considerations | 39 |
| Limitation and obstacles | 39 |

| | |
|---|----|
| Expected benefits and applications | 40 |
| CHAPTER IV. RESULTS AND DISCUSSION | 42 |
| The modified O.F.T. | 47 |
| The previous O.F.T. | 51 |
| Comparison between the modified and the previous O.F.T. | 55 |
| Total bilirubin level | 59 |
| MCV and/or MCH values | 62 |
| Hemoglobin A ₂ determination | 64 |
| Hemoglobin F determination | 65 |
| Hemoglobin electrophoresis | 66 |
| Ferritin level determination | 66 |
| RBC morphology | 67 |
| CHAPTER V. CONCLUSION AND RECOMMENDATION | 70 |
| REFERENCES | 75 |
| APPENDIX 1. Status penelitian (status of study)..... | 81 |
| APPENDIX 2. Surat Pernyataan (inform consent) | 82 |
| APPENDIX 3. Screening procedure for hemoglobinopathy... | 83 |
| APPENDIX 4. Data of the modified O.F.T. for calculation inter observer agreement | 84 |
| APPENDIX 5. Data of the previous O.F.T. for ICC calculation | 87 |
| APPENDIX 6. Data of Hb A ₂ level for ICC calculation ... | 88 |

| | | |
|-------------|---|-----|
| APPENDIX 7. | Data of MCV and MCH values as well as hemoglobin level for CV calculation | 89 |
| APPENDIX 8. | Data of MCV and MCH values among those who suffer from hemoglobin E trait | 90 |
| APPENDIX 9. | Test result the modified and the previous O.F.T. as well as the diagnosis | 91 |
| VITAE | | 111 |



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLE

| Table | Page |
|---|------|
| 3.1. Interpretation of test result of the modified O.F.T. | 24 |
| 3.2. Interpretation of Hb A ₂ level | 30 |
| 3.3. Interpretation of Hb F level | 32 |
| 3.4. Diagnostic criteria of hemoglobinopathies | 34 |
| 3.5. A validation of a test to the gold standard..... | 36 |
| 4.1. Frequency distribution of age | 41 |
| 4.2. Frequency distribution of hemoglobin level | 44 |
| 4.3. Laboratory data of microcytosis cases | 46 |
| 4.4. Characteristic of the study population | 47 |
| 4.5. A validation of the modified O.F.T. to the standard screening procedure | 48 |
| 4.6. Diagnostic performance of the modified O.F.T. at 95 % confidence interval | 49 |
| 4.7. Inter observer agreement of the modified O.F.T. | 51 |
| 4.8. Frequency distribution of hemolysis level | 51 |
| 4.9. A validation of the previous O.F.T. to the standard screening procedure..... | 53 |
| 4.10. Diagnostic performance of the previous O.F.T. at 95 % confidence interval | 53 |
| 4.11. Frequency distribution of hemolysis level among hemoglobinopathy cases | 55 |

| | | |
|-------|--|----|
| 4.12. | A comparison between the modified and the previous O.F.T. based on false positive result.. | 56 |
| 4.13. | A comparison between the modified and the previous O.F.T. based on false negative result.. | 57 |
| 4.14. | A comparison between the modified and the previous O.F.T. based on detection rate | 58 |
| 4.15. | A comparison between the modified and the previous O.F.T. | 59 |
| 4.16. | Frequency distribution of total bilirubin level | 60 |
| 4.17. | A comparison between total bilirubin level and test result of the modified O.F.T. | 61 |
| 4.18. | A comparison between total bilirubin level and test result of the previous O.F.T. | 61 |
| 4.19. | Frequency distribution of MCV value | 62 |
| 4.20. | Frequency distribution of MCH value | 63 |
| 4.21. | Prevalence of hemoglobinopathy among microcytic and hypochromic sub-group | 64 |
| 4.22. | Result of hemoglobin A ₂ determination | 65 |
| 4.23. | Ferritin level among those who had MCV value less than 80 fL. | 67 |

LIST OF FIGURE

| Figure | | Page |
|--------|---|------|
| 1.1. | The first and second modification of the modified O.F.T. | 2 |
| 1.2. | The third modification of the modified O.F.T. .. | 3 |
| 1.3. | Conceptual frame work | 6 |



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย