

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

# بسم الله الرحمن الرحيم





HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكرونيله



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



## Study the Role of Glutathione-S-Transferase Gene Polymorphism and its Relation to Aluminium level in Autistic Children (Interventional Study)

# Thesis Submitted for Partial Fulfillment of the Ph.D degree in Environmental Medical Science

## ${f Sara}$ Said Abdelkawi Elgammal

M.BBCh. Ain Shams University (2005) M.Sc.in Pediatrics-Ain Shams University (2011)

Faculty of Graduate Studies and Environmental Research Ain Shams University 2022

#### **APPROVAL SHEET**

### Study the Role of Glutathione-S-Transferase Gene Polymorphism and its Relation to Aluminium level in Autistic Children (Interventional Study)

Submitted By

### Sara Said Abdelkawi Elgammal

M.BBch.- Ain Shams University (2005)
M.Sc.in Pediatrics-Ain Shams University (2011)
A Thesis Submitted in Partial Fulfillment of The Requirement for the Ph.D. Degree In Environmental Sciences
Department of Environmental Medical Sciences

This thesis was discussed and approved by:

The committee

Signature

#### 1- Prof. Dr. Mostafa Hassan Ragab

Prof. of Community Medicine and Environment, Department of Environmental Medical Sciences Faculty of Graduate Studies and Environmental Research Ain Shams University

#### 2- Prof. Dr. Ehab Raga Abd El-Raouf

Prof. of Clinical Genetics, National Research Center

#### 3- Prof. Dr. Nevin Ezzeldin Sharaf

Prof. of Environmental Medicine, Environmental and Occupational Medicine Department National Research Center

#### 4- Prof. Dr. Prof. Dr. Hala Ibrahim Awadalla

Prof. of Community Medicine and Environment, Department of Environmental Medical Sciences Faculty of Graduate Studies and Environmental Research Ain Shams University



## Study the Role of Glutathione-S-Transferase Gene Polymorphism and its Relation to Aluminium level in Autistic Children (Interventional Study)

Thesis

Submitted for Partial Fulfillment of the Ph.D degree in Environmental Medical Science

Bv

#### Sara Said Abdelkawi Elgammal

M.BBCh.- Ain Shams University (2005) M.Sc.in Pediatrics-Ain Shams University (2011)

Supervised by

### Prof. Dr. Hala Ibrahim Awadalla

Professor of Community and Environmental Medicine Department of Environmental Medical Sciences Faculty of Graduate Studies and Environmental Research, Ain Shams University

### Prof. Dr. Mohamed Abd Elkader Al-khafif (Late)

Professor of Medical Biochemistry
Department of Environmental Medical Sciences
Faculty of Graduate Studies and Environmental Research,
Ain Shams University

#### Prof. Dr. Nevin Ezzeldin Sharaf

Professor of Environmental Medicine Environmental and Occupational Medicine Department National Research Centre

### Assist. Prof. Dr. Gehan Moubarz Mahdy

Assistant Professor of Environmental Biochemistry and Molecular Biology Environmental and Occupational Medicine Department National Research Centre

### **Dr. Ahmed Mamdouh AbdelGwad**

Lecturer of Environmental Medical Science
Faculty of Graduate Studies and Environmental Research,
Ain Shams University
Faculty of Graduate Studies and Environmental Research
Ain Shams University
2022

## Acknowledgment

First and foremost, all thanks and gratefulness are to **Almighty ALLAH** The most Merciful most Gracious and Giving, for His blessings, guidance, and His compassion.

The completion of this undertaking study could not have been possible without the participation, assistance and guidance of professionals whose names are glowing in the scientific field, their contributions are sincerely appreciated and gratefully acknowledged.

God bless the soul of *Prof. Dr. Mohamed Abd Elkader Al-khafif* (*late*), Professor of Medical Biochemistry, *Department of Environmental Medical Sciences*, Faculty of Graduate Studies and Environmental Research, Ain Shams University, for his continuous encouragement with kind guidance, he conferred his wide experiences on me. He was always supporting me from the very beginning of the study and was eager to get the best out of me through professional thoughtful guidelines. I would have been even happier if he could have reached this day and witness what we achieved. Allah bless his soul and may he rest in peace.

I would like to express my deepest thanks and profound gratitude to *Prof. Dr. Hala Ibrahim Awadalla*, Professor of Community and Environmental Medicine *Department of Environmental Medical Sciences*, Faculty of Graduate Studies and Environmental Research, Ain Shams University. It was such a great honor to work under her kind guidance. Her continuous help, combined with her sincere support obliged me to bear the responsibility towards this study. The statistics of the study as well as the review, could not have been fulfilled without her sincere effort.

I am also very indebted to *Prof. Dr. Nevin Ezzeldin Sharaf*, Professor of Environmental Medicine, Environmental and Occupational Medicine Department, National Research Centre, For her outstanding support, valuable time, and for her keen interest on me at every stage of this research. Her timely advice, meticulous scrutiny, scholarly advice and scientific approach have helped me to a very great extent to accomplish this task.

I am also immensely grateful to *Assist. Prof. Dr. Gehan Moubarz Mahdy*, Assistant Professor of Environmental Biochemistry and

Molecular Biology, Environmental and Occupational Medicine Department, National Research Centre, who was behaving with the utmost courtesy towards me throughout the whole work providing me the attention, care, and advice. She had an extremely important role in this study, specially in genetics and clinical process.

I am sincerely thankful to *Dr. Ahmed Mamdouh AbdelGwad*, Lecturer of Environmental Medical Science, Faculty of Graduate Studies and Environmental Research, Ain Shams University, for his continuous help throughout the course of this work.

My sincere thanks to *Prof. Dr. Noha Muhammad Hegazy Ibrahim*, Professor of Occupational Medicine, Department of Environmental and Occupational Medicine, for her kind and careful supervision, support, and valuable guidance all through the work.

With all the gratitude, I extend my thanks and appreciation to *Prof. Dr. Ehab Ragaa Abdel Raouf*, Professor of Clinical Genetics and NeuroPediatrics, founder and director of Learning Disability and Neuro Rehabilitation Clinic, Center of Excellence of Medical Research, National Research Center, who offered me the precious golden seed of this study, through allowing me to use his professionally organized clinic, creating great opportunities, that would have not arisen without his patience in supporting me overcoming the odds. I am very grateful for him, for adopting and embracing the study.

I am profoundly grateful to *Dr. Amal Elsaeid*, Researcher of Clinical Genetics at Department of Children with Special needs, for her constant support and encouragement throughout my study, specially during the clinic times, she sincerely helped and supported me through the work with the diagnosis and selection of children's cases.

I would like to extend my thanks to *Prof. Dr.* Sohair koraa, Egyptian Atomic Energy Authority for her effort in chemistry analysis. Appreciations.

I would like to express my appreciation to the patients, and their families, for their mindful cooperation.

Sara Said El Gammal

## Dedication

#### To:

This study is wholeheartedly dedicated to my beloved family members specifically my parents, who have been always my source of inspiration and strength, my kids (Nadin, Mahmoud and Farida) and my husband who continually provide their moral, spiritual, and emotional support.

## List of Contents

| Title   | Page No.     |
|---|--------------|
| List of Tables                                  | i            |
| List of Figures                                 | ii           |
| List of Abbreviations                           | iv           |
| Abstract  | viii         |
| Introduction                                    | 1            |
| Aim of the Study                                | 4            |
| Review of Literature                            |              |
| Chapter I: Aluminium and Pollution              | 5            |
| Aluminium                                       | 7            |
| Sources of Aluminium                            | 8            |
| Exposure to aluminium                           | 8            |
| Routes of aluminium exposure                    | 11           |
| Systemic distribution and excretion             | 13           |
| Aluminium toxicity                              | 14           |
| Aluminum and the brain                          | 16           |
| Effect of Aluminium exposure on glial and astro | cyte cells17 |
| Effect of aluminium on mitochondrial cells      | 20           |
| Blood brain barrier (BBB)                       | 23           |
| Oxidative stress                                | 24           |
| Role of oxidative stress in autism              | 26           |
| Nitric oxide                                    | 30           |
| Autism and nitric oxide                         | 33           |
| Malondialdehyde                                 | 34           |

| Autism and MDA  | 36 |
|---|----|
| Chapter II: Glutathione S-Transferases  | 39 |
| Definition  | 39 |
| Classification  | 39 |
| Structure   | 39 |
| Function  | 40 |
| Role of GST in autism   | 41 |
| GSTs gene polymorphism  | 42 |
| Mu Class  | 44 |
| Theta class   | 45 |
| Chapter III: Autism Spectrum Disorder   | 47 |
| Definition  | 47 |
| Prevalence  | 47 |
| Causes and Risk factors   | 49 |
| Conception Period, Prenatal and Perinatal Risk factors                            | 50 |
| Environmental chemical and toxicant factors                                       | 52 |
| Genetics  | 55 |
| Gene-Environment Interactions   | 56 |
| Clinical pictures   | 57 |
| Mechanism of autism   | 59 |
| Diagnostic assessment of autism   | 59 |
| Treatment of autism   | 63 |
| 1. Behavior and Communication Approaches  | 63 |
| 2. Complementary and Alternative Medicine Treatments (CAM) and Dietary Approaches | 66 |
| Autism and Antioxidants   | 69 |
| Vitamin E   | 70 |
| Vitamin C   | 72 |

| Zinc                 | 76  |
|----------------------|-----|
| 3. Medication        | 78  |
| Subjects and Methods | 81  |
| Results              | 101 |
| Discussion           | 120 |
| Summary              | 140 |
| Conclusion           | 145 |
| Recommendations      | 147 |
| References           | 148 |
| Appendices           | 206 |
| Arabic Summary       |     |

## List of Tables

| Table No          | . Title   | Page No.         |
|-------------------|---|------------------|
| <b>Table (1):</b> | General characteristics of the studied groups   | 101              |
| <b>Table (2):</b> | Classification of children with ASD according the Childhood Autistic Rating Scale (CARS)  | •                |
| <b>Table (3):</b> | Comparison between children with ASD a control group regarding the levels of Alum the oxidative stress markers and GST e activity   | ninum,<br>nzyme  |
| <b>Table (4):</b> | Comparison between children with ASD regarding Aluminium levels and the ox stress markers before supplements                        | idative          |
| <b>Table (5):</b> | Distribution of the GST gene polymorg among autistic and control children   |                  |
| <b>Table (6):</b> | Glutathione S-transferase (GST) enzyme a with respect to GSTM1 and GSTT1 gen among autistic and control children                    | otypes           |
| <b>Table (7):</b> | Blood levels of oxidative stress biom (MDA& NO) with respect to GSTM1 and Ogenotypes among autistic and control children            | GSTT1            |
| <b>Table (8):</b> | Aluminum mean level excreted in hair with a to GSTM1 and GSTT1 genotypes among a and control children                               | autistic         |
| <b>Table (9):</b> | The aluminum, the oxidative stress markers GST enzyme activity and CARS score children with ASD before and after antiox supplements | among<br>kidants |

## List of Figures

| Fig. No.            | Title Page   | No. |
|---------------------|--|-----|
| Figure (1):         | Sources and sinks of heavy metals  | 6   |
| Figure (1):         | Aluminium cooking utensils   |     |
| · ·                 | <u> </u>   |     |
| Figure (3):         | Shows four different types of glial cell found in the central nervous system |     |
| Figure (4):         | Effects of aluminium on astrocytes an  |     |
| rigure (4):         | microglia  |     |
| Figure (5):         | Mitochondria structure   |     |
| Figure (6):         | Segment A: the blood brain barrier of  |     |
| rigure (0).         | healthy individuals. Segment B: The bloo                                     |     |
|                     | brain barrier in ASD patients  |     |
| Figure (7):         | Schematic representation of NO signallin                                     |     |
| g                   | pathways in physiological conditions   | _   |
| Figure (8):         | Oxidation of the membrane and release of                                     |     |
| <b>3</b>            | malondialdehyde  |     |
| Figure (9):         | MDA formation and metabolism   | 37  |
| Figure (10):        | Structure of GST enzyme  | 40  |
| Figure (11):        | Structure of vitamin E   |     |
| Figure (12):        | The mechanism of vitamin E (alpha  |     |
| 8 /                 | tocopherol)-mediated low-densit  |     |
|                     | lipoprotein lipid peroxidation   |     |
| <b>Figure</b> (13): | Effects of vitamin C   | 73  |
| <b>Figure (14):</b> | Vitamin C and vitamin E antioxidants vs                                      | s.  |
|                     | oxidative damage   | 75  |
| <b>Figure (15):</b> | Antioxidants actions of zinc   | 78  |
| <b>Figure (16):</b> | Standard curve of MDA  | 97  |
| <b>Figure (17):</b> | Frequency distribution of children wit                                       | h   |
|                     | ASD according to birth order   |     |
| Figure (18):        | Frequency distribution of father   | s'  |
|                     | occupation of ASD and control children                                       |     |

## List of Figures Cont...

| Fig. No.            | Title   | Page No.                          |
|---------------------|---|-----------------------------------|
| Figure (19):        | Frequency distribution of occupation of ASD and control chil  |                                   |
| Figure (20):        | Source of drinking water of child   |                                   |
| Figure (21):        | Scatter plot correlation<br>Aluminum hair levels and glutar<br>transferase enzymatic activity<br>children with ASD. | thione–S-<br>among                |
| Figure (22):        | Scatter plot correlation between stress markers (MDA& NO) a enzyme activity.  | and GST                           |
| Figure (23):        | Scatter plot correlation between a hair levels and the Childhood Rating Scale (CARS) among child ASD.               | luminum<br>Autism<br>dren with    |
| Figure (24):        | PCR product for GSTT1, GSTM globulin genes separated by agreelectrophoresis stained with bromide                    | I1 and β<br>arose gel<br>ethidium |
| <b>Figure (25):</b> | Percentages of CARS scale among with ASD before and after an  |                                   |
|                     | supplements   |                                   |