# بسم الله الرحمن الرحيم

∞∞∞

تم رفع هذه الرسالة بواسطة / مني مغربي أحمد

بقسم التوثيق الإلكتروني بمركز الشبكات وتكنولوجيا المعلومات دون أدنى مسئولية عن محتوى هذه الرسالة.

| |
|---|
| ملاحظات: لا يوجد |

# Intralesional methotrexate for treatment of alopecia areata: clinical evaluation and effect on lesional TNF alpha

Thesis

*Submitted in Partial Fulfillment of the M.Sc. Degree in*
***Dermatology, Venereology and Andrology***

By

**Rodaina Mohamed Farag**
*M.B.B.Ch., 2017*

Supervised by

**Prof. Dr. Samar Abdallah Salem**
*Professor of Dermatology, Venereology and Andrology*
*Faculty of Medicine - Ain Shams University*

**Assist. Prof. Dr. Ahmed Abdelfattah Afify**
*Assistant Professor of Dermatology, Venereology and Andrology*
*Faculty of Medicine - Ain Shams University*

**Prof. Dr. Walid Abd Elhady Ahmed**
*Assistant Professor of Clinical Pathology*
*Faculty of Medicine – Ain Shams University*

Faculty of Medicine
Ain Shams University
2022


سورة البقرة الآية: ٣٢

# Acknowledgment

*First and foremost, I feel always indebted to* **ALLAH,** *the Most Kind and Most Merciful.*

*I'd like to express my respectful thanks and profound gratitude to* ***Prof. Dr. Samar Abdallah Salem,*** *Professor of Dermatology, Venereology and Andrology, Faculty of Medicine - Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

*I am also delighted to express my deepest gratitude and thanks to* ***Assist. Prof. Dr. Ahmed Abdelfattah Afify,*** *Assistant Professor of Dermatology, Venereology and Andrology, Faculty of Medicine - Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

*I am deeply thankful to* ***Prof. Dr. Walid Abd Elhady Ahmed,*** *Assistant Professor of Clinical Pathology, Faculty of Medicine – Ain Shams University, for her great help, active participation and guidance.*

*I would like to express my hearty thanks to all my family for their support till this work was completed.*

*Last but not least my sincere thanks and appreciation to all patients participated in this study.*

Rodaina Mohamed Farag

# List of Contents


| Title | Page No. |
|---|---|
| List of Tables | i |
| List of Figures | iii |
| Introduction | 1 |
| Aim of the Work | 5 |
| Review of Literature | |
| Overview of Alopecia Areata | 6 |
| Methotrexate | 36 |
| Patients and Methods | 42 |
| Results | 52 |
| Case Presentations | 86 |
| Discussion | 93 |
| Conclusion and Recommendation | 106 |
| Summary | 107 |
| References | 110 |
| Arabic Summary | — |

# List of Tables


| Table No. | Title | Page No. |
|---|---|---|
| **Table (1):** | Description of personal and clinical characteristics of alopecia areata patients | 52 |
| **Table (2):** | Risk factors among alopecia areata patients | 53 |
| **Table (3):** | Comparison of SALT score before treatment, 3 months and 6 months after intralesional methotrexate treatment | 54 |
| **Table (4):** | Percentage of improvement of SALT score 3 and | 57 |
| **Table (5):** | Comparison of lesional Tumor necrosis factor-alpha level and 3 months after intralesional after methotrexate treatment | 58 |
| **Table (6):** | Relation between SALT score before treatment with intralesional methotrexate with clinical characteristics | 61 |
| **Table (7):** | Relation between SALT score 3 months after intralesional methotrexate and personal and clinical characteristics | 63 |
| **Table (8):** | Relation between SALT score 6 months after intralesional methotrexate and personal and clinical characteristics | 65 |
| **Table (9):** | Relation between percentage of change of SALT score 3 months after intralesional methotrexate with the personal and clinical characteristics of alopecia areata patients | 67 |
| **Table (10):** | Percentage of SALT score improvement 6 months in relation to 3 months after intralesional methotrexate with personal and clinical characteristics of alopecia areata patients | 69 |
| **Table (11):** | Correlation between SALT score before treatment, 3 months and 6 months after intralesional methotrexate and personal and disease characteristics | 70 |

# List of Tables *(Cont...)*


| Table No. | Title | Page No. |
|---|---|---|
| **Table (12):** | Relation between lesional TNF-α tissue level before intralesional methotrexate and personal and clinical characteristics of alopecia areata patients | 74 |
| **Table (13):** | Relation between lesional TNF-α tissue level 3 months after intralesional methotrexate and personal and clinical characteristics of alopecia areata patients | 76 |
| **Table (14):** | Relation between percentage of change of lesional TNF-α tissue 3 months after intralesional methotrexate and personal and clinical characteristics of alopecia areata patients | 78 |
| **Table (15):** | Correlation between lesional TNF-α level before and 3 months after intralesional methotrexate and age, disease duration and age at disease onset of alopecia areata patients. | 79 |
| **Table (16):** | Correlation between intralesional methotrexate treatment and dermoscopic findings among alopecia areata patients | 84 |

# List of Figures


| Fig. No. | Title | Page No. |
|---|---|---|
| **Figure (1):** | Pathogenesis of alopecia areata and immune privilege | 14 |
| **Figure (2):** | Clinical types of alopecia areata | 17 |
| **Figure (3):** | Visual aid (Olsen/Canfield) for estimating percentage scalp hair loss SALT score | 18 |
| **Figure (4):** | Nail changes in alopecia areata | 20 |
| **Figure (5):** | Dermoscopic features of alopecia areata; yellow dots | 22 |
| **Figure (6):** | Microscopic aspects of alopecia areata. Longitudinal histological section of AA | 23 |
| **Figure (7):** | Algorithm for treatment of alopecia areata | 35 |
| **Figure (8):** | Dermlite dl3® dermoscope | 47 |
| **Figure (9):** | Comparison of SALT score before treatment, 3 months and 6 months after intralesional methotrexate treatment. | 55 |
| **Figure (10):** | Comparison of lesional TNF-α level before and 3 months after intralesional methotrexate | 59 |
| **Figure (11):** | Correlation between SALT score 3 months after intralesional methotrexate and the age of patients | 71 |
| **Figure (12):** | Correlation between SALT score 6 months after intralesional methotrexate and the age of patients | 71 |
| **Figure (13):** | Correlation between SALT score before intralesional methotrexate and the age at disease onset | 72 |
| **Figure (14):** | Correlation between SALT score 3 months after intralesional methotrexate and the age at disease onset | 72 |

# List of Figures *(Cont...)*


| Fig. No. | Title | Page No. |
|---|---|---|
| **Figure (15):** | Correlation between SALT score 6 months after intralesional methotrexate and the age at disease onset | 73 |
| **Figure (16):** | Correlation between TNF-α tissue level before treatment and duration | 80 |
| **Figure (17):** | 54 years old, female with AA of 8 years duration | 86 |
| **Figure (18):** | 37 years old, female with AA of one month duration | 87 |
| **Figure (19):** | 10 years old, male with AA of 2 months' duration | 88 |
| **Figure (20):** | 39 years old, female with AA of two years duration. Duration of current episode is two months | 89 |
| **Figure (21):** | 27 years old, male with AA of fifteen years duration. Duration of current episode is one month | 90 |
| **Figure (22):** | 11 years old, female with AA, of one-month duration. Patient relapses in the period of follow up | 91 |
| **Figure (23):** | 22 years old, female with AA, of 12 years' duration. Duration of current episode is one month | 92 |

# List of Abbreviations

| Abb. | Full term |
|---|---|
| *AA* | *Alopecia Areata* |
| *AF* | *Ablative fraction* |
| *AICAR* | *5-aminomidazole-4-carboxamide ribonucleotide* |
| *ATIC* | *Aminoimidazole-4-Carboxamide transformylase IMP cyclohydroxylase* |
| *CBC* | *Complete blood count* |
| *CTLA-4* | *Cytotoxic T lymphocyte –associated protein 4* |
| *DHFR* | *Dihydrofolate reductase* |
| *ELISA* | *Enzyme linked immunosorbent assay* |
| *FDA* | *Food and drug association* |
| *GM-CSF* | *Granulocye macrophage colony stimulating factor* |
| *GWAS* | *Genome wide association study* |
| *ICAM* | *Intercellular adhesion molecules* |
| *IFN-γ* | *Interferon gamma* |
| *IKZF4* | *Ikaros family zinc finger 4* |
| *IL* | *Intralesional* |
| *IL2RA* | *Interleukin 2 receptor subunit alpha* |
| *ILCS* | *Intralesional corticosteroids* |
| *IP* | *Immune privilege* |
| *JAK* | *Janus kinase* |
| *KA* | *Keratoacanthoma* |
| *MCH* | *Melanin- concentrating hormone* |
| *MCHR2* | *Melaning Concentrating hormone receptor* |
| *MF* | *Mycosis Fungoides* |
| *MICA* | *Major histocompatibility class I- related chain A* |
| *MTX* | *Methotrexate* |
| *MTZ* | *Microthermal treatment zone* |
| *NAF* | *Non-ablative fraction* |
| *NBUVB* | *Narrow band ultraviolet B* |

# List of Abbreviations *(Cont...)*

| Abb. | Full term |
|---|---|
| *NKG2D* | *Natural killer Group 2 D* |
| *OD* | *Optical Density* |
| *PCALCL* | *Primary Cutaneous anaplastic large cell lymphoma* |
| *PRDX5* | *Peroxiredoxin- 5* |
| *PRP* | *Platelet rich plasma* |
| *PTPN22* | *Protein tyrosine phosphate N22* |
| *PUVA* | *Psoralen ultraviolet A* |
| *RAET IL* | *Retinoic acid early transcript protein* |
| *RXR* | *Retinoid X receptor* |
| *SCC* | *Squamous cell carcinoma* |
| *STAT* | *Signal transducer and activator of transcription* |
| *STX17* | *Syntaxin-17* |
| *THF* | *Tetrahydrofolate reductase* |
| *TNF-α* | *Tumor necrosis factor alpha* |
| *TS* | *Thymidylate Synthetase* |
| *ULBP3* | *UL-16- binding protein* |

# INTRODUCTION

Alopecia areata (AA) is a nonscarring, autoimmune, inflammatory hair loss on the scalp and/or body ***(Rajabi et al., 2018).*** AA may occur as an acute self-limiting disorder with one to five patches that resolve within 6-12 months or as a chronic disorder with multiple patches relapsing and remitting over many years ***(Cranwell et al., 2018).***

Recognized subgroups of this disease include those patients with the complete absence of terminal scalp hair (alopecia totalis) and those patients with total loss of terminal scalp and body hair (alopecia universalis) ***(Pratt et al., 2017).***

Although few studies of incidence and prevalence have been performed, AA has a reported incidence of 0.1-0.2% with a lifetime risk of 1.7% with men and women being affected equally ***(Villasante et al., 2015).***

Alopecia Areata is the third most common childhood dermatosis with a female to male ratio of 1.5:1 and is most commonly associated with atopic dermatitis (32.7%) ***(Wohlmuth et al., 2018).*** It affects both sexes and all age groups ***(Majid et al., 2018).***

The pathogenesis of AA is unknown despite the presence of evidences suggesting the link between lymphocytic infiltration of the hair follicle and the disruption of the hair cycle provided by a combination of multiple factors including

cytokine release, cytotoxic T-cell activity, and apoptosis ***(Gohary et al., 2017).***

The persistence of AA lesions is attributed to disequilibrium in the production of cytokines, with a relative excess of pro-inflammatory and Th1 types cytokines versus anti-inflammatory cytokines, as shown in human scalp biopsies ***(Halilovic et al., 2018).*** Positive family history of AA has been reported in approximately 10-42% of patients ***(Darwin et al., 2018)***.

Some patients show hair regrowth spontaneously without medical intervention, many can be managed with topical or intralesional treatment alone, no systemic agents are currently approved for use by Food and Drugs Administration or Therapeutic Goods Administration ***(Lai et al., 2018).***

Tumor necrosis factor α (TNF-α, also known as cachectin) is a strong pro-inflammatory cytokine which plays a key role in the immune system during inflammation, cell proliferation, differentiation, and apoptosis. It was first described by Carwell et al., in 1975 as a cytokine which showed significant cytotoxic activity after stimulation of the immune system, and, thus, caused tumor necrosis ***(Kany et al., 2019)***.

This cytokine is synthesized in epidermal keratinocytes along with several other cytokines and is known to be a very potent inhibitor of proliferation. The changes in serum TNF-α levels were found in many diseases, such as psoriasis and systemic lupus

erythematosus. In some of these diseases, serum TNF-α concentration correlated with activity and intensity of the disease, and may be used as a prognostic factor ***(Baliwag et al., 2015).***

In addition to high serum level of TNF-α in alopecia areata patients, it was found that tissue level was significantly higher than controls. However, lesional and non-lesional skin tissue level was not significantly different in the same patient. This supports that alopecia areata is a systemic disease ***(Gohary et al., 2017).***

Methotrexate binds dihydrofolate reductase (DHFR) with high affinity for enzymes that require folate cofactors, including thymidylate synthetase (TS) and 5-aminoimidazole-4- carboxamide ribonucleotide (AICAR) transformylase. The inhibition of TS, induced by MTX, interferes with DNA synthesis in actively dividing cells, and the increase of AICAR enzyme system, which plays a key part in the purine metabolism of the cell, leads to enhanced release of adenosine into the blood ***(Markandeyan et al., 2016).***

Adenosine is released into the extracellular space and, among multiple anti-inflammatory actions, inhibits white blood cell accumulation, leads to a reduction in TNF-α and IFN-γ synthesis, and inhibits a variety of monocyte, macrophage, and T-cell activities. This action might explain the effect of MTX in AA ***(Alsufyani et al., 2017).*** In addition, MTX was found to have a role in inhibiting JAK/ STAT pathway ***(Thomas et al., 2017).***

Intralesional Methotrexate showed comparable results to intralesional triamcinolone in treatment of patchy alopecia areata with minor side effects compared to triamcinolone ***(Hamdino et al., 2018).***

Minor side-effects have been reported in the studies relating to IL-MTX we have reviewed, namely transient post-injection pain. These minor side-effects appear unrelated to the treated pathology or dose used. Serious side effects, if any, is likely due to pre-existing renal failure contributing to serum accumulation levels and subsequent toxicity ***(Searle et al. 2020).***