A clinical and immunological study of Phototoxic doses of ultraviolet A for treatment of alopecia areata:

A randomized controlled clinical trial

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By

Solwan Ibrahim AbdelRaouf ElSamanoudy

Supervised by

Prof. Dr. Hoda Mohamed Rasheed
Professor of Dermatology
Faculty of Medicine
Cairo University

Prof. Dr. Nermine Hamdy ElEishi
Professor of Dermatology
Faculty of Medicine
Cairo University

Dr. Vanessa Galal Hafez
Lecturer of Dermatology
Faculty of Medicine
Cairo University

Prof. Dr. Olfat Gameel Shaker
Professor of Medical Biochemistry
Faculty of Medicine
Cairo University

Faculty of Medicine
Cairo University
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ABSTRACT

BACKGROUND: Alopecia areata (AA) is a disease of the hair follicles with multifactorial etiology and a strong component of autoimmune origin. The mechanisms that lead to hair loss in AA are still unclear. An aberrant pattern of cytokine expression of the T-helper Th1cell type has been detected. So far, IFN-gamma, interleukins, TNF-alpha, are cytokines that are well known to play a major role in the pathogenesis of the disease**.**

OBJECTIVE: To determine the efficacy and safety of using the phototoxic doses of ultraviolet A(UVA) after application of topical 8- methoxypsoralen in the treatment of extensive and resistant cases of AA.

METHODS: **40** patients fitting the inclusion criteria were randomly allocated into one of two interventional groups: Group A (20 patients): receiving phototoxic doses of UVA after topical application of psoralen (test therapy group).Group B (20 patients): receiving potent intralesional corticosteroids (conventional therapy group).Each group was treated for 3 months and followed up for an additional 3 months. A biopsy was taken at baseline and at the end of treatment (3 months to compare the level of expression of IFN-γ, IGF-1 and TGF-ß1 before and after therapy

RESULTS: At 3 months, No significant difference was found between phototoxic and corticosteroid groups as regards the mean SALT nor the percent change of SALT from baseline (p= 0.808 and 0.204 respectively). Although not statistically significant, the percent change of IFN-γ showed a mean reduction in both groups, with patients receiving phototoxic doses of PUVA showing a tendency towards higher mean reduction **{**-35.97% ± 18.81, 95% CI: [2.37 to 3.83]} in comparison to patients receiving intralesional corticosteroids {-29.03 ± 17.80, 95% CI: [-37.36 to -20.7]}.At 6 months No significant difference was found between phototoxic and corticosteroid groups as regards the mean SALT nor the percent change of SALT from baseline (p= 0.808 and 0.735 respectively) Treatment success was achieved by 45% of patients in both groups.

CONCLUSION: The current study offers a proof that phototoxic regimen of PUVA exerts an immunomodulatory role and that it deserves to be placed among therapeutic tools used in the treatment of the of AA, owing to both its efficacy and safety.

(Key Words: Alopecia Areata, RCT, phototoxic therapy, IFN-γ, IGF-1 TGF-B1, and UVA**).**

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LIST of Abreviations

AA: Alopecia Areata
AAI: Alopecia areata incognito
ADTA: Acute diffuse and total alopecia
AIRE: Autoimmune regulator
APECED: Autoimmune PolyeEdocrinopathy Candidiasis Ectodermal Dysplasia
APS: Autoimmune Polyglandular Syndrome
AT: Alopecia Totalis
AU: Alopecia Universalis
BP: Binding Proteins
CD: Clusteral Differentiation
CGRP: Calcitonin Gene–Related Peptide
CKI: Cycline dependent kinase Inhibitor.
DNCB: Dinitrochlorobenzene
DPCP: Diphenylcyclopropenone
FFA: Frontal Fibrosing Alopecia
HF: Hair follicle
HLA: Human Leukocyte Antigen
ICAM: Inter Cellular Adhesion Molecule
IFN- γ: Interferon-gamma
IGF-1: Insulin like Growth Factor 1
IL: Interleukin
ILCs: Intralesional corticosteroids
IGFBP3: Insulin-like growth factor binding protein 3
IP: Immune privilege
IP-10: Interferon inducible Protein-10
IR: Immunoreactivity
IRS: Insulin receptor substrate
MCV: Mean Cell Volume
MHC: Major histocompatibility complex
MIC: Major histocompatibility complex class I chain-related gene A

MIG: Monokine induced by Interferone gamma
MOP: Methoxypsoralen
NGF: nerve growth factor
OPC: Outpatient clinic
ORS: outer root sheath
PUVA: Psoralen and Ultraviolet A
SADBE: squaric acid dibutylester syndrome
SALT: Severity of Alopecia areata Tool
TGF-β1:Transforming Growth Factor Beta 1
TNF-α: Tumor necrosis factor alpha
VIP: Vasoactive intestinal peptide
α-MSH: alpha Melanocyte Stimulating Hormone
J:Joule
cm^2 : centimeter squared
Rec: Research Ethical Committee

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INTRODUCTION

Alopecia areata (AA) is a disease of the hair follicles with multifactorial etiology and a strong component of autoimmune origin. It is characterized by non-scarring hair loss on the scalp or any hair-bearing surface. A wide range of clinical presentations can occur from a single patch of hair loss, to complete loss of hair on the scalp alopecia totalis (AT), or the entire body alopecia universalis (AU) ***(Alsantali, 2011).***Particularly severe, resistant and chronic cases of AA might cause considerable psychological distress for the affected individuals (***Garg & Messenger, 2009).***

The mechanisms that lead to hair loss in AA are still unclear. Several lines of evidence, however, point towards soluble mediators that are involved in the pathogenetic process. In AA, prominent features are a peri- and intrabulbar accumulation of mainly CD4+ and CD8+ T lymphocytes during the active stage of the disease. An aberrant pattern of cytokine expression of the Th1 T-helper cell type has been detected. So far, IFN-γ, interleukins, TNF-α, are cytokines that are well known to play a major role in the pathogenesis of the disease ***(Gregoriou et al., 2010).*** Those mediators may directly influence the growth and differentiation of hair follicle cells, thus producing a 'switch-off' signal in the hair cycle, eventually leading to hair growth arrest as long as the lymphocytic infiltrate persists ***(Hoffman et al., 1996).***

Another immunological aspect in the pathogenesis of AA is the immune privilege that is chiefly characterized by a very low level of expression of MHC class Ia antigens and by the local production of potent immunosuppressive agents, such as alpha-Melonocyte Stimulating Hormone (α MSH) , insulin like growth factor-1 (IGF-1) and transforming growth factor (TGF-ß1). It was proved that they can suppress the natural killer (NK)

CD8+ lymphocytes attacking the hair bulb ***(Ito et al., 2004)***, moreover they downregulate IFN-γ-induced ectopic MHC class I expression in human anagen hair bulbs in vitro ***(Paus et al., 2003).*** IFN-γ is known to be able initiate the disease process by destroying this immune privilege mechanism ***(Gilhar & Paus, 2007)***, also an essential requirement for IFN-γ-mediated Th1 activation in the induction of AA has been proven (***Freyschmidt-Paul et al., 2006)***

Various therapeutic agents have been described for the treatment of AA, but none are curative or preventive. Phototherapy in the form of topical psoralen and ultraviolet A (PUVA) has been a well documented therapy for AA since 1978 ***(Healy & Rogers, 1993).*** The success of such therapy might be attributed to the immunosuppression caused by PUVA mediated by several mechanisms, including depletion of Langerhan's cells and their antigen presenting capacity. Also induction of apoptosis of T lymphocytes and decreasing production of several cytokines might be other mechanisms of action ***(Freyschmidt-Paul et al., 2002)***

Aim of work

The aim of the current study is to determine the efficacy and safety of using the phototoxic doses of UVA after application of topical 8-methoxypsoralen (MOP) in the treatment of alopecia areata, in comparison to conventional therapy with intralesional corticosteroids (ILCs).

The study also aims at explaining the possible underlying immunological changes through measurement of tissue levels of IFN-γ, IGF-1 and TGF-beta1 both before and after treatment in both groups.

CHAPTER 1

ALOPECIA AREATA

Alopecia areata (AA) is a non scarring autoimmune, inflammatory scalp, and/or body hair loss condition. It affects up to 2% of the population and it is characterized by patchy hair loss. It can affect the entire scalp (alopecia totalis) or cause loss of all body hair (alopecia universalis***)(Alkhalifah et al., 2010).***

I. Epidemiology:

Alopecia areata is the most frequent cause of inflammation-induced hair loss, responsible for 0.7% to 3.8% of dermatology clinics visits ***(Alkhalifah et al., 2010)***. Depending on ethnic background and geographic area, the prevalence of alopecia areata is 0.1 to 0.2%, with a calculated lifetime risk of 2% ***(Safavi et al., 1995)***. Alopecia areata affects both children and adults and hair of all colors ***(Finner, 2011).*** There is generally no sex predilection ***(Wasserman , et al., 2007)***., but more men were found to be affected in one study involving a group of subjects who were 21 to 30 years of age ***(Kyriakis et al., 2009).*** Although the disorder is uncommon in children under 3 years of age, children constitute approximately 20% of patients with AA ***(Nanda et al., 2002)***. Most patients are relatively young: up to 66% of patients present with their first patch of AA before the age of 20 ***(Price et al., 1999)***, and only 20% are older than 40 years of age ***(Lu et al., 2006).***

II. Pathogenesis:

Alopecia areata is a complex disease ***(Fig. 1)*** arising from the concerted action of multiple genes and is possibly influenced by environmental factors as well ***(Petukhova et al., 2011***).

It has been hypothesized that AA is an organ-specific autoimmune disease with genetic predisposition and an environmental trigger ***(McDonagh & Tazi-Ahnini, 2002).***

Figure (1): **Multifactorial etiology of alopecia areata *(Amin & Sachdeva, 2013).***

A. Genetic Factor:

Alopecia areata may occur concurrently or sequentially in both monozygotic and fraternal twins. A concordance rate of 55% has been reported in monozygotic twins. This leaves much room for the role of the environment in AA pathophysiology ***(Jackow et al, 1998)***. It is likely that AA (similar to other autoimmune diseases) is polygenic, i.e. there are multiple susceptibility genes that interact with environmental factors ***(McDonagh & Tazi-Ahnini, 2002)***. A genetic study by ***Yang*** *and* ***colleagues (2004)*** found that 8.4% of the patients had a positive family history of AA, suggesting a polygenic additive mode of inheritance.

The family history of AA in patients ranges from10% up to 42% of cases. Alopecia areata may occur in diseases linked to chromosome 21 including Down's syndrome and autoimmune poly endocrinopathy. The risk of alopecia areata is increased to 30% in patients with autoimmune poly endocrinopathy candidiasis ectodermal dysplasia syndrome (APECED). This condition is associated with a mutation of the autoimmune regulator (AIRE) gene on chromosome 21q22 ***(McDonagh & Tazi-Ahnini, 2002)***.

AA AND THE MAJOR HISTOCOMPATIBILITY COMPLEX (MHC):

As with most autoimmune conditions, alopecia areata has an association with MHC antigens.

Human Leucocyte Antigen (HLA) class I molecules are expressed on virtually all nucleated cells and platelets and present antigens to CD8+ T cells. HLA class II molecules have three main subclasses (DR, DQ, and DP); they are found on specific immune cells, including B cells,

activated T cells, macrophages, keratinocytes and dendritic cell and present peptides to CD4+ T cells. Because class II molecules are associated with antigen presentation, many studies have focused on this area of the HLA molecule ***(Mari, 2004).***

An association between AA and HLA has been demonstrated. ***Kavak et al., (2000)*** reported patients with AA had HLAA1, HLA-B62, HLA-DQ1, and HLADQ3 ***(Ay Se et al., 2000)***.

HLA-DQ3 is associated with AA across multiple populations, and the frequency of HLA-DQ3 is increased in patients with early onset, severe disease. DR4 and DQ7 are associated with AT and AU, indicating that there is genetic heterogeneity between mild and severe disease ***(Wasserman et al., 2007)***.

These associations with HLA-DR and HLA-DQ suggest a role for T cells (both CD4 & CD8) in this disease as well as autoimmunity. Patients with AA have an increased frequency of auto antibodies to follicular structures; however, there is little consistency in which follicular structures are labeled by the antibodies ***(Gilhar and Kalish, 2006).***

It has been demonstrated that a Non-HLA molecule including the major histocompatibility complex class I chain-related gene A (MICA) is associated with AA. It could be a potential candidate gene and part of an extended HLA haplotype that may contribute to susceptibility and severity of this entity ***(Barahmani et al., 2006)***.