Effect of Different Types of Therapeutic Trauma on Vitiligo Lesions

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تأثير الأنواع المختلفة من الصدمات العلاجية على البهاق

رسالة مقدمة من

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ABSTRACT

Background:

Treatment of vitiligo represents a major dermatological challenge, therefore many efforts are exerted to introduce new treatment modalities. These modalities may act by changing certain cytokines and metalloproteinases.

Aim of work:

The aim of this study is to assess the efficacy of TCA chemical peel, dermapen and ablative fractional co_2 laser in treatment of stable non-segmental vitiligo and to detect their effects on IL-17 and MMP-9 levels using ELISA.

Patients and methods:

Thirty patients with stable, NSV and fulfilling the inclusion criteria were recruited in a randomized controlled study for a period of twelve months from March 2014- March 2015. Patients were subjected to complete medical history, detailed assessment of vitiligo and photography taking. They were randomly categorized into three equal groups. TCA peel (15% and 25% concentrations) was applied for the first group, dermapen machine (1mm and 2mm depths) was used for the 2^{nd} group and ablative fractional co_2 Laser (10 watt and 20 watt) was used for the 3^{rd} group. Skin biopsies were taken from treated areas and from control areas for which MMP-9 and IL-17 tissue levels were measured using ELISA technique.

Results:

It has been found that the thirty vitiligo patients had low basal tissue MMP-9 levels and high baseline IL-17 tissue levels. As regards the three different used modalities, all of them caused rise in MMP-9 as well as IL-17 levels and almost their levels were much more elevated with repetition of the previously mentioned traumatic procedures.

Conclusion:

Taking into consideration that elevated IL-17 levels is pathogenic, repeated trauma-based sessions are not preferred and single sessions will probably positively affect MMP-9 levels without marked elevation of IL-17.

TCA 25% peel proved to be the most effective modality both clinically and laboratory. These traumatic lines of treatment could be combined with other conventional therapies that may eliminate and control their possible harmful effect on inflammatory mediators and subsequently on vitiligo.

Keywords: ***Vitiligo, trauma, TCA, fractional CO_2 laser, dermapen, MMP-9 and IL-17.***

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Abbreviations

- **ACTH :** Adrenocorticotropic hormone
- **AIRE :** Autoimmune regulator
- **AMST :** Autologous melanocyte suspension transplant
- **ANA :** Anti-nuclear antibody
- **BB-UVB:** Broadband ultraviolet B light
- **Bcl-2:** B-cell lymphoma-2
- **BFGF :** Basic fibroblast growth factor
- **BG :** Blister graft
- **BSA :** Body surface area
- **cAMP :**cyclic Adenosine monophosphate
- **CAT:** Catalase gene
- **CD:** Cluster of differentiation
- **CIs:** Calcineurin inhibitors
- **CIT :** Collagen Induction Therapy
- **CLA :** Cutaneous lymphocyte-association antigen
- **CO2 :** Carbon dioxide
- **CRH :** Corticotrophin releasing hormone
- **CSs :** Corticosteroids
- **CTLA :** Cytotoxic T lymphocyte antigen
- **DNA :** deoxy ribonucleic acid
- **DOPA :** Dihydroxy-phenylalanine
- **DSCs :** Dermal stem cells
- **ECM :** Extracellular matrix
- **EGF :** Epidermal growth factor
- **ET :** Endothelin
- **FGF :** Fibroblast growth factor
- **FU :** Fluorouracil
- **GC-SF :** Granulocyte colony-stimulating factor
- **HGF:** Hepatocyte growth factor
- **HIV :** Human immunodeficiency virus
- **HLA:** Human leucocytic antigen
- **HMB 45 :** Human melanoma black 45
- **ICAM :** Intercellular adhesion molecules

- **IFN :** Interferon
- **IGF :** Insulin growth factor
- **IgG :** Immunoglobulin
- **ILs:** Interleukins
- **ILCSs:** Intralesional corticosteroids
- **IV :** Intravenous
- **kDa :** kilo Dalton
- **KGF :** Keratinocyte growth factor
- **KP :** Koebner's phenomenon
- **LM :** Laminin
- **LT:** Leukotrienes
- **MART :** Melanoma-associated antigen recognized by T cells
- **MAZ :** Microscopic ablation zone
- **MC1R :** Melanocortin 1receptor
- **MCHR :** Melanin-concentrating hormone receptor
- **MCZ :** Microscopic coagulation zone
- **MEL :** Monochromatic Excimer Laser
- **MENDs :** Microscopic epidermal necrotic debris
- **MHC-II :** Major histocompatibility complex
- **MITF :** Microphthalmia transcription factor
- **MMP :** Matrix metalloproteinases
- **MOP :** Methoxypsoralen
- **mRNA:** Methyl Ribonucleic acid
- **MSH :** Melanocyte Stimulating Hormone
- **MTZ :** Microthermal zones
- **Muse :** Multilineage Differentiating Stress Enduring
- **NALP1:** NATCH domain, Leucine-Rich Repeat-,and PYD-containing Protein 1
- **NB-UVB :** Narrowband-ultraviolet B light
- **NCC:** Neural crest cells
- **Nd-YAG :** Neodymium-doped: Yttrium Aluminium Garnet
- **NF-κβ :** Nuclear factor-Kappa beta
- **NGFR :** Nerve growth factor receptor
- **NK :** Natural killer
- **NSV :** Non-segmental vitiligo
- **PAR :** Protease-activated receptor
- **PCI:** Percutaneous collagen induction

- **PDGF :** Platelet derived growth factor
- **PG :** Punch graft
- **PGs :** Prostaglandins
- **Phe :** phenylalanine
- **PIH :** Post inflammatory hyperpigmentation
- **PL :** Polypodium leucotomos
- **Pmel17 :** Melanosomal matrix proteins
- **POMC :** Proopiomelanocortin
- **PRP :** Platelet rich plasma
- **PTPN22 :** Protein tyrosine phosphatase, non receptor type 22
- **PUVA :** Psoralen plus UVA
- **QOL:** Quality of life
- **RECK :** Reversion-inducing-cysteine-rich protein with kazal motifs inhibitor
- **ROS :** Reactive oxygen species
- **SCF :** Stem cell factor
- **SSRS :** Skin Stress Response System
- **STSG :** Split-thickness skin graft
- **SV :** Segmental vitiligo
- **TCA :** Trichloroacetic acid
- **TGF :** Transforming growth factor
- **Th cells:** T helper cells
- **TIMP :** Tissue Inhibitors of MetalloProteinases
- **TMP :** Trimethyl psoralen
- **TNF- α:** Tumor necrosis factor- α
- **Tregs :** T regulatory cells
- **TYR:** Tyrosinase
- **TYRP1& TYRP2 :** Tyrosinase-related protein 1 and 2
- **UVR:** Ultra violet rays
- **VEGF :** Vascular endothelial growth factor
- **VIDA :** Vitiligo disease activity

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Introduction

Vitiligo is an acquired, psychologically distressing disease with milky-white macules or patches ***(Ortonne and Passeron, 2012)***. Its pathogenesis is still obscure, inspite of multiple proposed mechanisms such as; cellular- and humoral-mediated changes, oxidative stress, apoptosis, melanocytorrhagia and a convergence hypothesis with simultaneous defects (***Falabella and Barona, 2008)***.

It has been found that there is a significant decrease in MMP-2 and 9 activities and expression in vitiligo which inhibit the migration of melanoblasts and melanocytes ***(Kumar et al., 2011)*.** Moreover, Th17 cells secrete IL-17, IL-6 and TNFα ***(Kolls and Linden, 2004)***. ***Swope et al., (1991)*** reported that TNF-α, IL-1 and IL-6 inhibit melanocyte proliferation and induce melanocyte apoptosis. IL-17 synergizes their inhibitory effect on melanocyte proliferation ***(Basak et al., 2009).***

Dealing with vitiligo is considered a stumbling block for many dermatologists and doctors as well as patients. While managing vitiligo, all available methods should be considered including; methods for repigmentation, inducing stability, camouflage and even depigmentation according to each individual case. Therefore, combination treatments and incorporation of new modalities are required. Treatment may be in the form of topical corticosteroids or calcineurin inhibitors, systemic steroids, antioxidants, phototherapy and Excimer laser. Surgical procedures as punch grafting for patients having localized, stable vitiligo refractory to other treatments ***(Hossani-Madani et al., 2011)***.

Koebner's phenomenon defined as the development of isomorphic lesions at traumatized uninvolved skin of patients with cutaneous diseases is reported in vitiligo ***(Gauthier, 1995)***. Others have reported that there is a reverse Koebner's phenomenon in vitiligo patients in which there is spontaneous repigmentation of vitiligo patches following skin grafts ***(Malakar and Dhar, 1998)***.

On the other hand, there is a wound- associated hyperpigmentation which could be explained by two processes; the first is pigmentary incontinence which occurs after destroying the basal cell layer resulting in accumulation of melanophages in the upper dermis, whereas the other process involves an epidermal inflammatory response induced by injury ranging from early tyrosine kinase induction to the late upregulation of growth factors, proteases, and extracellular matrix components, resulting in the release and oxidation of arachidonic acid to prostaglandins and leukotrienes. This leads to increase in

the synthesis of melanin and transfer of pigment to surrounding keratinocytes ***(Nicole et al., 2004)***.

These facts (wound-associated pigmentation together with the reverse Koebner's phenomenon) paved the way for the entrance of new modalities for vitiligo treatment:

Physical trauma induced by isolated dermabrasion or combination of dermabrasion and 5-Fluorouracil (5-FU) ointment led to perifollicular repigmentation ***(Sethi et al., 2007)***. This was explained by release of the inflammatory mediators and metalloproteinases, stimulating and facilitating melanocyte proliferation and migration following skin injury. Although, 5-FU has antimitotic activity on epidermal keratinocytes and tumoral cells, nontumoural melanocytes are much less vulnerable to 5-FU than keratinocytes are (***Tsuji and Karasek, 1986 & Gauthier et al., 2013)***.

Thermal trauma was performed using fractional laser therapy which induces secretion of various kinds of cytokines and growth factors which might play a role as mitogens for melanocytes during wound healing process. In addition, MMP-2 and MMP-9 known to enhance migration of melanocytes from adjacent normal skin are up regulated following laser therapy ***(Shin et al., 2012)***. Also, successful repigmentation of areas devoid of hair follicles (periungual areas and the dorsa of the feet) after ER: YAG laser skin ablation followed by topical application of 5-FU and Nb-UVB phototherapy has occurred ***(Anbar et al., 2006)***.

Chemical trauma (Chemical peeling) by phenol ***(Savant and Shenoy, 1999)*** and TCA (trichloroacetic acid) ***(Puri and Puri, 2012)*** showed perifollicular pigmentation in hairy areas and perilesional repigmentation in non hairy areas.

Objective & Aim of work

Aim of work is to evaluate the efficacy of TCA chemical peeling, dermapen and ablative fractional CO_2 laser in treatment of stable, non-segmental vitiligo and to compare their results, hence conclude the best method of cutaneous trauma in treatment of vitiligo. Evaluation is based on clinical assessment as well as performing ELISA for MMP-9 and IL-17. Other objective is postulating possible mechanisms by which TCA chemical peeling, dermapen and ablative fractional CO_2 laser induce pigmentation.

Chapter I: Vitiligo

▪ INTRODUCTION

Vitiligo is an acquired, multifactorial, depigmenting disorder which may have devastating psychological and social consequences and is characterized by the appearance of circumscribed white macules due to progressive loss of functional melanocytes in the epidermis ***(Mehta et al., 1973)***.

The word *'vitiligo'* may have evolved from the Latin word *vitium*, meaning a defect, ***(Carter, 1992)*** or *vitelius* signifying a calf's white patches ***(Fitzpatrick, 1964)***.

▪ EPIDEMIOLOGY

It affects 1–2% of the population worldwide, with no gender or racial predilection ***(Halder and Chappell, 2009)*** and the apparent female predominance was explained by their greater willingness to express concern about cosmetic issues. It usually starts during childhood or young adulthood ***(Kyriakis et al., 2009)*.**

▪ MELANOCYTES and MELANOGENESIS

Human body melanocytes arise from the neural crest cells (NCC) which originate from the embryonic germ layer named neuroectoderm. NCC are initially multipotent cells but gradually become lineage-restricted in developmental potential. Several populations of NCC (cranial, dorsal trunk, ventral trunk) give origin for melanoblasts of the skin ***(Betters et al., 2010 & Ernfors, 2010)***.

Thereafter, melanoblasts migrate over very long distances throughout the embryo aided by the presence of extracellular matrix formed by fibrillar proteins such as collagen. They also adhere to one another using adhesion molecules such as cadherins and adhere to matrix by integrins. They reside in the epidermis, hair follicles, oral mucosa, choroid of the eye, iris, and some internal sites, such as meninges and the inner ear (the stria vascularis) ***(Tachibana, 1999 & Brito and Kos, 2008)***. Finally, they proliferate and differentiate into melanocytes which in turn undergo maturation, acquire a dendritic morphology and start melanogenesis ***(Ernfors, 2010)***.