

# Clinical Correlation and Immunological Study about Th1 Cytokines Profile in Patients with Alopecia Areata

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## Abstract

Several studies have shown that within the cascade of pathogenesis of alopecia areata, cytokines and other molecules that coordinate cyclical hair growth play a crucial role. In this research, we attempt to review the immunological role of some of Th1 cytokines namely  $\gamma$ -INF and TNF- $\alpha$ , in the pathogenesis of alopecia areata, alongside with the exploration of their clinical correlation to disease severity. This is a case controlled study conducted upon fifty four patients (twenty nine males and twenty five females, mean age 32 years) complaining from alopecia areata, and thirty apparently healthy subjects (who are age and sex-matched with the patients group). All patients and the control subjects were investigated for serum concentrations of  $\gamma$ -INF, and TNF- $\alpha$  through a solid phase sandwich enzyme amplified sensitivity immunoassay (EASIA). Results about the serum concentrations of  $\gamma$ -INF and TNF- $\alpha$  evaluated in this study shows that there is a significant difference in the concentrations of these two cytokines between patients of alopecia areata and control group ( $P < 0.05$ ). Serum concentrations of  $\gamma$ -INF and TNF- $\alpha$  were also found to be logically clinically correlated to the size of affected area of hair loss among patients with severe type of alopecia areata.

**Keywords:** alopecia, cytokines, immunology, interferon, tumor necrosis factor.

## الخلاصة

أظهرت عدة دراسات أن السيتوكينات وجزيئات أخرى تنسق نمو الشعر الدوري وتلعب دوراً حاسماً داخل تتالي الحالة المرضية من الحاصة البقعية. في هذا البحث، نحاول استعراض الدور المناعي لبعض سيتوكينات Th1 هي  $\gamma$ -INF و TNF- $\alpha$  في الآلية المرضية الثعلبية، جنباً إلى جنب مع استكشاف ارتباطها السريري لشدة المرض. هذه دراسة للحالة والحالة المسيطرة أجريت على أربعة وخمسين مريضاً (الذكور تسعة والعشرين و الإناث خمسة وعشرين، متوسط عمر 32 عاماً) يشكون من الحاصة البقعية، ومجموع ثلاثين حالة سيطرة (الذين هم العمر والجنس متطابق مع مجموعة المرضى). تم التحليل المختبري في جميع المرضى و السيطرة الاصحاء لتركيزات المصل من  $\gamma$ -INF و TNF- $\alpha$  عن طريق الاختبار المناعي (EASIA) وكانت النتائج عن تركيزات مصل  $\gamma$ -INF و TNF- $\alpha$  التي تم تقييمها في هذه الدراسة تبين أن هناك فرق كبير في تركيزات هذه السيتوكينات بين المرضى من الحاصة البقعية ومجموعة السيطرة ( $P < 0.05$ ). كما وجدت تركيزات مصل  $\gamma$ -INF و TNF- $\alpha$  تكون مرتبطة ارتباطاً منطقياً سريرياً بحجم المنطقة المتأثرة من تساقط الشعر بين المرضى مع النوع الشديد من الحاصة البقعية.

**الكلمات المفتاحية:** الحاصة، السيتوكينات، علم المناعة، الانتروفيرونات، عامل الورم التثخري.

## Introduction

Alopecia areata is a skin condition that causes a sudden loss of patches of hair on the scalp or other hairy parts of the body [King, et.al.2008]. Patients with alopecia areata represent approximately 0.7% to 3.8% of all the patients attending dermatology clinics [Taisuke Ito.2013]. About one person in 50 will suffer from alopecia areata at some point in their life. It occurs in men and women of all races equally. In 1–2% of cases, the condition can spread to the entire scalp (alopecia totalis) or to the entire epidermis (alopecia universalis) [Maqdasi and Waiz .2014]. Initial presentation most commonly occurs in the late teenage years, early childhood, or young adulthood, but can happen with people of all ages [Petukhova, et.al.2010].

The genetics of alopecia areata is still poorly understood [McDonagh and Tazi-Ahnini.2002], some people have a genetic predisposition to the disease [Messenger, et.al.2012]. There are many findings confirm the association between alopecia areata and other auto-immune diseases [Friedmann .1981], common

autoantibodies shared by other autoimmune diseases and follicular components have been detected [Hordinsky and Ericson .2004].

The pathophysiology of alopecia areata is not fully identified yet [Arca ,*et.al.*2004], although most evidence supports the hypothesis that it is a T-cell mediated autoimmune disease of the hair follicle and that cytokines play an important role[Kasumagic-Halilovic and Prohic Karamelic .2010]. Alteration in T-lymphocyte function and cytokines secreted by T-cell subsets has been proposed in the immunopathogenesis of alopecia areata[Tembhre and Sharma.2013]. Previous data suggested that the initiation phase of alopecia areata is a heavily Th1-cytokines profile immune response [Bakry ,*et.al.*2014].

Over the past two decades, it has been suggested that normal hair follicles represent an immune privilege site [Gilhar and Kalish .2006; Amos Gilhar.2010]. Perhaps the most intriguing features of the normal hair follicle immune system are the very low expression of MHC class I antigens and the absence of MHC class II antigen expression in the proximal epithelium of anagen hair follicles [Amos Gilhar.*et.al.*2007]. It was further proposed that alopecia areata results from a collapse of hair follicle immune privilege[Islam.*et.al.*2014]. Th1 cytokine bias may be implicated in the pathogenesis of alopecia areata[Amos Gilhar.*et.al.*2005], CD8+ T cells act as the effector cells, with CD4+ T cell help[Gilhar .*et.al.*2002]. It is evident that the mechanism of hair follicle dysfunction in alopecia areata is immunological, controlled by activated T cells [David Norris.2014], and hair loss is associated with a perifollicular lymphocytic infiltrate, induced by interferon-gamma produced by T cells [Gilhar ,*et.al.*1999]. Strongly supports a role for Th1 cells in the pathogenesis of this condition [Kalish and Gilhar .2003].

Autoimmune attack of the bulbar region of anagen phase hair follicles by CD8+ T cells and Th1 cytokines has been proposed to result in hair loss in alopecia areata [Ghoreishi, *et.al.*2010]. Interferon (IFN)- $\gamma$  is a key cytokine implicated in the pathogenesis of alopecia areata [Taisuke Ito.2013]. It is secreted from Th1 lymphocyte, NK cells and macrophage. It is aberrantly expressed in alopecia areata through a Th1 mediated response. Among several actions IFN- $\gamma$  also deprives dermal papilla cells of their ability to maintain anagen hair growth [Stamatis Gregoriou, *et.al.* 2010], with consistent production of IFN- $\gamma$  within lesional skin [John Harris.2010]. It promotes the differentiation of naïve CD4+ T-cells to the Th1 subset and inhibits the proliferation of Th2 cells beside activating neutrophils and stimulates the cytolytic activity of NK cells [Mohammed Al-Saadi, *et.al.*2011]. This aberrant expression of IFN- $\gamma$  as a result of antigen dependent immune response suggesting that IFN- $\gamma$  is involved in the pathogenesis of alopecia areata [Katagiri .*et.al.*2006].

Tumor necrosing factors (TNF) are lymphokines which are capable of causing in vivo hemorrhagic necrosis of certain tumor cells. They are produced due to activation of T-cells and mast cells[Mufeed Ewadh .*et.al.*2014]. TNF- $\alpha$  is synthesized in epidermal keratinocytes along with several other cytokines and is known to be a very potent inhibitor of proliferation. TNF- $\alpha$  causes vacuolation of matrix cells within the follicle bulb and a decrease in the size of the hair matrix [Stamatis Gregoriou,*et.al.*2010]. TNF- $\alpha$  is expressed in many types of cells but primarily in macrophage cells in response to immunological challenges [Ware,*et al.*,1998]. It is a dangerous cytokine that incites the immune system to attack healthy tissues throughout the body. TNF- $\alpha$  plays a central role in the genetically programmed death (apoptosis) of hair cells in alopecia areata. And it is considered by

several leading researchers to be the most significant factor in hair cell death [Ruckert ,*et.al.*2000].

The target of this study is to reveal the immunopathological role of Th1 cytokines profile represented by  $\gamma$ -INF and TNF- $\alpha$ , in local Iraqi alopecia areata patients in comparison with healthy controls along with their clinical correlations.

## Materials and Methods

This is a case controlled study conducted at the Outpatient Clinic Department of Dermatology & Venereology- Merjan Teaching Hospital, Babylon, Iraq, between February and November 2014.

Fifty four patients (twenty nine males and twenty five female, mean age 32 years) complaining from alopecia areata were enrolled in this study. This group of alopecia areata patients was auxiliary divided into three subgroups according to the size of the affected area (lesional size): group 1 is composed of those patients who had affected area measuring less than 20 cm, group 2 surface area between 20 and 30 cm, group 3 affected surface area more than 30 cm.

Thirty apparently healthy subjects (who are age and sex-matched with the patients group) were selected as a control group in the study.

A detailed medical history regarding age, duration of the disease, family history of a same disease, family history of other autoimmune diseases was taken from each of the patients. Full clinical examination for each patient was performed including the hair texture, variability of hair shaft diameters, grading of alopecia, the alopecia areata patch's surface area.

All patients and the control group were investigated for serum concentrations of  $\gamma$ -IFN, and TNF- $\alpha$  according to principle and manual procedure of BioSource Company (Biosource, Europe, S.A, 8 B-1400, Nivelles, Belgium), which is a solid phase sandwich enzyme amplified sensitivity immunoassay (EASIA). Blood sample of about 3 ml was taken from each of the participants, deposit in sterilized plane tubes then allowed to be clotted, serum was separated (centrifuged at 3000 rpm for 15 min) in order to be used for the immunological evaluation. Cytokines serum concentration levels were measured in picogram / ml.

The statistical analysis was performed by using SPSS version 18 for windows. Data were expressed as Mean and SD. t-test was used to evaluate the difference between cytokines serum levels of alopecia areata patients and control individuals. P values less than 0.05 is considered significant.

This study was approved by the local ethical committee.

## Results

During the period from February to November 2014, a total of 54 patients with alopecia areata were incorporated into this study.

Twenty nine males and twenty five females age range 17-58 years, mean age 32 years. Apparently healthy control group of thirty individuals, their ages ranged between 20-59 years with mean age 30 years as shown in table(1).

**Table (1) age and gender distribution among patients with alopecia areata and controls.**

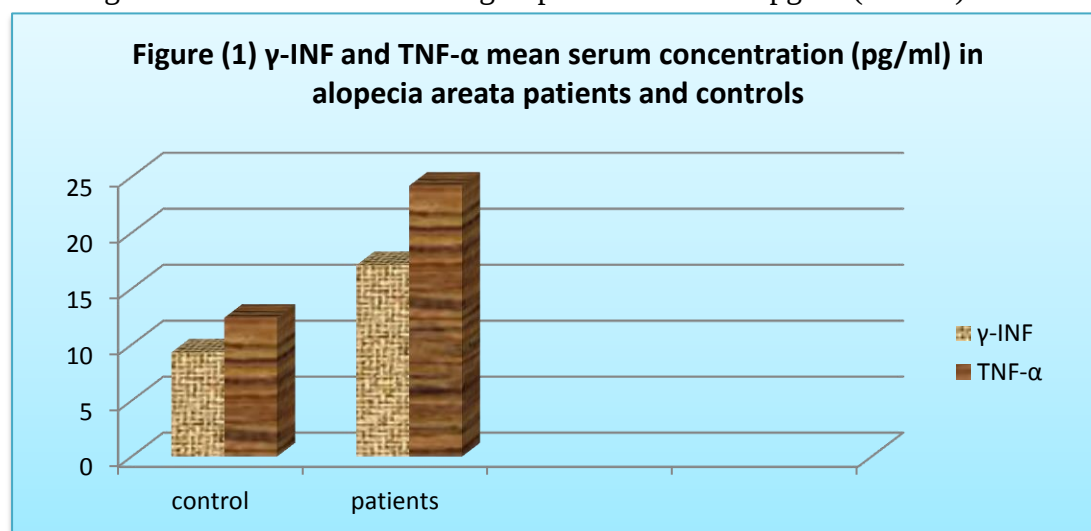
| Age interval   | Patients total no.(%)-♂;♀    | Controls total no. (%) - ♂;♀ |
|----------------|------------------------------|------------------------------|
| 10-19 years    | 3(5.5%)-2;1                  | 0                            |
| 20-29 years    | 16(29.6%)-10;6               | 7(23.3%)-4;3                 |
| 30-39 years    | 19(35.1%)-11;7               | 12(40%)-7;5                  |
| 40-49 years    | 9(16.6%)-4;5                 | 9(30%)-3;6                   |
| 50-59 years    | 7(12.9%)-2;6                 | 2(6.6%)-1;1                  |
| Mean age/years | 32                           | 30                           |
| Total no.(%)   | 54(100%)-29(53.7%);25(46.2%) | 30(100%)-15(50%);15(50%)     |

In the present work alopecia areata patients have been further subdivided into three smaller groups according to the size of affected area of their alopecia lesions. Group 1, group 2, and group 3 having affected area of <20 cm, 20-30 cm, and >30 cm respectively as shown in table(2).

**Table (2) subgroups of alopecia areata patients**

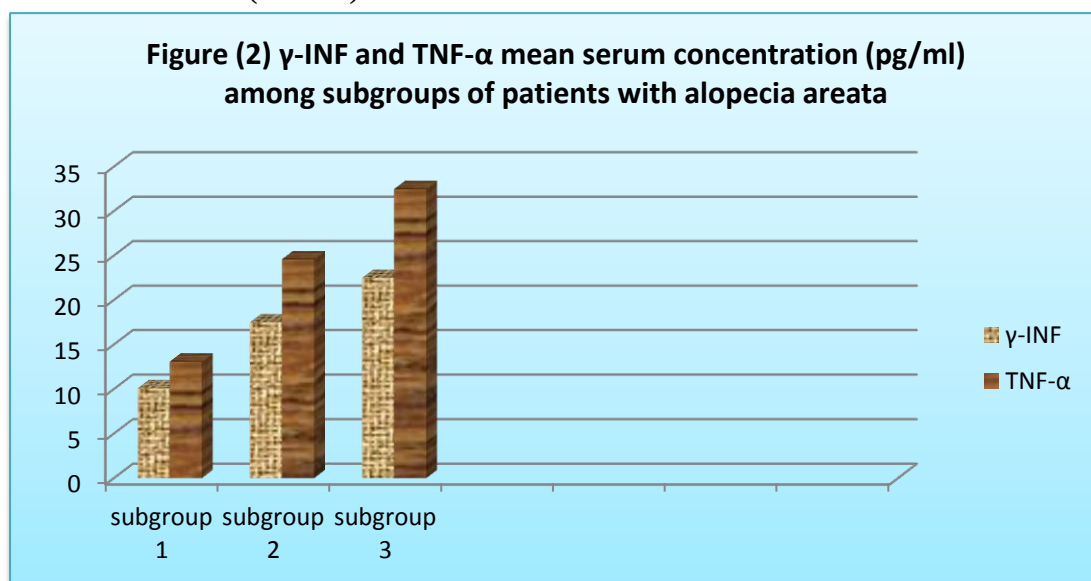
| Parameter             | Group 1 | Group 2  | Group3 |
|-----------------------|---------|----------|--------|
| No. of patients       | 16      | 18       | 20     |
| Size of affected area | <20 cm  | 20-30 cm | >30 cm |
| ♂-♀                   | 9-7     | 11-7     | 10-10  |

Results about the serum concentrations of  $\gamma$ -INF and TNF- $\alpha$  evaluated in this study illustrated in Figure(1) show that there is a significant difference in the concentrations of these two cytokines between patients of alopecia areata and control group ( $P < 0.05$ ).  $\gamma$ -INF mean serum concentration evaluated in patients with alopecia areata was 17.1 pg/ml, whereas in control individual its mean value was 9.3 pg/ml. TNF- $\alpha$  mean serum concentration in alopecia areata patients was 24.2 pm/ml, this is much higher than its value in control group which was 12.4 pg/ml ( $P < 0.05$ ).



Serum concentrations of  $\gamma$ -INF and TNF- $\alpha$  were also found to be clinically correlated to the size of affected area of hair loss among patients with alopecia areata subgroups. Figure(2) illustrates that the more the size the affected area (hence the more the severity of alopecia areata), the higher the serum concentration of these two

cytokines.  $\gamma$ -INF and TNF- $\alpha$  serum concentrations were found to be statistically nonsignificant when compared between patients in subgroup 1 with affected area of less than 20 cm and healthy controls ( $P>0.05$ ). While both subgroups 2 and 3 patients had serum concentrations of  $\gamma$ -INF and TNF- $\alpha$  significantly higher than that of the control individuals ( $P<0.05$ ).



## Discussion

Even though the pathogenesis of alopecia areata is poorly understood, the latest evolution in the understanding of immune-pathogenesis of other autoimmune diseases has shown that the essential role is attributed to the regulation of local and systemic cytokines[Kasumagić-Halilovic, and Ovcina-Kurtovic.2012]. However, few studies about this entity have been finished[Kuwano .*et.al.*2007].

The importance and the pathological role of cytokines in dermatology are accumulating dramatically. In the present study, in order to confirm the potential pathological role of Th1 cytokines  $\gamma$ -INF and TNF- $\alpha$  in alopecia areata, serum concentration of these two cytokines was measured using solid phase sandwich enzyme amplified sensitivity immunoassay (EASIA) among fifty four patients with alopecia areata and thirty apparently healthy control individuals. Data illustrated in figure (1) show that, the mean serum levels of these two cytokines were significantly dissimilar between patients of alopecia areata and healthy control group( $P<0.05$ ).  $\gamma$ -INF and TNF- $\alpha$  mean serum concentration were found to have higher levels among alopecia areata patients than healthy control persons.

Recent studies have focused on the role of some cytokines in the pathophysiology of alopecia areata; however, no information is available regarding the difference in cytokine profiles according to the severity of this disease. In the present work, the serum levels of  $\gamma$ -INF and TNF- $\alpha$  were non-significantly elevated in patients with the localized form of alopecia areata represented by patients in subgroup1 if they were compared to healthy control subjects. In contrast, the serum levels of these cytokines were significantly elevated in patients with the extensive form (subgroup2 and 3) as shown in figure (2). Several studies have shown that within the cascade of pathogenesis of alopecia areata, cytokines and other molecules that coordinate cyclical hair growth play a crucial role[Stamatis Gregoriou,*et.al.*2010]. In this case-control study, our results show that the significant differences in the mean serum concentration of these two cytokines is parallel to the

size of affected surface area, the larger the lesion, the higher the significant difference in the mean serum cytokines level between the two studied groups ( $P < 0.05$ ) as demonstrated in figure(2). Result's data also demonstrate that there is no significant differences found in the mean serum levels of  $\gamma$ -IFN and TNF- $\alpha$  between healthy controls and those alopecia patients with localized lesions in group 1, while both subgroup 2 and 3 showed marked difference in their serum levels in comparison to healthy persons ( $P > 0.05$ ). This finding might be attributed to that the elevated serum levels of Th1 type of cytokines may mirror the inflammatory symptoms in alopecia areata, especially in the extensive form of the disease, which might be regarded as prognostic tool for the severity of the disease [Arca *et.al.*2004].

A restricted number of revisions in the literature have assessed the mutual serum levels of both  $\gamma$ -IFN and TNF- $\alpha$  in patients with alopecia areata. And less more studies took into account the prognostic significance of these two cytokines in response to the severity of the disease. The results obtained in our study reveal that the mean serum levels of  $\gamma$ -IFN and TNF- $\alpha$  were significantly prominent in alopecia areata patients in comparison with healthy subjects, which may illustrate the important role these two cytokines play in the development of this disease, and seems to be a useful indicator of the severity of alopecia areata.

There is evidence to advocate that the link between lymphocytic infiltration of the follicle and the interruption of the hair follicle cycle in alopecia areata may be delivered by a mixture of factors, comprising cytokine release, cytotoxic T-cell activity, and apoptosis [Kasumagic-Halilovic, *et.al.*2011]. Gathering of T lymphocytes in perifollicular, perivascular and peribulbar spaces obstructing the hair cycle and provide evidence that an immune process is tangled, leading to hair loss [Kasumagić-Halilovic, and Ovcina-Kurtovic.2012]. While targeted cell killing by cytotoxic T cells with the help of T helper cells reflects a Th1-mediated immune response, which is habitually dependent on the production of  $\gamma$ -IFN and TNF- $\alpha$  to drive that response. These two highly inflammatory cytokines, are consistently elevated in Th1 mediated diseases and appear to be required for their pathogenesis [John Harris, 2010].

A solid premise on the pathogenic mechanisms of alopecia areata become patent through the role of  $\gamma$ -IFN and TNF- $\alpha$  established in this work. The pathogenic mechanisms so far known indicate a rather complex process that sustains an inflammatory reaction [Stamatis Gregoriou *et.al.*, 2010]. The increased levels of serum cytokines secreted by T-cell subsets suggested altered T-helper cell function [Tembhre *et. al.*, 2013]. This immunopathology might have a genetic base [Galbraith and Pandey .1995], which is why most autoimmune diseases shared by the family members [John Harris.2010]. This work findings came in parallel with previously issued data confirming that the Th1 type cytokine  $\gamma$ -IFN and TNF- $\alpha$  are crucial considerations in alopecia areata [Kasumagic-Halilovic *et.al.*2010], and their levels are connected to the disease severity [Barahmani *et.al.* 2010], more supporting the hypothesis of Th1 immunopathogenesis for hair loss in alopecia areata [Amos Gilhar.*et.al.*2003].

In recent years, a growing number of biological mediators have been introduced in the entity of immunopathogenesis of various diseases, including alopecia areata, although their principal pathological role yet to be fully elucidated [Le Bidre *et.al.*2011]. However, alopecia areata aetiology is probably multifactorial, and the exact cause remains unknown [Maqdasi and Waiz.2014]. The high serum levels of  $\gamma$ -INF and TNF- $\alpha$  founded in our study illuminate the activation and the possible

immunopathological role of the immune system in the establishment of alopecia areata.

## Conclusions

Within the challenge of this study, it is established that the elevated serum levels of  $\gamma$ -INF and TNF- $\alpha$  may mirror the inflammatory hair loss of alopecia areata patients, especially in its sever form. Therefore, the control of these cytokines production may be important in the management of this autoimmune disease. We courage that more studies are essential for enhanced understanding of the pathogenic role of immune mediators in alopecia areata, and the development of novel treatments in the future.

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