“ESTIMATION OF SERUM ZINC LEVELS IN 40 CLINICALLY CONFIRMED CASES OF ALOPECIA AREATA PATIENTS IN COMPARISON WITH EQUAL NUMBER OF GENDER AND AGE MATCHED CONTROLS-A CROSS SECTIONAL STUDY”

REG NO. BT0110002

Dissertation

Submitted to the
KLE University, Belgaum, Karnataka

In Partial Fulfillment
of the requirements for the degree of

M. D.
in
DERMATOLOGY, VENEREOLOGY AND LEPROSY

DEPARTMENT OF DERMATOLOGY, VENEREOLOGY, AND LEPROSY, JAWAHARLAL NEHRU MEDICAL COLLEGE, BELGAUM, KARNATAKA

APRIL - 2013

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ENDORSEMENT BY THE HOD, PRINCIPAL / HEAD OF INSTITUTION

This is to certify that the dissertation entitled **"ESTIMATION OF SERUM ZINC LEVELS IN 40 CLINICALLY CONFIRMED CASES OF ALOPECIA AREATA PATIENTS IN COMPARISON WITH EQUAL NUMBER OF GENDER AND AGE MATCHED CONTROLS-A CROSS SECTIONAL STUDY"** is a bonafide research work done by **THE CANDIDATE REGISTER NO. BT0110002**

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LIST OF ABBREVIATIONS USED

AA	-	Alopecia aereata
APCs	-	Antigen presenting cells
CD	-	Cluster of Differentiation
CGRP	-	Calcitonin gene related peptide
CMV	-	Cytomegalovirus
DEB	-	Dundee experimental bald
HLA	-	Human leukocyte antigen
ICAM	-	Intercellular Adhesion Molecule
IFN-α	-	Interferon gamma
IgM	-	Immunoglobulin M
IL	-	Interleukin
MHC	-	Major histocompatibility complex
NIAMS	-	National Institute of Arthritis and Musculoskeletal and Skin Diseases
SCID	-	Severe combined immunodeficiency
SP	-	Substance P
TH	-	T helper
TNF	-	Tumor necrosis factor
UK	-	United Kingdom
USA	-	United States of America

ABSTRACT

Background and objectives

Alopecia areata is an unpredictable, usually patchy, nonscarring hair loss condition. Any hair-bearing surface may be affected. Iron and zinc are the well-known trace elements that are associated with hair shedding. Objectives of this study were to assess serum zinc levels of clinically confirmed cases of Alopecia Areata in comparison with equal number of gender and age matched controls and to compare the relation of these values with demographic data.

Methodology

This one year cross sectional study was conducted in the Department of Dermatology, Venereology and Leprosy, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre attached to Jawaharlal Nehru Medical College, Belgaum during the period of January 2011 to December 2011. The study comprised of 40 patients with alopecia areata and equal number of age and gender matched healthy controls. Serum immunoturbidometry method was the method used for estimation of serum zinc levels.

Results

In the present study among cases 65% were males and 35% were females with male to female ratio of 1.85:1. 42.5% of patients with alopecia areata were aged between 19 to 30 years with the mean age of 26.85±11.99 years. 97.50% patients presented with patchy and 2.50% with generalized alopecia areata with most commonest site being scalp in 70%. Most (72.5%) of patients had single patch of alopecia aereata. All the patients had asymptomatic alopecia areata

(100%). However, one patient (2.5%) had nail changes associated with alopecia areata. The serum zinc levels were found to be normal in healthy controls as well as patients with alopecia areata. The mean serum zinc levels among those with alopecia areata were slightly high (103.21±10.17 mg/dL) compared to healthy controls (98.60±22.51 mg/dL) and this difference was statistically not significant.

Conclusion

The results of the present study showed no significant relation between serum zinc levels and AA.

Key words:

Alopecia aereata; Trace elements; Serum zinc;

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Chapter 1

Introduction

INTRODUCTION

Alopecia areata (AA) is a disorder where there is a loss of hair with no scarring to the affected area. The cause of alopecia areata is not yet fully understood.[1]

It can start at any age, although the majority (60%) of individuals develop alopecia areata before 20 years of age.[2]

Both sexes are affected equally and there is no known racial preponderance. The severity and pattern of hair loss can vary considerably between individuals. Alopecia areata is typically multi-focal, occurring on the scalp or any other hair-bearing region of skin.[3]

The bald areas are commonly oval or circular in shape and smooth to the touch. Hair shaped like an exclamation mark can be present around the margins of the patch. The condition can affect the entire scalp (alopecia totalis) or can cause loss of all body and scalp hair (alopecia universalis).[4]

Alopecia areata is a relatively common condition which occurs all over the world.[2] It accounts for approximately 2% of new cases attending dermatology outpatient clinics in the United Kingdom (UK) and United States of America (USA).[2]

A population-based study in Minnesota, USA, found an overall incidence of alopecia areata was 20.2 per 100,000 person-years. The lifetime risk has been estimated at 1.7%.[5]

The majority of people affected experience only the occasional bald area, which spontaneously resolves within a year, but most will suffer a relapse at some stage in their life. The prognostic factors for a less favourable outcome include: a family history of alopecia areata; childhood onset of alopecia areata; severe hair loss, as in alopecia totalis or universalis; a history of atopic diseases such as eczema, asthma or hay fever; other autoimmune conditions, particularly thyroid disease.[2]

The number of people with alopecia areata who progress to develop alopecia totalis or universalis is not known, but estimates range from 7 to 30%.[6] Vitiligo and autoimmune thyroid disorders are sometimes associated with alopecia areata.[2]

Alopecia areata can cause significant psychosocial problems. In an image orientated society, hair loss can be psychologically devastating for people affected and their families. Hair defines individuality and appearance; therefore, alopecia may result in reduced self esteem and may negatively affect the quality of life.[7] The unpredictable nature of the disease and the possibility of long-term, potentially unpleasant, therapy all add to the anxiety experienced when living with this condition.

The pathophysiology of AA is considered to be T-cell mediated autoimmunity that occurs mostly in genetically predisposed individuals.[8] In addition to disturbance of immune function, complex interactions between predisposing genetic and environmental factors act as triggers for disease progression.[9] Also, perifollicular nerves and vasculature, viruses, trace element

alterations,[10] endocrine disorders, and thyroid dysfunction[11] have been hypothesized. There are claims that imbalance of trace elements may trigger the onset of AA. Clinically, AA can present with many different patterns. A flat alopecic plaque with normal skin color, involving the scalp or any other pilar region of the body is the characteristic lesion of AA.[12]

In common with a number of inflammatory autoimmune diseases, genetic factors including human leukocyte antigen (HLA) class II associations have been identified in alopecia areata. No consensus has been reached on the identity of a specific disease target within the hair follicle in alopecia areata. Suggested candidate cell types include the dermal papilla cells, the keratinocytes of the matrix and presumptive cortex and the hair bulb melanocytes, but these need not be mutually exclusive. The pathogenesis is known to involve disturbance of immune function but there is no proof that an autoimmune mechanism is fundamental.[9]

Iron and zinc are the well-known trace elements that are associated with hair shedding.[13] Various studies[10,11,13] have shown varied results of the levels of magnesium, copper, and zinc in different population.

Hence the present study was an attempt to assess serum zinc levels of clinically confirmed 40 cases of AA in comparison with equal number of gender and age matched controls and to compare the relation of these values with demographic data like gender and age in equal number of controls.

Chapter 2

Objectives

OBJECTIVES

The objectives of the present study were;

Primary

To study the serum zinc levels of clinically confirmed 40 cases of Alopecia Areata in comparison with equal number of gender and age matched controls.

Secondary

To compare the relation of these values with demographic data like gender and age in equal number of controls.

Chapter 3

Review of Literature

REVIEW OF LITERATURE

Alopecia areata is a recurrent nonscarring type of hair loss that can affect any hair-bearing area. Clinically, alopecia areata can manifest many different patterns. Although medically benign, alopecia areata can cause tremendous emotional and psychosocial distress in affected patients and their families.

Alopecia areata is an unpredictable, usually patchy, nonscarring hair loss condition. Any hair-bearing surface may be affected. AA is hypothesized to be an organ-specific autoimmune disease mediated by T lymphocytes directed to hair follicles. Although genetic predisposition and environmental factors may trigger the initiation of the disease, the exact cause is still unknown. There have been significant advances in understanding and treatment of this condition over the past decade. Much of the most recent research and future directions in AA originate from three research workshops cosponsored by the National Alopecia Areata Foundation and the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS) in 1990, 1994, and 1998. At these meetings, numerous subspecialties, including immunologists, molecular biologists, biochemists, dermatologists, pathologists, and geneticists, discussed AA in an open forum.[2]

Historical aspects

- Although Hippocrates first used the term alopecia (literally translated as "fox's disease"), the characteristics of the hair loss disease AA were first described by Cornelius Celsus in 30 A.D.[14]

- The first clinical description of alopecia areata is attributed to Celsus (14 to 37 B.C.) and the designation alopecia areata is by Sauvages.[12]

- Hebra demonstrated the incorrectness of the hypothesis of fungal etiology as proposed by Willan and Gruby (1843).[12]

- Von Baresprung proposed the neurotrophic theory, and Jacquet elaborated the dystrophic theory, considering the disease to be caused by infectious focuses, particularly dental, a hypothesis today that has been totally discarded.[12]

- Celsus described two forms of alopecia. The first he described as complete baldness occurring in people of all ages. The second he called "ophiasis", which literally translates as "snake", due to the winding way the bald region spread across the skin. He suggested ophiasis was only seen in children.[14]

- Alopecia areata is sometimes known as "area celsi" in tribute to Cornelius Celsus. Alopecia areata has been given many different names throughout history.[14]

- However, the actual term "alopecia areata" was first used by Sauvages in his "Nosologica Medica", published in 1760 in Lyons, France.[12]

- From the 1800's onwards there was considerable debate about the cause of alopecia areata. Two main hypotheses were put forward, one based on parasitic infection, the other based on a nervous disorder.[12]

- The parasitic hypothesis drew support from the way the hair loss lesion developed - slowly expanding in size just as a local infection would. Even more significant were the apparent epidemics of alopecia areata reported to occur in institutions such as orphanages and schools.[15-17]

- However, many attempts to isolate an infective organism and to transfer alopecia areata by inoculation have failed.[18,19]

- The initiation of alopecia areata by a nervous disorder, variously known as the trophoneurotic, neurotrophic, or neuropathic hypothesis, eventually gained the support of most dermatologists of the day. This vague hypothesis could be supported by the apparently frequent clinical observations of emotional or physical stress and trauma that were associated with the onset of alopecia areata and often reported in the medical journals at that time.[20,21]

- Emotional stress and physical damage were believed to adversely affect hair follicles via the nervous system and Joseph (1886) showed that patchy hair loss could apparently be induced by cutting nerves in the necks of cats (it was later suggested that the hair loss was actually due to the cats scratching themselves). This highly circumstantial, but compelling evidence drew popular support for the trophoneurotic hypothesis. The idea circulated among dermatologists for many years because it was (and still is) very difficult to fundamentally prove or disprove that alopecia areata was a nervous disorder. The hypothesis is still supported by some dermatologists today.[22]

- One of the more unusual variations on the neuropathic origin of alopecia areata was put forward by Jacquet (1902) who suggested alopecia areata was initiated by sources of nerve irritation such as defective and diseased teeth.[23]

- Jacquet's hypothesis was apparently confirmed by others.[24] Although Baily (1910) showed dental disease to be equally frequent in people without alopecia areata.[25] Eye strain was another suggested cause of alopecia areata.[26]

- With the start of the twentieth century alopecia areata was known to be associated with disorders of the endocrine glands, particularly the thyroid.[27] As such, some believed the underlying cause of alopecia areata was due to a hormone dysfunction.

- By the 1920's most dermatologists had abandoned the parasitic theory of alopecia areata and favored variations on the trophoneurotic and endocrine theories - often combining the two.[12]

- Sufferers of alopecia areata were certainly under extensive mental stress, not least from fear that they would be suspected of having ringworm or syphilis. Until the advent of antibiotics, syphilis was a widespread, contagious disease. It often manifests itself by sudden, rapid loss of hair in well defined patches, just like alopecia areata,[18] before progressing to development of lesions and sores. Syphilis in the secondary stage can also affect finger nails.[28]

- To further complicate the matter, some dermatologists suggested that alopecia areata could be found in increased association with syphilis - as distinct from the direct action of syphilis on hair follicles. Syphilis was believed to induce alopecia areata by the mental distress it created and its possible upset of the endocrine system.[29] These clearly visible symptoms of syphilis were often confused with alopecia areata by the general population and resulted in social ostracism for the sufferer.[18]

- The early 20th century saw the development of another hypothesis of alopecia areata induction based on toxic agents. An unknown poison was believed to be introduced to the hair follicle via the blood system inducing hair loss.[30]

- The sudden remission and relapse of alopecia areata and its action simultaneously over the body was believed to support the idea. Also in support, injection of thallium acetate (rat poison) was shown to induce alopecia areata like hair loss in some people,[18,30] with expression of exclamation mark hairs - a diagnostic feature of alopecia areata.[28]

- The toxic origin of alopecia areata never gained widespread popularity against the neuropathic and endocrine hypotheses and has long since fallen from grace.

- It is now widely believed that alopecia areata is an autoimmune disease. Even though studies more than 100 years old showed that alopecia areata affected hair follicles were invaded by inflammatory cells,[31] the

inflammatory autoimmune disease hypothesis did not become popular until the 1960s.

- The idea was first proposed by Rothman in a discussion of a paper by Van Scott (1958).[32] Autoimmune disease occurs when a patient's immune system mistakes part of his or her own tissue for a foreign invading organism, attacks, and attempts to destroy, that part of the tissue. Increasingly, hard evidence is being produced in support of this hypothesis over the previous suggestions.

Epidemiology

Alopecia areata is a benign condition and most patients are asymptomatic; however, it can cause emotional and psychosocial distress in affected individuals. Self-consciousness concerning personal appearance can become important. Openly addressing these issues with patients is important in helping them cope with the condition.

Frequency

Prevalence in the general population is 0.1-0.2%. The lifetime risk of developing AA is estimated to be 1.7%. Alopecia areata is responsible for 0.7-3% of patients seen by dermatologists.[5,33] Worldwide prevalence of AA is the same as that in the US.

Alopecia areata is a common disease encountered by dermatologists, with a frequency ranging from 0.7% to 3.8% of patients attending dermatology

clinics.[34,35] In the US, AA was estimated to occur in 0.1% to 0.2% of the general population,[33] with a lifetime risk of 1.7%.[5]

Race

Alopecia areata occurs in populations worldwide. All races are affected equally by AA; no increase in prevalence has been found in a particular ethnic group.

Sex

Data concerning the sex ratio for alopecia areata vary slightly in the literature. In one study including 736 patients, a male-to-female ratio of 1:1 was reported.[6]

AA likely affects males and females equally.[36] Some studies show a significant male preponderance in the adult age group, although others identify contrasting results.[37,38]

A study observed male preponderance (1.4:1): in those with severe involvement.[46] Two studies showed a higher proportion of girls.[40,41] A male preponderance was inconsistent with findings in another study carried out in Singapore, which showed a more severe alopecia in girls.[35] Therefore, it is not clear whether gender affects the extent of disease.

Age

Alopecia areata can occur at any age from birth to the late decades of life. Congenital cases have been reported. Peak incidence appears to occur from age

15-29 years. As many as 44% of people with alopecia areata have onset at younger than 20 years. Onset in patients older than 40 years is seen in less than 30% of patients with alopecia areata.[42]

Pediatric AA constitutes approximately 20% of AA cases, and as many as 60% of patients with AA will present with their first patch before 20 years of age.[43]

One study suggests that 85.5%of Asian patients with AA have disease onset before 40 years of age.[35] The disease prevalence peaks between the second and fourth decades of life.[43]

In a study the differences in the mean age of onset or mean duration of AA between boys and girls were not significant.[39] The median age of onset was 10 years old, which was lower than 11.2 years old in a report from Singapore,[39] but higher than 5.7 years old in another report from Kuwait.[44] Authors[39] also reported that, earlier the age of onset, the more severe the extent of AA, which was consistent with other report.[35]

A study[39] aimed to review the epidemiologic profile of childhood AA in China found that, childhood AA accounted for 12.80% of the total number of those with AA seen during the same period and 0.12% of the total number of patients seen. Local incidence of 12.80% was lower than that reported previously, but similar to the 11.1% in one report from Singapore.[35]

Epidemiologic studies of AA are available from USA, Japan and European countries.[45-47]

The statistical data registered in the literature are variable. The disease can begin at any time of life, but with a peak incidence between 20 and 50 years of age, and other articles affirm 60% of the patients present the first episode of the disease before 20 years of age.[12]

In material from the Hospital das Clínicas (teaching hospital of the University of São Paulo Medical School-FMUSP) Pimentel verified that 70% of the cases occurred between 10 and 25 years.[48] Both sexes are equally affected,[49,50] although at the Hospital das Clínicas it has been observed that with relation to the severe forms, 63% occur in men and 36% in women.[12]

Alopecia areata is fairly common and can be seen at all ages. Its lifetime risk has been estimated at 1.7%. Although some studies stated that 20% to 50% of AA develop during childhood, and it has been estimated that the risk of AA occurring in children is around 10-fold greater than that in the general population, there have been few reports focusing on AA in children.[12]

A study[51] aimed at evaluating the prevalence of thyroid function abnormalities in patients with AA and its association with other autoimmune diseases and various autoimmune antibodies retrospectively analyzed medical records of 123 patients with AA. The main site of involvement, pattern, and extent of alopecia as well as presence of the similar disease in first-degree family members and serologic status of patients were recorded. Participating in the study were 57 males and 66 females (6 to 59 years old). In the majority of patients (69.9%) the disease was manifested in the first two decades of life. Patients with family members having alopecia were recorded in 24.4%. Thyroid function

abnormalities were found in 8.9% of patients. Positive autoimmune antibodies were associated with AA in 51.4% of patients with no significant association between the severity and duration of disease and presence of these antibodies. The incidence of positive auto-immune antibodies in Iranian patients is higher than previous reports. This study results were compatible with previous data obtained from different ethnic populations. Previous reports documented that a greater severity and longer duration of AA were seen in the early onset forms.

Another study[41] to assess the clinical and epidemiologic profile of childhood alopecia areata performed a survey in which a total of 226 childhood patients less than 16 years old were enrolled. The majority of patients (84.96%) presented with limited alopecia. The male:female ratio was 1.4:1. Boys appeared to have more severe involvement. The earlier the age of onset, the greater the severity of the disease. Sixty-seven patients (29.65%) had previous episodes of alopecia areata. Greater severity and longer duration were seen in the relapsing patients than in the primary patients. Six patients (2.65%) had an associated disease. A positive family history was reported in 25 patients (11.06%). The prevalence figures for alopecia areata in first-, second-, and third-degree relatives of the probands were 2.87%, 0.40%, and 0.13%, respectively. The heritabilities of AA in first-, second and third-degree relatives were 51.20%, 46.25%, and 25.65%, respectively. It can be speculated that the effect of genetic factors is important in the occurrence of this disease.

Epidemiologic studies of AA are available from USA, Japan and European countries,[45-47] but there is a paucity of literature on AA from Asian countries, especially from the Indian subcontinent.

In a prospective, hospital-based study[34] lasting for a decade (1983-1992), the epidemiology of AA was studied, including associated diseases and risk factors for development of severe AA. Simultaneously a similar study was carried out in age and sex-matched controls. Eight hundred and eight patients (532 men, 276 women) and 572 age- and sex-matched controls (370 men, 202 women) were studied. The incidence of AA was 0.7% of new dermatology outpatients. Alopecia areata in North Indians showed a preponderance in men (M:F=2:1) and the majority of persons with disease (88%) were below 40 years of age. Onset in childhood was more frequent in girls or women, but the incidence of severe alopecia was higher in boys or men with onset at an earlier age. Diseases associated with autoimmunity were seen in only 5% of patients. Atopy was found to be associated in 18% of patients, but its reported association with younger age of onset and severe alopecia was not confirmed. Presence of vitiligo in family members and onset before 20 years of age, especially in boys or men, were found to be risk factors for severe alopecia.

Clinical aspects

In general, the patients report an important loss of hair and abrupt presence of an alopecic area or areas. The characteristic lesion of AA is a flat alopecic plaque with normal skin coloration involving the scalp or any other pilar region of the body. In acute phases, the lesions can be slightly erythematous and edematous, and appear in the periphery of the plaques are exclamation mark hair, presenting a thinner and less pigmented appearance at the point of emergence from the scalp and with greater thickness in the distal extremity.[12]

Besides the above, these hairs demonstrate deposition of melanin pigment in the distal extremity (Widy's sign) and, although not absolutely pathognomonic, strongly suggest the diagnosis when present. The plaques of alopecia areata are usually asymptomatic, although several patients may complain about paresthetic sensations with discreet pruritus, pain or sensation of local ardor. Various symptomatological aspects can aid in the diagnosis of AA: positive gentle traction test - in the acute phases of the disease, the hair is plucked easily by gentle traction, whether from the periphery of the plaques in localized forms, or from several areas of the scalp in diffuse forms. In the most chronic phases, this test is negative, since the hair is not plucked as easily as in the acute phases; presence of cadaverous hair - these are hairs in which there occurs a fracture of the shaft inside the pilar follicle, producing blackened points inside the follicular ostia that resemble the comedos; development of white fluff about half a centimeter in length along the alopecic area.[12]

As the lesions course to more chronic phases, the presence of those signs is no longer detected and a mild follicular hyperkeratosis can appear in the alopecic area. Finally, the surface of the alopecic areas can become slightly atrophic, but never with a cicatricial aspect.[12]

In agreement with number of lesions, extension of involvement and topography of hair loss, alopecia areata is clinically classified into several types.[12]

Classification[52]

Classification of alopecia areata based on Ikeda's types or on pattern of hair loss

Based on Ikeda's types

- Common
- Atopic
- Pre-hypertension
- Autoimmune

Based on pattern of alopecia

- Restricted to scalp
    - Patchy
    - Ophiasis
    - Sisaphio
    - Reticulate
    - Diffuse
    - Subtotal
    - Alopecia totalis
- Generalized
    - Alipecia universalis

Alopecia can be classified either based on Ikeda's types or based on the pattern of hair loss. An extensive clinical survey conducted over 19 years, Ikeda classified AA patients based on associated conditions and course of the disease into the four types.

The atopic type (10%)

Begins during childhood or adolescence and progresses slowly over many years with individual patches lasting more than one year. Ophiasic and reticular patterns were commoner and the chances of developing total alopecia were very high (30-75%).

The autoimmune type (5%)

It affected the middle aged, ran a prolonged course, and led to alopecia totalis in l0% to 50%. Autoimmune diseases, diabetes mellitus and at peptic ulcer were associated.

The pre-hypertensive type (4%)

This occurred in young adults whose one or both parents were hypertensive, progressed faster, and led to total alopecia in about 40%. Configuration of patches in a reticular pattern was common. The separate status of this group has been questioned as some studies have found the prognosis to be similar to the common type of AA.

The common type (81%)

It is the prototype of the fast- progressing form of the disease that affects adults aged between 20 and 40 years. Patients are free of associated disorders, and individual patches last less than six months, with spontaneous regrowth occurring within three years. Alopecia totalis may develop in 5% to 15%. Heterogeneity also explains the racial variation in clinical and immunologic

features which could have occurred because of differing proportion of the various subtypes.

In a study[53] by Sharma VK et al, 356 patients (234 males, 122 females) with alopecia areata were classified according to Ikeda's classification. The common type of alopecia areata was most frequently seen in 239 (67.13%) patients, followed by atopic in 60 (16.85%), prehypertensive in 48 (13.4%), and autoimmune/endocrine in 9 (2.52%) patients. Severe alopecia did not occur with a higher frequency in atopic or endocrine/autoimmune alopecia areata than in the common type ($p>0.05$). Prehypertensive alopecia areata had the lowest frequency of severe alopecia in the above study. The odds for developing severe alopecia were highest (2.6) when onset was before 16 years of age, followed by female sex (2.12), atopy (0.86), autoimmune/endocrine (0.53), and prehypertensive (0.28) types. Alopecia areata should be broadly classified as childhood (< 16 years) and adult onset with subtypes of atopic, autoimmune/endocrine, and common type under both. The prehypertensive type should be combined with the common type of alopecia areata.

Classic forms[12]

Alopecia areata in single or unifocal plaque

In this form there is a single, round or oval, smooth alopecic plaque, in which the skin coloration is normal, with hair of a normal appearance in the periphery of the plaque that is easily plucked by traction (demonstrating activity of the process) typical exclamation mark hair can be present.

Alopecia areata in multiple or multifocal plaques

In this form typical alopecic plaques occur that affect the scalp or other pilar areas.

Ophiasic alopecia areata

In this presentation, the hair loss occurs along the line of temporo-occipital implantation, giving rise to an extensive alopecic area, in a band that reaches the inferior margins of the scalp.

Alopecia totalis

There is total loss of terminal hair of the scalp without affecting other body hair, there can also be ungual involvement.

Alopecia universalis

There is total loss of body hair, involving the scalp, eyelashes, eyebrows, beard and moustache, armpits and genital areas. In general, it occurs in association with a variety of ungual lesions. Besides these forms that are considered classic, there are atypical presentations of alopecia areata:

Atypical forms[12]

Sisaphio type alopecia areata (inverse ophiasis)

In this form, the hair loss involves the entire scalp except for the lower margins, along the line of temporo-occipital implantation. It is the inverse clinical image of the ophiasis form.

Reticular alopecia areata

In this form, multiple alopecic plaques occur separated by narrow bands of preserved hair, conferring a reticulated aspect to the picture.

Diffuse alopecia areata

In this form, the hair loss is acute and widespread. It can be the initial form, mainly among children and adolescents, or can develop from plaque forms. Most of these cases develop into the more serious alopecia totalis or universalis forms. It is the most difficult form to diagnose, demanding a differential diagnosis with acute telogen effluvium, androgenetic alopecia and also alopecia syphilitica. Thus necessitating complementary exams in general and even histopathological exam by biopsy.

Extrafollicular involvement in alopecia areata[12]

Extrafollicular involvement can be observed in alopecia areata, particularly in its more severe forms, comprising ungual alterations, ocular alterations, and reports of a possible relationship with salmon patch on nape of the neck.

Ungual alterations[12]

Particularly in the most serious forms of alopecia areata, there can occur several types of ungual alterations the most frequent form of onychopathy being the presence of cupuliform depressions that can be so intense they produce true trachyonychia. There can also occur longitudinal and transverse furrows,

koilonychia, onycholysis, onychomadesis, onychorrexis, pachyonychia, punctate leukonychia or transverse and red lunula. The ungual alterations are more frequent in children (12%) in relation to adults (3.3%). The prevalence of ungual alterations is greater in the more severe forms: alopecia universalis 15.4%; alopecia totalis 3.7%; and alopecia areata in plaque 2.25%. The ungual alterations can precede, accompany or succeed the alopecia lesions.

Ophthalmologic alterations[12]

Various ocular alterations have been reported in alopecia areata, apparently related to dysfunctions of the pigment epithelium of the retina. The presence has been described of clusters of crystals (hyaline excretions in the choroid), focal hypopigmentation of the retina, opacities of the crystalline lens, posterior subcapsular cataracts, decrease in the visual acuity, Horner's syndrome, papillary ectopia, heterochromia and atrophy of the iris, miosis and palpebral ptosis.

Salmon patch on the nape[12]

Some works have attempted to correlate the presence of flat hemangioma with severity of the alopecia areata. Flat hemangioma on the nape was registered in 95.8% of the cases of areata universalis, in 86.7% of the cases of areata totalis and in 55.5% of the cases of ophiasic areata. These data showing a higher frequency of flat hemangioma on the nape in the most severe forms lead to the hypothesis that the presence of this lesion is an indication of a less favorable prognosis, though this correlation has yet to be proven in function of the high

frequency of salmon patch in the nape of the general population, thus further studies are necessary with a greater number of cases and statistical significance.

Pathophysiology

The exact pathophysiology of alopecia areata remains unknown. The most widely accepted hypothesis is that alopecia areata is a T-cell–mediated autoimmune condition that is most likely to occur in genetically predisposed individuals.[54]

AA is a lymphocyte cell mediated inflammatory form of hair loss with research evidence suggesting an underlying autoimmune etiopathogenesis. The development of hair loss involves aberrant modulation of the hair growth cycle, resulting in dystrophic anagen hair follicles and/or increased frequency of telogen state follicles. Genetic susceptibility to the development of AA involves specific alleles of the HLA region though other non-HLA genes are also likely to be involved. Susceptibility to the development of AA may be modified by environmental factors, including exposure to proinflammatory agents and possibly other modulators, including stress and diet.[43]

Hair follicle growth cycling modulation in alopecia areata

There are three key phases of the hair cycle: the growth phase (anagen), the regression phase (catagen), and the resting phase (telogen). The cycling of these phases is finely coordinated by the expression of hormones, cytokines, transcription factors, and their corresponding receptors and is carefully regulated through endocrine, paracrine, and autocrine routes. The disruption of these finely

tuned pathways can result in the development of hair diseases. Exogen is a hair follicle cycle event that describes the controlled shedding of club hair fibers.[43]

In healthy individuals, shedding normally occurs during the subsequent anagen growth phase as a new hair fiber is produced. In the development of alopecias, exogen occurs in advance of renewed anagen growth, leaving a hair follicle devoid of visible hair fiber a state called kenogen.[43]

In AA, significant disruption of the hair growth cycle clearly occurs, but different perturbations in hair growth occur depending on the pattern, severity, and duration of AA in each patient. There are several possible presentations of AA. First, the anagen phase of a hair follicle can become inflamed and maintained in a dystrophic anagen state, unable to produce hair fiber of significant size or integrity. When there is a greater intensity of inflammation, the hair follicles may be forced into a telogen phase and may then cycle through multiple anagenetelogen phases of brief duration. Correspondingly, inflammatory cell infiltration occurs in early anagen follicles without migration to draining lymph nodes as follicles capitulate and return to telogen.[43]

Finally, when AA is chronic, the hair follicles tend to persist in a prolonged telogen phase without an apparent attempt to return to an anagen growth phase.[43]

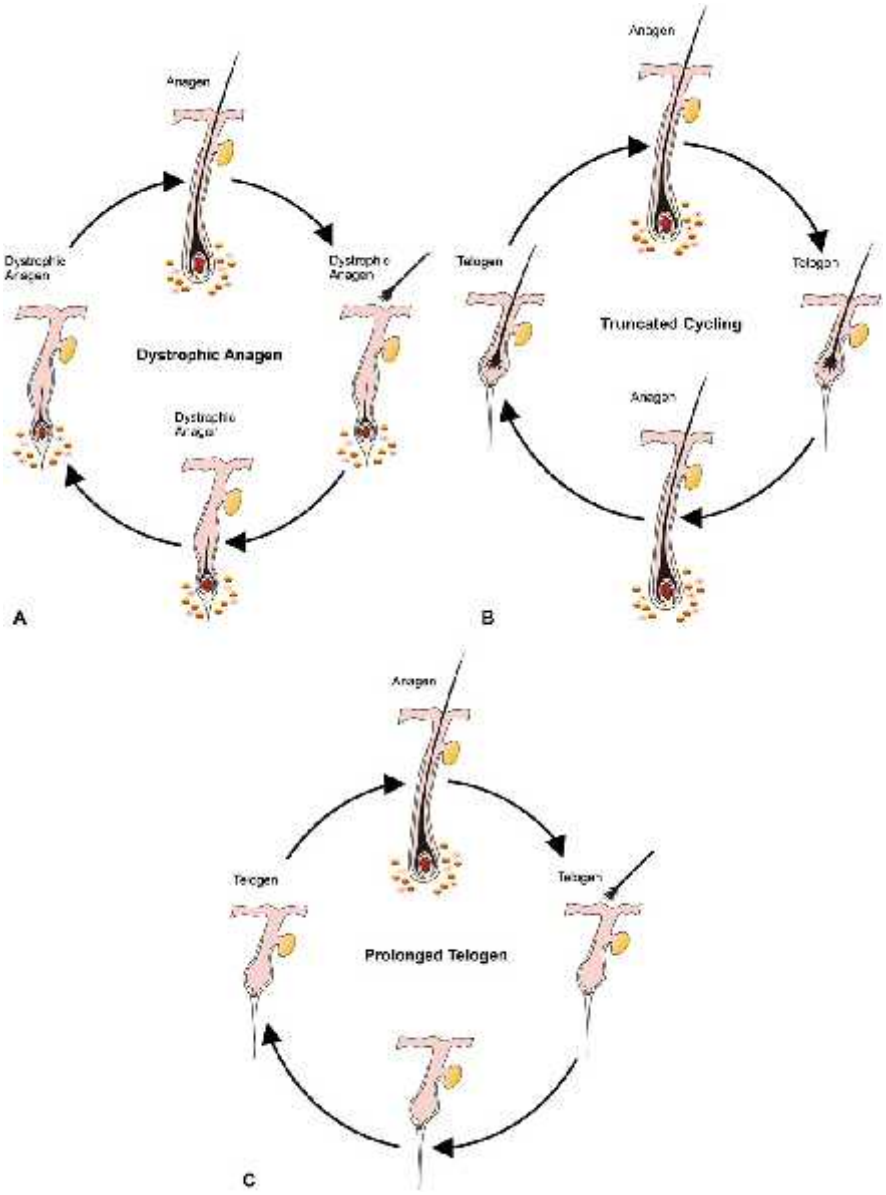


Figure 1. Hair growth cycle patterns in alopecia areata. A, Hair follicles held in dystrophic anagen by mild inflammatory insult unable to produce significant hair fiber. B, Anagen growth phases truncated by moderate inflammatory insult resulting in rapid cycling and brief hair fiber growth. C, Hair follicles enter prolonged telogen dormancy with development of chronic alopecia areata.[43]

Hypothesis for alopecia areata development

Although many hypotheses to explain autoimmune disease development have been suggested by immunologists, most of these scenarios have not been seriously considered in the context of AA. Currently, AA development hypotheses focus on hair follicle immune privilege collapse or the inappropriate presentation of antigens to the immune system during normal hair follicle cycling. Anagen stage hair follicles retain immune privilege, and a breach in immune privilege and exposure of unique hair follicle antigens may result in targeting by the skin immune system.[43]

This popular hypothesis is highly ‘‘skin-centric’’ and largely ignores current immunologic dogma, although it is attractive in its simplicity. An alternative hypothesis is based on the knowledge that hair follicle immunoprotection is transient, limited to the anagen growth cycle stage. Regression of the hair follicle in catagen involves significant apoptosis and immune cell infiltration.[43]

This normal hair follicle cycling event may continuously expose the immune system to low levels of hair follicle derived antigens. Hair follicle specific auto-antibodies found in humans and animal models in the absence of AA may be a consequence of this constant low level exposure.[43]

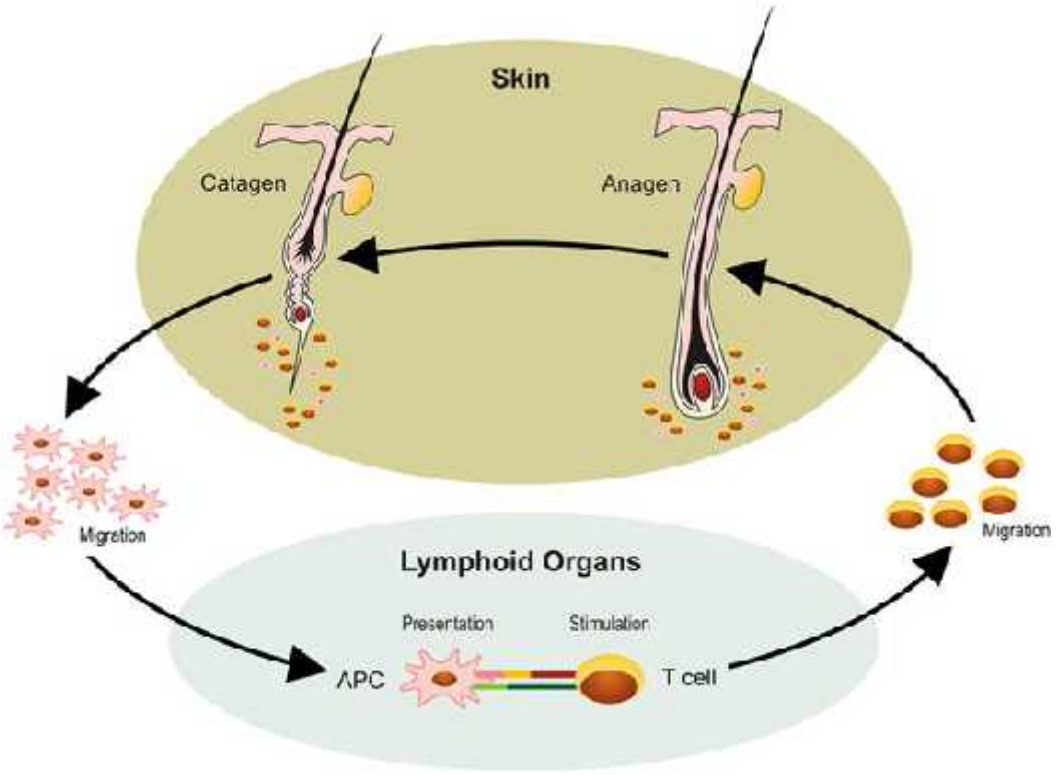


Figure 2. Alopecia areata pathogenesis. Inappropriate excitation of antigen presenting cells during disordered catagen and migration of cells to draining lymph nodes may lead to hair follicle antigenespecific lymphocyte activation, migration, and infiltration of anagen stage hair follicles.[43]

Langerhans cells and dendritic cells are capable of presenting cell apoptosis derived antigens to lymphocytes and stimulating autoimmunity. If catagen regression became disordered, the associated immune cell infiltrate might inappropriately coexpress antigenic peptides and costimulatory molecules and induce an immunologic response.

Mouse studies have revealed that significant proinflammatory events occur in the lymph nodes in advance of lymphocyte infiltration into the skin and subsequent hair loss onset.[55,56] As such, the onset of AA may be initially triggered during routine catagen because of inappropriate skin immune system signaling.[43]

Genetic factors

These are important in the genesis of alopecia areata, as demonstrated by the high frequency of positive family history in the patients, varying from 10 to 42% in the series studied. Positivity in the family history is higher in individuals with early onset of the alopecia, reaching 37% among patients in whom the process began before 30 years of age and 7.1% when the disease begins after 30 years of age.[12]

Analysis of the material from Hospital das Clinicas (FMUSP) indicates familial occurrence of 4.1%.8 Also demonstrating the importance of genetic factors is the occurrence of AA in twins with concordance of 55% when identical.[57-59]

Likewise indicating the participation of genetic factors, is the higher incidence of alopecia areata in individuals with Down's syndrome (8.8%) in relation to the general population, signaling a possible participation of genic alteration in chromosome 21 as a causal factor for alopecia areata.[12]

The possible correlations between alopecia areata and histocompatibility antigens have been studied. The correlations with HLA class I have not been established, however it has been demonstrated that there is a correlation between susceptibility and severity with class II HLA. There is a significant association between alopecia areata and various class II HLA, HLA DR4, DR5 and DQ3, while HLA DR5 is related to the more severe forms with early onset.[12]

The HLA-DQB1*03 alleles appear to represent susceptibility markers for all forms of alopecia areata, whereas alleles HLA DR B1*0401, HLADQB1 *0301 seem to be markers for the more severe forms: areata totalis and areata universalis.[12]

Another indication for participation of genetic factors in alopecia areata is the frequent association with atopy, a condition known to be hereditary. There are correlations between the presence of atopy and the severity of alopecia areata, in that the condition is frequently present in more serious forms of alopecia areata.[12]

In the material from Hospital das Clinicas (FMUSP) the presence of atopy was observed in 42.8% of the alopecia areata patients against 26.6% of the paired controls.[48]

Therefore, innumerous studies have demonstrated an important participation of genetic factors in the genesis of alopecia areata, which is possibly a polygenic disease with participation of related genes both in terms of susceptibility and disease severity.

The genetics of an individual can play a role in the development of AA. That AA can be inherited is based on observations on monozygotic twins; AA in twins can have similar times of onset and patterns of hair loss. Some patients with AA have a strong family history that spans many generations, and this also suggests that AA can be inherited. Between 4% and 28% of patients who have AA will have at least one other affected family member.[43]

Gene association studies have indicated that HLA genes play a role in AA and multiple HLA class I (A, B, and C) alleles conferring susceptibility have been identified in different cohorts of AA patients. Major histocompatibility complex (MHC) HLA class I molecules are present on almost all nucleated cells and they present antigens to CD81 T cells. There is an aberrant expression of HLA antigens found within the follicles of AA affected patients. However, the evidence in support of an association of HLA class I with AA is inconsistent with different susceptibility alleles identified in different cohorts, while some studies were unable to confirm any significant association.[43]

A much more consistent and stronger association between HLA class II alleles and AA development has been observed. HLA class II antigens are constitutively expressed on specific immune cells and may be upgregulated on nonimmune cells in response to injury. They are responsible for presenting antigenic peptides to CD41 T cells. HLA class II antigens are aberrantly highly expressed on AA affected hair follicles. Specific alleles, such as DQB1*03 and DRB1*1104, have been reported as markers for susceptibility to AA. These findings suggest the importance of specific HLA class II alleles in the onset and progression of AA and imply that antigen presentation to CD41 cells plays a significant role in AA development. Several association studies have investigated non-HLA gene alleles, indicating that multiple genes may contribute to AA susceptibility.[43]

A genome-wide study[60] was completed on extended human families with multiple AA patients. Intervals on human chromosomes 6, 10, 16, and 18 were identified as potential AA susceptibility loci. This study[67] further confirmed that

the HLA region on human chromosome 6 is associated with genetic susceptibility to AA. However, there was at least one significant genetic determinant of AA found at 6q.23.3 outside of the HLA gene cluster. Large scale, genome-wide screens using the AA registry and DNA bank[61] are nearing completion and should provide significant new information on the potential gene activity in AA development beyond the HLA region.

Immunological factors

There is considerable evidence for the participation of immunological mechanisms in the pathogenesis of alopecia areata: the association with autoimmune diseases, the presence of circulating antibodies of several types and the presence of immunologically active cells in the inflammatory infiltrations that constitute the histopathological expression of alopecia areata.[12]

Association with autoimmune diseases

There are countless reports of an association between alopecia areata and autoimmune diseases, mainly autoimmune diseases of the thyroid and vitiligo,[6,62] but also pernicious anemia,[63] lupus erythematosus,[64] myasthenia gravis,[65] rheumatoid arthritis, polymyalgia rheumatica, ulcerative colitis,[62] diabetes,[66] and candidiasis-endochrinopathy syndrome.[67]

The incidence of thyroid disease among patients with alopecia areata varies from 8 to 11.8%, against 2% in the normal population.[6,62] Some studies have shown that the incidence of vitiligo in alopecia areata patients is four times greater in relation to the normal population.[6,62]

Autoantibodies

The presence of autoantibodies in alopecia areata patients is quite frequent, particularly thyroid antimicrosomal antibodies. Anti-gastric parietal cell antibodies are also frequently detected.[12]

These antibodies probably represent a phenomenon secondary to the initial immunological phenomenon involving the pilar follicles. The presence of autoantibodies against follicular structures has been described initially by detection with immunofluorescence and, more recently, by Western blot analysis. Some authors[68,69] have detected class Immunoglobulin M (IgM) and C3 antibodies in the follicular structures by direct immunofluorescence.

Various studies, however, have demonstrated total negativity of those antifollicular antibodies by immunofluorescence.[70,71]

Using Western blot technique, some authors have reported the detection of antifollicular antibodies in 100% of the alopecia areata patients, against 44% of normal controls.[72]

The same authors have also demonstrated, by indirect immunofluorescence, the presence of circulating autoantibodies directed to multiple follicular structures, matrix, internal membrane and hair shaft.[73]

The controversial findings relative to the presence of these antibodies and their occurrence in normal controls suggests that these antibodies represent an event secondary to the inflammatory follicular phenomenon, that possibly exposes follicular antigens, with these antibodies appearing afterwards. Cellular

Immunity Although there is controversy in the studies on cellular immunity, there is already uncontestable evidence for the participation of immune cellular mechanisms in the pathogenesis of alopecia areata. Studies[63] relative to circulating lymphocytes in alopecia areata patients have shown varying results, from normal rates to even a reduction in their number. Other works have shown a correlation between the reduction in T lymphocytes and severity of alopecia.

Furthermore, studies[74] have demonstrated that CD8 positive T cells from patients previously cultivated with follicular homogenates are capable of producing alopecia areata in scalp fragments from patients transplanted into mice with combined immunodeficiency. The same experiment using CD4 T cells from patients does not produce alopecia areata lesions.

Some cytokines Interleukin(IL)-1 alpha, IL-1 beta and tumor necrosis factor (TNF) alpha are potent growth inhibitors of the pilar follicle and *in vitro* produce follicular alterations morphologically identical to those observed in alopecia areata.[12]

In alopecia areata patients, abnormalities have been detected in the expression of type TH1 and IL-1 beta cytokines in lesions of alopecia areata in the scalp.[12]

Therefore, there is concrete evidence in relation to the participation of cellular immunity and this allows the hypothesis to be formulated that in alopecia areata patients, sensitization to follicular antigens occurs in T lymphocytes and especially those CD8 positive. The activation of lymphocytes that comprise the perifollicular infiltrate characteristic of alopecia areata leads to the release of

cytokines capable of inhibiting growth of the pilar follicle, thereby interrupting hair synthesis.[12]

Apparently, follicular aggression exposes other antigens that act as immunogens and stimulate the secondary and variable production of the circulating antibodies that are sometimes detected in these patients.[12]

Other etiopathogenic factors

Atopy

When alopecia areata occurs in atopic individuals, it has an earlier onset and tends to develop into the more severe forms. It is possible that the immune deregulation characteristic of atopic state contributes to intensifying the tissue-specific autoimmune phenomena.[12]

Psychological trauma

Some studies have suggested that emotional stress contributes to the appearance of alopecia areata, given the observation that emotional trauma precedes the process together with the high prevalence of psychological disorders occurring in these patients. While, on the contrary, other studies have demonstrated that there is no participation of emotional phenomena in the development of alopecia areata.[12]

Scientific demonstration of the participation of psychiatric phenomena in the genesis of alopecia areata is very difficult. A possible explanation of the pathogenic mechanisms provoked by emotional conditions lies in the production

of neuromediators capable of interfering in the immunity. Some studies have revealed a decrease in the expression of calcitonin gene related peptide (CGRP) and substance P in the scalp of alopecia areata patients.[75,76] CGRP has an anti-inflammatory action, and its decrease in alopecia areata could favor the characteristic follicular inflammatory phenomena. Substance P is capable of inducing hair growth in mice, and its decrease in alopecia areata could be a contributing factor to the reduced proliferation of pilar follicles.[12]

These are examples of possible biochemical means by which emotional phenomena could influence the genesis of alopecia areata lesions through the action of neuromediators. Furthermore, in these types of disease, in that the disease itself produces evident psychological alterations through detriment to the self-image, it is necessary to consider not only the possibility of emotional phenomena interfering in the disease, but also that the disease itself can produce important psychological alterations.[12]

In spite of the progress regarding the etiopathogenesis of alopecia areata, the real etiology of the illness remains unknown and continues to be a matter for investigation. Currently, there are animal models that develop alopecia areata spontaneously or in which the disease can be induced. As is the case of the mouse C3H/HeJ62 and the Dundee experimental bald (DEB) rat, that will certainly contribute to an explanation of the illness and its pathogenic and therapeutic aspects.[12]

Histopathological aspects

The presence of peribulbar lymphocytic inflammatory infiltrate is a histopathologic characteristic, found in most of the terminal hair in one evolutionary stage: catagen or telogen. The follicles become smaller during the course, forming miniaturized hair and are substituted by fibrous tracts. Eosinophils are also found in all the stages of alopecia areata, both in the peribulbar infiltrate and in the fibrous tracts.[12]

It has been demonstrated by immunohistochemistry that the cellular infiltrate is composed above all by T lymphocytes with CD4 T lymphocytes in greater number than CD8 T lymphocytes. There is an increase in the expression of HLA-DR. The CD4 and CD8 lymphocytes are in a varied proportion, from 2:1 to 8:1, and the CD4/CD8 ratio is slightly higher in the acute phase in relation to the chronic phase. Both CD4 and CD8 lymphocytes invade the follicular epithelium. There is also an increase in the expression of Intercellular Adhesion Molecule (ICAM)-1 in the dermal papillae, in the keratinocytes and in the external sheath of the hair root.[12]

Cell-mediated immunity

Studies[64-67] of cell-mediated immunity in AA have given conflicting results. Circulating total numbers of T lymphocytes have been reported as reduced or normal.[64-67] The number of circulating T cells are reduced in AA and the level of this reduction is related to disease severity. In addition, the impairment of helper T-cell function and the change in suppressor T-cell numbers may also reflect changes in disease activity.[63] A slight increase in helper T cells

(CD4) and decrease in number of suppressor T cells (CD8) resulting in an increase in the ratio of helper to suppressor cells is correlated with the amount of hair loss.[64] Successful treatment of AA with immunomodulatory agents such as oral cyclosporine and systemic steroids also supports the immune-mediated pathogenesis in AA.[12]

A study[74] reported that AA can be induced in human scalp explants from AA patients transplanted on severe combined immunodeficiency (SCID) mice by transfer of autologous T lymphocytes isolated from involved scalp. In this study, the T lymphocytes that had been cultured with hair follicle homogenate along with antigenpresenting cells were capable of inducing the changes of AA. These changes include hair loss, perifollicular T-cell infiltration, as well as HLA-DR and intercellular adhesion molecule–1 expression of follicular epithelium. T cells that had not been cultured with follicular homogenate were not able to induce AA. The necessity of the follicular homogenate to induce AA suggests that T cells recognize a follicular autoantigen. Furthermore, AA induction was followed by injection with CD8+ cells cultured with follicular homogenate but not the cultured CD4+ cells. This study also suggests that AA is mediated by T cells, particularly CD8+ cells. Shared hereditary susceptibility, increased frequency of organ-specific antibodies, antibodies to pigmented hair follicles, high levels of autoantibodies to multiple structures of anagen hair follicles in AA patients, an increase in the ratio of helper to suppressor cells, and induction of AA on SCID mice by transfer of T lymphocytes cultured with follicular homogenates are evidence supporting the hypothesis that AA is an organ-specific autoimmune disease.

The hair follicle has a distinct immune system[77] that differs from its surrounding skin.[70] The cellular components of the hair follicle immune system are composed of intrafollicular T lymphocytes and Langerhans cells, located exclusively in the distal outer root sheath, and perifollicular mast cells and macrophages. There is also a unique expression of follicular MHC class Ia/Ib, and ICAM-1.[71,72] Human hair follicles may even serve as a Langerhans cell reservoir. The epithelium of the proximal anagen hair follicle is immune privileged since the inner root sheath and hair matrix do not express MHC class I molecules. This immune privilege may collapse in AA.[12]

A recent theory[54] for AA proposed by Paus involves the upregulation of MHC antigens and/or downregulation of locally produced immunosuppressants (melanocyte-stimulating hormone, adrenocorticotropin, and transforming growth factor), allowing the immune system to recognize the immune-privileged hair follicle antigens leading to onset of AA.

Cytokines

It appears that cytokines have a significant pathogenic role in AA. Cytokines are immunomodulators mediating inflammation and regulating cell proliferation. Cytokines derived from epidermal keratinocytes, IL-1α and IL-1β and tumor necrosis factor alfa (TNF-α) are potent inhibitors of hair follicle growth and in vitro produce changes in hair follicle morphology similar to those in AA. T helper cells also produce cytokines and they are divided into two subgroups depending on the pattern of cytokine production. Type 1 T helper (TH1) cells produce interferon gamma (IFN- α) and IL-2.[12]

Infection

A report has been published regarding the possibility of cytomegalovirus (CMV) infection found within the patches of scalp AA. This initial report showed a convincing positive association with CMV, but this has not been confirmed because other investigators have reported negative findings. The whole concept of molecular mimicry of the hair follicle with a virus is intriguing, but the evidence for a viral origin of AA at this time is not conclusive.[77]

Emotional stress

Several studies suggest that stress may be a precipitating factor in some cases of AA. Acute psycho trauma before the onset of AA, higher number of stressful events in the six months of preceding hair loss, higher prevalence of diagnosed psychiatric disorders, and psychologic factors and family situations in patients with AA have been reported. In contrast, there are reports revealing that emotional stress does not play a significant role in pathogenesis of AA.[12]

Intrinsically abnormal melanocytes or keratinocytes

Morphological analysis of follicles in active AA lesions have revealed regressive changes in the hair bulbs of anagen hair follicles. Abnormal melanogenesis and melanocytes are common findings. This evidence together with the presence of antibodies to pigmented hairs of AA may explain some of the associated pigmentary anomalies seen clinically in acute AA and the preferred effect of AA on pigmented hairs. In addition, degeneration of precortical keratinocytes has been shown in follicles of active AA lesions.

Abnormal melanosomes in clinically normal regions, together with degenerative changes including vacuolation in the outer root sheath of all hair follicles from nonbalding lesions of AA, correspond well with the hypothesis of a subclinical condition of the disease in clinically normal areas of AA.[2]

Neurologic factors

It has been suggested that local changes in the peripheral nervous system at the level of the dermal papilla or bulge region may play a role in the evolution of AA because the peripheral nervous system can deliver neuropeptides that modulate a range of inflammatory and proliferative processes. This theory has revealed a decrease in CGRP and substance P (SP) expression in the scalp of patients with AA. The neuropeptide CGRP has a potent anti-inflammatory action, and neuropeptide SP is capable of inducing hair growth in the mouse.[2]

In addition, application of capsaicin, which causes neurogenic inflammation and releases SP, to the entire scalp of two AA patients revealed an enhanced presence of SP in AA perifollicular nerves and induced vellus hair growth.[2]

Environmental impact on alopecia areata

Environmental factors may also contribute to AA development. Specific gene alleles might provide an innate degree of susceptibility to AA for an individual, but environmental factors likely cumulatively determine the actual onset, hair loss pattern, and severity of the disease. However, the exact environmental stimuli required for AA expression are yet to be determined.

Hormonal fluctuation, infectious agents, and vaccinations have all been cited as possible triggers for AA. In the mouse model, dietary soy oil increases resistance to AA development, suggesting that diet might also play a role in AA susceptibility. It is likely that there are many potential environmental inputs with different factors involved in AA development for different individuals.[12]

Stress is commonly cited as a cause for AA onset, but controlled clinical studies have been inconclusive. Some did not find a significant correlation of hair loss onset with stressful life events, while others confirm stressful events in AA patients before the onset of disease. In contrast, several studies have shown that individuals with AA are more likely to exhibit aberrant psychosocial traits, such as increased anxiety, depression, and aggression.[12]

Investigations on the skin of mice and humans have indicated increased presence of multiple factors associated with stress mediation.[12]

Trace elements can greatly affect the human health. They are the components of enzyme, hormones, vitamins and nucleic acid. If people do not supplement adequate trace elements like zinc, calcium, iron and copper, they can be seriously troubled by various problems like hair loss.

Animal models

In the past, understanding of the pathogenesis of AA was slow to progress because of the lack of animal models for this disease. Recently, investigations of AA have been facilitated by the use of animal models with either spontaneous or induced AA. Animal models with spontaneous AA include the C3H/HeJ mouse,

DEBR and Smyth chickens. AA can be induced in normal C3H/HeJ mice using full-thickness skin grafts donated from affected C3H/HeJ mice. Animal models with AA like hair loss are significantly useful in investigations regarding pathogenesis, disease behavior, efficacy, and side effects of available or future treatments.[2]

Animal models of alopecia areata two inbred rodent models have been developed for use in AA research: the DEBR and the C3H/HeJ mouse. These two models have shown AA to be an autosomal polygenic trait with incomplete penetrance. By studying the breeding patterns of C3H/HeJ mice, it was found that the segregation pattern of the AA phenotype is under the control of one or more dominant gene alleles. In C3H/HeJ mice, AA develops after 4 months in females and 10 months in males and affects up to 20% of a colony. DEBR females develop AA after 5 to 8 months; DEBR males develop AA in 7 to 10 months. More than 40% of a DEBR colony will be affected, and the condition is more likely to affect females than males with a 3:1 ratio. Both rodent models express an AA phenotype as multifocal sites of hair loss, often with symmetrical distribution. Histologically, the skin of mice and rats shows an infiltration of CD41 and CD81 T cells, macrophages, and dendritic cells in and around the hair follicles.[2]

Functional studies in animal models further support the notion of AA as an autoimmune disease and provide information on the mechanisms of AA development. AA can be transferred from spontaneously affected mice to healthy mice with an almost 100% success rate by using a skin grafting technique. This allows the study of AA with predictable onset and progression. The grafting of

affected skin promotes an induction of anagen stage hair follicle inflammation in a skin graft recipient. This infiltration is progressive over time and begins several weeks in advance of hair loss onset. Infiltration of the hair follicles and inhibition of AA onset can be blocked by targeting activated lymphocyte markers or by blocking costimulatory receptors.[2]

Most significantly, subcutaneous injection into normal haired mice of lymphocyte cell subsets isolated from AA affected mice shows that these cells induce the disease. CD81 cells quickly induced localized patches of hair loss. CD41 cells did not induce local hair loss, but they were shown to activate the host's immune system to promote multiple AA patches after several weeks. It was concluded that CD81 cells are the direct modulators of hair loss while CD41 cells play a classic ''helper'' role in AA onset.[2]

Diagnosis

Routine testing is not indicated in AA. Routine screening for autoimmune disease (thyroid disease in particular) is not generally indicated because of insufficient clinical evidence. Older patients, patients with long disease duration, females, patients with persistent patchy AA (as compared to transient patchy AA), and patients with AT/AU have been found to be more likely to have thyroid abnormalities. However, because AA severity and thyroid disease are neither correlated nor causal, routine thyroid testing was not recommended.[43]

Potassium hydroxide, fungal culture, lupus serology, syphilitic screening, and a scalp biopsy may be necessary in ambiguous or difficult to diagnose cases.

However, most presentations of AA are obvious, and further laboratory tests are not indicated in the vast majority of cases.[43]

The diagnosis is clinical and generally simple in the common cases. It is only difficult in the diffuse chronic forms, necessitating subsidiary exams, trichogram and biopsy of the scalp.[12]

Trichogram

It should be performed on hair removed from the border of the alopecic area and can reveal exclamation mark hair, as well as a smaller number of anagen hairs and larger number of telogen hairs than normal (90% anagen, 10% telogen).[12]

Biopsy

Biopsy reveals the histopathologic findings described above that enable differentiation from trichotillomania and other types of alopecia, such as the androgenetic form. It is underscored that biopsy should be done with a bistoury not a punch, in order that a significant sample of the pilar follicles is obtained. Currently, transversal sections of the material are used as these enable exam of a larger amount of pilar follicles cut at different levels. Besides the characteristic peribulbar infiltrate a significant decrease is demonstrated in the terminal hair in association with an increase in the vellus hair type, registering a proportion of 1:1, when the normal ratio is 7:1.[12]

Pathology

The histopathologic picture varies depending on disease duration. A peribulbar lymphocytic infiltrate ‘‘swarm of bees’’ characterizes the acute phase of AA. In subacute cases, large numbers of catagen and telogen hairs will be present. Hair follicle miniaturization with minimal or no inflammation is seen in chronic cases. There is abnormal hair cycling in AA. Anagen follicles may enter telogen prematurely, or some may survive for some time in a dystrophic anagen state.[43]

Consequently, the histopathologic appearance of AA varies depending on disease duration. However, increased numbers of eosinophils can be present in regions of AA affected skin in any stage of AA and are a useful diagnostic feature.[43]

In the acute stage, a peribulbar lymphocytic infiltrate ‘‘swarm of bees’’ preferentially targets anagen stage follicles. The infiltrate is composed of both CD41 and CD81cells with the CD41/CD81 ratio being higher in clinically active disease. As a consequence, edema, microvesiculation, apoptosis, necrosis, macrophages, and foreign body giant cells can be seen in and around the affected hair follicles.[43]

The root sheaths and hair matrix are infiltrated by lymphocytes and there may be hair follicle pigment incontinence, keratinocyte cell necrosis, and vacuolar damage. Focal matrix cell vacuolization and necrosis, a relatively uncommon event, is claimed to be a characteristic feature of AA.[43]

Ultrastructural studies showed that keratinocytic degeneration may affect layers of matrix cells in AA, unlike the apoptosis of scattered outer root sheath cells in normal catagen.[43]

Anagen arrest, shortly followed by catagen, weakens the hair shaft and causes breakage at the surface of the skin. As the follicle goes into telogen, the fractured widened tip will further extrude, resulting in the typical exclamation point hair.[43]

Trichomalacia with marked narrowing of the hair shafts (‘‘pencil point hair’’) results in fragile hairs that fall from the scalp in great numbers. In the subacute stage, large numbers of catagen hairs, followed by telogen hairs, can be observed. The percentage of catagen/telogen is markedly increased and often exceeds 50% of the total follicles. Some remnant inflammation may persist in or around fibrous streamers as the follicles ascend to telogen level.[43]

In the chronic stage, there is marked hair follicle miniaturization. The terminal to vellus scalp hair follicle ratio is reduced and is likely to be 1:1. These miniaturized anagen follicles are situated slightly deeper than normal vellus follicles. Chronic lesions are characterized by the presence of nanogen follicles (an intermediate stage between terminal and vellus anagen). Nonsclerotic fibrous tracts (streamers) extend along the original site of the previous terminal follicles into the subcutis.[43]

The inflammatory infiltrate, if present, is likely to be in the papillary dermis around miniaturized follicles. In the recovery stage, the terminal to vellus

ratio reverts to normal, the percentage of anagen hairs increases, and there is little or no inflammation.[43]

The total number of follicles are normal or decreased in AA compared to normal scalp. Autoimmune activity in alopecia areata, the pathogenesis of AA and the molecular mechanisms that lead to hair loss are poorly understood. In the past, AA was believed to be of infectious or neurotrophic origin. Recent research studies have indicated that AA is an inflammation-driven disease and is likely an autoimmune disorder.[43]

Circumstantial evidence in support of an autoimmune mechanism underlying AA comes from several sources. The association of AA with other autoimmune diseases has been reported. The presence of inflammatory lymphocytes around and within affected hair follicles and the ability to promote hair regrowth with the use of immunosuppressive agents is consistent with an autoimmune hypothesis.[43]

The infiltration of antigen presenting cells (APCs) such as macrophages and Langerhans cells both around and within the dystrophic hair follicles has also been observed. This is potentially consistent with a response to autoantigens within the hair follicles and attraction of these APCs. A major proinflammatory event in AA is suggested to be the abnormal expression of class I and II major histocompatibility complex antigens in hair follicle epithelia.[43]

There is also an increase in proinflammatory markers such as intercellular cell adhesion molecule and endothelial cell selectin in the blood vessels around the hair follicles. The presence of hair follicle specific IgG autoantibodies in the

peripheral blood of AA patients also further reinforces the hypothesis that the development of AA could be autoimmune related.[43]

Role of trace elements

Recently it is hypothesized that, serum levels of zinc, copper and magnesium as the lack of these three elements is often associated with hair loss. It was found out that the serum zinc levels were significantly decreased in alopecia areata patients whose disease was extensive, prolonged and resistant to any treatment, whereas serum copper and magnesium levels showed insignificant rise compared to controls. They concluded that copper and magnesium levels are not altered in alopecia areata patients, but the decreased zinc levels found in their study may merit further investigation of the relationship.

Zinc

Incidentally, zinc happens to be the most frequently found element used in hair growth boosting supplement pills so this may not be for no reason. Further clinical studies enrolling a larger number of patients, using more sophisticated techniques and involving samples of blood, erythrocytes and hair would be needed to better understand the role of these trace elements in alopecia areata. Also, an exclusive treatment with zinc supplements could be tried in such patients to see the outcome. There are claims that imbalance of trace elements may trigger the onset of AA.[79-82]

Zinc is the essential material for the formation of more than 300 kinds of enzyme. At the same time, it plays a vital role in accelerating the synthesis of

nucleic acid and protein. The lack of zinc can be caused by the inadequate supplementation and the disturbed absorption. According to the research, the lack of zinc can seriously affect the growth of hair and even cause hair loss.

Oral zinc compounds have been used for decades for treating disorders such as telogen effluvium[79,80] and alopecia areata.[81,82] Reports have also been published on oral zinc sulfate therapy with encouraging results for some cases of alopecia areata. In 1976 Wolowa and Jablonska[83] reported that two patients with alopecia areata regrew their hair after treatment with oral zinc sulfate. It has been reported that some alopecia areata patients have zinc deficiency.[84-86] Zinc is an essential cofactor for multiple enzymes and it is involved with important functional activities in the hair follicle.[87]

Further, zinc is a potent inhibitor of hair follicle regression and it accelerates hair follicle recovery.[85,86] There are several reports stating that the serum zinc level is low in alopecia areata patients.[10,87-89]

However, the pathogenesis of this reduced serum zinc level is unknown. As cofactors of metalloenzymes, zinc has considerable effects on nearly all aspects of the metabolism that takes place in the organs of the body, including the skin. In fact, congenital and acquired zinc deficiencies are usually expressed as a variety of skin manifestations such as acrodermatitis enteropathica, psoriasis-like eruptions, blisters, onychopathy and loss of hair.[84,90]

Several reports have shown that oral administration of zinc compounds improved hair growth.[83] Yet in 1981, Ead[91] reported that oral administration of zinc compounds had no therapeutic effect on hair loss. Ead[91] found that after zinc

supplementation, the serum zinc level changed from 77.5 µg/dl to 112.2 µg/dl and the serum zinc level increased by 34.7 µg/dl, but the patients did not show a positive therapeutic effect. In this study, 6 out of 15 patients belonged to the negative response group. Among this negative response group, 4 patients' serum zinc levels increased and 2 patients' serum zinc levels decreased.

Zinc is a metal moiety of many enzymes and it is indispensable for normal cellular function and it has important roles in bone formation, cell-mediated immunity, the general immunological defense of the host and tissue growth. Zinc provides structural integrity to enzymes and/or it participates directly in catalysis. Examples of zinc metalloenzymes include DNA and RNA nucleotidyl transferases, alcohol dehydrogenase, glutamic, lactic and malic dehydrogenase and δ-aminolevulinic acid dehydratase.[92]

The etiology of alopecia areata is still unknown. Several kinds of treatments have been tried with various results, that is, oral and topical corticosteroids, triamcinolone intralesional injection, photochemotherapy, topical irritants and allergens, immunosuppressants and cryotherapy. Checking the serum zinc level is necessary to evaluate hair loss of an unknown cause, and zinc supplementation may be needed in the alopecia areata patients who have a low serum zinc level.[87]

In a study, after adhering to zinc supplementation for twelve weeks, the patients' mean serum zinc level changed from 56.9 µg/dl to 84.5 µg/dl, and the level increased by 27.60 µg/dl. When analyzing the differences between the positive and negative response groups, the positive response group increased their

serum zinc level by 40.9 μg/dl, and the negative response group increased their serum zinc level by 7.7 μg/dl. This difference was statistically significant. Those patients with mild alopecia areata and those with a single alopecia areata patch had a greater positive response than the patients with moderate alopecia areata and those with multiple alopecia areata patches. The positive response group maintained zinc supplementation for at least six months with no recurrence of their hair loss being seen during their follow-up. The positive response group also showed continuous terminal hair regrowth during follow-up. Although these patients had a mild type of long term alopecia areata, zinc supplementation can become a possible adjuvant therapy when combined with other therapeutic methods, and especially for those alopecia areata patients with a low serum zinc level.

Prior to this study, there has only been one report of alopecia areata patients having a low serum zinc level in the Korean medical literature[81] and there has been no report about the therapeutic effects of zinc supplementation in Korea. This study was the first in Korea to evaluate the therapeutic effects of twelve weeks of oral zinc supplementation in alopecia areata patients with a low serum zinc level and reported on the changing serum zinc levels after oral zinc supplementation. Positive therapeutic effects were seen in 9 out of 15 patients, but because of the small numbers of patients, the therapeutic effects were not statistically significant. Subsequent studies with a large number of alopecia areata patients are needed to clarify the therapeutic effects of oral zinc supplementation.

Mussalo Rauhama et al.[10] did not find any difference in serum trace element concentrations of alopecia patients compared to the normal population,

but showed a statistically significant difference between the copper content of serum in AA and alopecia universalis patients.

Naginiene et al[11] found a lower level of zinc in blood and urine of children with alopecia and increased levels of copper and chromium concentrations in their hair compared to healthy individuals. This study showed statistically significant lowered levels of zinc in AA patients compared to controls, but no significant change in copper and magnesium levels. Also, the decreased levels of zinc was seen more in those patients with prolonged duration, extensive lesions, and lesions resistant to treatment, but no statistical correlation could be made because of the small number of these patients. Although the difference of mean zinc levels in AA patients and controls is only 10 µg/dL, it can be of quite significant clinical importance since the trace elements act at molecular level and are active at very minute concentrations. The zinc deficiency induced by trace element replacements with heavy metals can cause the onset of alopecia besides other factors.

Bruske and Salfeld[93] interpreted the statistical association of blood and serum levels of zinc, magnesium, and copper in patients with many dermatological disorders including AA and after comparing with healthy people did not find any change in serum levels of zinc and copper, but found a significantly higher level of magnesium. The varied results of the levels of magnesium, copper, and zinc in various studies can be explained on the basis of sample size, methodology, and population variation.

Chapter 4

Methodology

METHODOLOGY

The present study was conducted in the Department of Dermatology, Venereology and Leprosy, attached to KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum on 40 clinically confirmed cases of Alopecia Areata.

Study design

The study design was one year cross sectional study.

Study period and duration

The present one year study was conducted during the period of January 2011 to December 2011.

Place

This study was carried out at Department of Dermatology, Venereology and Leprosy, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum attached to Jawaharlal Nehru Medical College, Belgaum.

Method of collection of data

Source of Data

Patients with alopecia areata attending Out Patients Department, Department of Dermatology, Venereology and Leprosy, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum were studied.

Sample size

The present study was comprised of 40 patients with alopecia areata and equal number of age and gender in healthy controls.

Sampling procedure

The sample size was calculated based on 80% of average cases of Alopecia areata in 3 years based on hospital statistics.

1. Total number of cases in 2007 - 54
2. Total number of cases in 2008 - 48
3. Total number of cases in 2009 - 52

Total cases in last three years - 154

Average of three years - 51

80% of average of last three years - 40

Selection criteria

Inclusion Criteria

- 40 clinically confirmed (old and new) cases of alopecia areata irrespective of age, sex, duration of illness and associated diseases, willing to participate in the study.

Exclusion criteria

- Patients with disorders like Trichotillomania, Tinea capitis and Traction Alopecia.
- Patients not willing to participate in the study.
- Patients already taking zinc as supplement therapy.

Procedure

Prior to the commencement of the study, approval from Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belgaum was obtained.

Patients with alopecia areata attending Out Patients Department, Department of Dermatology, Venereology and Leprosy, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum were evaluated based on selection criteria. After finding the suitability as per inclusion and exclusion criteria they were requested to participate in the study and briefed about the nature of the study, the interventions used and written informed consent was obtained (Annexure–I).

The consented patients were enrolled in the present study. Further descriptive data of the participants like name, age, sex, detailed history, were obtained by interviewing the participants. A thorough clinical examination was conducted for dermatological and systemic examination and findings were recorded on predesigned and pretested proforma (Annexure-II). Equal number of gender and age in controls were taken (1:1 ratio) by above mentioned method.

Zinc Estimation

Collection of Blood Samples

About five ml of venous blood was collected from antecubital vein under aseptic precautionary measures using sterile disposable syringe. The blood was centrifuged at 2000 rpm for 15 minutes and the serum was collected in a plastic

container with an air tight cap and labelled. Glass containers were deliberately avoided to prevent zinc contamination from glass.

The serum samples were analysed for zinc levels at Department of Biochemistry, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum.

Method of estimation

Serum immunoturbidometry method was the method used for estimation of serum zinc levels.[94]

Statistical analysis

Data obtained was coded and entered into Microsoft excel spreadsheet (Annexure IV). The demographic and clinical data was expressed as rates, ratios and percentage and the continuous data was expressed as mean and standard deviation (SD). The comparison between study and control group was done using unpaired ‘t’ test. A ‘p’ value of less than 0.05 was considered as statistically significant.

Figure 3. Zinc Estimation Kit

Figure 4. Photometer

Chapter 5

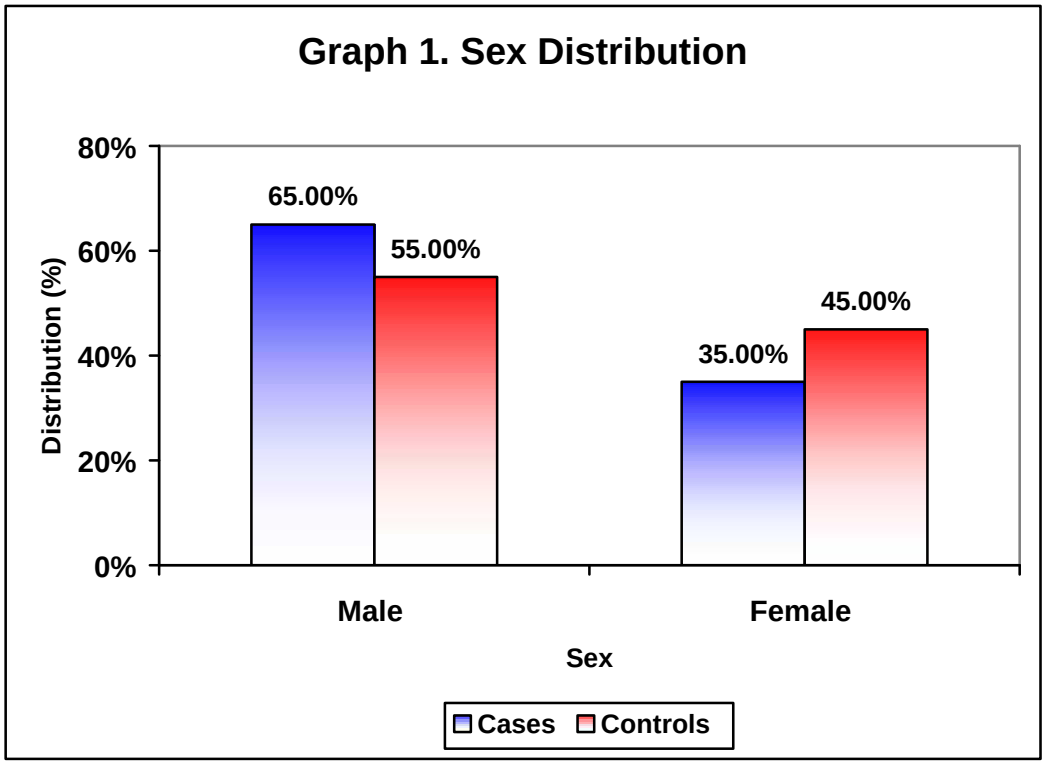
Results

RESULTS

The present one year cross sectional study was conducted in the Department of Dermatology, Venereology and Leprosy, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre attached to Jawaharlal Nehru Medical College, Belgaum during the period of January 2011 to December 2011. The study was comprised of 40 patients with alopecia areata and equal number of age and gender in healthy controls. The data obtained was coded and entered into Microsoft excel spreadsheet (Annexure IV) and analysed. The results and observations are as below.

Table 1. Sex Distribution

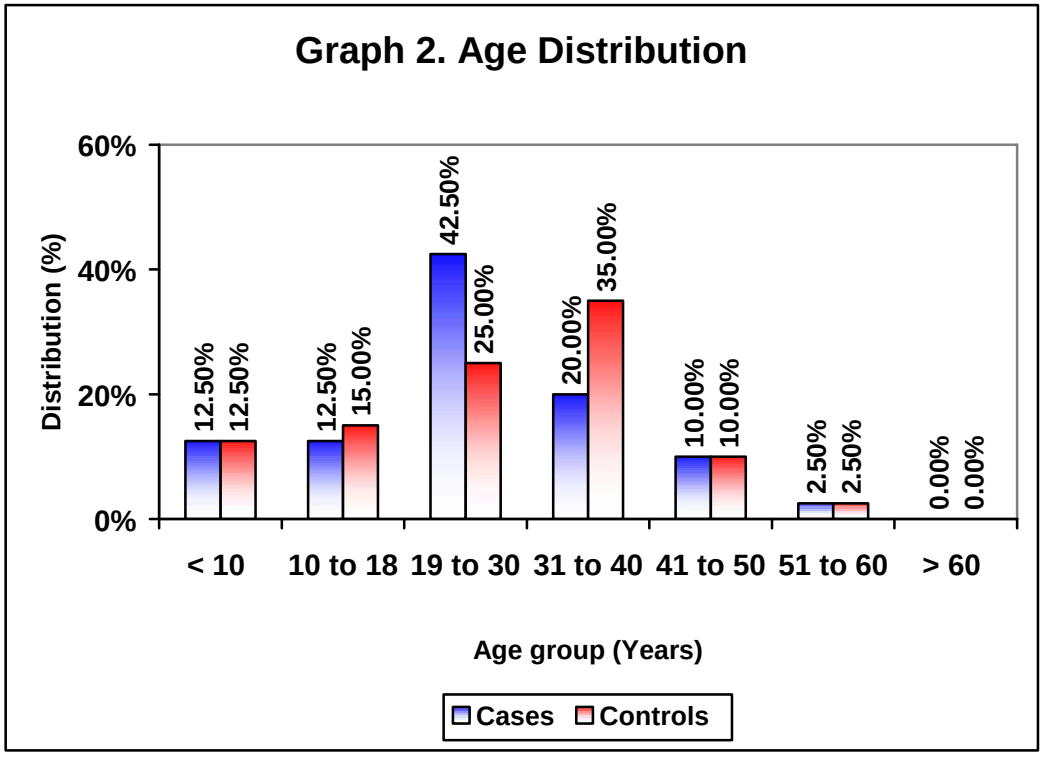
Sex	Cases (n=40)		Controls (n=40)	
	Number	**Percent**	**Number**	**Percent**
Male	26	65.00	22	55.00
Female	14	35.00	18	45.00
Total	**40**	**100**	**40**	**100**



In the present study among cases 65% were males and 35% were females with male to female ratio of 1.85:1. In controls 55% were males and 45% were females with male to female ratio of 1.22:1. Though there was slight male preponderance in both groups the comparison between cases and controls was statistically not significant suggesting equal distribution.

Table 2. Age Distribution

Age group (Years)	Cases (n=40)		Controls (n=40)	
	Number	Percent	Number	Percent
< 10	5	12.50	5	12.50
10 to 18	5	12.50	6	15.00
19 to 30	17	42.50	10	25.00
31 to 40	8	20.00	14	35.00
41 to 50	4	10.00	4	10.00
51 to 60	1	2.50	1	2.50
> 60	0	0.00	0	0.00
Total	**40**	**100**	**40**	**100**

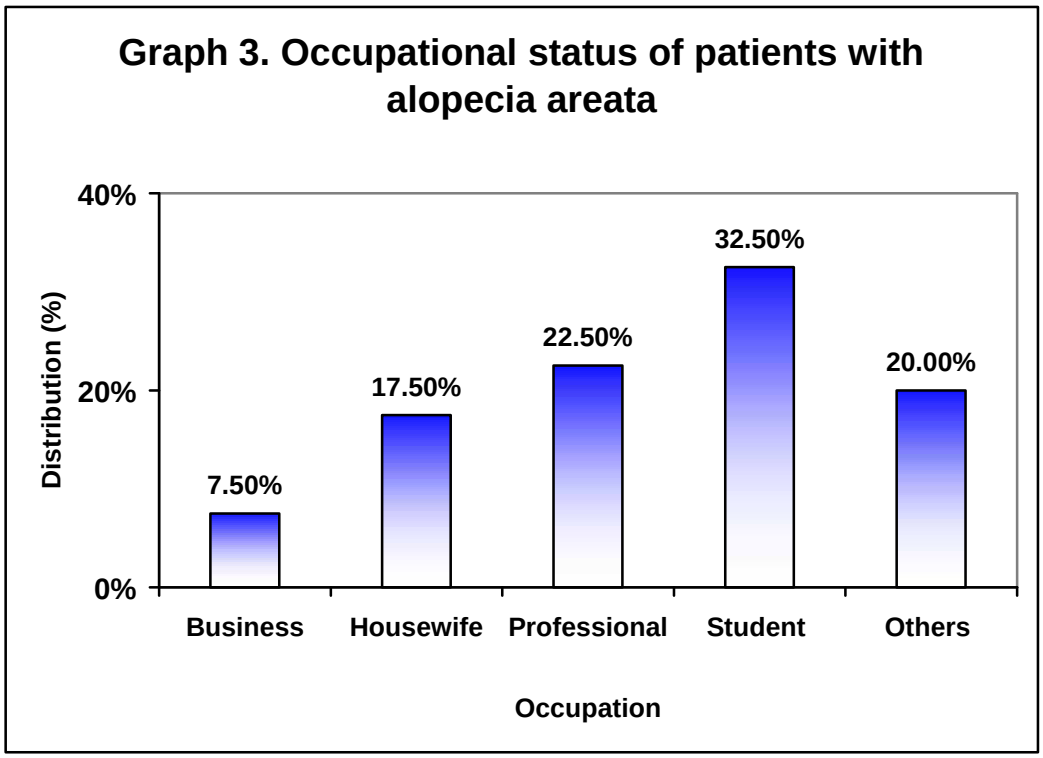


In this study 42.5% of patients with alopecia areata were aged between 19 to 30 years followed by 20% between 31 to 40 years, 10% between 41 to 50 years and 2.5% between 51 to 60 years. However, 12.5% cases each were in the age of less than 10 years and between 10 to 18 years.

The mean age among cases was 26.85 ± 11.99 years with range minimum being 6 years and maximum being 57 years. Among controls the mean age was 28.03 ± 12.43 years with range being 6 to 59 years. The comparison between the two groups showed no statistically significant difference suggesting equal distribution of age in both the groups.

Table 3. Occupational status of patients with alopecia areata

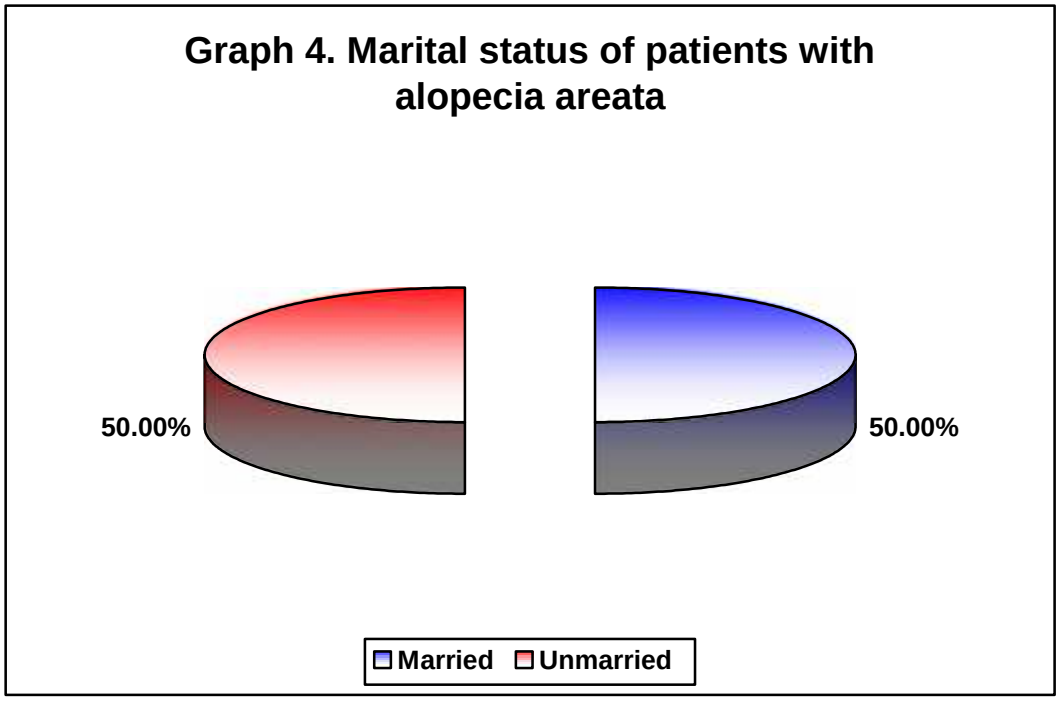
Occupation	Cases (n=40)	
	Number	**Percent**
Business	3	7.50
Housewife	7	17.50
Professional	9	22.50
Student	13	32.50
Other	8	20.00
Total	**40**	**100**



In the present study among the patients with alopecia areata 32.50% were students, 22.5% were professionals, 17.5% were housewives and 7.5% were engaged in business activities.

Table 4. Marital status of patients with alopecia areata

Marital status	Cases (n=40)	
	Number	**Percent**
Married	20	50.00
Unmarried	20	50.00
Total	**40**	**100**



In the present study equal number of patients with alopecia areata were married (50%) and unmarried (50%).

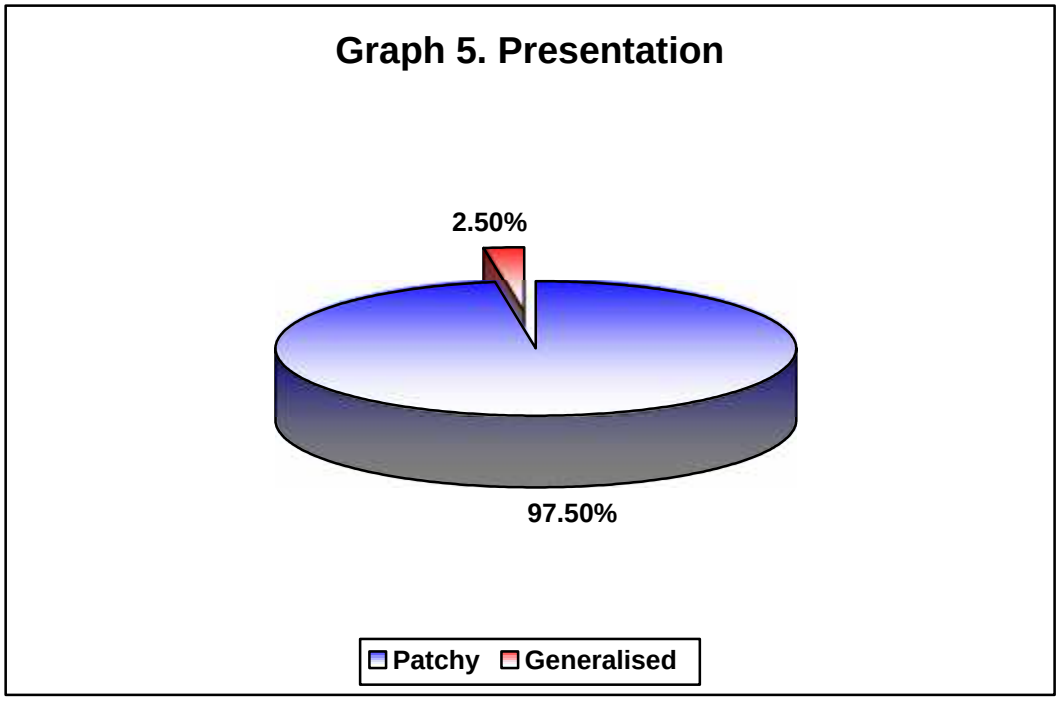
Table 5. General physical examination findings

Parameters	Mean values	
	Mean	**SD**
Pulse (bpm)	79.15	7.27
Systolic blood pressure (mm Hg)	113.25	12.65
Diastolic blood pressure (mm Hg)	73.85	6.49

The mean pulse rate among patients with alopecia areata was 79.15 ± 7.27 bpm. The systolic blood pressure was noted as 113.25 ± 12.65 mm of Hg and the diastolic blood pressure was noted as 73.85 ± 6.49 mm Hg.

Table 6. Presentation

Presentation	Cases (n=40)	
	Number	**Percent**
Patchy	39	97.50
Generalized	1	2.50
Total	**40**	**100**

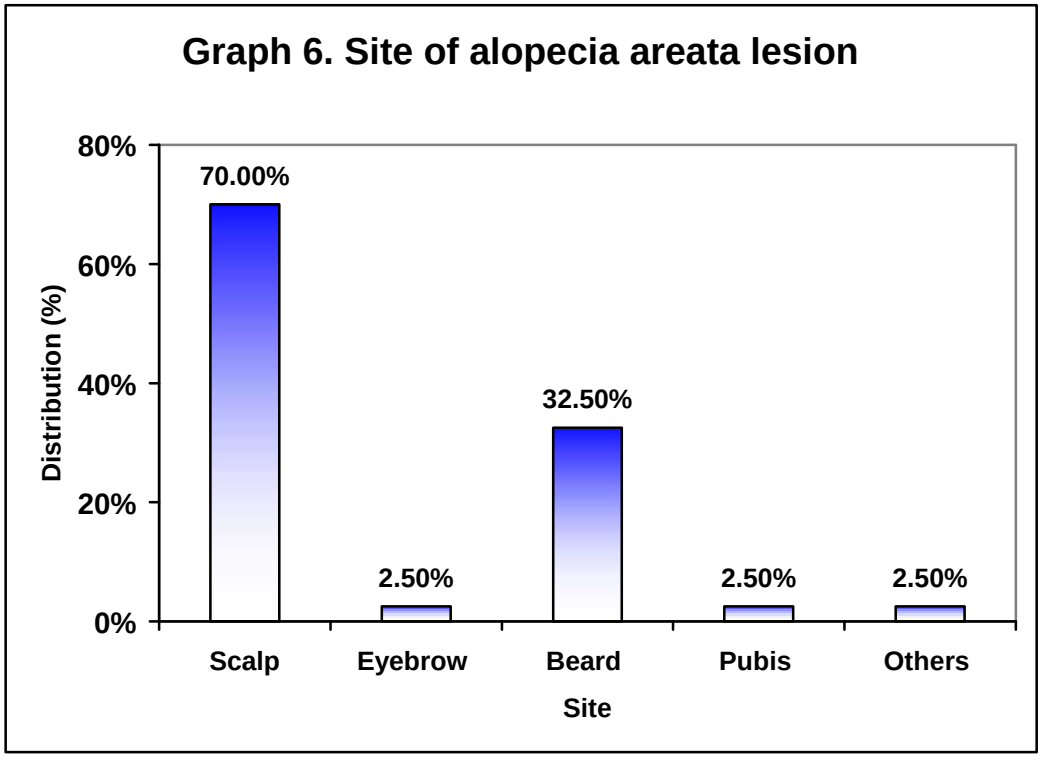


In the present study 97.50% patients presented with patchy and 2.50% with generalized alopecia areata.

Table 7. Site of alopecia areata lesion

Site of lesion	Cases (n=40)	
	Number	**Percent**
Scalp	28	70.00
Eyebrow	1	2.50
Beard	13	32.50
Pubis	1	2.50
Others	1	2.50

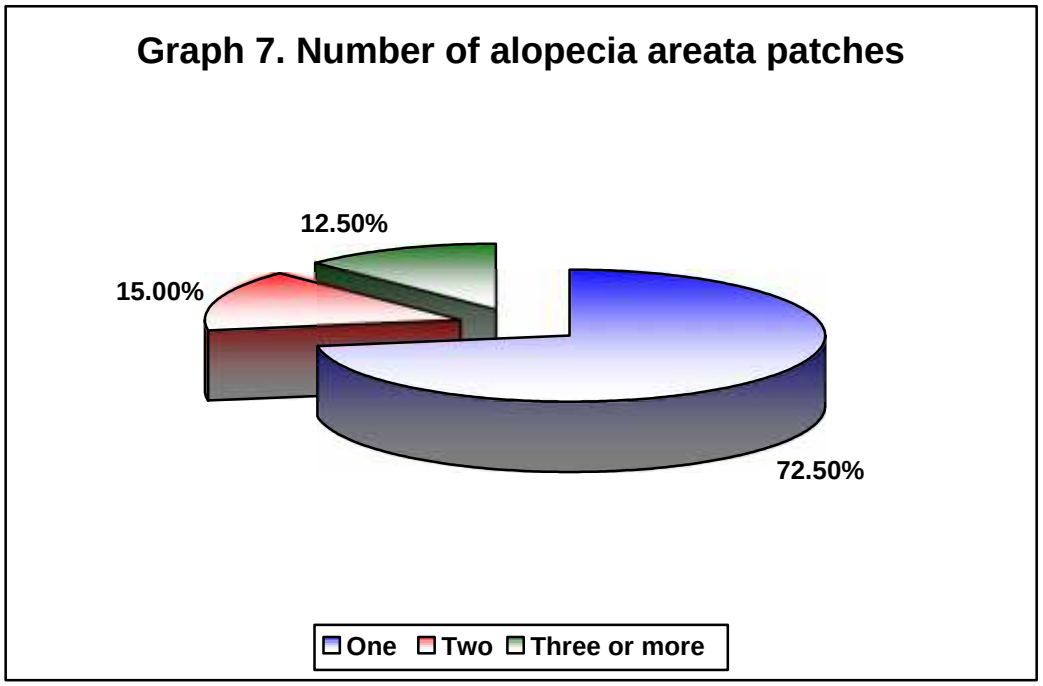
Multiple presentations



The most commonest site was scalp in 70% followed by 32.50% on beard and 2.5% each on eyebrow, pubis and other sites.

Table 8. Number of alopecia areata patches

Patches	Cases (n=40)	
	Number	**Percent**
One	29	72.50
Two	6	15.00
Three or more	5	12.50
Total	**40**	**100**

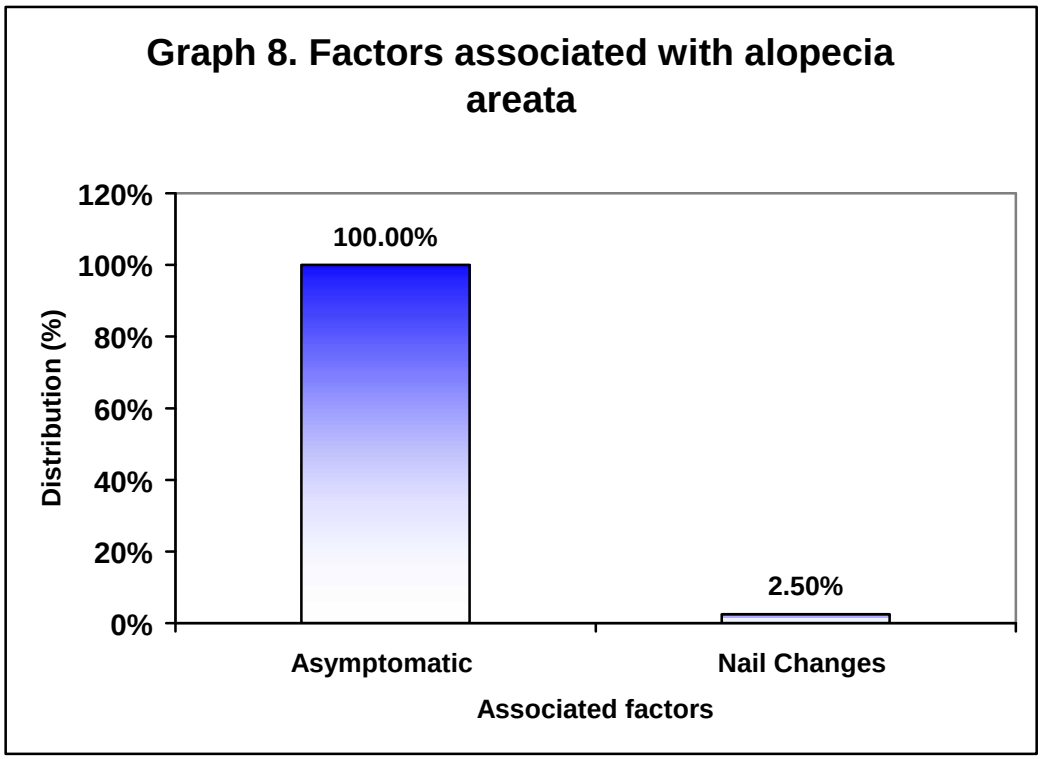


In this study 72.5% of patients had single patch of alopecia aereata. However, 15% of patients had two and 12.5% had three or more patches of alopecia areata.

Table 9. Factors associated with alopecia areata

Factors	Cases (n=40)	
	Number	**Percent**
Asymptomatic	40	100.00
Nail changes	1	2.50

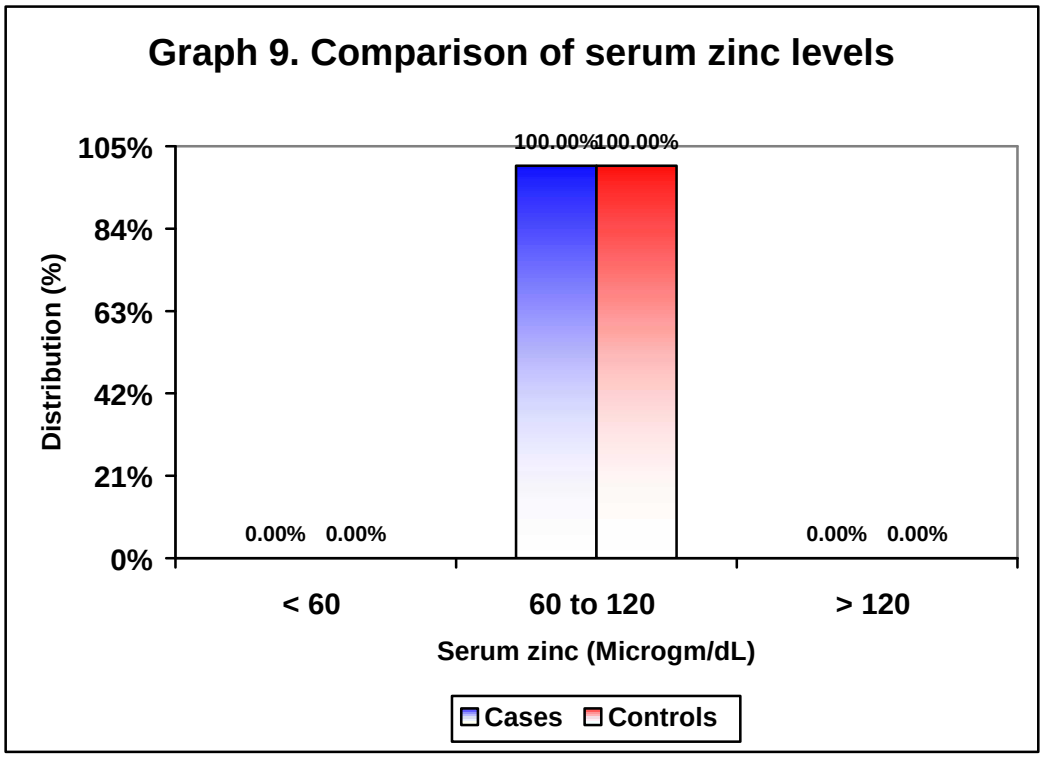
Multiple conditions



In this study all the patients had asymptomatic alopecia areata (100%). However, one patient (2.5%) had nail changes associated with alopecia areata.

Table 10. Comparison of serum zinc levels

Serum Zinc (mg/dL)	Cases (n=40)		Controls (n=40)	
	Number	**Percent**	**Number**	**Percent**
< 60	0	0.00	0	0.00
60 to 120	40	100.00	40	100.00
> 120	0	0.00	0	0.00
Total	**40**	**100**	**40**	**100**

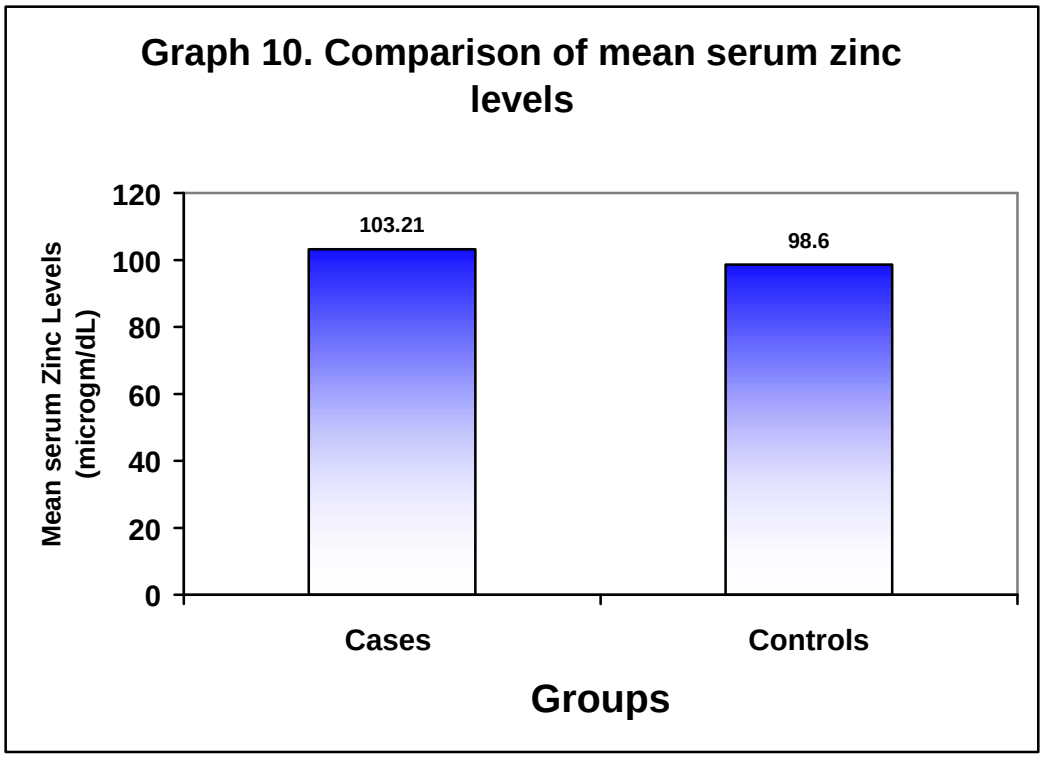


In the present study serum zinc levels were found to be normal in healthy controls as well as patients with alopecia areata.

Table 11. Comparison of mean serum zinc levels

	Cases (n=40)		Controls (n=40)	
	Mean	**SD**	**Mean**	**SD**
Serum zinc (mg/dL)	103.21	10.17	98.6	22.51

p=0.160



In this study the mean serum zinc levels among those with alopecia areata were slightly high (103.21 ± 10.17 mg/dL) compared to healthy controls (98.60 ± 22.51 mg/dL). However this difference was statistically not significant.

Chapter 6

Discussion

DISCUSSION

The pathophysiology of AA is considered to be T-cell mediated autoimmunity that occurs mostly in genetically predisposed individuals. In addition to disturbance of immune function, complex interactions between predisposing genetic and environmental factors act as triggers for disease progression. Also, perifollicular nerves and vasculature, viruses, trace element alterations, endocrine disorders, and thyroid dysfunction have been hypothesized. There are claims that imbalance of trace elements may trigger the onset of AA. Clinically, AA can present with many different patterns. A flat alopecic plaque with normal skin color, involving the scalp or any other pilar region of the body is the characteristic lesion of AA. Complex interactions between predisposing genetic and environmental factors play a likely role in the induction of immune-mediated responses in AA. Iron and zinc are the well-known trace elements that are associated with hair shedding.[78]

Hence, the present study was undertaken to assess serum zinc levels of clinically confirmed 40 cases of AA in comparison with equal number of gender and age in controls and to compare the relation of these values with demographic data like gender and age in equal number of controls.

The present one year cross sectional study was conducted in the Department of Dermatology, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum attached to Jawaharlal Nehru Medical College, Belgaum during the period of January 2011 to December 2011. The study was

comprised of 40 patients with alopecia areata and equal number of age and gender matched healthy controls.

In the present study there was slight male preponderance that is, males constituted 65% among cases and 55% among healthy controls. The male to female ratio was 1.85:1 among cases and 1.22:1 in healthy controls. However the comparison between cases and controls was statistically not significant suggesting equal distribution.

Wasserman D, et al[36] reported that, AA likely affects males and females equally. Some studies[37,38] by Kyriakis KP, et al[37] and Kavak A,[38] show a significant male preponderance in the adult age group, although others identify contrasting results. Data concerning the sex ratio for alopecia areata vary slightly in the literature. In a study by Muller SA, et al[6] including 736 patients, a male-to-female ratio of 1:1 was reported. In another study Xiao FL et al[39] observed male preponderance (1.4:1): in those with severe involvement whereas the male preponderance was inconsistent with findings in another study by Tan E, et al[35] carried out in Singapore, which showed a more severe alopecia in girls. Other two studies by Sharma VK, et al[40] and Tan E, et al[41] showed a higher proportion of females. A study by Bhat YJ, et al[78] from Shinagar, India reported increased incidence of AA in men compared to women (34:16). Therefore, it is not clear whether gender affects the extent of disease.

In this study 42.5% of patients with alopecia areata were aged between 19 to 30 years followed by 20% between 31 to 40 years, 10% between 41 to 50 years and 2.5% between 51 to 60 years. However 12.5% each had age less than 10

years and between 10 to 18 years. The mean age among cases was 26.85 ± 11.99 years with range of minimum being 6 years and maximum being 57 years. Among controls the mean age was 28.03 ± 12.43 years with range being 6 to 59 years. The comparison between the two groups showed no statistically significant difference suggesting equal distribution of age in both the groups.

Alopecia areata can occur at any age from birth to the late decades of life. Congenital cases have been reported. The statistical data registered in the literature are variable. The disease can begin at any time of life, but with a peak incidence between 20 and 50 years of age, and other articles affirm 60% of the patients present with the first episode of the disease before 20 years of age.[12] Peak incidence appears to occur from age 15 to 29 years.[42] Pediatric AA constitutes approximately 20% of AA cases, and as many as 60% of patients with AA will present with their first patch before 20 years of age.[43] One study by Tan E, et al[35] suggests that 85.5% of Asian patients with AA have disease onset before 40 years of age. Alkhalifah A, et al[43] reported that, the disease prevalence peaks between the second and fourth decades of life.[43]

In a study the differences in the mean age of onset or mean duration of AA between boys and girls were not significant. The median age of onset was 10 years old, which was lower than 11.2 years old in a report by Bolduc C et al[42] from Singapore, but higher than 5.7 years old in another report from Kuwait.[44] Authors also reported that, earlier the age of onset, the more severe the extent of AA, which was consistent with other report by Tan E et al.[35] A study by Bhat YJ et al[78] from Srinagar, India reported majority of the patients below 40 years of

age which was comparable to the another study by Sharma VK, et al[34] from India.

In the present study among the patients with alopecia areata 32.50% were students, 22.5% were professionals and 17.5% were housewives. Equal number of patients with alopecia areata were married (50%) and unmarried (50%).

The mean pulse rate among patients with alopecia areata in this study was 79.15 ± 7.27 bpm, systolic blood pressure was 113.25 ± 12.65 mm of Hg and the diastolic blood pressure was 73.85 ± 6.49 mm Hg.

In the present study 97.50% patients presented with patchy alopecia areata and only 2.50% with generalized alopecia areata. The most commonest site was scalp in 70% followed by beard in 32.50% cases, and 2.5% each on eyebrow, pubis and other sites. Most of patients (72.5%) had single patch of alopecia areata. However, 15% of patients had two and 12.5% had three or more patches of alopecia areata. All the patients had asymptomatic alopecia areata (100%). However, one patient (2.5%) had nail changes associated with alopecia areata.

In a study by Bhat YH et al[78] from Srinagar, India onset of the lesions was sudden in majority of the patients, followed by slow progression, and two patients had recurrent lesions after complete remission.

In the present study serum zinc levels were found to be normal in healthy controls as well as patients with alopecia areata. In this study the mean serum zinc levels among those with alopecia areata were slightly high (103.21 ± 10.17

mg/dL) compared to healthy controls (98.60 ± 22.51 mg/dL). However this difference was statistically not significant.

There are several reports stating that the serum zinc level is low in alopecia areata patients.[10,85,88,89] However, the pathogenesis of this reduced serum zinc level is unknown. As cofactors of metalloenzymes, zinc has considerable effects on nearly all aspects of the metabolism that takes place in the organs of the body, including the skin. In fact, congenital and acquired zinc deficiencies are usually expressed as a variety of skin manifestations such as acrodermatitis enteropathica, psoriasis-like eruptions, blisters, onychopathy and loss of hair.[84,90]

Several reports have shown that oral administration of zinc compounds improved hair growth.[83] Yet in 1981, Ead et al[91] reported that oral administration of zinc compounds had no therapeutic effect on hair loss. Ead et al[91] found that after zinc supplementation, the serum zinc level changed from 77.5 µg/dl to 112.2 µg/dl and the serum zinc level increased by 34.7 µg/dl, but the patients did not show a positive therapeutic effect.

Another study by Al-Jaff ANE et al[95] from Iraq revealed that serum Zn level was significantly lower than those of control ($p<0.001$), while serum Cu was significantly higher than that of control group ($p=0.002$).

A study by Bhat YJ et al[78] from Srinagar, India reported the mean of serum zinc levels in AA patients and controls as 78 ± 7.45 µg/dl and 88 ± 8.78 µg/dl, respectively and this difference in mean of serum zinc levels was found to be significant ($p<0.05$) standard error of difference between two means using students '*t*' test.

Factors responsible for this decline in Zn levels are unknown**.** However, decreases in plasma Zn content has been attributed to reductions in intake or absorption in small intestine, or to increases in urinary loss, or to redistribution from plasma to tissue. Furthermore, tissues with high cellular turnover (skin**)** are characteristically affected by Zn deficiency calling the attention to the possibility that some dermatological manifestations, such as alopecia areata, may be attributed to Zn deficiency.[10]

Zn plays an important role in achieving proper function of the immune system in the body. Also, it is required for the enzyme activities necessary for cell division, cell growth; and wound healing. Immunologic defects of T-cell function are typical in Zn deficiency. Experimentally, suboptimal intake of Zn has rapid adverse effects on the immune system of humans, including T cell mediated responses critical for host protection against parasitic infection. Beck FWJ et al[96] concluded that mild zinc deficiency lead to an imbalance between TH1 and TH2 lymphocytes, decreases the recruitment of T native cells, and decreases the percentage of T cytolytic cells.

Therefore, the presence of a significant reduction in Zn level of alopecic patients may lead to impaired immune function of those patients. Further study may be required to evaluate the role of Zn supplementation in alopecic patients. Oral Zn supplementation has been reported to stimulate both T and B-cell activity and improves the immune system in elderly people.[95] The results of the present study were contradictory to these findings.

However few studies have reported no significant difference in zinc levels among patients with alopecia areata compared to healthy individuals. A case-control study by Omidian M, et al[97] from Imam Khomeini Hospital, Jundishapour University of Medical Sciences in Ahwaz, reported the serum zinc level as 873±154.9 micg/1 and in alopecia areata patients 831.8±155.8 micg/1 in control group (P>0.05). Study concluded no significant difference between the serum zinc levels of patients with alopecia areata and controls.

Further clinical studies enrolling a larger number of patients, using more sophisticated techniques, and involving samples of blood, erythrocytes, and hair are needed to better understand the role of these trace elements in AA. Also, exclusive treatment with zinc supplements can be tried in these patients to see the outcome.

Chapter 7

Conclusion

CONCLUSION

The present study was aimed to assess serum zinc levels of clinically confirmed 40 cases of AA in comparison with equal number of gender and age in controls and also to compare the relation of these values with demographic data like gender and age. The results of the present study showed no significant relation between serum zinc levels and AA. Further clinical studies enrolling a larger number of patients, using more sophisticated techniques, and involving samples of blood, erythrocytes, and hair are needed to better understand the role of these trace elements in AA. Also, exclusive treatment with zinc supplements can be tried in these patients to see the outcome.

Chapter 8

Summary

SUMMARY

Alopecia areata is an unpredictable, usually patchy, nonscarring hair loss condition. Any hair-bearing surface may be affected. Iron and zinc are the well-known trace elements that are associated with hair shedding. The present study was an attempt to assess serum zinc levels of clinically confirmed 40 cases of Alopecia Areata in comparison with equal number of gender and age in controls and to compare the relation of these values with demographic data like gender and age in equal number of controls.

This one year cross sectional study was conducted in the Department of Dermatology, Venereology and Leprosy, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre attached to Jawaharlal Nehru Medical College, Belgaum during the period of January 2011 to December 2011. The study comprised of 40 patients with alopecia areata and equal number of age and gender in healthy controls. Serum immunoturbidometry method was the method used for estimation of serum zinc levels.

In the present study among cases 65% were males and 35% were females with male to female ratio of 1.85:1. 42.5% of patients with alopecia areata were aged between 19 to 30 years with the mean age of 26.85 ± 11.99 years. 97.50% patients presented with patchy and 2.50% with generalized alopecia areata with most commonest site being scalp in 70%. Most (72.5%) of patients had single patch of alopecia aereata. all the patients had asymptomatic alopecia areata (100%). However, one patient (2.5%) had nail changes associated with alopecia areata. The serum zinc levels were found to be normal in healthy controls as well

as patients with alopecia areata. the mean serum zinc levels among those with alopecia areata were slightly high (103.21 ± 10.17 mg/dL) compared to healthy controls (98.60 ± 22.51 mg/dL). However this difference was statistically not significant.

The results of the present study showed no significant relation between serum zinc levels and AA.

Chapter 9

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Annexures

Annexure I

ANNEXURE I – CONSENT FORM

I.DO.NO. ☐☐☐

Estimation of serum zinc levels in 40 clinically confirmed cases of Alopecia Areata patients in comparision with equal number of gender and age matched controls – A Cross Sectional study conducted by Dr. ***** ****** Post graduate student in M.D Dermatology under guidance of Dr. ***** ******, Professor Department of Dermatology, J N Medical College, Belgaum.

Respected Sir/Madam, we invite you to participate in our study as, you are eligible for the same. During the study you will be asked some questions in detail regarding your present complaints.

The purpose of this study is **Estimation of serum zinc levels in 40 patients of Alopecia Areata**. You are being asked to participate in this research because you have been diagnosed as a patient of Alopecia areata. All patients attending the hospital, who are diagnosed to have this disease, will be requested to participate in this study during the period of one year.

Should you choose to participate, you will be asked to give a detailed history of your disease, undergo a physical examination, and consent to a few routine blood and urine investigations.

You may undergo some amount of discomfort during the process of investigations, which may include slight pain and bleeding. However all necessary steps and precautions will be taken to ensure your safety. The result of

you taking part in this research would help health care providers towards a better understanding of this disease, and thus we will be able to provide improved patient care.

If you decide not to participate in this study, you will still be receiving the usual standard care for your disease.

Your privacy will be respected and all information collected about you during the course of this study will be kept confidential. Your identity will remain undisclosed.

The J N Medical College will provide, within the limitations of the laws of the State of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in this project. In the event of an emergency, you should contact KLE'S Dr. Prabhakar Kore Hospital and MRC on Telephone No. ***** *******.

You shall not be receiving any payment or any financial incentives for participating in this study.

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however, will be maintained confidential at all times.

Your participation in this study is voluntary. Your decision whether or not to participate will neither affect the care of your current disease,nor your future relations with the doctor or the hospital. You are free to discontinue participation in this study at any time and for any reason. In case you need further information

regarding your rights as a study participant, you may please contact Principal and Chairman of the Ethical Committee, J N Medical College, Belgaum on telephone No. *** ******

Statement of Consent:

I.D.NO: | | | |

I Mr/Ms/Mrs _____ volunteer and consent to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I accept to participate in this study. All the information regarding this study is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

Participants's name:

Signature or left thumb print of participant:

Witness name:

Signature of witness:

Signature of Investigator:

Date:

If the participants are Minors (under 18), the parents sign the form, rather than the participants.

Annexures

Annexure II

ANNEXURE II – PROFORMA

Estimation of serum zinc levels in 40 clinically confirmed cases of Alopecia Areata patients in comparison with equal number of gender and age matched controls-A Cross Sectional study

Case No. ☐☐ **OP/IP No.** ☐☐☐☐☐☐

Name: First name ☐☐☐☐☐☐ Middle name ☐☐☐☐☐☐☐

Last name ☐☐☐☐☐☐

Age: ☐☐

Sex:

1. Male
2. Female

☐

Occupation:

1. Business
2. Housewife
3. Professional
4. Any other

☐

Income: Monthly income (In Rs.):

a. Above poverty line b. Below poverty line

☐

Address with phone number:

Presenting complaints and duration:

History of present illness:

1. a. Onset:

1.Sudden
2.Gradual

☐

☐

b 1. Patchy 2.Generalised

Site of lesion:

Scalp
1. Present ☐
2. Absent

Beard/Moustache ☐
1. Present
2. Absent

Eye brows/Eye lashes ☐
1. Present
2. Absent

Axilla
1. Present ☐
2. Absent

PUBIS
1. Present ☐
2. Absent

Others
1. Present ☐
2. Absent

History of

1.Trauma
2.Itching ☐
3.Atopy
4.Hypertension
5.Diabetes
6.Exposure
7.Vitiligo
8.Thyroid
9.Others

Any associated factors:
1. Itching ☐
2. Pain
3. Burning
4. Asymptomatic

Past History:

History of similar illness:
1. Present ☐
2. Absent

History of Diabetes Mellitus: ☐
1. Present
2. Absent
3. If Yes,Duration _____months/years

History of Hypertension: ☐
1. Present
2. Absent
3. If yes,Duration_____months/years

History of vitiligo
1.Present
2.Absent
3.If yes Duration
History of Atopy
1.Present
2.Absent
3.If yes Duration

History of anyother medical disorders:_____

Family History:

Marital Status**:** ☐
1. Married
2. Unmarried
3. Divorcee

Treatment History
1-On treatment
2-Not on treatment

Personal History:
Diet ☐
1. Veg
2. Mixed

Appetite ☐
1. Normal
2. Poor

Bowel/ Bladder ☐
1. Normal
2. Altered

Sleep ☐
1. Normal
2. Altered

Alcohol
1. Present
2. Absent

Smoking
1. Present
2. Absent

General Physical Examination:

Built
1. Poor
2. Moderate
3. Good

Vitals

Pulse / min

BP(mm/hg):Systolic
Diastolic

Temperature ^{0}F

Weight Kg

Pallor
1. Present
2. Absent

Icterus
1. Present
2. Absent

Cyanosis
1. Present
2. Absent

Clubbing
1. Present
2. Absent

Lymph nodes
1. Palpable
2. Non palpable

Edema
1. Pitting
2. Non Pitting
3. Absent

Mucocutaneous Examination

Site involved

SCALP	**No.of patches**	**Sides**	**Sizes**	**Exclamation Hair**	**Scarring/ Non Scarring**
1.Vertex					
2.Temporal					
3.Occipital					
4.Parietal					

EYE BROWS

EYE LASH

1.Upper
2.Lower

BEARD/MOUSTACHE **NO.OF PATCHES**

AXILLA

PUBIS

TOTALIS

UNIVERSALIS

OTHERS

NAIL CHANGES

1.Pitting
2.Longitudnal ridges
3.Beaus lines
4.Onychodystrpohy
5.Onychorrhexis

Systemic Examination:

Cardiovascular system: Heart sounds

1. Normal ☐
2. Abnormal;if abnormal specify the finding_____

Respiratory system: Breath sounds

1. Normal ☐
2. Abnormal;if abnormal specify the finding_____

Per abdomen:

1.Normal ☐

2.Abnormal;if abnormal specify the finding_____

Central nervous system: Neurological examination

1. Normal ☐
2. Abnormal;if abnormal specify the finding_____

Investigations

1.Hb%
2.Total count
3.ESR
4.Differential Count
5.Serum ZINC levels

Diagnosis:-

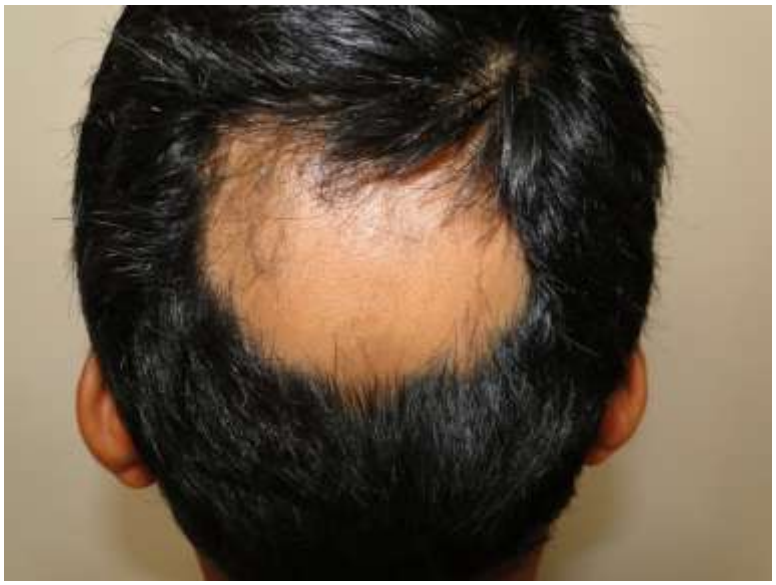
Signature:

Guide's Signature:

Annexures

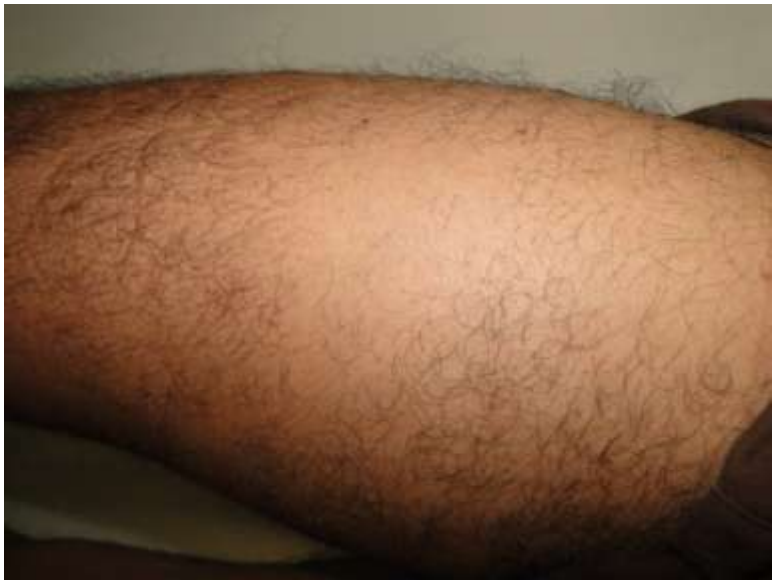
Annexure III

ANNEXURE III – PHOTOGRAPHS

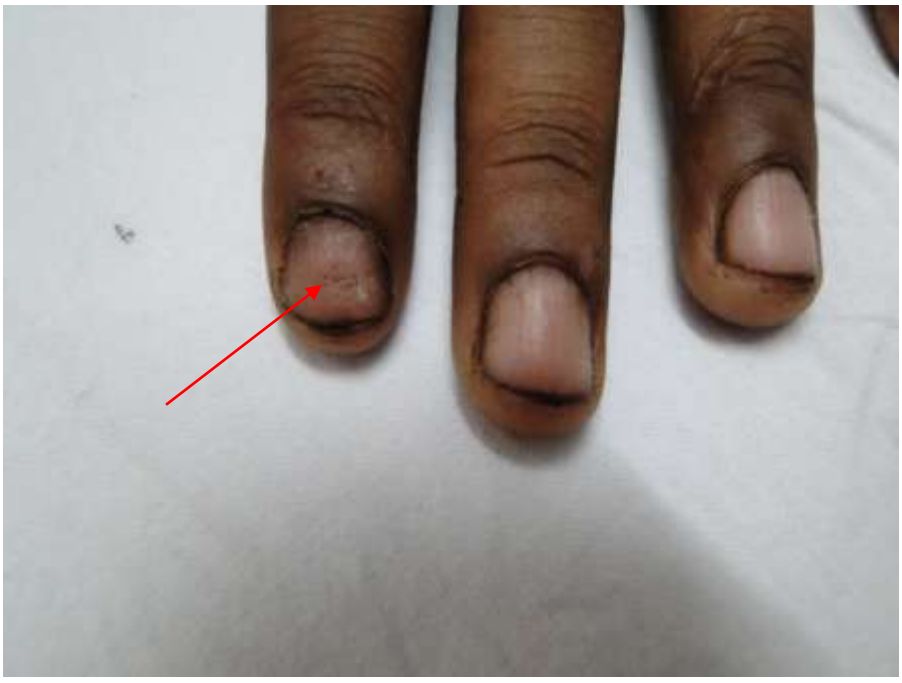


Photograph 1. Non scarring single hairless patch on occiput

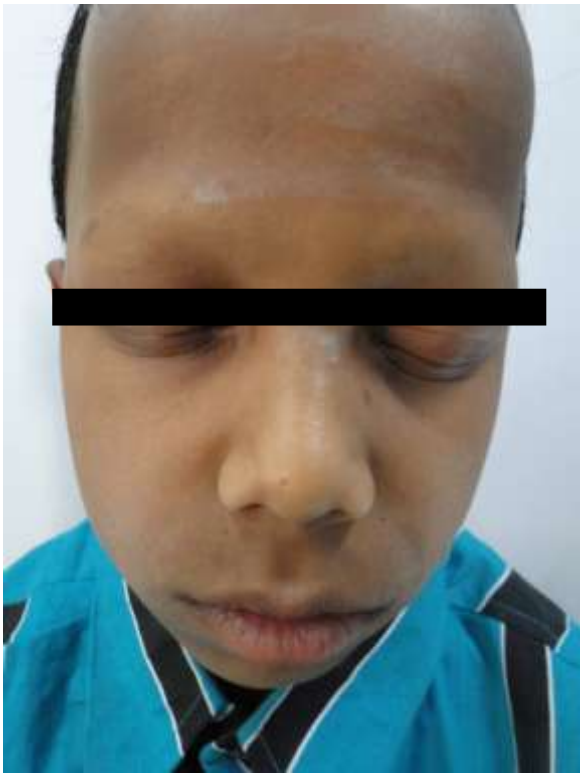
Photograph 2. Ophiasic alopecia aereata



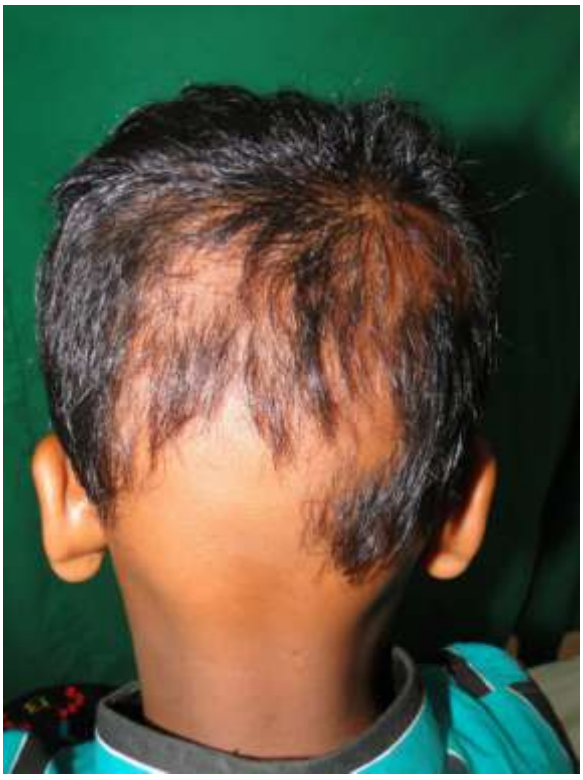
Photograph 3. Single non scarring hairless patch over left thigh



Photograph 4. Nail showing pitting



Photograph 5. A case with loss of eyebrows and eye lashes



Photograph 6. A case with non scarring alopecia patch

Photograph 7. A single non scarring patch present over the beard

Annexures

Annexure IV

ANNEXURE IV – MASTER CHART

A	-	Any other
B	-	Business
F	-	Female
Fr	-	Frontal
G	-	Generalised
H	-	Housewife
M	-	Male
Mr	-	Married
N	-	No
Ns	-	Non scarring
O	-	Occipita
P	-	Patchy
Pl	-	Parietal
Pr	-	Professional
S	-	Student
T	-	Temporal
U	-	Unmarried
V	-	Vertex
Y	-	Yes
BP	-	Blood pressure
GPE	-	General physical examination
mg	-	Milligram
dL	-	Deci Litre

ANNEXURE IV - MASTER CHART - CASES

Serial Number	Out patient number	Demography				Presentation	Associated disease	Duration of disease (Years)	GPE			Presentation																				
										B.P. (mm Hg)		Site of lesion															Associated factors				
												Site																				
		Sex	Age (Years)	Occupation	Marital status				Pulse (/Minute)	Systolic	Diastolic	Scalp	Eyebrow	Eyelash	Beard	Mustache	Axilla	Pubis	Totalis	Universalis	Others	Number of patches	Size	Side	Exclamation hair	Scarring	Itching	Pain	Burning	Asymptomatic	Nail changes
1	1233446	M	30	B	U	P	N	-	84	130	80	V	N	N	N	N	N	N	N	N	N	1	1X0.5		+	NS	N	N	N	Y	N
2	1954621	F	12	S	U	P	N	-	90	100	70	O	N	N	N	N	N	N	N	N	N	1	1.5X1.5		+	NS	N	N	N	Y	N
3	1920083	M	28	S	U	P	N	-	78	110	80	O	N	N	N	N	N	N	N	N	N	1	2X1		+	NS	N	N	N	Y	N
4	1852442	M	32	S	U	P	N	-	80	120	80	-	N	N	Y	N	N	N	N	N	N	2			+	NS	N	N	N	Y	N
5	2082321	F	6	S	U	P	N	-	96	90	70	O	N	N	N	N	N	N	N	N	N	1	2X2		+	NS	N	N	N	Y	N
6	1738043	M	30	S	U	P	N	-	86	110	80	-	N	N	Y	N	N	N	N	N	N	2			+	NS	N	N	N	Y	N
7	1588592	M	10	S	U	P	N	-	80	100	70	O	N	N	N	N	N	N	N	N	N	1	2X1		+	NS	N	N	N	Y	N
8	1739376	F	42	H	MR	P	N	-	80	130	80	V	N	N	N	N	N	N	N	N	N	1	1X1.5		+	NS	N	N	N	Y	N
9	1915261	F	6	S	U	P	N	-	100	80	60	O	N	N	N	N	N	N	N	N	N	1	2X2		+	NS	N	N	N	Y	N
10	1954621	F	12	S	U	P	N	-	78	110	70	O	N	N	N	N	N	N	N	N	N	1	3X2	1	+	NS	N	N	N	Y	N
11	1957507	M	36	B	MR	P	N	-	90	120	70	V,O	N	N	N	N	N	N	N	N	N	2,4	1X1,1X2;1.5X1,2X1,1X1	.5X	+	NS	N	N	N	Y	N
12	1894735	F	46	H	MR	P	N	-	80	120	70	O	N	N	N	N	N	N	N	N	N	1	1.5X2		+	NS	N	N	N	Y	N
13	1406331	F	9	S	U	P	N	-	64	90	60	O	N	N	N	N	N	N	N	N	N	1	2X2		+	NS	N	N	N	Y	N
14	1618703	M	41	A	MR	P	N	-	84	126	80	-	N	N	Y	N	N	N	N	N	N	2			+	NS	N	N	N	Y	N
15	1633672	M	30	A	MR	P	N	-	84	126	80	V	N	N	N	N	N	N	N	N	N	1	2X2		+	NS	N	N	N	Y	N
16	1675095	M	50	A	MR	P	N	-	80	140	80	-	N	N	Y	N	N	N	N	N	N	1			+	NS	N	N	N	Y	N
17	1309130	M	22	PR	U	P	N	-	82	120	80	-	N	N	Y	N	N	N	N	N	N	3			+	NS	N	N	N	Y	N
18	1745083	F	25	H	MR	P	N	-	72	100	70	V	N	N	N	N	N	N	N	N	N	1	2X1	-	+	NS	N	N	N	Y	N
19	1458310	M	35	B	MR	P	N	-	82	120	80	V	N	N	N	N	N	N	N	N	N	1	1X1.5		+	NS	N	N	N	Y	N
20	1459631	M	40	PR	MR	P	N	-	78	110	70	-	N	N	Y	N	N	N	N	N	N	2,1			+	NS	N	N	N	Y	N
21	1721791	M	30	A	MR	P	N	-	74	110	80	O	N	N	N	N	N	N	N	N	N	1	1.5X1		+	NS	N	N	N	Y	N
22	1538947	M	57	PR	MR	P	N	-	76	130	80	V	N	N	N	N	N	N	N	N	N	1	1X1		+	NS	N	N	N	Y	N
23	1638964	M	14	S	U	P	N	-	70	100	70	V	N	N	N	N	N	N	N	N	N	1	1X1		+	NS	N	N	N	Y	N

ANNEXURE IV - MASTER CHART - CASES

Serial Number	Out patient number	Demography				Presentation	Associated disease	Duration of disease (Years)	GPE			Presentation																				
										B.P. (mm Hg)		Site of lesion															Associated factors				
												Site																				
		Sex	Age (Years)	Occupation	Marital status				Pulse (/Minute)	Systolic	Diastolic	Scalp	Eyebrow	Eyelash	Beard	Mustache	Axilla	Pubis	Totalis	Universalis	Others	Number of patches	Size	Side	Exclamation hair	Scarring	Itching	Pain	Burning	Asymptomatic	Nail changes
24	1589755	M	14	S	U	P	N	-	64	100	60	V	N	N	N	N	N	N	N	N	N	1	1X1		+	NS	N	N	N	Y	Y
25	1557820	M	32	A	MR	G	N	-	82	120	70	O	Y	N	Y	N	N	Y	N	N	Y	2	1X1,1X1.5		+	NS	N	N	N	Y	N
26	1545071	M	28	PR	MR	P	N	-	74	124	70	-	N	N	Y	N	N	N	N	N	N	2			+	NS	N	N	N	Y	N
27	1544076	M	21	PR	U	P	N	-	74	110	70	-	N	N	Y	N	N	N	N	N	N	2			+	NS	N	N	N	Y	N
28	1515414	M	32	A	MR	P	N	-	80	124	80	-	N	N	Y	N	N	N	N	N	N	1			+	NS	N	N	N	Y	N
29	947154	M	28	PR	MR	P	N	-	80	124	80	V	N	N	N	N	N	N	N	N	N	1	2X1		+	NS	N	N	N	Y	N
30	1148220	M	29	PR	U	P	N	-	82	120	80	-	N	N	Y	N	N	N	N	N	N	3			+	NS	N	N	N	Y	N
31	1810782	M	30	PR	MR	P	N	-	80	126	80	-	N	N	Y	N	N	N	N	N	N	3			+	NS	N	N	N	Y	N
32	1458160	M	31	A	MR	P	N	-	80	116	80	V	N	N	N	N	N	N	N	N	N	1	2X3		+	NS	N	N	N	Y	N
33	181077	M	30	A	U	P	Y	3	80	114	80	FR	N	N	N	N	N	N	N	N	N	1	3X2		+	NS	N	N	N	Y	N
34	854846	F	27	H	MR	P	N	-	78	110	70	V	N	N	N	N	N	N	N	N	N	1	1.5X2		+	NS	N	N	N	Y	N
35	1588382	F	23	H	MR	P	N	-	78	110	70	O	N	N	N	N	N	N	N	N	N	1	2X2		+	NS	N	N	N	Y	N
36	1515414	F	32	H	MR	P	N	-	70	114	64	V	N	N	N	N	N	N	N	N	N	1	1X1		+	NS	N	N	N	Y	N
37	1029888	F	30	H	U	P	N	-	74	110	80	V	N	N	N	N	N	N	N	N	N	1	1X1.5		+	NS	N	N	N	Y	N
38	1479627	M	21	PR	U	P	N	-	74	116	70	-	N	N	Y	N	N	N	N	N	N	1			+	NS	N	N	N	Y	N
39	1764345	F	9	S	U	P	N	-	68	100	70	V	N	N	N	N	N	N	N	N	N	1	1X1		+	NS	N	N	N	Y	N
40	1476144	F	14	S	U	P	N	-	80	100	70	O	N	N	N	N	N	N	N	N	N	1	1X1`		+	NS	N	N	N	Y	N

ANNEXURE IV - MASTER CHART - CASES

Serum zinc (µg/dL)
114
102
116
118
97
106
88
99
84
86
94
99
99
114
112
118
98
102
119
109
101
118
106

ANNEXURE IV - MASTER CHART - CASES

Serum zinc (µg/dL)
98
99
99
89
96
120
106
84
92
110
97
106
103
110
117
106
104

ANNEXURE IV - MASTER CHART - CONTROL

Sl No	IP No.	Age	Gender	Zinc level
1	2071621	30	M	85
2	2071571	30	M	92
3	2071567	32	F	98
4	2071679	36	F	86
5	2071680	31	F	88
6	2071682	33	M	99
7	1852519	6	F	110
8	2042635	59	M	93
9	2076325	9	F	115
10	2076322	7	F	94
11	1943706	15	M	96
12	1886169	48	M	98
13	2078039	34	M	118
14	2079349	36	M	105
15	1521542	30	M	80
16	1978930	36	M	95
17	1978930	42	F	100
18	2065478	6	F	101
19	1579895	30	M	98
20	1579919	32	M	86
21	1582747	16	F	80
22	1583245	32	F	84
23	1326650	26	M	100
24	1535448	18	M	118
25	1583275	40	M	109
26	1602464	25	F	190
27	1621103	37	F	70
28	579525	26	F	86
29	1590828	40	M	98
30	1652979	25	F	102
31	1753642	12	F	104
32	1647079	9	M	78
33	1553814	30	M	84
34	1885975	31	F	96
35	2055783	41	M	101
36	2057139	20	F	110
37	2067457	31	M	91
38	1854274	18	F	99
39	1915773	14	M	86
40	1634367	48	M	114