SERUM GRANULYSIN LEVELS IN PATIENTS WITH ALOPECIA AREATA IN A TERTIARY CARE CENTRE IN SOUTH INDIA – A CASE CONTROL STUDY

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE RULES AND REGULATIONS FOR THE M.D. (BRANCH XX) DERMATOLOGY, VENEREOLOGY AND LEPROSY EXAMINATION OF THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY TO BE HELD IN MAY, 2020.

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CERTIFICATE

This is to certify that the dissertation entitled " **Serum granulysin levels in patients with alopecia areata in a tertiary care centre in South India – A case control study** " is a bonafide original work of **Dr. Anju Liya Thomas.**

This study was undertaken at Christian Medical College and Hospital, Vellore from January 2019 to August 2019, under our direct guidance and supervision, in partial fulfillment of the requirement for the award of the MD degree (Branch XX) in Dermatology, Venereology and Leprosy of the Tamil Nadu Dr. M.G.R Medical University.

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DECLARATION

I hereby declare that this M.D. dissertation entitled **" Serum granulysin levels in patients with alopecia areata in a tertiary care centre in South India –A case control study**" is a bonafide work done by me under the guidance of Dr. Susanne A. Pulimood, Professor & Head, Department of Dermatology, Venereology and Leprosy, Christian Medical College, Vellore. This work has not been submitted to any other university in part or full.

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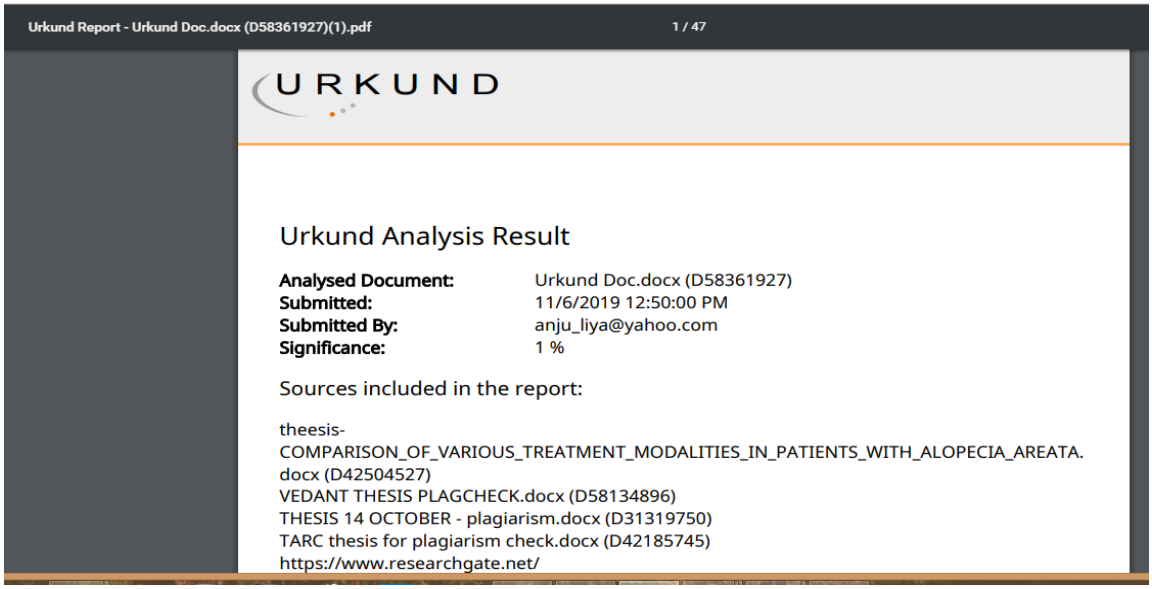
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Abbreviations

AA	Alopecia areata
AU	Alopecia universalis
AT	Alopecia totalis
AAI	Alopecia areata incognito
HF	Hair follicle
TARC	Thymus and activation regulated chemokine
NK cell	Natural killer cell
GVHD	Graft versus host disease
SJS	Stevens -Johnson Syndrome
TEN	Toxic epidermal necrolysis
SAPLIP	Saposin like lipid - binding proteins
IFN	Interferon
IL	Interleukin
IBD	Inflammatory bowel disease
TSH	Thyroid stimulating hormone
ELISA	Enzyme linked immunosorbent assay
CXCL	CXC subfamily chemokine
CRH	Corticotropin releasing hormone
NKG2D	Natural killer cell G2D receptor
ULBP	UL16 binding protein
RANTES	Regulation upon activation of normal T cell expressed and secreted protein

MIF	Macrophage migration inhibitory factor
MSH	Melanocyte stimulation factor
MICA	MHC class 1 polypeptide related sequence A
AD	Atopic dermatitis
LE	Lupus erythematosus
TGF	Transforming growth factor
Th	T helper cell
DVL	Dermatology, Venereology and Leprosy
NA	Not available
SD	Standard deviation
SALT	Severity of alopecia tool
AAPI	Alopecia areata progression index
DLQI	Dermatology life quality index
QoL	Quality of life
IRB	Institutional Review Board
OPD	Outpatient department

INTRODUCTION

Alopecia areata (AA) is an autoimmune process that is directed against the hair follicles. It is the most common hair loss disorder in patients presenting to the dermatology outpatient clinic. The clinical features of alopecia areata can range from a localized single patch of alopecia to an extensive disease causing loss of hair of the scalp, eyebrows, beard and body hair. While a single patchy alopecia is a self-limiting condition, the severe form of the disease can gradually progress to alopecia totalis and universalis and significantly affect the quality of life of the affected individuals. (1)

Diagnosis of alopecia areata is mostly clinical and biopsy is only considered when other conditions with non-scarring alopecia need to be ruled out. (2)

At present, identifying some features at dermatoscopy help to assess severity. (3) These however, are not specific for alopecia areata. Hair pull test and SALT scoring to assess degree of hair loss and thereby severity is another measure. (8)

The pathogenesis of alopecia areata follows a collapse of immune privilege of the hair follicle with cell-mediated cytotoxicity playing an important role. (23) Genetics, viral infections, cytokines, chemokines, interleukins and autoantigens also have a role in the etiopathogenesis. All these factors can cause activation of the natural killer cells and cytotoxic T cells and granulysin is produced by these activated cells. Its role in immunity and as an inflammatory agent is known but the concept of granulysin as an inflammatory marker in alopecia areata is new. Serum granulysin levels may help in

determining the disease severity especially in the acute phase (6) and it indirectly measures the activity of the natural killer cells and T cells. As high granulysin levels have been found to be associated with an acute rather than a chronic disease, (6) granulysin levels may also be useful in disease prognosis. It would also help to study the clinical profile and trichoscopy features of AA patients and correlate the disease severity scores like Severity of Alopecia Tool (SALT) score (4) and Alopecia Areata Progression Index (AAPI) score (5) with serum granulysin levels. Currently, there are only a few studies on biochemical markers like TARC and IL-15 to assess the disease severity of alopecia areata. (44,57) A study by Ono et al (6) has evaluated granulysin levels as a marker of disease severity and found that it correlates with severity in acute AA. There have been no studies reported from India. Since there is a paucity of studies looking into various biomarkers which causes hair loss in this disease and is known to affect the quality of life, we plan to measure the serum granulysin levels in patients with acute and chronic AA and see its correlation with disease severity.

OBJECTIVES AND AIM OF THE STUDY

Aim :

To study serum granulysin levels in adult patients with alopecia areata in a tertiary care centre in South India.

Objectives :

a) Primary - To study serum granulysin levels in adult patients with acute and chronic alopecia areata as compared to healthy controls.

b) Secondary- To study the correlation of serum granulysin levels with disease severity in alopecia areata.

REVIEW OF LITERATURE

BACKGROUND:

Alopecia areata (AA) is a non-scarring alopecia caused by an autoimmune process occurring at the hair follicles. (8) It can occur in children and adults and it affects both sexes. It has varied clinical manifestations from presenting as a single patch to multiple patches and even complete loss of hair from the body. (8) There are many medical conditions associated with AA. Natural killer cells and cytotoxic T cells play an important role in the etiopathogenesis. They become activated and produce a cytolytic protein called granulysin which has inflammatory, immune and apoptosis – inducing actions. (3) The severity of AA, based on the extent of hair loss, can be assessed by scores like SALT score (4) and AAPI score. (5)Measurement of the serum concentration of granulysin helps in assessing the activity of natural killer and cytotoxic T cells and it is an inflammatory marker. (4) In the acute stage of the disease, there is an increased inflammation with CD 4^+ and CD 8^+ cells observed around the hair bulb and granulysin levels are found to be elevated. (6) Hence, the potential of granulysin as an inflammatory marker and marker for disease severity needs to be explored.

HISTORY :

The term “Alopecia areata’’ has its origin from the Greek word “Alopex” which means fox due to the fur loss seen in foxes and “Areata” a Latin word which means a vacant space. (7) The term alopecia areata was first coined by the French physician Sauvages De Lacroix in 1763. (7) The first clinical description of alopecia areata was given by Thomas Bateman in 1817. The presence of positive family history and association of

AA with other diseases and autoimmune conditions were first described by the French dermatologist Raymond. (7) The presence of peribulbar infiltrates in histopathology and the beneficial effects of steroids led to the consideration of AA as an autoimmune condition in 1958. (7)

EPIDEMIOLOGY:

The prevalence of AA in India as reported in the studies by Sharma et al is 0.7% (8) and a similar prevalence was observed by Mishra et al, (9) while the prevalence as reported by Safavi et al (10) was 158/100,000 in US, 2.45% in Japan and 3.8% in Singapore. (12)

Gender distribution :

In certain studies in literature, the males were more commonly affected than females. The male:female ratio ranged from 1.1:1 to 2:1.(2) Few studies that have reported a higher prevalence in males are by Sharma et al, (8) a prospective study done in Chandigarh, North India, Yang et al, (17) an epidemiological study done in China from 2001-2003 and Kavak et al, (16) a multicentre, prospective study from 1995-2007. A female predominance was noted in few studies with a range of 1:1.2 to 1:2. These studies were by Seyrafi et al, (11) a retrospective study done in Iran from 2002-2004, Tan et al, (12) a single centre study from Singapore during the period 1998-1999 and Lundin et al (13) from the USA –a multicentre study. The gender distribution in different studies is shown in table 1A and table 1B.

Age :

Alopecia areata is commonly seen in young adults (2) (14). The most common age group to be involved is 20-40 years followed by 1-20 years and the least commonly involved is the 60-80 year age group (10) and the age distribution is shown in table 1A.

Table 1 A– A comparison of demographic profile, studies from Asia

	You et al (15)	Kavak et al (16)	Tan et al (12)	Ono et al (6)	Yang et al (17)
Country	Korea	Turkey	Singapore	Japan	China
Study duration	9 years	12 years	1 year	-	2 years
Study design	Retrospective case control	Prospective case control	Prospective study	Prospective case control study	Prospective study
Age(years) (mean ±SD	32.63 ±17.40	24.32 ± 10.54	Median 25.2 years	Acute 34.7±10.9 Chronic 35.3±15.5	28.98 ±13.43
M:F ratio	1.05 :1	1.6 :1	1:1.3	0.47 :1	1.1:1

Table 1B – A comparison of demographic profile, studies from India

	Siddappa et al (18)	Sharma et al *(8)	Mane et al (19)	Thomas et al (20)	Peter et al (21)
Study duration	1 year	9 years	-	1 year	1 year
Study design	Retrospective case control	Prospective case control	Prospective study	Prospective case control	Prospective study
Age(years) (mean ±SD	24.52 ±10.06	-	26.85± 9.3	-	29.12±12.07
M: F ratio	2.57: 1	2:1	2.3:1	2.5 : 1	-

*(children included in study)

PATHOGENESIS OF ALOPECIA AREATA

1. Breakdown of immune privilege of hair follicles :

Alopecia areata is an autoimmune condition that is directed against the hair follicles (HFs). Immune privilege is a defense mechanism by which organs like hair follicles, central nervous system, testes, eye are protected from potential harm of immune recognition by creating an anergic state. (22) The factors that maintain immune privilege are the absence of lymphatics in the hair follicles and the extracellular matrix. (23) The extracellular matrix functions like a barrier to the invasion by immune cells. Alopecia areata occurs when there is a breakdown of the immune privilege of the hair follicles. (24) (25)

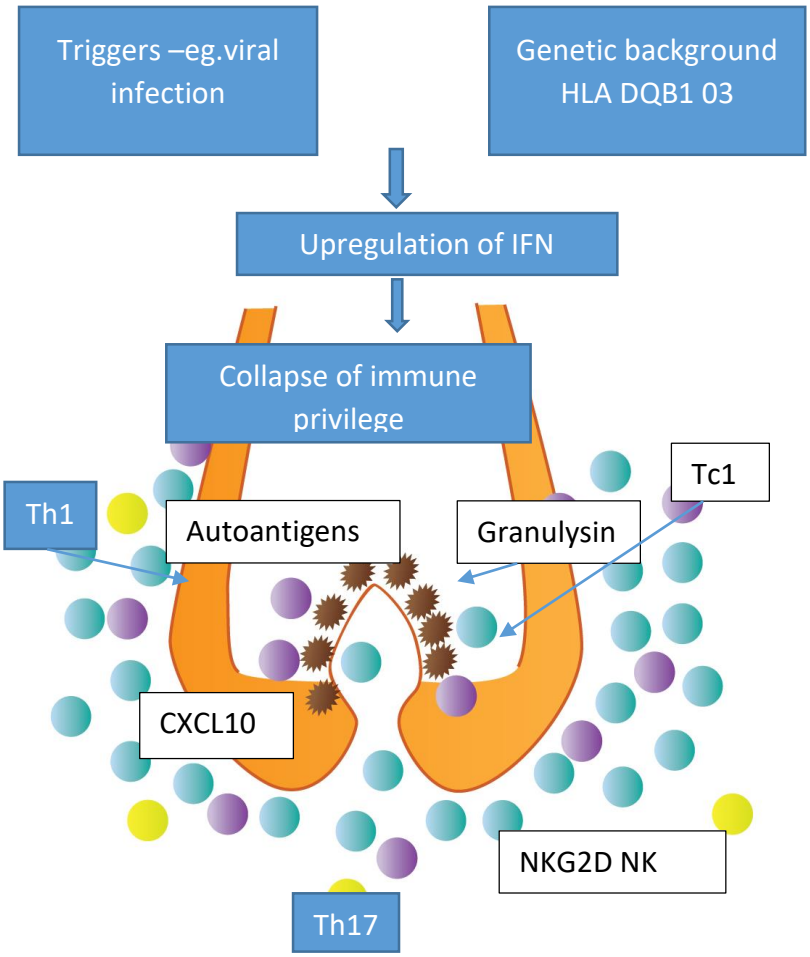

Figure 1 – Summary of pathogenesis of AA (Adapted from Taisuke Ito –Role of cytokines and chemokines in T cell mediated autoimmune process in AA)(26). Th1 chemokine –CXCL10, Th1/Tc 1 -T helper cytokines,chemokines .NKG2D NK cell-NKG2D receptor positive natural killer cell , Th 17 –cytokine

Maintenance of immune privilege of hair follicles :

Under normal circumstances, immune privilege is maintained in the cells other than the hair follicles by the MHC- 1 protein in maintaining self - tolerance by binding to the NK cell Ig –like receptor (KIR) and prevents its activation and

keeps the innate immunity in check. Under those circumstances when cells are experiencing an oxidative stress, there is an expression of molecules called MICA (MHC class 1 polypeptide related sequence A) and ULBP (UL 16 binding protein) These molecules bind to a specific receptor on the NK cell, the NKG2D activating receptor. Activation of this receptor leads to activation of NK cells and innate immunity. (22) These activated NK cells produce granulysin.

Hair follicles normally lack the above mentioned MHC I protein and therefore NK cells are kept in an inactivated state in the HFs by three ways-decreasing the expression of MICA and NKGD and by increasing the production of an inhibitory substance called macrophage migration inhibitory factor (MIF). (27)

There are few molecules that maintain immune privilege in hair follicles (IP guardian molecules)-transforming growth factor β (TGF β), α melanocyte stimulating hormone (MSH), indoleamine 2, 3 diooxygenase (IDO), protein red encoded by IK gene, interleukin IL-10, calcitonin gene related peptide, (28) insulin like growth factor-1. (29) There are two main theories considered in the breakdown of immune privilege at the site of hair follicles. (22)

a) Local defects in the hair follicles b) Dysregulated immune system

a) Local defects in the hair follicles:

Environmental factors cause a build up of oxidative radicals (30)(31). Regulation upon Activation of Normal T-cell Expressed and Secreted protein (RANTES) is a member of the chemokine family produced by the activated T lymphocytes in alopecia areata. It acts a chemoattractant for Th 1 cells, eosinophils and also

induces the proliferation and activation of natural killer cells suggesting a role in the pathogenesis of AA.

b) Dysregulated immune system :

The other hypothesis considered in the breakdown of immune privilege is a dysregulated immune system. The initial step occurs in the lymphatic system with activation of the immune cells. These activated immune cells attack the hair follicles (HFs) under the influence of Interferon gamma (IFN gamma) (32) and the autoimmune process is activated in the hair follicles. (24)

Genetics in the pathogenesis of AA :

Genetics is considered to play a major role in the pathogenesis of AA. Specific HLA alleles like QB1*03, DRB1 * 1104 increases the susceptibility to AA. Patients with alopecia unversalis (AU) and alopecia totalis (AT) are more likely to have HLA DRB1 * 1104 and DQB1 *0301. There are particular regions in the genetic loci that are susceptible to the development of AA .These include the genetic loci that contain ULBP genes. These genes encode ligands that are present on the NK cell receptor NKG2D. ULBP causes activation of the NK cells by acting on NKG2D receptor. (33) (34)

Viral infections in AA :

There are few viral infections that are implicated in the pathogenesis of AA. These include cytomegalovirus (35), hepatitis B, hepatitis C and Epstein Barr viral infections and have been recognised as triggers in the pathogenesis of AA. (26) Some vaccinations against these infections have also been considered as triggers.

(36) Following viral infections, Th-1 mediated immune responses are activated by interferons which in turn activate the autoimmune process in AA. (37) Among the interferons, IFN –alpha has been considered to play a major role in the implication of AA following viral infections. (33)

Role of autoantigens in AA :

Melanogenesis associated peptides are produced by the anagen hair follicles. These are the autoantigens on which the cytotoxic T cells act. The role of these peptides in the pathogenesis of AA is proven by the fact that non pigmented hairs are spared in alopecia areata, association with vitiligo and in the condition with overnight graying where only the pigmented hairs are involved. (34)

Role of stress hormone in AA:

The outer root sheath of the hair follicle has an increased expression of corticotropin - releasing hormone (CRH) and its receptor CRH – 1. In normal circumstances, in response to stress, there is an increased production of corticosteroids through the action of CRH on its receptor. In AA, this response to stress is altered and there is no increased production of corticotropin hormone. Hence in patients with AA, as the normal response to stress is altered, stress can affect patients with AA and is considered to play a role in the etiopathogenesis. (33)

Th1 cytokines in AA:

IFN-γ is thought to play an important role in the immunopathogenesis of AA. (42,43) Mice that were deficient in IFN-γ did not develop AA in experiments with

animal models. (38) Cells producing IFN-γ were seen to be increased around the hair follicles in AA. (39) Besides IFN-γ, the other cytokines found to be involved in the pathogenesis were IL-2, IL-12, IL-13, IL-17A.(40)

Th1 chemokines and AA:

Several studies have found chemokines to be increased in the early pathogenesis of AA (41). The chemokines found to be significantly elevated in AA are CXCL -9, CXCL-10 (42) (43). An upregulation of CXCL-10 was observed in the lesional skin of patients with AA while CXCL-9 was found to chemoattract inflammatory cells. (26)

Role of interleukin 15 and AA:

Interleukin 15 is a cytokine. It is produced by mononuclear phagocytes and its function is to cause activation and proliferation of natural killer cells and CD 8 + T cells. IL-15 levels have been found to be increased in patients with alopecia areata. It was found to be elevated in the chronic than in the acute disease. IL-15 and its receptor 1L-15 R α have been found to be upregulated in patients with AA.(44)

Role of granulysin in the pathogenesis :

Granulysin is a granule protein with cytolytic action. It is produced by the activated cytotoxic T cells and natural killer cells (NK) cells. (45) Along with granulysin, these cells produce perforin and granzymes. The concentration of granulysin in serum correlates with the activity of these cells. It belongs to the family of saposin-like lipid binding proteins (SAPLIP). (46)

It exists in two forms – a 15 kDa precursor and a 9 kDa effector form. (47) The 15 kDa precursor form is constitutively secreted and how it functions is not well known.(3) On the other hand, the 9 kDa form is released by a calcium dependent granule exocytosis pathway and not detected in the serum. Cleavage at the amino and carboxy terminal of the 15kDa form results in the formation of 9 kDa effector form. The effector form is sequestered in the granules and released by receptor mediated cytolysis.

Granulysin has a cytolytic action. Its role in immunity is as a chemoattractant and it also causes the immune cells to produce inflammatory cytokines. The cytolytic action of granulysin is seen at a higher concentration while its action as as a chemoattractant is seen at a lower concentration. It causes the immune cells to move to the site of injury and mount up an immune response and increases the inflammatory infiltrates at that site. (48)

In a study looking at a biomarker for acute and chronic AA as well as looking at the severity of AA, serum granulysin levels were found to be elevated in acute than chronic AA. Serum granulysin was shown to reflect the extent of damage of hair follicles by the cytotoxic T cells. Serum granulysin in the acute phase can be a useful marker for the disease activity of AA. (6) The serum concentration of granulysin is maintained in a steady state in comparison to the other cytokines and the half - life of granulysin is found to be greater than that of the other cytokines.

Granulysin has been shown to be elevated in a number of conditions such as acute viral vesicular infections, (49) malignancy, (50) transplantation (51) and cutaneous diseases such as psoriasis, (52) acne,(53) lichen planus (54) and

folliculitis. (55) It has also been identified as a useful marker for graft versus host disease (GVHD) in allogenic stem cell transplant as its serum levels correlate with the clinical severity. Chung et al (56) identified granulysin as an important mediator for generalised keratinocyte apoptosis in SJS/TEN.

Other biomarkers in AA :

Besides granulysin, literature shows few other markers for disease severity in AA

a) **Serum Thymus and Activation Regulated Chemokine (TARC**):

Serum TARC was found to exert its pathogenic roles in AA. (57) $CD68^+$ histiocytes are the main source of TARC around the alopecia areata hair follicles and therefore TARC can induce chemotaxis of $CCR4^+$ cells. It has been reported that TARC does not accelerate the migration of AA patient's $CD4^+$ T cells. Natural killer (NK) cells express CCR4 and subsequently, TARC induces their chemotaxis and NK cells were found to have a role in immune privilege collapse. Hence TARC also has a role as a marker for disease activity in AA. (57)

b) **IL -15** :

It is a cytokine and functions in maintaining innate and adaptive immunity. It has been found to be increased in patients with AA and the levels were found to correlate with the disease activity. IL -15 could become a possible biomarker for AA. (44)

c**) Micronutrients**:

Micronutrients like vitamins and minerals play a role in normal hair follicle development and in immune cell function. (58) Few studies have sought to

determine whether serum levels of these nutrients might differ in AA patients and whether supplementation of these nutrients might represent a therapeutic option for AA.(50)

It has been observed that serum vitamin D tend to be lower in patients with AA as compared to controls. (59) Study by Darwish et al (60) and d'Ovidio et al (61) showed vitamin D deficiency more commonly associated in males while study by Cerman et al (62) showed AA to be more common in females in view of decreased exposure to sunlight and religious customs. Darwish et al observed that levels of vitamin D did not correlate with the severity of the disease, duration or with SALT score. A study conducted by Fattah et al (63) pointed to the reduced levels of serum zinc in patients with AA and in those with a resistant disease. However, an inverse correlation was observed between zinc levels and duration, severity of the disease. A similar observation was found in a study done in a Thai population where zinc levels were low in the study subjects as compared to controls with a p value of 0.04. (64)

Mouse models in understanding AA & disease progression:

The two most common animal models that have been used in animal experiments to understand the pathogenesis, disease progression are C3H/HeJ mouse strain and Dundee experimental Bald Rat. Both these animal models were preferred as they have a tendency to develop alopecia areata spontaneously and in increased frequency. These animal models were used to study the immunological and genetic basis in AA, the progression and evolution of the disease and also to test the efficacy of drugs. (38)

COMORBIDITIES ASSOCIATED WITH AA:

a) **Atopy** :

Patients with AA showed association with atopy which included atopic dermatitis, allergic rhinitis, asthma. (65) This association with atopy was observed in most studies and ranged from 8.4% -38.2%. (2) However, in the study done by Kaur et al, (67) a greater frequency of atopy was observed (50% of the patients), allergic rhinitis in 42 %, bronchial asthma in 6% and atopic eczema in 2% of the patients. Atopy is considered as a risk factor for developing AA and it was found to be commonly associated with AT or AU. (66) Atopic dermatitis is two to three times more common in AA. (67) Association with atopy is considered to have a poor prognosis. Atopic eczema association with AA is observed to be less common in Asians and it is less commonly found in tropical countries. (68)

b) **Thyroid dysfunction** :

The association with thyroid dysfunction was observed to be between 1-3% in patients with AA.(8) In a study by Thomas et al, (20) a higher frequency of hypothyroidism was noted. (14.6%) Of the other thyroid disorders, Hashimoto's thyroiditis was observed in 2.7% and Grave's disease 1% in the study done by Safavi et al. (10) Hypothyroidism was the most common thyroid disorder in AA while Hashimoto's thyroiditis and Grave's disease are less commonly seen. Hypothyroidism causes a change in hair texture and affects the anagen hairs where a delay in resuming their growth was observed. Thyroid autoantibodies were noted in 51% of the patients in a study done by Safavi et al. (10) It was also found

that presence of thyroid autoantibodies did not have any significant correlation with the severity of the disease in AA.(69)

c) **Vitiligo:**

Vitiligo was seen in a frequency ranging from 0.3% - 4.1%. Association with autoimmune disorders carries a poor prognosis. Treatment for AA was shown to cause regrowth of white hairs. (53) A similarity in the structure of the antibodies directed against the endothelial cells in the vascular plexus of the hair bulb and the antibody against the melanocytes is considered to be likely cause for the association. (70) (71) The frequency of vitiligo in different studies is shown in table 3.

d) **Psoriasis vulgaris** :

Psoriasis was reported in 1.7 % of the patients in the study by Chu et al (147) and 0.6% of the patients in the study by Sharma et al. (8) "Renbok or Reverse Koebner phenomenon" was seen where AA patches within psoriatic plaques showed resolution.(72)

e) Lupus erythematosus:

An association with collagen vascular disorders was noted in a frequency of 0.5-0.7%. A higher frequency of association was noted in a study done by Bu Werth et al (73) in 39 patients with lupus erythematosus, AA was found in 10% of patients One of the four patients in this study had an ophiasis pattern of AA. Lupus erythematosus profundus patients was found to have an association with linear type of AA.(74)

g) **Inflammatory bowel disease** :

AA has been found associated with ulcerative colitis and Crohn's disease, more common with ulcerative colitis than Crohn's disease. (64) AA is considered as an extraintestinal manifestation of Crohn's disease or secondary to treatment with anti-TNF alpha agents used in inflammatory bowel disease (IBD). (64) AA is seen to occur along with exacerbation of intestinal lesions of Crohn's disease.(75) In the study from Minnesota by Safavi et al, (10) 0.7% of patients had inflammatory bowel disease and 2% of patients in the study done by Huang et al. (65)

h) **Diabetes mellitus**:

The frequency of type 2 diabetes reported in studies was 0.4%-4.7%. Type 1 diabetes mellitus was found associated with AA. AA is considered to arise as a result of multiple gene defects. Family members of patients with AA were found to be at risk of developing type1 diabetes. (76)

i) **Systemic hypertension**:

In a study conducted by Lee et al (77) in 871 Korean patients to assess the comorbidities in AA, hypertension was seen in 5.8% of the study patients. It is not known if hypertension was caused due to treatment with steroids used in AA. The other studies showed a frequency of 1-2 %. A higher frequency of 21.9 % was observed in the study by Huang et al. (65)

j) **Anemia**:

A study done by White et al (146) in 21 female patients with AA, showed 14 patients with iron deficiency anemia, i.e, 66% of patients while Thomas et al (20) showed a lower frequency of iron deficiency anemia (6.8% of patients). Studies showed low ferritin and low iron levels in patients with AA compared to controls. Pernicious anemia was seen in 0.3 % -2.8% of patients with AA.(79) In the study conducted by Friedman et al, a higher frequency of pernicious anemia was seen in patients (11.4%).

k) **Psychiatric disorders** :

In the study done by Huang et al, (65) 25.5% of the patients had psychiatric disorders. Depression and anxiety disorders were the most common association noted. However a study done by Bashir et al (80) showed 34.11% of the patients reported to have depression. Ruiz-Doblado (81) et al observed psychiatric disorders in 66% of the patients. While most patients were seen to adapt well to AA, those with AT and AU had issues in their family, work and sexual life due to the disease and reduced quality of life.

In a retrospective study done with data obtained from Cleveland Clinic Alopecia Registry by Conic et al (82) on comorbidities associated with AA, the most commonly associated conditions were atopy, IBD, diabetes mellitus, autoimmune disorders, psychological disorders and vitamin D deficiency.

IKEDA CLASSIFICATION (based on associations with AA) :

Based on the associated comorbid conditions, Ikeda has classified AA into atopic, autoimmune, prehypertensive and the common types. (83) The atopic type begins

in the early part of life and 30-75 % of cases develop AT later. (4) The autoimmune type is seen in patients who are middle aged and 10 -50% of patients have a risk of developing AT. The prehypertensive type of AA is seen in the young adult groups and 40% of patients develop into AT in the later part of their life. The common type is seen in the 20-40 year age group and there is a 5-15% risk of cases developing AT.(4,83)

CLINICAL FEATURES :

Alopecia areata presents with well demarcated patches of hair loss. In most of the patients AA is asymptomatic while in some patients AA may present with mild pruritus or burning sensation. It can involve the hair of the scalp and or the body hair. Scalp is involved in 90% of the cases. (4) It may also involve the eye brows, beard area and body hair including the axillary and pubic hair. Sharma et al (8) reported beard involvement in 28%, eyebrows in 5.8% and body in 4.3% while Mishra et al (9) reported scalp involvement in 62.5% and facial hair in 24% of patients. It may present as a single or multiple patches of alopecia. Smaller patches may coalesce with each other to form larger patches. Alopecia areata can be classified depending on the extent and the pattern of hair loss.

Based on the extent, alopecia areata can be classified into

a) Patchy AA b) Alopecia totalis (AT) c) Alopecia universalis (AU):

In a study conducted by Mishra et al (9) in India, patchy type was the most common, found in 88% of the patients, AU in 7% of the patients and ATs in 2% of the patients. In the study done by Sharma et al, (8) AT was observed in 14.9%

of the patients, AU in 27.27% of patients and multiple patchy AA was observed in 57.8%. In the study done by Hegde et al, (84) single patchy type AA was found in 38.7%, multiple patchy AA in 34.7%, AT in 1.3% and AU in 2.7% of the patients.

Patchy AA :

Hair loss is seen as circumscribed patches and hair loss can spread peripherally. It is the most common presentation of alopecia areata seen in 75% of cases. (85) Five to ten percent of patients with patchy AA can progress to AT or AU. (4)

Alopecia totalis (AT) :

Complete or nearly total absence of hair over the scalp is called as alopecia totalis. Alopecia totalis carries a poor prognosis and does not have a satisfactory response to treatment.

Alopecia Universalis (AU) :

Complete loss of scalp and body hair including eye brows, eye lashes, beard, axillary and pubic hair is called as alopecia universalis. This type carries a bad prognosis.

Based on the patternAA is classified into six types: (86)

a) **Reticular :**

In this pattern of AA, the hair loss is in a “net like pattern” and there are active and resolving patches. (87) In a study done by Hegde et al, (84) reticular pattern was seen in 2.7% of the patients.

b) **Ophiasis** :

This pattern of AA derives its name from the Greek word named “Ophis” which means snake and hair loss is seen as a band of alopecia involving the frontal, temporal and occipital areas of the scalp. (85) In a study done by Munoz et al, (88) this pattern was found in 2.68% of the cases and in 12 % of patients by Hegde et al. (84)

c) **Sisaipho** (Ophiasis inversus):

In this pattern, hair loss is seen as a band of alopecia that spares the temporal and occipital areas of the scalp, i.e.,hair in the periphery of the scalp is spared. This pattern is reportedly found to be associated with conditions like atopy, vitiligo and thyroid disorders and nail abnormalities especially trachyonychia. Sisaipho has a better prognosis compared to the ophiasis pattern. (89). Munoz et al (88) observed Sisaipho in 0.18% of the patients (90) while Hegde et al, (84) observed it in 4% of the patients.

d)**Acute diffuse and total alopecia**:

A new variant of alopecia areata has been described, which is usually seen in women. Patients present with a rapidly progressing hair loss involving extensive areas. Ironically this variant has a good prognosis and hair starts regrowing completely within a period of about six months. (91) In a study done by Sato –Kawamura, (92) 9 out of 112 patients (8.03%) showed acute diffuse and total alopecia type AA. Tendency of this variant to occur in females, tissue eosinophilia in biopsy and a good prognosis in that the hair regrowth happens in a short period of time is what distinguishes this variant from the other types of AA.(93)

e)**Perinevoid AA**:

In this pattern of AA, a patch of alopecia is seen around a nevus. This pattern of alopecia is similar to halo nevus but in contrast to halo nevus there is no spontaneous resolution of nevus or a regrowth of hair in the surrounding areas of alopecia. (94) The occurrence of AA is considered to be due to an inflammatory response that is mounted against the nevus or melanocytic cells. (95)

f) **Linear** :

Four cases of linear AA were reported in literature. They were found in patients with lupus erythematosus profundus. (96) This pattern of AA has to be differentiated from the non - scarring alopecia seen with lupus erythematosus profundus. Histopathology helps in differentiating AA from other conditions. As "swarm of bees '' appearance can be absent in the chronic stage of AA, presence of mucin deposits will help in differentiating lupus profundus from AA .(88)

Marie Antoinette syndrome:

This is a condition where patients present with a single patch of alopecia which is followed within a few weeks by the sudden onset of graying of hairs. This is also referred to as canities subita. (97) It is described as overnight graying of hairs in patients with AA. There is a preferential loss of pigmented hairs and sparing of gray hairs which makes the scalp appear like it has turned gray overnight. The exact etiopathogenesis of this condition is not known. (97)

Alopecia areata incognito (AAI):

This type of AA is acute in onset and characterised by diffuse hair loss (98). This is most commonly seen in individuals aged 20-40 years. It is also most commonly seen in females. Clinically it can be differentiated from the other types of AA. The time of onset of the disease to diffuse hair loss can occur over an average period of 2 months. The classical presentation of patchy hair loss is not seen in AAI and patients classically present with diffuse hair loss. When the disease is in the acute and subacute stages, the density of the hair follicles is preserved but it begins to decrease later. The finding of an inflammatory infiltrate around the terminal hair bulb is a specific feature seen in the histopathology of AAI. (99) As the disease duration progresses there is a reduction in inflammatory infiltrates.

Nail changes in AA:

The most commonly observed nail findings are nail pitting, trachyonychia, longitudinal ridges, when comparing the data mentioned in published studies given in (Table 4). Nail pits were observed in 7.2% -79.6%, followed by trachyonychia 8-18%, longitudinal ridges 2.3 -10% in most studies.

The other nail findings include red lunula, longitudinal ridging, pigmented bands, punctate leukonychia. Less commonly reported nail findings seen in AA include onychomadesis, onychorrhexis, lamellar pitting, Beau's lines, distal notching, ragged cuticles.

As children tend to have more severe presentation like AT and AU (107) and nail changes are more common in severe AA, these changes are seen more frequently in

children. Trachyonychia, red spotted lunulae, pitting are commonly seen in children. Pitting in AA are more superficial and have a grid like distribution while in psoriasis pits are more deeper and randomly arranged. (100) Trachyonychia was initially thought to be a classical nail feature of AA, but it can be seen in other conditions like nail lichen planus, nail psoriasis and in some patients, trachyonychia was idiopathic. In AA, trachyonychia is classically seen with alopecia universalis. Nail changes can be seen earlier than the onset of hair loss, along with hair loss or following hair loss in AA. Finger nails are commonly involved and toe nails are rarely involved. The number of nails involved may be different in each patient. Nail findings are usually seen associated with AT and AU. Hence nail changes are considered to carry a poor prognosis. (101) The following table 2 shows the nail changes that are seen in the various Indian and Western studies in literature.

Table 2 - Various studies on nail changes in alopecia areata

	Thomas et al (20)	Gandhi et al (102)	Sharma et al (103)	Chelidze et al (100)	Tosti et al (104)	Roest et al (105)	Tan et al (12)	Kasumagic et al (106)
Country	India	India	India	USA	Italy	Netherland	Singapore	Bosnia
Overall nail changes		44%	29%	-	46%	64.1%	10.5%	24.5%
Pits	7.2%	28%	14%	11.4%	34%	29.7%	11.4%	79.6%
Longitudinal ridges	4.2%	10%	8%	-	-	9%	2.3%	-
Lamellar splits	-	18%	-	-		-	-	-
Trachyonchia	-	9%	8%	8-14%	11%	18%	8.2%	14.3%
Distal notching	-	2%	-	-	-	-	-	-
Ragged cuticles	-	2%		-	-	-	-	-
Beau ‘s lines	-	2%	-	-	-	0.8%	-	-
Mottled lunulae	-	-	-	-	2%	5.1%	-	-
Punctate leukonychia	1.4%	-	3%	-	1%	24.6%	-	-
Onychomadesis	-	-	-	-	1%	0.4%	-	-
Onychorhexis	-	-	-	-	-	-	-	6.1%

Differential diagnosis of AA:

There are several other conditions that can clinically resemble AA. The following are considered in the differential diagnosis- trichotillomania, androgenetic alopecia, secondary syphilis, tinea capitis and triangular alopecia (107).

a) Trichotillomania

This clinically presents as non- scarring alopecia caused by repeated pulling of hairs and dermoscopy can show features like tulip hairs, hair powder, broken hairs showing hair shafts in different lengths and histopathology is necessary to differentiate it from AA.(91) (34)

b) Androgenetic alopecia

The diffuse hair loss in androgenetic alopecia and AA presenting with diffuse hair loss instead of a patchy presentation can be difficult to differentiate from each other clinically. Dermatoscopy becomes useful in such a case to differentiate between the disorders. (91-92)

c) Secondary syphilis

The patchy hair loss in syphilis is often described as " motheaten alopecia" whereas in AA the patches are smooth and well circumscribed. Serological parameters help in differentiating between the two conditions. (91-92)

d) **Tinea capitis**

Tinea capitis is the closest differential for AA in the pediatric population. Fungal scrapings and culture will help in confirming tinea capitis. (91-92)

e) Temporary triangular alopecia-

A triangular patch of alopecia seen in infants. (91-92)

Trichoscopy in AA :

Trichoscopy is the study of hair and scalp disorders using the instrument called dermatoscope. It can be done using a hand held dermatoscope with 10 X magnification or using a videodermatoscope 20 x to 120 x magnification. Trichoscopy can be done to assess the hair changes in AA that predict disease activity and also to detect response to treatment. (115)

The trichoscopic findings seen in AA are shown in table 3A.

Table 3A –Studies on trichoscopy in AA from countries other than India

	Mahmoudi et al (108)	Waskiel et al (109)	Inui et al (110)	Kibar et al (111)
Country	Iran	Poland	Japan	Turkey
Yellow dots	10.4%	62%	63.7%	69.23%
Black dots	14.8%	53%	44.3%	66.7%
Broken hairs	33.3%	49%	45.7%	56.4%
Exclamation mark hairs	28.2%	39%	31.7%	61.5%
Circle or pig tail hairs	-	21%	-	-
Short vellus hairs	16.5%	61%	72.7%	51.2%
Upright regrowing hairs	-	23%	-	-
Coudability hairs	-	51%	-	20.5%
Pohl pinkus constriction	-	4%	-	-

Table 3B- Indian studies on trichoscopy in AA

	Mane et al (19)	Peter et al (21)	Guttikonda et al (112)	Hegde et al (84)	Bapu et al (113)	Ankad et al (114)
Yellow dots	81.8%	42%	88%	57.33%	89.6%	50%
Black dots	66.6%	75%	58%	84%	31%	20%
Broken hairs	55.4%	67%	56%	37.33%	12.9%	30%
Exclamation mark hairs	12.1%	33%	26%	18.67%	19.8%	60%
Circle or pig tail hairs	-	-	14%	-	-	-
Short vellus hairs	40.9%	56%	66%	68%	78.4%	10%
Upright regrowing hairs	-	-	-	-	-	-
Coudability hairs	-	-	14%	-	-	-
Pohl pinkus constriction	-	-	2%	-	-	

Yellow dots :

Yellow dots in dermatoscopy are the follicular infundibula that are filled with sebum or keratotic material. (109) They appear as regular or polycyclic yellow or yellowish - brown dots. (115) It has been hypothesized that yellow dots are usually difficult to detect in people with yellowish or dark skin colour. (115) Yellow dots are not usually seen in the pediatric population as the sebaceous glands are not developed in them. In AA, yellow dots are seen to be evenly distributed. (111) They are more easily seen with polarised dermatoscope than in a non - polarised dermatoscope. (116) Yellow dots are not specific for AA. They are also seen in dissecting cellulitis of the scalp, chronic telogen effluvium, (117) trichotillomania, discoid lupus erythematosus, traction alopecia (118) and traumatic alopecia. Yellow dots are also seen in kerion celsi. (119). Trichoscopic features of yellow dots in different types of hair loss shown table 3C.

Table 3C- Trichoscopy features of yellow dots in different types of hair loss

Yellow dots	Condition
Evenly distributed	Alopecia areata
Sparse yellow dots (124, 125, 127)	Trichotillomania, traction or traumatic alopecia, chronic telogen effluvium
Large, " 3 D ''soap bubble –like, imposed over dark dystrophic hairs (121)	Dissecting cellulitis scalp
Large, dark yellow dots (121)	Discoid lupus erythematosus

Black dots :

Black dots refer to the pigmented hairs that have been broken at the scalp level. (116) Black dots are seen in the scalp of AA patients who have active disease. They are markers of disease activity along with broken hairs and exclamation mark hairs. (122) The incidence of black dots varies between 0-84 %. (133) They are not specific for AA. Black dots are also seen in trichotillomania, tinea capitis, lichen plano pilaris, traction alopecia, discoid lupus erythematosus (118), traumatic alopecia and dissecting cellulitis of the scalp. (123) Loose anagen hair presents with black rectangular granular spots in children. (124) Hence this condition needs to be ruled during trichoscopy of AA in children.

Exclamation mark hairs (tapering hairs) :

These are hairs that are tapered and hypopigmented at their proximal ends and are thickened and hyperpigmented at the distal ends. (125)They are seen in 12-71% patients with AA. (126) Exclamation mark hairs are considered as markers of disease activity. (127) They are seen when the disease is active and when it is progressing . Exclamation mark hairs disappear after successful treatment. (125) Hence exclamation mark hairs help to predict the response to treatment. These are usually 1 to 3 cm and can be visualised by the naked eyes while some tapering hairs are only 1-5 mm long and dermatoscope is required to identify them. (110) These are known as "micro - exclamation mark" hairs. (125) Exclamation mark hairs are also seen in other conditions like tinea capitis, (116) trichotillomania, (128) anagen effluvium and alopecia secondary to chemotherapy. (129)

Coudability hairs:

These are hairs that have thin proximal ends and a thickening of the distal ends and these distal ends are not visible within the view of the dermatoscope. (137,138) These are sometimes thought of as long exclamation mark hairs. These hairs are seen in 5% - 80% of patients with AA. (139-141) They are considered as markers of disease activity and severity. (130) (131) Even though coudability hairs are considered as a pathognomonic feature of AA, (130) they are also seen in other conditions like trichotillomania, (127,141) chronic intoxication and malnutrition. (125)

Broken hairs:

These are hairs that have been broken transversely or they are caused when hairs that have regrown rapidly in an incompletely destroyed hair that had previously formed a black dot (116) They were observed in 0-71% of patients with AA. (110,111) Broken hairs are seen when the disease is active and these are markers of disease activity in AA. These are also seen in trichotillomania but the broken hairs can be differentiated from AA because in trichotillomania, hairs are broken at different lengths. These are also observed in traumatic or traction alopecia, tinea capitis, androgenetic alopecia, primary cicatricial alopecia. (116)

Short vellus hairs :

These are short hairs that are 10 mm in length, seen in people with dark skin. Short vellus hairs appear as thin, non pigmented hairs. They carry a good prognosis as they are markers of hair regrowth. They may appear straight or twisted in dermatoscopy. They usually appear after treatment. Detection of short hairs that are transforming into terminal hairs are visible as short hairs with increase in thickness and pigmentation in the proximal ends. (115) The percentage of vellus hairs seen in AA ranged between 34 %-100%. (117,13) These are also seen in other conditions like trichotillomania, (120) androgenetic alopecia, (132) acute and chronic telogen effluvium. (133)

Upright regrowing hairs :

These are hairs that are pigmented and grow straight and are seen to have tapering distal ends. As children tend to have greater hair regrowth, these hairs are commonly seen in the pediatric population. These hairs were observed in 11%-96% of patients with AA.

(127,140,144) Upright regrowing hairs are also seen in temporal triangular alopecia, (134) tinea capitis, (120) trichotillomania, (120) and acute telogen effluvium.

Circle hairs or pigtail hairs :

These are short coiled hairs with ends that are tapering. (125) They indicate hair regrowth and are seen again in the pediatric population as this group tend to have a greater tendency for regrowing hairs. They usually disappear after a few weeks. (115) They are usually visible in 4%-61% of patients with AA. (21) Circle hairs can also be seen in trichotillomania, (134) tinea capitis, (135) chemotherapy induced alopecia.

Pohl –Pinkus constrictions :

These are hairs that show constrictions in the shaft along its length. (125)These are produced in the shafts due to repeated arrest of the mitotic and metabolic activity of the hair follicles. (116) These are seen in 2-10% of the patients with AA. (136) These hair changes in dermatoscopy reflect disease activity and are observed in patients who have active loss of hair. Hairs with multiple Pohl-Pinkus constrictions resemble hair changes that are seen with monelithrix. (116) There are other conditions where Pohl-Pinkus constrictions are seen .These include infections, nutritional disorders, alopecia following chemotherapy ,cicatricial alopecia. (137)

Flame hairs :

Flame hairs are considered to be broken hairs that are produced in response to damage caused due to mechanical pulling of hairs, trauma or due to drugs (138). These are described as short hairs showing pigmentation, having a tip that is wavy at the distal end. These were so named because of their close resemblance to the flame on a match

point. Flame hairs were considered to be specific for trichotillomania (120) but the study done by Tosti *et* al (138) showed that flame hairs are also seen in other conditions like alopecia areata (21% of cases), traction alopecia (4% of cases), central centrifugal cicatricial alopecia. (3% of cases)

Trichoscopy of AAI:

Trichoscopy can be used to diagnose patients with this condition. Yellow dots were found in about 95% -100 % of the patients and it was also associated with the findings of follicles that are hair bearing or empty follicles. Presence of large number of regrowing, tapered hairs are another specific feature in patients with AAI. (99)

Correlation of trichoscopy with disease activity, severity, AA subtypes

Disease activity – Black dots, broken hairs, exclamation mark hairs (115-117)

Disease severity – Yellow dots, black dots (110)

Alopecia subtypes –Yellow dots, vellus hairs (AU) (115 -117)

Black dots, exclamation mark hairs (Patchy multiple) (108)

HISTOPATHOLOGY OF AA:

The diagnosis of AA is mostly clinical. Histopathology helps in differentiating it from other non scarring disorders. The histopathology of AA varies depending on the stage of the disease. In the acute stage of the disease a dense inflammatory infiltrate is seen involving the anagen hair bulbs. The infiltrate is mostly composed of Langerhans cells, lymphocytes and eosinophils which give rise to the " swarm of bees'' appearance in histopathology. (4) This appearance is mostly due to the presence of $CD4^+$ (60-80% of cells) and $CD8^+$ (20-40%) cells. The dermal papilla shows the presence of foreign

body giant cells and macrophages while other changes like apoptosis and necrosis of the cells are also seen. The epidermis and dermis in between the hair follicles are normal. Miniaturisation of hair follicles is also observed in AA. As the diseases becomes chronic, the $CD4^+$ cells become reduced while the $CD8^+$ cells persist. (44)

SCORING SYSTEMS IN AA:

a) Severity of Alopecia Tool score (SALT) :

The SALT score is done to assess the percentage of hair loss in AA. (86) It was developed by the National Alopecia Areata Foundation working committee. The total area of the scalp is divided into four quadrants –the posterior, right, left and top quadrants of the scalp and the percentage of hair loss involving the scalp is assessed. (93)

The SALT score represents the area of the scalp that is involved in alopecia areata. Hence a higher SALT score is seen in AT and AU and a lower score in patchy AA. It does not take into account the factors that determine disease activity or disease severity in AA.

SALT II score - a modification of the original SALT score was done to study the hair loss in those conditions where only a small patch of hair loss is predominantly seen such as in AA and it finds its use in those conditons where only specific areas of the scalp are involved such as in frontal fibrosing alopecia and male androgenetic alopecia. (139)

ALOPECIA AREATA PROGRESSION INDEX (AAPI) SCORE

The Alopecia Areata progression index (AAPI) score, a new scoring system was developed to assess the complete hair loss activity in patients with AA. At present, the

methods that are used to assess disease activity are hair pull test, detection of black dots, exclamation mark hairs and broken hairs in dermatoscopy and scalp biopsy. The AAPI score is a modification of the SALT score. The SALT score is deficient in that it accounts only for the area of the scalp involved in AA but does not take into account the activity of the disease and this led to the development of the AAPI score. (5)

Most evaluation methods to assess hair loss in AA tend to assess disease activity in focal lesions. In AAPI score, the overall disease activity of the entire scalp is determined and not just the activity of individual areas of alopecia. As detecting disease activity markers like black dots, broken and exclamation mark hairs are necessary in calculating AAPI score, detection of black dots in Caucasians is difficult owing to the light hair color. In AAPI, scoring is determined by the detection of markers of disease activity at a single point in time.

Quality of life in AA :

The quality of life (QOL) is significantly affected in patients with AA. The degree to which alopecia areata can impact the QOL is underestimated as they can present with depression, low self esteem, anxiety disorders. Dermatology Life Quality index score is a commonly used questionnaire to assess the QOL. (140) The DLQI score has a minimum score of 0 and a maximum score of 30 and the impact on life is greater with higher scores of DLQI. In a cross sectional study done in Serbia, the QOL was assessed using short form 36 health form (SF 36), DLQI score and Skindex -29 questionnaires were used. (141) In the Skindex- 29 score, three aspects of life are assessed-degree of severity of symptoms, social functions and emotional health. (141) The SF 36 score assesses eight domains of physical health and score between 0-100, higher QOL is seen

with higher scores. A study done in Iran to assess QOL in AA showed that greater impact was seen in patients with a severe disease and those with acute onset disease of less than six months duration. (142)

Prognostic factors for AA:

There are few factors that determine the prognosis of patients with AA. The following factors indicate poor prognosis in AA. (12) (66) (125-127)

a) Younger age of onset b) Family history c) AT/AU d) Presence of atopy

e) Duration of more than 1 year f) Ophiasis g) Nail disease

h) Association with autoimmune diseases

Course of AA :

Most patients with AA have a spontaneous regrowth of their hairs. Eighty percent of patients with patchy AA had complete resolution of alopecia within a duration of one year. (86) Most people will have more than one episode of AA in their lifetime. A study done by Lyakhovitsky et al (149) from Israel showed prior episodes of hair fall in about 40.3 % of people in the study population. Five to ten percent of patients with AA progress to develop AT or AU .The chance of regaining normal hair growth in AT or AU is less than 10%. (4)

In conclusion, assessing the serum granulysin levels is useful in alopecia areata for the following reasons:

a) Alopecia areata has a clinical course that is unpredictable where patients may have resolution or have multiple recurrent episodes of hair loss. In such patients it may have a prognostic value.

b) Granulysin being an inflammatory marker and marker for the cytotoxic action of CD 8+ cells may be useful to assess the degree of inflammation in AA.

c) It would also help to study the clinical profile of patients with high serum granulysin levels when compared to those with normal levels of granulysin.

MATERIALS AND METHODS

Study design:

This study was done as a single centre, prospective, case control study in patients with alopecia areata.

Study setting :

The study was conducted at Christian Medical College, Vellore, Tamil Nadu. This is a tertiary care centre, with 2900 beds, with an average outpatient strength of about 8000 per day. The Department of Dermatology, Venereology and Leprosy had an annual outpatient registration of 60,543 during the period 2018 to 2019 in the unit II OPD. The study patients were recruited from among those attending the unit II OPD.

Study Period :

Patients for this study were recruited from January 2019 to August 2019 (8 months).

Study subjects :

Adult patients with AA seen during the study period were eligible for inclusion in the study.

Inclusion criteria for cases :

i) All adult patients with AA who are 18 years & above

ii) Patients who were willing to participate in the study

Exclusion criteria for cases:

i) Patients less than 18 years of age

ii) Pregnant women

iii) Patients with viral exanthems, malignancy, transplant, GVHD, drug rash

iv) Preceding skin diseases like psoriasis, lichen planus, acne, folliculitis, inflammatory skin conditions

v) Patients not willing to participate in the study

vi) Patients with alopecia areata without the involvement of the scalp

Inclusion criteria for controls:

i) Healthy volunteers above the age of 18 years were recruited as controls for comparison

Exclusion criteria for controls:

i) Patients less than 18 years of age

ii) Pregnant women

iii) Patients with viral exanthems, malignancy, transplant,GVHD, drug rash

iv) Preceding skin diseases like psoriasis, lichen planus, acne, folliculitis, inflammatory skin conditions

v) Patients not willing to participate in the study

Sample Size :

Based on the data by Ono et al, (6) a correlation between the serum granulysin level and the percentage of AA patches in acute disease was observed. (Spearman's r= 0.59) Assuming that we would get a similar correlation which should be significant at 5% level with the power of 80 and with one sided test, the minimum sample size needed

was about 16 in each group. However, we proposed to study a minimum of 20 study subjects with acute and chronic AA separately.

As the primary objective was to compare serum granulysin levels in acute and chronic AA with controls we proposed to study a minimum of 20 healthy controls.

Recruitment of cases :

All patients with AA who were satisfying the inclusion criteria and seen in the dermatology outpatient clinic of Unit II were included in the study. The diagnosis of AA was based on the clinical and dermoscopic pictures. Patients with AA of less than six months duration were recruited as acute alopecia areata and those with disease of more than six months as chronic AA respectively. The details of the disease and the investigations to be done were explained to the patient and relatives using the patient information sheet in their respective language. (Annexure 1) An informed consent was obtained from all patients and controls before they were recruited into the study. (Annexure 2)

Data collection:

The demographic details, history of the disease, associated comorbidities, treatment details with response to treatment, clinical features, vital signs, dermatoscopic examination findings and investigations were entered in a clinical research proforma. (Annexure 3)

Demographic details :

The name, gender, age, hospital number and contact information of the patients were documented in the clinical research proforma.

History :

- A detailed history of the disease and the presenting symptoms of the patient were recorded. Duration of hair loss was recorded in months.
- Family history of AA
- History of other medical comorbid conditions like thyroid dysfunction, anemia, hypertension, coeliac disease, other autoimmune conditions were recorded
- History of atopy
- History of previous episodes of hair loss
- History of prior and current treatment of hair loss –use of topical steroids, intralesional steroids, systemic treatment (steroids/ methotrexate/ azathioprine)
- History of alternate methods of treatment
- History of prior and current response to treatment (improved / status quo/ worsened)

Blood pressure measurement :

Blood pressure of all the study patients was measured using the sphygmomanometer and the values were documented in the clinical research proforma.

Cutaneous examination:

The following cutaneous features were documented in the research proforma.

- Site of involvement –scalp, eyebrows, beard, body
- Number of patches of alopecia –single / multiple
- The extent of the disease –patchy/ AT / AU
- The pattern of the disease –reticular/ sisaipho/ ophiasis/ others

- Presence of nail changes –pits/ trachyonychia/ punctate leukonychia/ Beau's lines / longitudinal ridges

Dermatological Life Quality Index (DLQI) score :

The quality of life of all the patients who were recruited in the study was assessed using the DLQI questionnaire. (annexure 4)

The DLQI has a minimum score of 0 and a maximum score of 30.

Trichoscopy :

A trichoscopic examination of the scalp was done for all the study patients, clinical photographs and dermoscopic images were obtained using a FotoFinder System, GmbH Medicam 800 (software version from 2.1) at 20 x magnification. Trichoscopy was done in patients who presented with both single and multiple patches. In those who presented with multiple patches, a representative area with the maximum trichoscopic findings and features of disease activity was chosen. The following trichoscopic features as described by Tosti et al (157) were recorded in the clinical research proforma.

The following features were recorded as suggestive of disease severity –yellow dots, black dots

The following features were recorded as suggestive of disease activity in AA

-Black dots, broken hairs, exclamation mark hairs

The following features were recorded as suggestive of regrowth in AA

-Vellus hairs, Upright regrowing hairs, Circle or pig tail hairs

Besides these findings, the following trichoscopic features were also recorded:

-Coudability hairs, Pseudomonelithrix, Flame hairs, Pohl Pinkus constrictions

Assessment of disease severity in alopecia areata :

The severity of hair loss was assessed by using two scores namely the SALT score and AAPI score.

a) SALT SCORE

The entire scalp is divided into 4 parts based on the surface area, top (40% - 0.4), posterior (24% - 0.24), right side (18% - 0.18), and left side of scalp (18% - 0.18) . The amount of hair lost in each area is determined separately and is multiplied by the percentage of scalp covered in that region of the scalp. Summing the products of each area will give the SALT score

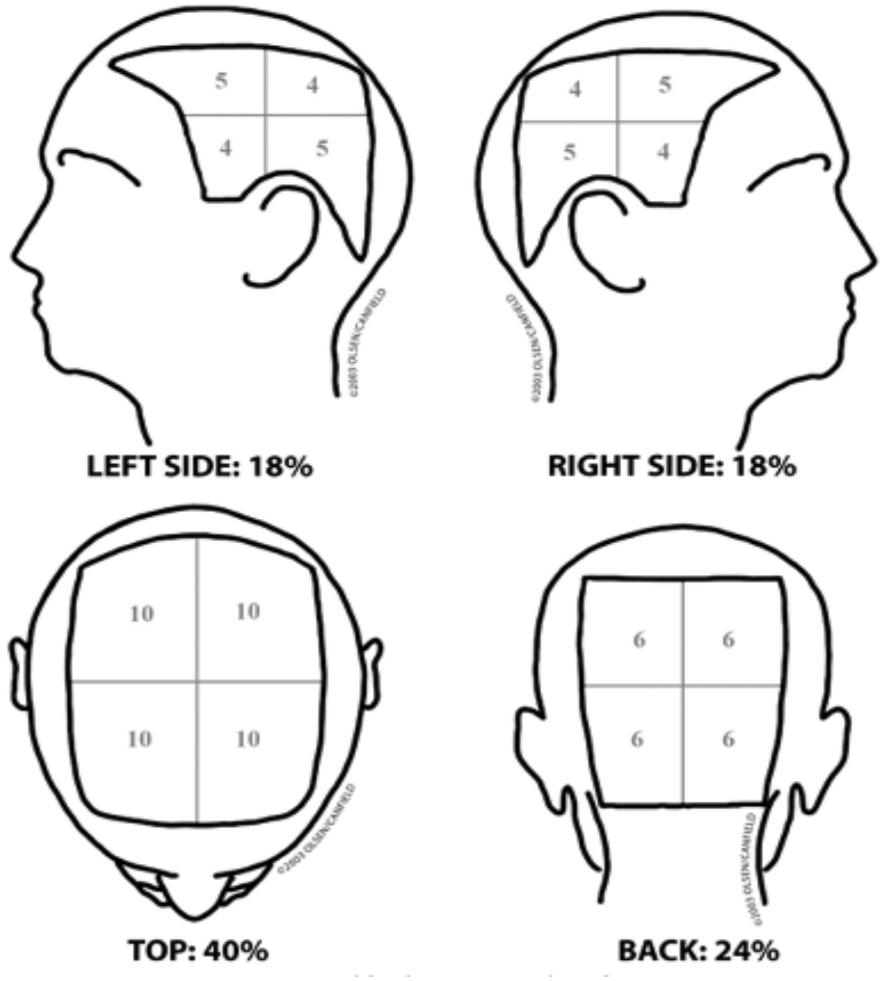

Figure 2 – Severity of Alopecia tool score

Total SALT score = Left side x 0.18 + Right side x 0.18 + Top x 0.40 + Back x 0.24

AAPI SCORE:

The AAPI score has the following 3 components (5).

a) Percentage of area of alopecia in each quadrant of the scalp

b) Score of hair loss

c) Surface area represented by the specific quadrant of the scalp

Percentage of area of alopecia in each quadrant of the scalp :

The percentage of the area of involvement of the scalp is calculated as in the SALT score.

Score of hair loss:

The score of hair loss (SL) is calculated for each quadrant of the scalp. The hair pull test (first component of SL) is performed by holding a bundle of fifty to sixty hairs with the index, middle finger and thumb close to the scalp. Then a gentle traction is given and the fingers are moved down the hair shafts and the number of pulled hairs are counted while the broken hairs are not counted. The test is done at three regions of the scalp namely the vertex, occipital and the parietal regions of the scalp. Hair pull test is given a score according to the number of grasped hairs that are pulled out, score of 2 for >20 % , 1 for 10-20% and 0 for < 10% of grasped hairs that were pulled out. The second component of SL is determined by detecting the presence of broken hairs, black dots, exclamation mark hairs and dystrophic vellus hairs and instead of the magnifying findings with 3x magnifier, trichoscopy with 20x magnification was used. The trichoscopic findings were graded as grade 4 with >50% of black dots / broken hairs/ exclamation mark hairs / dystrophic vellus hairs, grade 2 with < 50% of black dots / broken hairs/ exclamation mark hairs / dystrophic vellus hairs, grade 0: 0%

For each quadrant of the scalp, a hair pull test was done and based on the number of grasped hairs pulled out, a grading was given. Trichoscopic examination was done in each quadrant of the scalp and we looked for black dots, broken hairs, exclamation mark hairs and vellus hairs and based on whether any of these trichoscopic features were involving less than or greater than 50 % of the field, a grading was given. The total SL

was calculated by adding the grade for hair pull test with the grading obtained with trichoscopy. The total score for each quadrant was calculated by multiplying the percentage of the area of involvement, the SL and surface area represented by that specific quadrant . The total AAPI score is the sum of the scores of all the four quadrants.

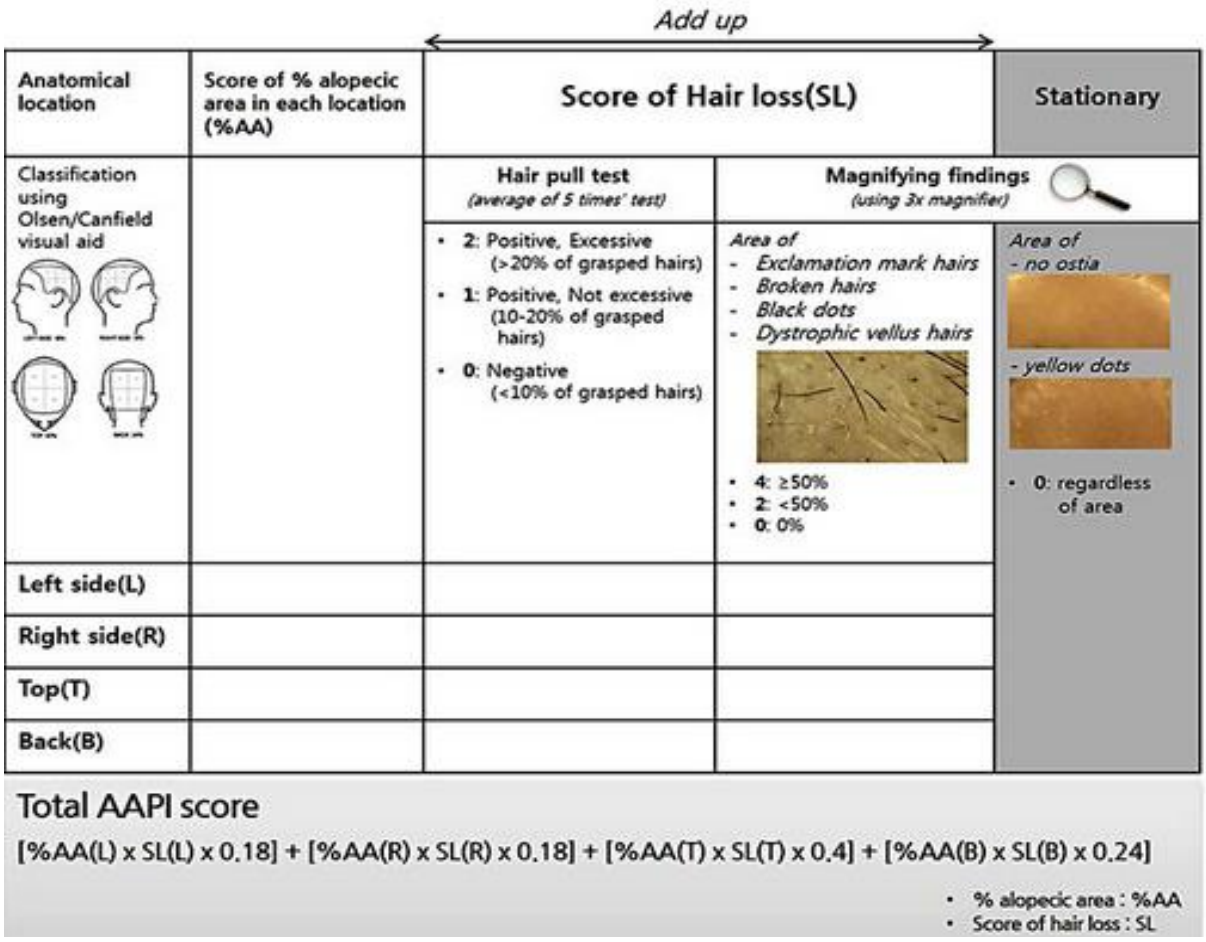

Figure 3 – Alopecia Areata Progression Index Score

Recruitment of Controls:

Twenty healthy volunteers above the age of 18 years were included in the study as controls. Patients who attended the dermatology outpatient clinic with minor skin conditions like dermatosis papulosa nigra, melasma, xanthelasma, acrochordons were included in the study.

Investigations:

Routine blood investigations that were done in the study patients were hemoglobin, iron, total iron binding capacity, TSH, thyroid antibodies, blood sugars.

Measurement of serum granulysin :

A 2 ml of whole blood sample was collected for the estimation of serum granulysin for all the study patients at the first visit to the outpatient clinic. All the blood samples were collected in EDTA tubes and taken to the Department of Clinical biochemistry at Christian Medical College, Vellore, by the investigator. Serum was separated from the collected samples, it was frozen and stored at -70 degree Celsius in the biochemistry laboratory. The granulysin levels of the serum samples obtained from the patients and control subjects were measured using Biovendor RD191327200R Human Granulysin ELISA kit. This is an enzyme linked immunosorbent assay. The sensitivity of the ELISA kit is 0.03 ng/ml.

Standards (recombinant protein based) and samples were incubated in micro-titration wells which were pre-coated with polyclonal anti-human granulysin antibody. The period for incubation was 1 hour. After incubation, the samples were washed and biotin-labelled polyclonal anti-human granulysin antibody was added. This was further

incubated with the captured granulysin for about 1 hour. Streptavidin-HRP conjugate was added after washing the wells. The samples were further incubated for about half an hour. This was further followed by washing and the remaining conjugate was allowed to react with the substrate, tetramethylbenzidine.

The concentration of granulysin in the samples was directly proportional to the level of absorbance of the final yellow product. A standard curve was created by plotting absorbance values against granulysin concentrations of standards. The concentration of granulysin in the patient samples was obtained using this curve. The serum granulysin levels were finally verified by a senior biochemist at the end of the procedure.

Statistical analysis:

Statistical Methods:

The mean and standard deviation (SD) was calculated for continuous variables like age, duration of the disease, SALT and AAPI scores. The mean (SD) or median of serum granulysin levels between acute and chronic versus healthy controls was done using t test or Mann Whitney U test. The graphical presentation of these was done using Box Cox plots or scatter plots. The Spearman rank correlation was done to estimate the correlation between serum granulysin level and the SALT score in AA and also between SALT and AAPI scores. A graphical representation of the same using scatter plots for SALT score, AAPI score and serum granulysin levels in acute and chronic AA was also done.

IRB Approval :

This study was approved by the Institutional Review Board of Christian Medical College, Vellore, with IRB Min number 11716 on January 25, 2019 and was funded by the Fluid research grant of the institution (Annexure 5).

RESULTS

In this prospective case control study, 46 patients with AA who fulfilled the inclusion criteria were recruited. Patients with disease of less than six months duration were recruited as acute cases and those with disease of more than six months were recruited as chronic cases. There were 22 acute and 24 chronic cases of AA in this study. The study was done over a period of 8 months (from January 2019 to August 2019).

Twenty healthy volunteers were recruited as controls.

1.DEMOGRAPHIC PROFILE

1.1 Age

In our study, 36 patients belonged to the age group of 18-40 years (78.26 %), 9 patients belonged to the age group of 40 -60 years (19.56%) and only one patient was aged above 60 years. The age distribution of the study population is shown in figure 4 and age distribution in the acute and chronic groups is shown in figure 5.

The mean age at presentation in the overall study subjects was 32.24 ± 9.77 years while it was 33.26 ± 8.64 years in the acute group and 30.79 ± 11.26 years in the chronic group.

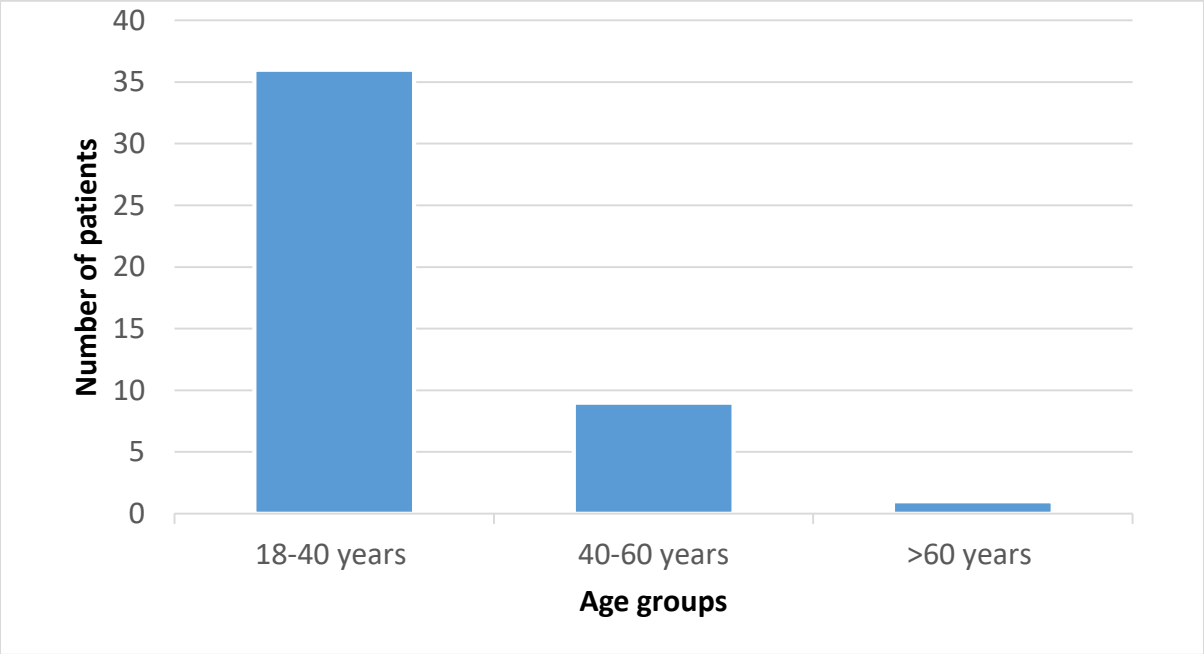

Figure 4 - Age distribution of study patients with AA

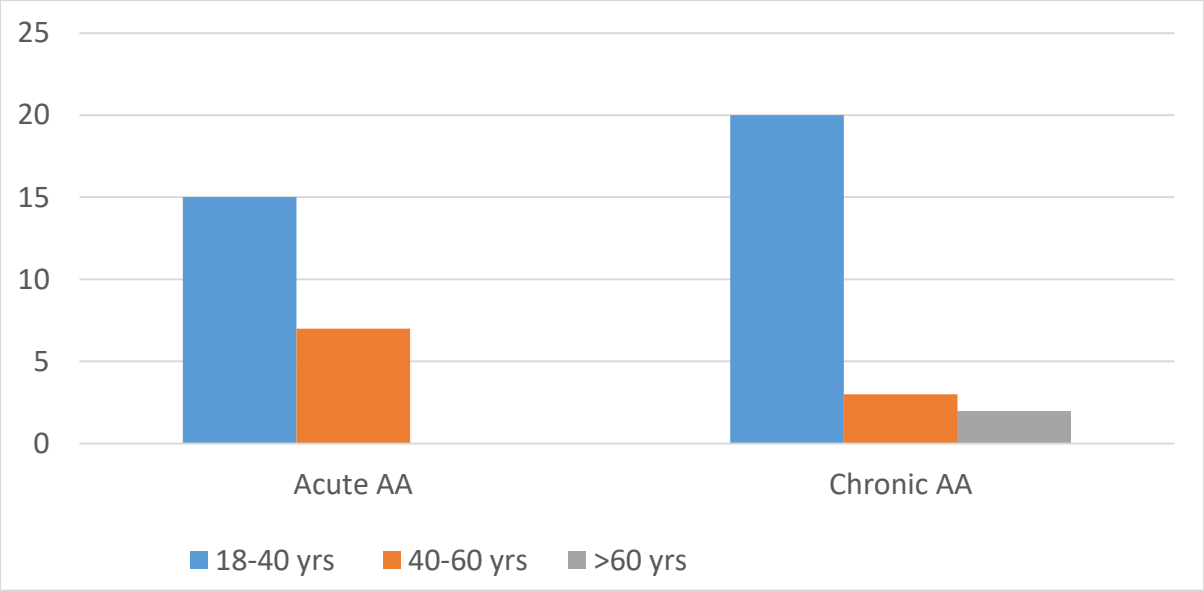

Figure 5- Age distribution of patients in the acute AA and chronic AA group

1.2 Gender :

The overall gender distribution in the study patients was equal with a male is to female ratio of 1:1. In the acute AA group, there were 10 males and 11 females while in the chronic AA group there were 12 males and 12 females. The gender distribution in the acute and chronic groups is shown in figure 6.

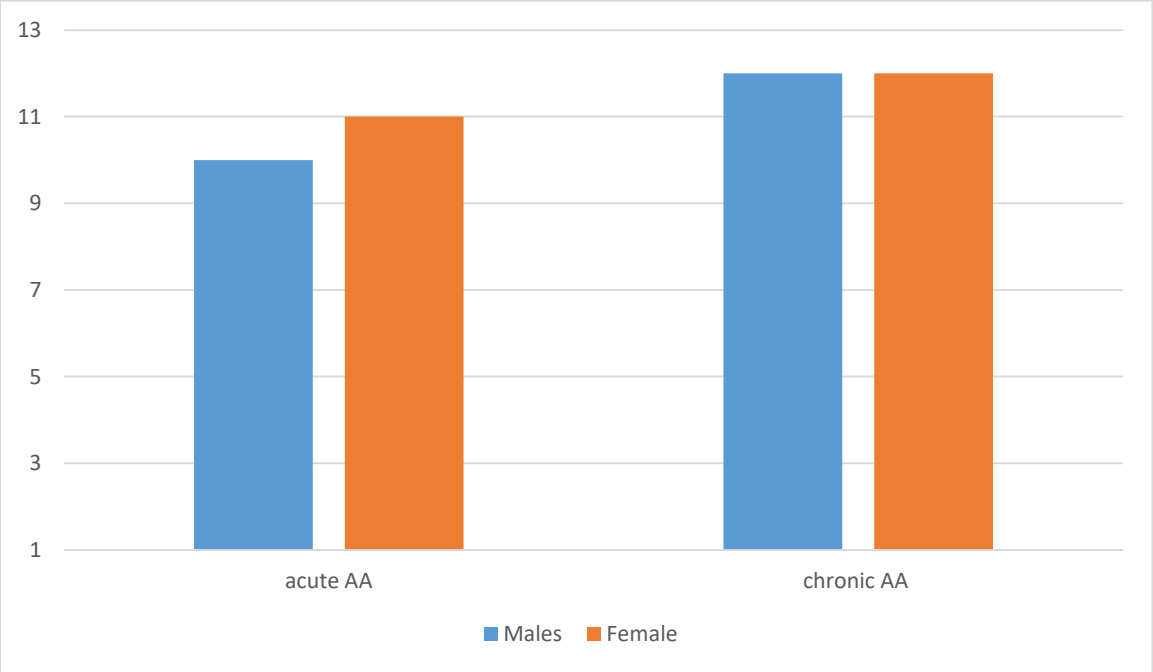

Figure 6 - Gender distribution in acute and chronic AA patients

2. Duration of the disease :

Patients who presented with a disease duration of less than six months were considered as acute AA and those with a disease duration of more than six months were considered as chronic AA. The mean duration of disease in the acute AA was 2.22 ± 1.10 months and it was 34.3 ±38.5 months in the chronic group.

3. Family History:

A family history was present in 9/46 (19.56%) patients, of which 8 patients belonged to the chronic AA and one patient to the acute AA group. The frequency of family history in both the acute and chronic groups is shown in figure 7.

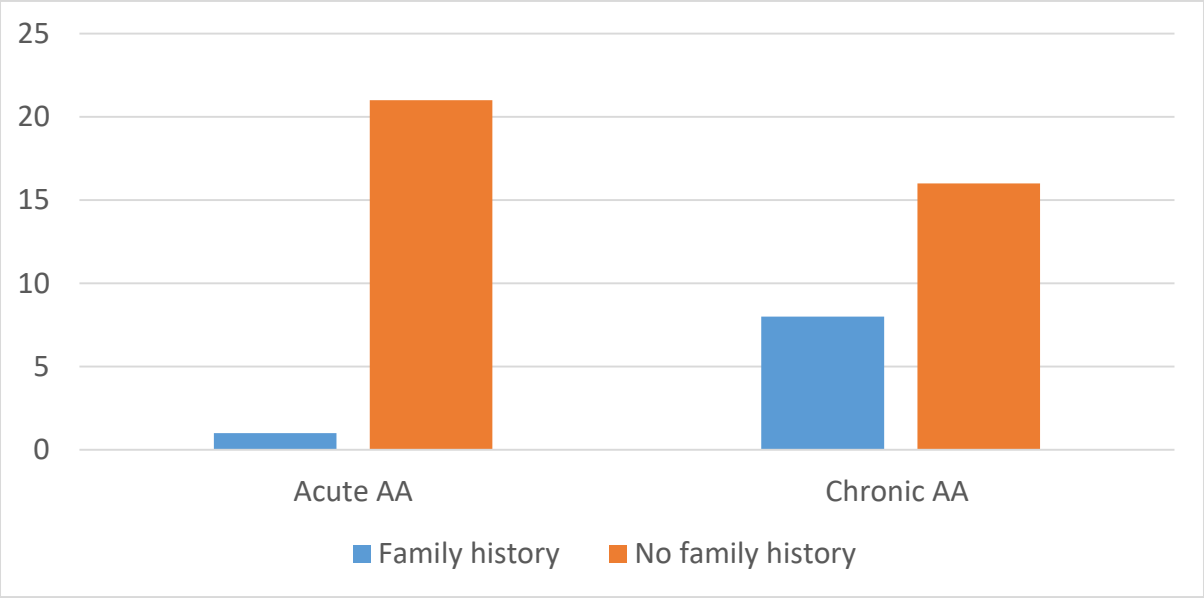

Figure 7- Frequency of patients with family history in acute and chronic AA group

4. Medical Comorbidities :

The medical comorbid conditions were found in 11/46 (23.9%) patients. These were thyroid dysfunction, anemia, vitiligo and hypertension. The frequency of medical comorbidities are shown in figure 8.

Thyroid dysfunction was seen in 4/46 (8.7%). All four patients had hypothyroidism. There were no patients with autoimmune thyroiditis or hyperthyroidism. Patients with anemia were 4/46 (8.7%). Systemic hypertension was seen in only one out of the 46 patients (2.17%). Two out of the 46 patients had vitiligo (4.34%). None of the patients

in our study had diabetes mellitus, coeliac disease, rheumatoid arthritis or any other autoimmune conditions.

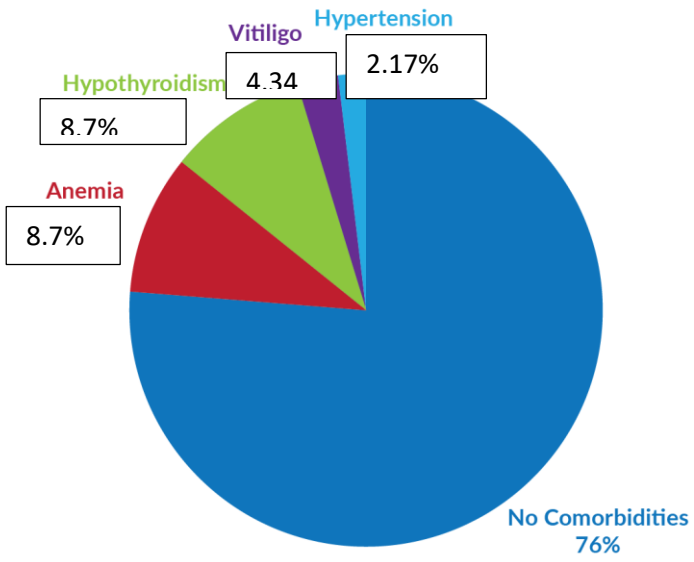

Figure 8 –Medical comorbidities associated with AA

5. Association with atopy:

Five patients had an associated atopy 5/46 (10.87%). Among the atopic conditions, three patients had bronchial asthma, one patient had allergic rhinitis and only one patient had atopic eczema. The atopic conditions seen in our study patients is depicted in figure 9. The atopic conditions seen in the acute AA and chronic AA group is depicted in figure 10.

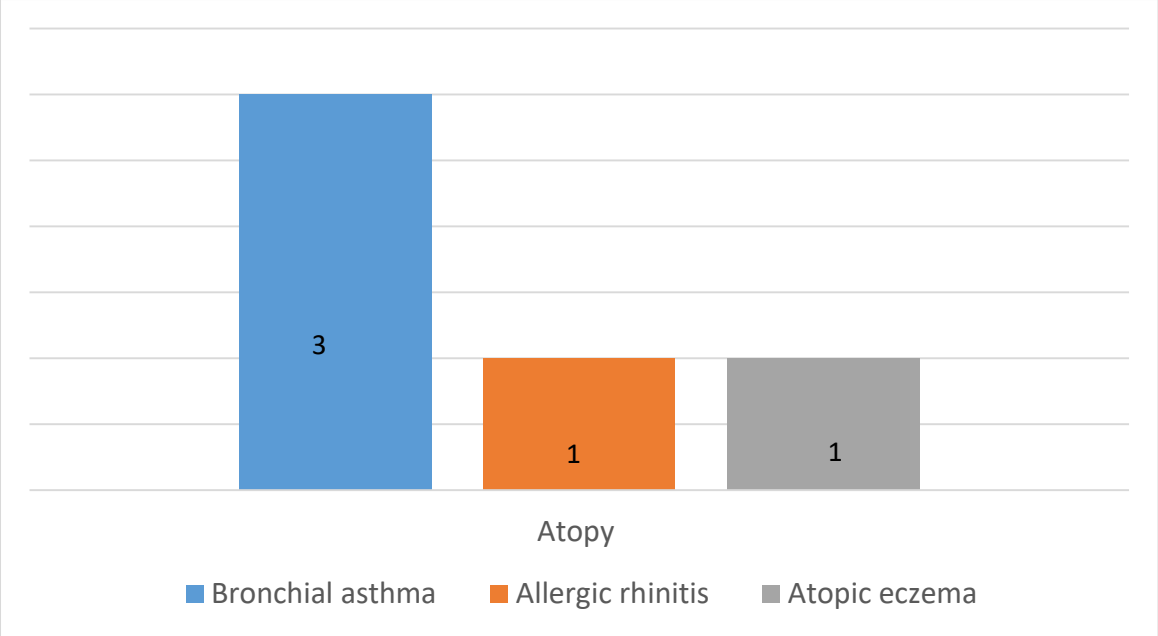

Figure 9- Atopic conditions associated with AA

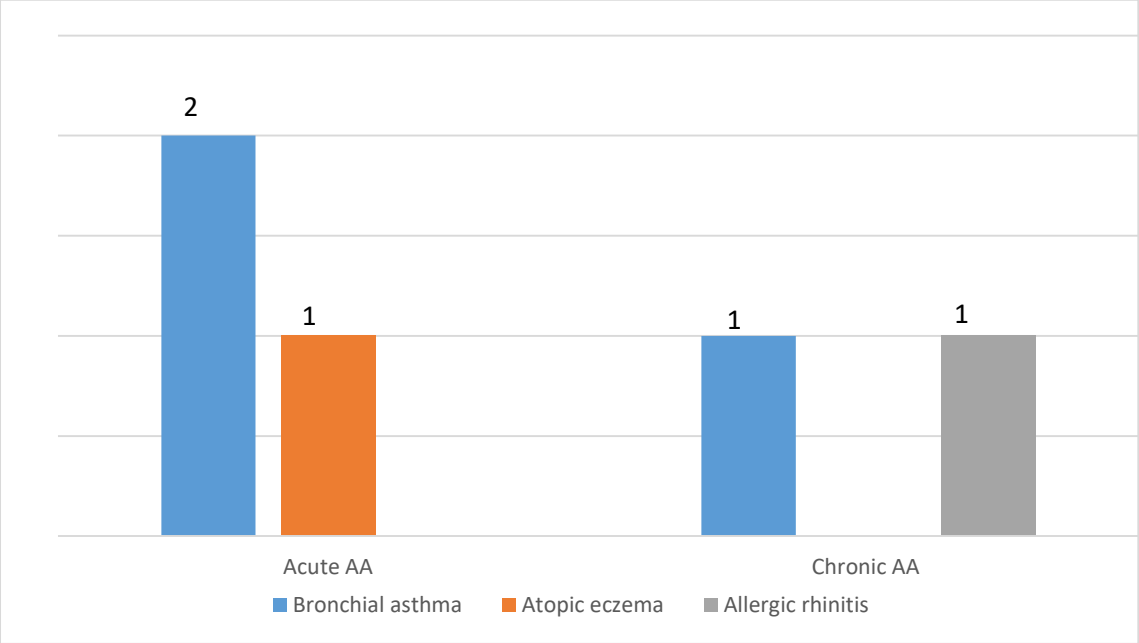

Figure 10-Atopic conditions seen in the acute and chronic AA groups

6. History of prior episode of hair fall :

Previous episodes of hair loss were seen in 19/46 (41.30%). Out of these 19 patients, 9 patients (47.37%) had acute AA while 10 patients(52.63%) had chronic AA. The remaining 27/46 (58.7%) patients were having their first episode of AA.

7. Treatment History :

A history of having received previous treatment was noted in 31 patients.The treatment received by them included topical steroids, intralesional injection with steroids and systemic treatment. Among these patients, 17/31 (54.83 %) were treated with topical steroids, 18/31 (58.06%) received intralesional injection with triamcinolone , 12/31 (22.58%) received systemic treatment, of which 6 patients received treatment with oral steroids, one each received treatment with azathioprine, methotrexate and cyclosporine and three patients received treatment with systemic homeopathy and other native medication. A combination treatment was received by 15/31 (48.39%) of the patients. The number of patients who received the different modalities of treatment is shown in figure 11.

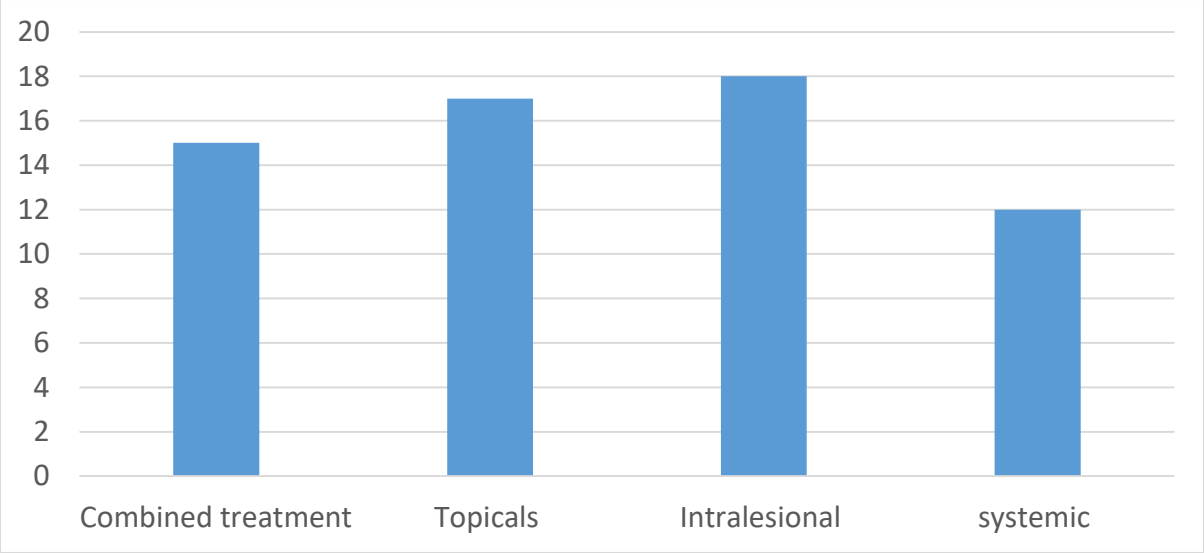

Figure 11- Treatment history of patients with AA

7.1 Response to treatment:

Of the 31 patients who had received treatment, 14/31 (45.1%) had progression of the disease while on treatment, 13/31 (41. 9%) reported good response to the treatment while 4 /31 (12.9%) of study patients reported that their disease was status quo. Figure 12 depicts the response to treatment.

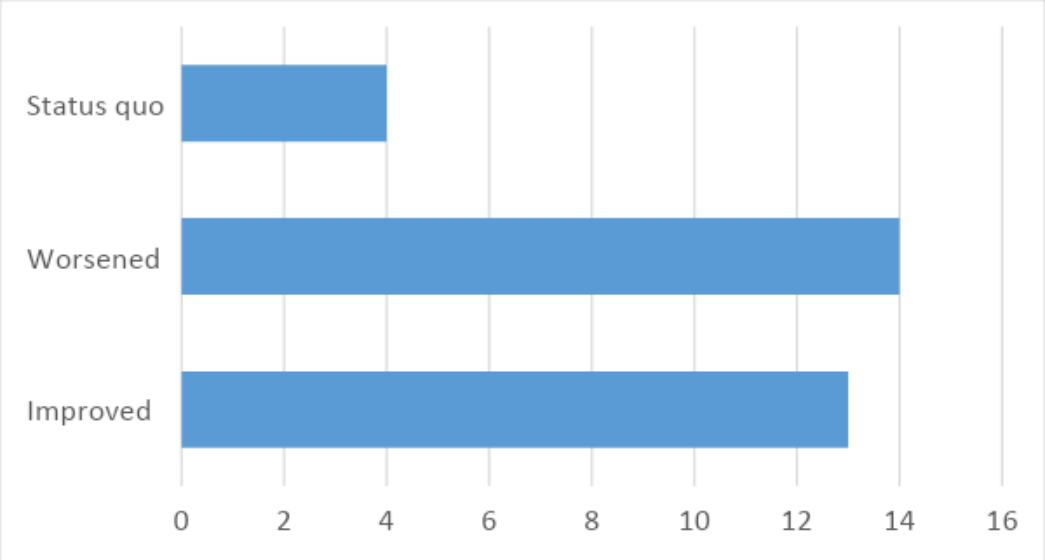

Figure 12- Response to prior treatment, with x - axis showing the number of patients

8. Clinical profile :

8.1 Site of involvement :

Patients with AA not involving the scalp were not recruited in the study as SALT score and AAPI score required the involvement of the scalp hair. Hence all the 46 patients had scalp involvement (100%), 13/46 (28 %) had involvement of the beard area, 9/46 (19%) had involvement of the body hair and 8/46 (17%) had involvement of the eyebrows. Among the 22 cases of acute AA, all 22 patients had scalp involvement (100%), 4 /22 (18 %) had involvement of their beard area, 3/22 (13%) patients had involvement of their eyebrows, 2/22 (9%) had involvement of the body hair. Among the 24 patients with chronic AA, all 24 patients had scalp involvement, 9/24 (37%) patients had beard area involved 5 /24 (20%) patients had involvement of their eyebrows and 7/24 (29%) patients had body hair involved. The various sites of involvement is shown in figure 13.

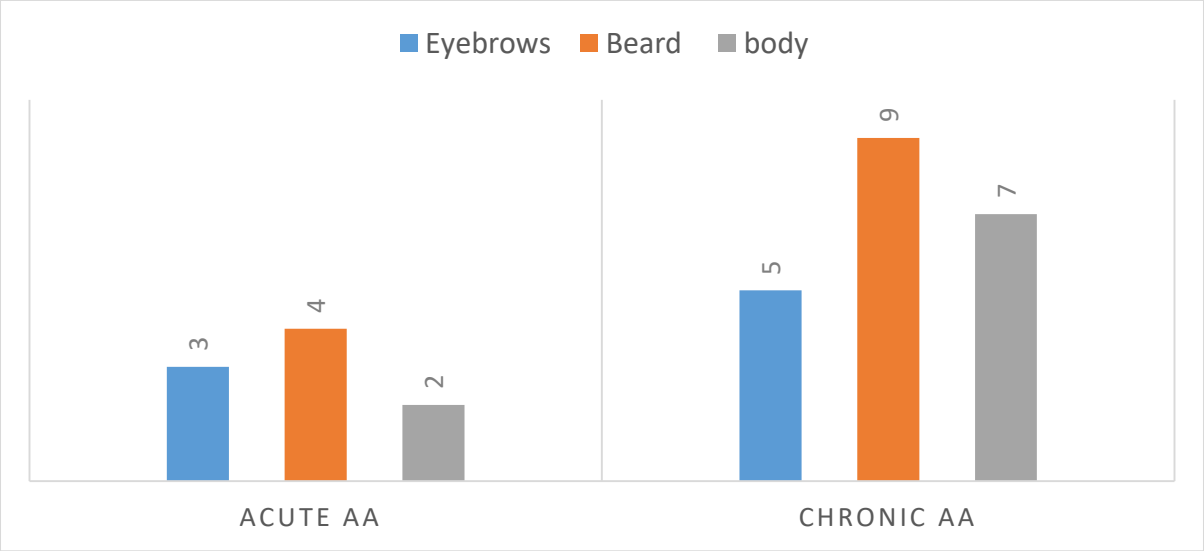

Figure 13- Additional site of involvement other than scalp in acute and chronic AA (numericals above bars indicating the number of patients)

8.2 Number of alopecic patches :

Patients recruited in the study presented with either single or multiple patches of the scalp. The following table 4 shows the number of patches in acute and chronic AA. As shown, 40.7 % of patients with acute AA and 10.5% of patients with chronic AA had single patch of alopecia while 59.3 % of patients with acute AA and 89.5% of patients with chronic AA presented with multiple patches.

Table- 4 Association of number of alopecic patches with duration of disease

Variables		Hair loss Duration				p value
		Acute AA		Chronic AA		
		N	%	N	%	
Patches	Single	11	40.7	2	10.5	0.025
	Multiple	16	59.3	17	89.5	

8.3 Extent of AA :

The extent of AA was studied to see if our patients had patchy involvement, AT or AU. In patients with acute AA, 90.91% of the patients had a patchy involvement and 9.09% of the patients had AU. In the chronic AA group, 70.83 % of the patients had a patchy involvement while 29.17% of patients presented with AU. There were no patients with AT in the acute or the chronic group. The extent of AA and the frequency of patients in each group is shown in table 5.

Table 5: Extent of AA in acute and chronic cases

Variables		Hair loss Duration				p value
		Acute AA		Chronic AA		
		n	%	n	%	
Extent	Patchy AA	20	90.91	17	70.83	0.022
	AU	2	9. 09	7	29.17	

8.4 Pattern of AA:

The ophiasis pattern of AA was seen in three patients in the study while the remaining 43/46 patients had the classical pattern. None of the study subjects had reticular or the sisaipho pattern of clinical presentation.

8.5 Nail changes:

Nail changes were observed in 14/46 (30.43%) of the patients. The common nail findings seen were nail pits in 5/46 (10.8%) and longitudinal ridges in 9/46 (19.6%) of patients. In the acute AA group, 3/22 (13.64%) patients had pits while 5/22 (22.73%) had longitudinal ridges. In the chronic AA group, 2/24 (8.33%) patients had nail pits while 4/24 (16.7%) had longitudinal ridges. The other nail changes like red lunula, trachyonychia, Beau's lines, punctate leukonychia were not seen in our study subjects.

We also looked at the nail changes seen in patients with patchy AA and AU. In the patchy AA group, 2/37 patients had nail pits and 6/37 patients had longitudinal ridges. In the AU group, 4/9 patients showed nail pits and 2/9 patients showed longitudinal ridges.

8.6 Trichoscopy findings :

The trichoscopy findings seen in our study patients were yellow dots, black dots, broken hairs, exclamation mark hairs, circle hairs, short vellus hairs, upright regrowing hairs and flame hairs. Pseudomonelithrix and coudability hairs were not seen in our study patients. The trichoscopy findings in the acute and chronic AA group are shown in table 6.

Table 6- Trichoscopy findings in acute and chronic AA

	Acute AA (n= 22)	Chronic AA (n=24)	Total n=46
Yellow dots	12(54.55%)	10 (41.7%)	22(47.8%)
Black dots	17 (77.3%)	13(54.16%)	30(65.2%)
Broken hairs	16(72.73%)	8 (33.33%)	24(52.17%)
Exclamation hairs	5 (22.73%)	3(12.50%)	8(17.3%)
Circle hairs	7(31.82%)	2(8.33%)	9(19.6%)
Short vellus hairs	11(50%)	6 (25.0%)	17(37%)
Upright regrowing hairs	6(27.3%)	3 (12.50%)	9(19.6%)
Flame hairs	0	2(8.33%)	2(4.34%)
Coudability hairs	0	0	0
Pseudomonelithrix	0	0	0

TABLE 7 - Trichoscopy findings in AA patients with bad prognostic factors

	Yellow dots	Black dots	Broken hairs	Exclamation mark hairs	Circle hairs	Vellus hairs	Regrowing hairs	Flame Hairs
FAMILY HISTORY N=9	6	6	6	2	2	2	0	0
AT/AU N=9	6	6	6	2	1	1	0	0
ATOPY N=5	3	4	3	2	2	1	2	0
OPHIASIS N=3	2	1	2	1	0	0	0	0
NAIL DISEASE N=13	9	9	9	3	1	6	1	1
AUTOIMMUNE ASSOCIATION N=3	2	2	2	0	0	2	2	0

Trichoscopic findings in patients with bad prognostic factors is shown in table 7. Yellow dots, black dots and broken hairs were seen most commonly in those patients who presented with bad prognostic factors. In those who had atopy, most common findings were black dots seen in 4 out of 5 patients followed by yellow dots and broken hairs. In those who presented with the ophiasis pattern of AA, the most common trichosopic findings were yellow dots and broken hairs whereas black dots were seen in a lesser frequency in them.

Table 8 –Trichoscopy findings seen in patients with single and multiple alopecic patches

	Single Patch (n=16)	Multiple Patches (n=30)(representative patch)
Yellow dots	8 (50%)	17 (56.7%)
Black dots	11(68 .8%)	23 (76.6%)
Broken hairs	8(50%)	20 (66.67%)
Exclamation mark hairs	4(25%)	8 (26.67%)
Circle hairs	4 (25%)	8(26.67%)
Short vellus hairs	9(56.25%)	13 (43.33%)
Upright regrowing hairs	2 (12.50%)	8 (26.67%)
Flame hairs	0	2(6.67%)
Coudability hairs	0	0
Pseudomonelithrix	0	0

We looked at the trichoscopy findings seen in the patients who presented with single and multiple patches. The findings are shown in the above table 8**.**

In patients with single and multiple alopecic patches, the most common trichoscopy findings were black dots, broken hairs, exclamation mark hairs and they indicated disease activity while short vellus hairs, upright regrowing hairs were the most common features that indicated hair regrowth and yellow dots which indicated disease severity. Flame hairs were seen in only 2 out of the 30 patients with multiple patches and were not seen in patients with single patches. Short vellus hairs were seen in increased

frequency in patients with single patches as compared to those who presented with multiple patches (56.25% vs 43.33%).

8.7 SALT score :

Severity of alopecia tool score was done for all the study patients. SALT scoring was done for the 22 acute cases and 24 chronic cases. The mean ± SD of SALT score was 7.14 ± 10.84 in acute AA cases and 31.11 ± 33.48 in chronic AA cases.

8.8 AAPI score :

The AAPI score was calculated for all the 22 acute cases and 24 chronic cases of AA. The mean ±SD of AAPI index score in acute cases was 15.53 ± 27.9 and in chronic cases it was 29.31 ± 37.89.

8.9 SALT score vs AAPI score :

There is a high positive correlation between SALT and AAPI score (r =0.774) and it is statistically significant with a p value of < 0.001 shown in figure 14 and table 9.

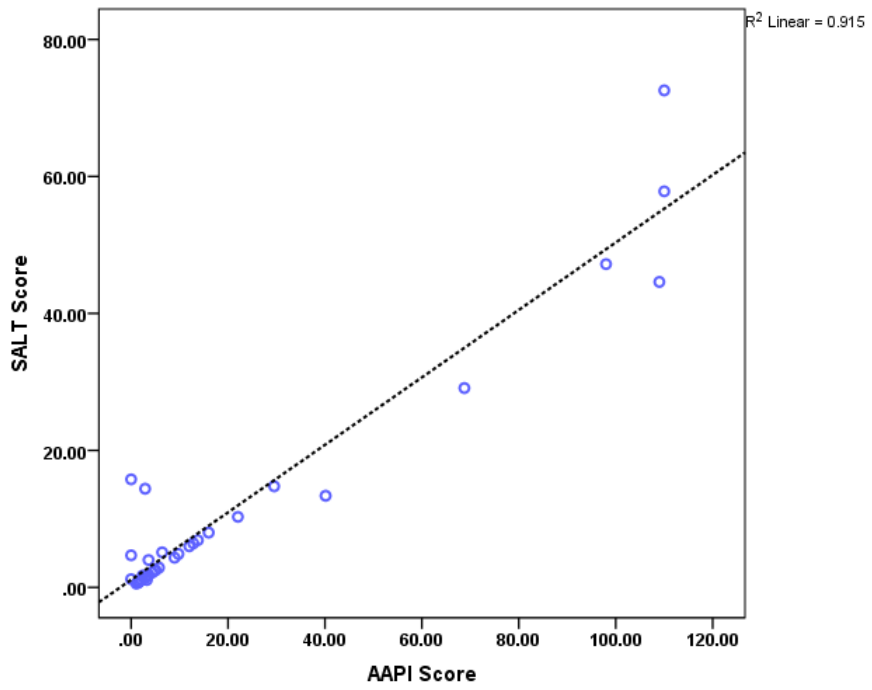

Figure 14- Scatter plot for AAPI vs SALT score

Table 9- SALT vs AAPI score with Spearman's correlation

Correlations			
Variables		Salt score	AAPI score
SALT score	Correlation Coefficient	1.000	.774
	Sig. (2-tailed)	.	.000
	N	36	36
AAPI score	Correlation Coefficient	.774	1.000
	Sig. (2-tailed)	.000	.
	N	36	36

9. DLQI score :

The DLQI was calculated for all the acute and chronic cases. The following table 10 shows the mean comparison of DLQI scores in acute and chronic AA.

Table 10 - Mean comparison of DLQI Score in acute and chronic cases

Variables	Acute AA	Chronic AA	p value
	Mean ± SD	Mean ± SD	
DLQI Score	3.85 ± 3.95	7.58 ± 5.93	0.023

The quality of life was significantly worse in patients with chronic AA as compared to acute AA.

10. Serum granulysin levels :

Serum granulysin levels in acute AA was 0.28 ± 0.13 ng/ml and in chronic AA was 0.21 ± 0.13 ng/ml and among controls was 0.18 ± 0.06 ng/ml. These values are shown in the table 11 below and the values are depicted in the box plot in figure 15.

Table 11: Descriptive statistics for serum granulysin of controls, acute and chronic AA patients

Variable	Control			Acute			Chronic		
	Mean (SD)	Min	Max	Mean(SD)	Min	Max	Mean (SD)	Min	Max
Serum Granulysin ng/ml	0.18 (0.06)	0.10	0.28	0.28 (0.13)	0.10	0.69	0.21 (0.08)	0.10	0.40

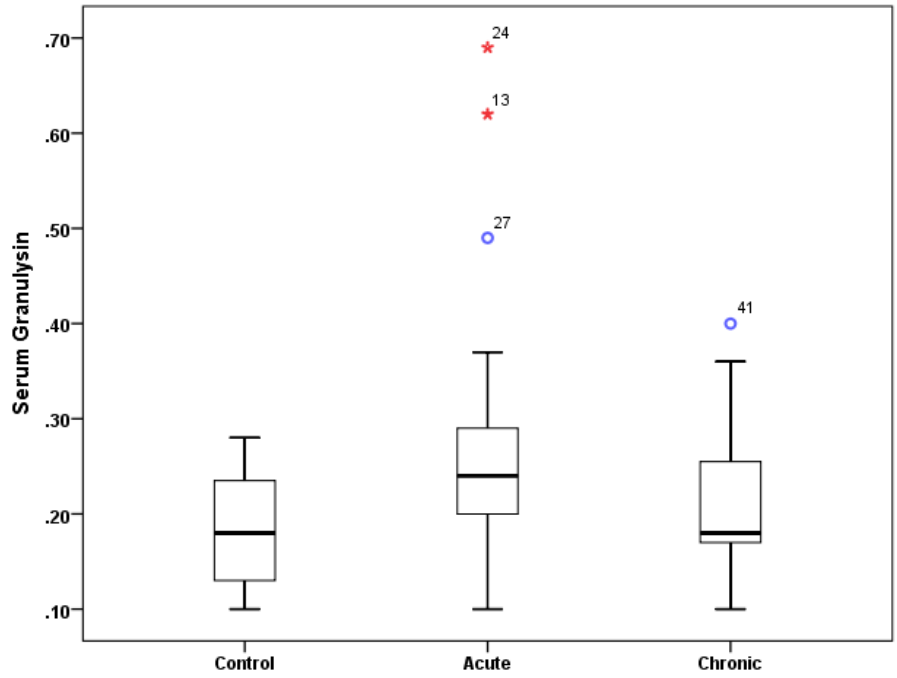

Figure 15 –Box plot showing serum granulysin in controls, acute and chronic AA

10.1 Serum granulysin in acute AA vs controls :

The mean serum granulysin levels in acute AA was 0.28 ± 0.13 ng/ml and in controls it was 0.18 ± 0.06 ng/ml. The serum granulysin levels in acute cases were significantly elevated compared to the controls and it is shown in table 12.

Table 12- Comparison of mean serum granulysin level of acute AA with controls

Variables	Acute AA	Controls	p value
	Mean ± SD	Mean ± SD	
Serum granulysin level ng/ml	0.28 ± 0.13 ng/ml	0.18 ± 0.06 ng/ml	0.005

10.2 Serum granulysin in chronic AA vs controls :

The mean serum granulysin was 0.21 ± 0.08 ng/ml in chronic AA compared to 0.18 ± 0.06 ng/ml in controls. The serum granulysin levels in chronic cases did not show any statistical significance compared to the controls and it is shown in table 13.

Table 13 - Comparison of mean serum granulysin level of chronic AA with controls

Variables	Chronic AA	Controls	p value
	Mean ± SD	Mean ± SD	
Serum granulysin level (ng/ml)	0.21 ± 0.08	0.18± 0.06	0.166

10.3 Serum granulysin in acute vs chronic AA:

Patients with acute AA had higher serum granulysin levels compared to patients with chronic AA and serum granulysin levels (p value of 0.08) is shown in table 14.

Table 14 -Comparison of mean serum granulysin levels in acute and chronic AA

Variables	Acute AA	Chronic AA	p value
	Mean ± SD	Mean ± SD	
Serum Granulysin level ng/ml	0.28 ± 0.13	0.21± 0.08	0.080

10.4 Correlation of serum granulysin and SALT score (overall cases):

The serum granulysin levels and its relation to the SALT score in overall AA cases is shown in table 15. There was no correlation of serum granulysin levels with the overall cases of AA.

Table 15- Serum granulysin levels vs SALT score in overall cases

Variable	Serum Granulysin (overall cases)	p value
	Correlation Coefficient	
Salt Score	0.002	0.989

10.5 Correlation of serum granulysin and SALT score (acute AA cases) :

In acute AA, a moderate correlation was seen between serum granulysin levels and SALT score which was significant as shown in figure 16 and table 16.

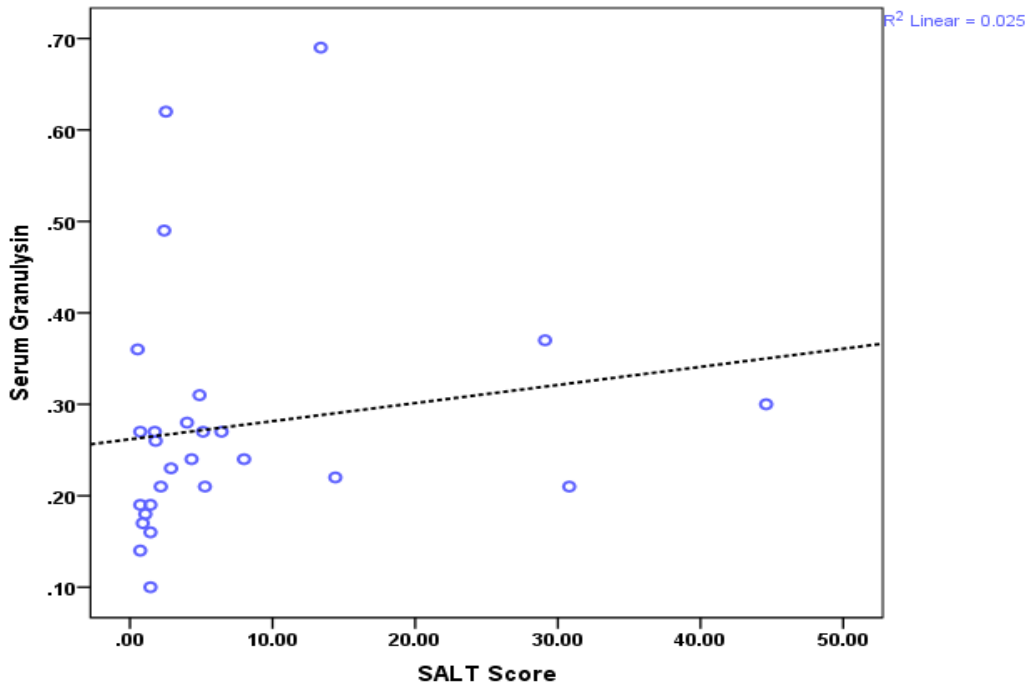

Figure 16 - Scatter plot showing correlation SALT score -versus serum granulysin level in acute AA

Table 16- Serum granulysin levels vs SALT score in the acute AA

Variable	Serum Granulysin level (Acute AA)	p value
	Correlation Coefficient	
Salt Score	0.410	0.034

10.6 Correlation of serum granulysin and SALT score (chronic AA cases) :

There was no correlation demonstrated between serum granulysin levels and SALT score among patients with chronic alopecia areata as shown in table 17.

Table 17: - Serum granulysin levels vs SALT score in chronic AA

Variable	Serum Granulysin level (Chronic AA)	p value
	Correlation Coefficient	
SALT Score	-0.065	0.799

The correlation of serum granulysin vs AAPI score in the overall cases, acute and chronic AA is shown below.

10.7 Correlation of serum granulysin and AAPI score (overall cases) :

Serum granulysin levels vs AAPI score in overall group of AA patients showed no correlation. (Table 18)

Table 18 –Serum granulysin vs AAPI score in overall AA patients

Variable	Serum Granulysin (Overall Cases)	p value
	Correlation Coefficient	
AAPI Score	-0.057	0.711

10.8 Correlation of serum granulysin and AAPI score (acute AA cases) :

In the acute AA group, the AAPI score showed a modest correlation with serum granulysin levels which was significant and is shown in Figure 17 and table 19.

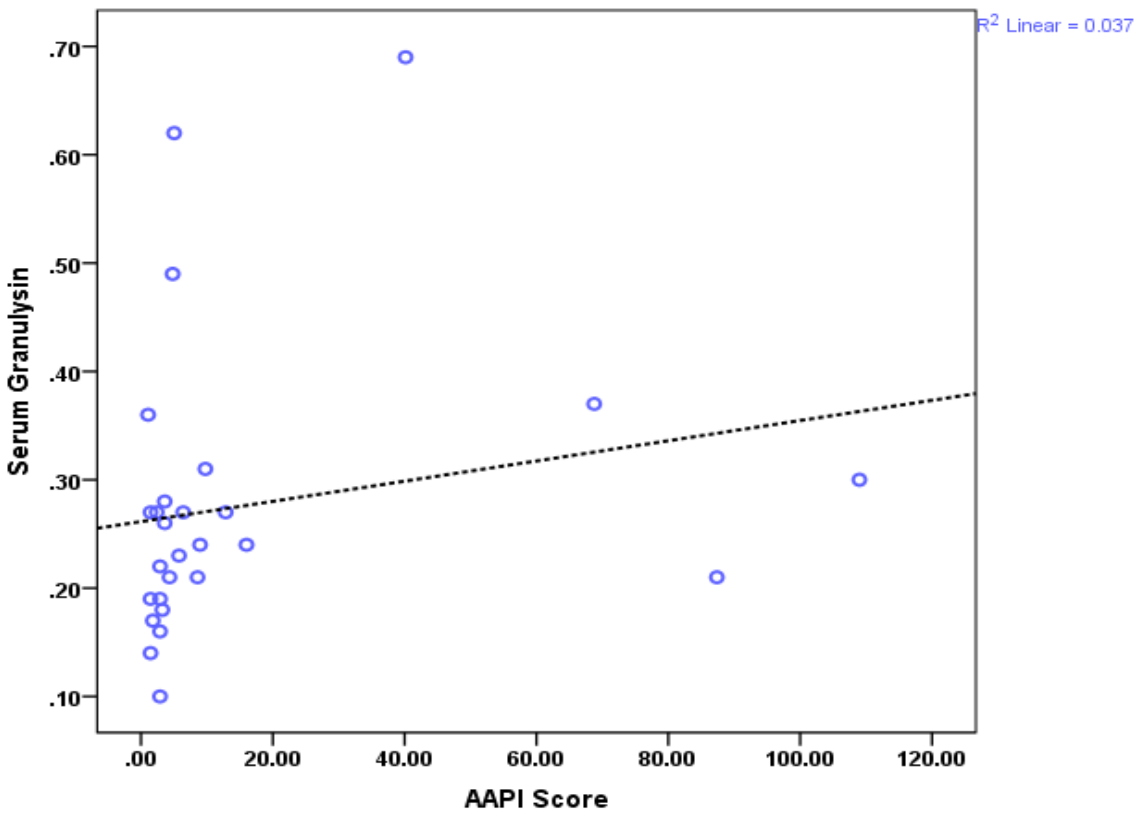

Figure 17- Serum granulysin levels and its correlation to the AAPI score in acute AA

Table 19-Serum granulysin vs AAPI score in acute AA patients

Variable	Serum Granulysin level (Acute AA)	p value
	Correlation Coefficient	
AAPI Score	0.425	0.027

10.9 Correlation of serum granulysin levels and AAPI score (chronic AA cases) :

The serum granulysin levels vs AAPI score in the chronic AA group is shown in table 20.

Table 20-Serum granulysin vs AAPI score in chronic AA patients

Variable	Serum granulysin levels (Chronic AA)	p value
	Correlation Coefficient	
AAPI Score	-0.429	0.076

There was no correlation seen between serum granulysin levels and AAPI score among the patients with chronic AA.

10.10 Serum granulysin vs number of alopecic patches :

Serum granulysin levels and its correlation with the number of patches is depicted in the following table 21.

Table 21 - Comparison of mean serum granulysin levels with the number of alopecic patches

Variables	Alopecic Patches		p value
	Single	Multiple	
	Mean ± SD	Mean ± SD	
Serum granulysin level in ng/ml	0.23 ± 0.10	0.26 ± 0.12	0.459

The mean serum granulysin levels was not significantly different when compared in patients with single and multiple patches.

CLINICAL PHOTOGRAPHS

Figure A -Alopecia areata with single patch

Figure B- Alopecia areata with multiple patches

Figure C,D - Alopecia areata - Ophiasis type

Figure E- Alopecia universalis

Figure F -Alopecia areata (patchy type) involving beard area

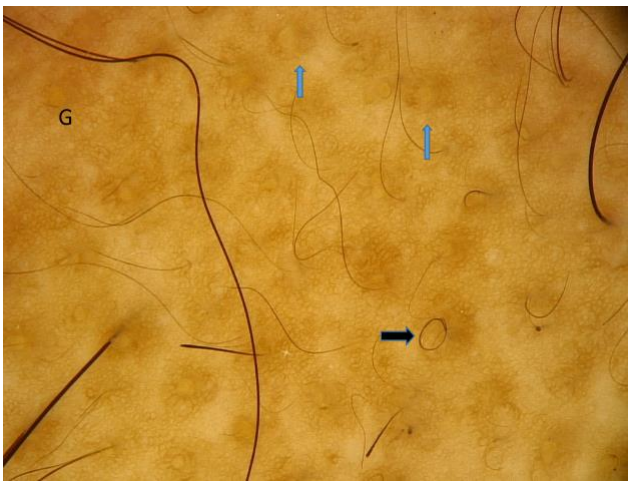

Figure G - Trichoscopy showing yellow dots (blue arrows) circle or pig tail hairs (black arrow [20x, non polarised, Fotofinder, GMBH]

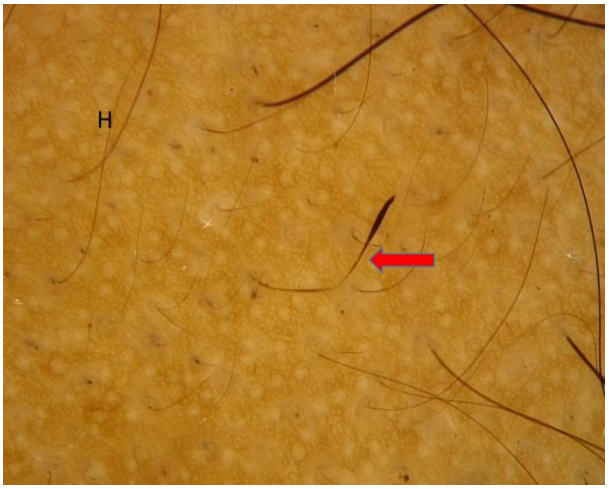

Figure H- Trichoscopy showing exclamation mark hair (red arrow) [40x, non polarised, Fotofinder,GMBH]

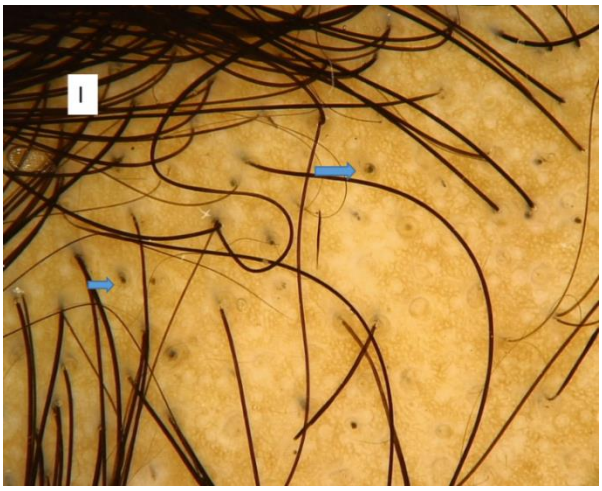

Figure I- Trichoscopy showing black dots (blue arrows) [20x, non polarised, Fotofinder, GMBH]

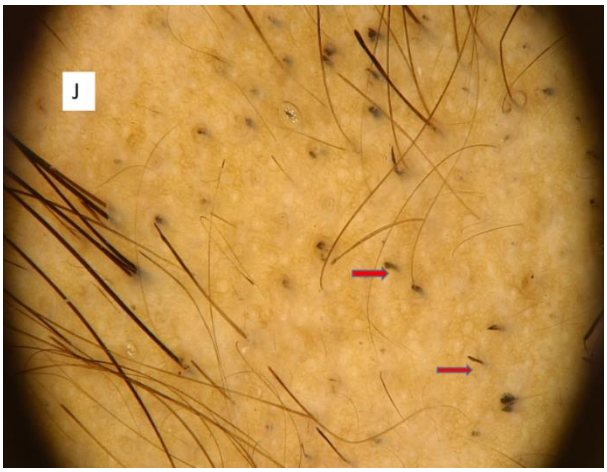

Figure J- Trichoscopy showing broken hairs (red arrows) [20 x ,non polarised, Fotofinder, GMBH]

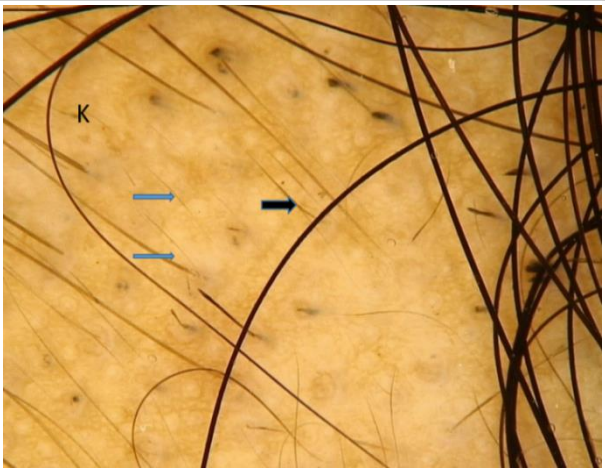

Figure K -Trichoscopy showing non pigmented vellus hairs (blue arrow) and upright regrowing hair (black arrow) [20x, non polarised, Fotofinder, GMBH]

Figure L and M- Human Granulysin ELISA kit

DISCUSSION

Serum granulysin is a cytolytic protein that is known for its inflammatory and immunological functions. (3) Its role in infections and in transplantation is also well known. (148) Granulysin has also been studied for its role as a marker in severe adverse cutaneous drug reactions. (56) However the levels of granulysin in AA and its role as a marker for disease activity has not been studied in the Indian population. Our aim in this study was to look at the serum concentration of granulysin in AA in the Indian population and to see if the levels of granulysin correlate with the disease duration and severity.

1. DEMOGRAPHIC PROFILE

1.1 Age :

The mean age at presentation was 32.24 ± 9.77 years in the overall study subjects and 42 ± 14.65 years in controls. In the acute AA group, it was 33.26 ± 8.64 years and in the chronic group, 30.79 ± 11.26 years. The mean age at presentation in the study done by Ono et al (6) was similar to that observed in our study. We observed that 78.26% of patients belonged to the 18-40 year age group and this was similar to other studies where AA was commonly seen presenting in the 20-40 year age group. (18, 19, 21) The comparison of the demographic profile to other studies is shown in table 22**.**

Table 22- Demographic profile of patients with AA- comparison of our study with other Indian studies

	Siddappa et al(18)	Sharma et al *(8)	Mane et al (19)	Thomas et al (20)	Peter et al (21)	Present study
Study duration	1 year	9 years	-	1 year	1 year	8 months
Study design	Retrospective case control	Prospective case control	Prospective study	Prospective case control	Prospective study	Prospective case control
Age(years) (mean ±SD	24.52 ±10.06	-	26.85± 9.3	-	29.12±12.07	32.24 ± 9.77
M: F ratio	2.57: 1	2:1	2.3:1	2.5 : 1	-	1: 1

*children included with adults

1.2 GENDER :

There were an equal number of males and females with male : female ratio of 1: 1. In most of the other studies, AA was found in an increased frequency in males compared to females. A male preponderance was observed in the studies by Sharma et al, Kavak et al and Yang et al. However, a greater female preponderance was observed in the study by Ono et al, male: female ratio was 0.47: 1. (4) In the studies done by Guzman et al (1) and Shellow et al (144) also, females were found to be more commonly affected.

2. FAMILY HISTORY:

A positive family history was seen in 9 out of the 46 patients (19.56%) in the present study. We also observed that 8 of the 9 patients with a family history had a chronic disease. A higher frequency of family history was observed in the present study when compared to other studies where the family history ranged between 8.7-14%. (11,12,18) A few studies showed a higher frequency of family history when compared to ours and it ranged between 24.1-42%. (18,154)

3. MEDICAL COMORBIDITIES:

The comorbid conditions that we observed with AA were hypothyroidism in 8.7% , anemia in 8.7% and vitiligo in 4.34%. Hypertension was observed in only one patient in our study. We did not observe other thyroid disorders like hyperthyroidism or autoimmune thyroiditis among the study patients. Diabetes mellitus, coeliac disease or other autoimmune disorders were also not seen in our patients. The frequency of thyroid disorders in alopecia areata in other studies ranged between 2.3-14.6% as reported in a systematic review by Fricke and Miteva. (2) Muller et al (18) reported an overall thyroid association in 8% and Chu et al (147) in 7.2% which is similar to what was observed in our study. The frequency of vitiligo has ranged from 1.8 -7 % (11,12,15,) in other studies while a lower frequency compared to our study was reported by Chu et al (147) Mishra et al (9) and Sharma et al. (8) A similar frequency was reported by Muller et al. (14) Anemia was observed in 8.7% patients in the present study while a much larger percentage of patients with anemia was reported by White et al. (78) A higher frequency of hypertension of 21.9% was noted in the study by Huang et al (65) while in our study only one patient had hypertension. The other comorbid conditions

mentioned in other studies were inflammatory bowel disease, rheumatoid arthritis, psoriasis, diabetes mellitus, psychiatric disorders, vitamin D deficiency and lupus erythematosus. (68,85,89,158,159) The comorbid conditions found associated with AA is shown in table 23.

Table 23- A comparison between the studies from India and abroad showing the comorbidities associated with AA

	Chu et al (147)	Sharma et al * (8)	Safavi et al (10)	Tan et al (12)	Presnt study
Country	Taiwan	India	Minnesota	Singapore	India
Atopy	AD 4.6% Asthma 5.1 % Rhinitis 13.6%	8.4%	Asthma 9.2% Hay fever 14% AD -8.9%	27.8% # 32.9% **	10.87%
Thyroid dysfunction	3%	1%	Hashimoto"s disease (2.7%) Grave's disease(1%)	2.3%	8.7%
Diabetes mellitus	4.7%	0.4%	1%	3.2%	-
Vitiligo	0.3%	1.8%	2.1%	4.1%	4.34%
Inflammatory bowel disease	-	-	0.7%	-	-
Rheumatoid arthritis	0.8%		1%	0.9%	-
Psoriasis	1.7%	0.6%	-	-	-
Collagen vascular disease	0.5% (LE)***	0.6%	0.7% (LE) ***		
Hypertension	-	1%	-	-	2.17%
Down 's syndrome	-	-	0.7%	1.4%	-
Anemia	-	-	Pernicious anemia 0.3%	-	8.7%

** severe alopecia, # limited alopecia, *(children and adults included) ,*** Lupus erythematosus

4 .1. ASSOCIATION WITH ATOPY :

Association with atopy was seen in 10.87 % of patients which was similar to the observation by Ikeda et al (83) and Mishra et al. (12) Among the atopic conditions, bronchial asthma was most commonly associated with AA in our study. Allergic rhinitis and atopic eczema association were also observed in a few of our patients. The overall atopy associated with AA in other studies ranged from 8.4-38.2%. (11,14,61) Compared to our study, a higher proportion of atopy was noted in the studies by Kaur et al (67) and Huang et al (65) and a lower frequency was observed by Sharma et al. (18) Kaur et al (67) also showed that association with atopy was not associated with increased severity of AA although previous studies by Ikeda (83) and Muller et al (14) considered atopy to be a bad prognostic factor. Kaur et al (67) had also observed allergic rhinitis in 42% of patients followed by bronchial asthma in 6% of patients and atopic eczema in 2% of the patients.

5. HISTORY OF PRIOR EPISODE OF HAIR FALL :

Among our study population, 41.30 % had history of prior episodes of AA while 58.7% experienced their first episode of hair fall. A study done by Lyakhovitsky et al (149) from Israel showed a similar frequency of first and prior episodes of hair fall in the study population.

6. TREATMENT HISTORY :

The patients had a history of different types of treatment before recruitment into study. The various treatment modalities included topical steroids, intralesional triamcinolone injection and systemic treatment. The most common treatment received by our patients

was intralesional injection in 58.06% patients. This could be explained by the number of patients with single patch AA who received intralesional injection as treatment. A study by Lyakhovitsky et al (149) reported that 55 out of the 73 (75.3%) patients received treatment with intralesional steroids while 40 (54.8%) patients received treatment with topical steroids and 11 (15.07%) received systemic treatment while in our study 18 out of 31 (58.06%) received treatment with intralesional , 54.83% of the patients received treatment with topical steroids followed by 22.58% of the patients who received systemic treatment. Of the 31 patients who had received treatment, 15 had received combination treatment with topicals, intralesional injection and systemic treatment.

6.1 Response to treatment :

Majority of patients in our study in whom the response was assessed reported a worsening or progression of the disease while on treatment. This could be explained by more patients with poor prognostic factors like those with a family history (9 patients), association with autoimmune disorder (3 patients), those with alopecia universalis (9 patients) and ophiasis pattern (3 patients) seeking medical care in this tertiary care centre.

7. CLINICAL PROFILE :

7.1 Site of involvement :

In addition to scalp,we looked at the other sites of involvement in our study and found that beard (28 %) was the second most common site of involvement followed by body hair (19%) and eyebrows (17%). Beard was the common site of involvement after scalp

according to the studies by Mishra et al, (9) Sharma et al (8) and Manzoor et al. (150) Eyebrows was the third common site of involvement as observed by Sharma et al and body hair as the third common site was observed by Manzoor et al. (150)

7.2 Number of alopecic patches :

Single patch AA usually undergoes spontaneous resolution and sometimes resolve without the patient's knowledge. In the study done by Ono et al (6) on serum granulysin levels, single patch AA was excluded. We observed single patch alopecia in 28.26% of the patients and Jain et al (151) observed a higher frequency of single patches in (35.34%) of the patients. Multiple patches were noted in 71.74% of the patients similar to 70.6% of the patients presenting with multiple patches in the study by Ono et al. (6)

7.3 Extent of AA :

We observed patchy type of AA as the most common presentation seen. When compared to our study Mishra et al (9) observed a higher frequency of patchy AA in 88% and Yang et al (17) in 96.8% of patients while a lower frequency was observed by Miteva et al (3) and Sharma et al (8) in 58% of patients. Our study did not have any patients with AT. When compared to our data on AU patients, a lower frequency of AU was reported in some studies and it ranged between 7-7.3% (8,12) while a higher frequency was reported by Ono et al in 20.6% (4) and Sharma et al (11) in 27.2 % of the patients. We also observed that patchy AA was more commonly associated with acute disease and AU with chronic disease. (p- value = 0.022)

7.4 Pattern of AA:

The classical pattern of AA was observed in 93.5% of our patients while ophiasis pattern was seen in 6.5% of the patients. In most studies the classical pattern was the most common presentation. Reticular and sisaipho pattern were not observed in our study. The ophiasis pattern was observed in 2.7% of the patients in the study by Tan et al (12) and in 13.6 % of patients in the study by Kavak et al. (16) Patients with ophiasis pattern carry a bad prognosis. Sisaipho has a more benign course compared to the ophiasis pattern.

7.5 Nail changes:

Nail changes were seen in 30.43% (14/46) of the study patients. They were noted in 10.5- 38% of the patients as reported in a systematic review by Fricke and Miteva. (2) Studies done by Gandhi et al, (102) Sharma et al, (8) Tosti et al (104) and Chelidze et al (100) showed nail pits to be the most common nail change followed by trachyonychia. Longitudinal ridges and nail pits were the only nail changes that we observed of which longitudinal ridges were more common. It was also noted that longitudinal ridges were more common than nail pits in both the acute and chronic groups.

Compared to our study, nail pits were observed in a higher frequency by Tosti et al (104) and Sharma et al (8) and in a similar frequency by Chelidze et al. (100) They were associated with AU. Longitudinal ridges were seen associated with the patchy type of AA and were observed in a lesser frequency in the studies by Sharma et al (8) and Gandhi et al. (102) Distal notching, punctate leukonychia, ragged cuticles, Beau's lines and onychomadesis were the nail changes observed in other studies.

A comparison of the frequency of nail changes in various studies are depicted in Table 24**.**

Table 24 – Comparison of studies on nail changes in AA with present study

	Gandhi et al (102)	Sharma et al (8)	Chelidze et al (100)	Tosti et al (104)	Present study
Overall nail changes	44%	29%	NA*	46%	30.43%
Pits	28%	14%	11.4%	34%	10.8%
Longitudinal ridges	10%	8%	0	0	19.6%
Lamellar splits	18%	0	0	0	0
Trachyonchia	9%	8%	14%	11%	0
Distal notching	2%	0	0	0	0
Ragged cuticles	2%	0	0	0	0
Beau ‘s lines	2%	0	0	0	0
Mottled lunulae	0	0	0	2%	0
Punctate leukonychia	0	3%	0	1%	0
Onychomadesis	0	0	0	1%	0

*Not available

7.6 Trichoscopic findings:

Yellow dots were seen in 47.8% of our patients and they are usually found in lesser frequency in Asians due to their skin type. (117) Yellow dots are considered as markers in trichoscopy that indicate disease severity. (110) A higher frequency of yellow dots was observed by Mane et al in 81.8% (19) and by Dias et al (153) in 86.6%. Yellow dots in a lesser frequency were observed by Mahmoudi et al (108) in 10.4%. The variation in the proportion of yellow dots could be explained by different shampooing habits, the type of trichoscope used and the skin type. (112) Black dots were observed in 0-84% of patients with AA as reported in an update by Waskiel et al. (109) A higher

frequency of black dots compared to our study were observed by Peter et al (21) and a lesser frequency by Mahmoudi et al. (108) Mane et al have reported similar frequency of black dots as in our study. Broken hairs are usually reported at a frequency of 0-71%. (116) Peter et al (21) observed a higher frequency of broken hairs compared to ours, while Mahmoudi et al (108) observed a decreased proportion of broken hairs. Black dots, broken hairs and exclamation mark hairs are considered as dermoscopic markers of activity in alopecia areata (110) while yellow dots and black dots were observed to have correlation with severity. (110) Short vellus hairs were reported in a greater frequency by Inui et al (110) (72.7%) and lesser proportion by Mahmoudi et al. (108) Short vellus hairs indicate hair regrowth and carry good prognosis as observed by Laccaruba et al. (154) In all the studies shown in table 25, most common trichoscopic findings were yellow dots, black dots, broken hairs and exclamation mark hairs.

Table 25 –Comparison of studies on trichoscopy with the present study

	Mahmoudi et al (108)	Waskiel et al (109)	Inui et al (110)	Mane et al (19)	Peter et al (21)	Present study
Country	Iran	Poland	Japan	India	India	India
Yellow dots	10.4%	62%	63.7%	81.8%	42%	47.8%
Black dots	14.8%	53%	44.3%	66.6%	75%	65.2%
Broken hairs	33.3%	49%	45.7%	55.4%	67%	52.17%
Exclamation mark hairs	28.2%	39%	31.7%	12.1%	33%	17.3%
Circle or pig tail hairs		21%				19.6%
Short vellus hairs	16.5%	61%	72.7%	40.9%	56%	37%
Upright regrowing hairs	-	23%	-	-	-	19.6%
Coudability hairs	-	51%	-	-	-	-
Pohl pinkus constriction	-	4%	-	-	-	-
Flame hairs	-		-	-	-	4. 34%

Circle hairs, upright regrowing hairs, coudability hairs and Pohl Pinkus constrictions were not observed in the other studies except in the study by Waskiel et al.(109) Flame hairs were observed in our study but was not reported in most other studies while Miteva and Tosti (138) observed flame hairs in 21% patients with AA.

7.7 SALT score :

The severity of alopecia tool scoring was done for our patients. The mean SALT score in patients who presented with an acute disease was 7.14 ± 10.84 while in those who presented with a chronic disease it was 31.11 ± 33.48. A higher SALT score and therefore more severe disease was observed in the chronic group and this could be due to the increased frequency of single patch patients in the acute AA group.

7.8 AAPI score :

The AAPI score was 15.53 ± 27.9 in the acute group and 29.31 ± 37.89 in the chronic group. AAPI score may be useful as a prognostic tool by assessing the disease progression in AA. It can be useful to assess response to newer treatment modalities in AA. However, we found that the AAPI score was not applicable to patients with AT or AU as hair pull test was part of SL in AAPI.**.** Instead of the magnifying findings with a 3x magnifier, trichoscopy findings with 20x magnification was used in our study. We observed that AAPI scoring system required modification to make it applicable to patients with a severe disease like AT or AU in whom the hair pull test is not possible. At present, the AAPI score was applicable only to patients with a patchy type of AA. (5)

Correlation of SALT vs AAPI score: We compared the SALT and AAPI scores and found that there is a high positive correlation between the scores with ($r = 0.774$) and it was statistically significant with a p -value of < 0.001.

7.9 DLQI score :

The DLQI score was done for all patients and we observed that patients in the acute group with less than six months of disease had a better quality of life compared to the chronic group. A greater impact on the quality of life was noticed in our patients with chronic disease in contrast to the observation by Abedini et al (142) where patients who presented with less than six months of disease duration had a higher DLQI score. Al Mutairi and Eldin (155) observed that DLQI scores and duration of the disease did not show a positive correlation. An observation similar to our study with increased DLQI scores with greater disease duration was noted by Qi et al (156) from China.

8. Serum granulysin levels :

The serum granulysin levels were assessed in the acute and chronic disease groups. In the acute group the serum levels of granulysin were 0.28 ± 0.13 ng/ml (range 0.10-0.69), in chronic group it was 0.21 ± 0.08 ng/ml (range 0.10-0.40) and in controls it was 0.18 ± 0.06 ng/ml. The values observed in our study were lower than the serum granulysin concentration observed in the study by Ono et al. (6) The kits used in both the studies were different and therefore the serum granulysin levels could not be compared. The kit we used was a human granulysin kit, Biovendor from the Czech Republic while Biolegend from San Diego was used in the study by Ono et al. The sensitivity of detection for our kit was 0.03 ng/ml whereas the normal serum concentration of granulysin detected by Biolegend kit was on an average 5 ng/ml and maximum values were 80 ng/ml. It has been recommended that each laboratory should include its own normal and pathological reference ranges of granulysin levels.

The serum granulysin levels in acute AA were found to be elevated compared to the controls and it was statistically significant (p -value 0.005) while in chronic cases it did not show any statistically significant elevation compared to the controls (p -value 0.166). We observed that serum granulysin levels in acute AA were higher compared to those who had chronic disease but it showed only a trend to significance with p -value of 0.08 when the acute and chronic groups were compared.

We had aimed to see if granulysin levels were correlating with disease severity. We looked at the serum granulysin levels and its relation to SALT score in patients with acute and chronic groups and found that in acute cases there was a moderate correlation of serum granulysin levels with SALT score (r =0.410) with a p -value of 0.034.

We also looked at the correlation of serum granulysin levels with AAPI score and found that there was a moderate correlation with AAPI score in the acute groups with r =0.425 and a p- value of 0.027.

The serum granulysin levels and its correlation with the number of alopecic patches were studied and we observed that granulysin levels were not significantly different when compared in patients with single and multiple patches.

In the study done by Ono et al, the serum concentration of granulysin was found to be increased in both acute and chronic AA with values of 26.2 ± 21.3 ng/ml in the acute group, 12.1 ± 5.8 ng/ml in the chronic group and 5.9 ± 4.4 ng/ml in the controls. The serum concentration of granulysin levels was observed to be higher in the acute group in the present study similar to the findings in the study by Ono et al and increased levels were associated with a bad prognosis and the values indicated increased disease severity in acute AA. However, in view of time constraints, patients in the acute group could

not be followed up to assess their prognosis. A comparison of the study characteristics of our study with the Ono et al study is shown in Table 26

Table 26- Comparison of characteristics of the present study with Ono et al

	Ono et al study	Present study
Country	Japan	India
Study period	-	January 2019 -August 2019
Duration	-	8 months
Study design	Single centre, prospective, case control	Single centre, prospective, case control
Number of cases (n)	n=34, acute =16, chronic =18	n=46, acute-22, chronic= 24
Controls	14 healthy controls	20 healthy controls
Single patch	Excluded from the study	Included in the study
Immunohistochemical analysis and histopathology	Done	Not done
Trichoscopy	Not done	Done
SALT score , AAPI score	Not used in study. Disease severity assessed by the number of bald areas	SALT & AAPI score done to assess disease severity
Quality of life assessed by DLQI score	No	Yes

Further studies with a large sample size are required to study the effect of serum granulysin levels and the use of a different granulysin kit may be needed in the future to further study the serum granulysin levels in AA in the Indian population.

CONCLUSION

In this hospital based prospective case control study done in India on serum granulysin levels in patients with AA for a period extending from January 2019 to August 2019 with healthy controls for comparison we observed the following findings:

- The serum granulysin levels in acute AA were found to be elevated compared to the controls and it was statistically significant (p-value =0.005).
- The serum concentration of granulysin in chronic cases did not show significant elevation compared to the controls (p-value =0.166).
- There was a moderate correlation of serum granulysin levels with SALT score (r=0.410) in the acute AA group with a p -value of 0.034.
- There was a moderate correlation of serum granulysin levels with the AAPI score (r=0.425) in the acute AA group (p-value of 0.027).
- The mean serum granulysin levels were not significantly different when compared in patients with single and multiple patches.

The other significant findings observed in our study are the following :

- The mean age at presentation was 32.24 ± 9.77 years in the overall cases.
- We found an equal gender distribution while most studies had shown a male preponderance.
- A higher frequency of family history of 19.6% was observed. Of the patients who had a positive family history, eight had a chronic disease as against only one patient with an acute disease.

- The association with comorbidities like hypothyroidism in 8.7%, anemia in 8.7%, vitiligo in 4.34% were observed while hypertension was seen in only one patient.
- Treatment with intralesional triamcinolone in 58.06% of patients was the most common treatment modality received by our patients followed by topical steroids in 54.83% of patients and then systemic treatment in 22.58% of patients.
- The most common site of involvement other than the scalp was beard area in 28% of patients, followed by the body in 19% of patients and eyebrows in 17% of patients.
- The most common extent of presentation was multiple patchy type AA seen in 80.4% of patients followed by AU in 19.6% of patients. There were no patients with AT.
- We observed a lesser frequency of nail changes of 30.43%. Nail pits and longitudinal ridges were the only nail findings in our study. We observed a higher frequency of longitudinal ridges, 9 out of 46 patients as compared to 5 out of 46 patients who showed pits.
- The most common trichoscopy findings were black dots in 65.2% of patients, broken hairs in 52.17%, yellow dots in 47.8% and short vellus hairs in 37% of patients. We observed flame hairs in 4.34% of patients which is only rarely reported in AA. In the acute AA group where higher serum granulysin levels were observed, we noted a higher frequency of black dots, broken hairs and yellow dots compared to the chronic AA group.

- There was a high positive correlation between SALT and AAPI scores (r=0.774) and it was statistically significant with a p value of < 0.001.

LIMITATIONS

- In view of time constraints, we had a small sample size. Larger sample size would have helped to further study the correlation of serum granulysin levels with disease severity.
- We could not follow up the patients in our study. A follow up would have helped to see the prognosis of patients with high serum granulysin levels.
- The level of detection of serum granulysin levels in our kit was low and hence serum granulysin levels were lower in our study subjects.
- The AAPI score was not practically applicable to patients who presented with total loss of hair from the scalp.

RECOMMENDATION

- Larger studies with follow up would help in studying whether serum granulysin is a prognostic marker for AA.
- To use a human granulysin kit that has a higher level of detection of granulysin levels in future studies.
- We recommend a modification of the AAPI score so that it is applicable in patients with AT and AU.

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ABSTRACT

Title of the abstract : Serum granulysin levels in patients with alopecia areata in a tertiary care centre in South India - a case control study

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Name of the candidate: Anju Liya Thomas

Degree and subject: MD, Dermatology, Venereology and Leprosy

Name of the guide: Dr. Susanne A. Pulimood

Background :

Alopecia areata (AA) is an autoimmune process that is directed against the hair follicles. At present, identifying some features at dermatoscopy, hair pull test and Severity of Alopecia Tool (SALT) score helps to assess disease severity. The role of granulysin as a biomarker for the activity of cytotoxic T cells and natural killer cells and therefore as a marker for disease severity and prognosis needs to be explored.

Objective :

Our primary objective was to study serum granulysin levels in adult patients with acute and chronic alopecia areata as compared to healthy controls and our secondary objective was to study the correlation of serum granulysin levels with disease severity.

Materials and methods :

The present study was a prospective, case control study conducted at the dermatology outpatient department of Christian Medical College, Vellore from January 2019 to

August 2019. After obtaining informed consent from patients aged above 18 years, we recruited 22 acute AA cases, 24 chronic AA cases and twenty healthy controls. The demographic details, history of the disease, associated comorbidities, treatment details with response to treatment, clinical features, vital signs, dermatoscopic examination findings, SALT score and AAPI score were assessed. The serum granulysin levels were estimated in the acute and chronic AA cases and compared to 20 healthy controls. The mean (SD) was calculated for all the continuous variables and for serum granulysin levels in acute, chronic AA cases and controls. The Spearman rank correlation was done to estimate the correlation between serum granulysin levels, SALT and AAPI scores.

Results :

The mean age at presentation in the study subjects was 32.24 ± 9.77 years and a 1:1 male to female ratio was noted. The associated comorbidities that were observed were hypothyroidism in 8.7%, anemia in 8.7%, vitiligo in 4.34% of patients and hypertension was seen in only one patient. The most common trichoscopy findings in were black dots, seen in 65.2%, broken hairs in 52.17%, yellow dots in 47.8% and short vellus hairs in 37% of patients. The most common site of involvement other than the scalp was beard area in 28%, followed by body in 19% and eye brows in 17%. The most common extent of presentation was multiple patchy type AA in 80.4% followed by alopecia universalis in 19.6% of patients. There were no patients with alopecia totalis. The mean serum granulysin levels was 0.28 ± 0.13 ng/ml in acute AA, 0.21 ± 0.08 ng /ml in chronic AA and 0.18 ± 0.06 ng/ml in controls. The serum granulysin levels in acute AA were elevated as compared to the controls and it was statistically

significant (p-value =0.005) while chronic AA cases did not show any significant elevation. There was a moderate correlation of serum granulysin in acute AA with SALT score in acute AA with r = 0.410 and a p -value of 0.034 and with AAPI score, r = 0.425 and a p - value of 0.027.

Conclusion :

Serum granulysin may be a biomarker in patients presenting with acute disease and it may also be useful as a prognostic marker.

Key words – Alopecia areata, granulysin.

ANNEXURE 1 – PATIENT INFORMATION SHEET

Study title (for lay public): Study of blood levels of granulysinin alopecia areataand association of granulysin levels with the severity of disease.

We are inviting you to take part in a research study. Please read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you want more information.

Purpose of research:

We would like to see if granulysin levels are increased in the blood of patients with alopecia areata.We would be doing the study to see if increased levels are associated with increased severity of the disease

Expected duration of the Subject's participation: You will be examined by the doctor only once in the study period.

Description of the procedures

Patients with alopeciaareata will be participating in the study.All the patient demographic details will be taken and clinical examination of the pattern and extent of hair losswill be done which will be recorded in a special form .Two ml of blood will be collected and clinical photograph will be taken and hair examination under magnification will be done using an instrument called video dermoscope.

Risks or discomforts to the Subject: Nil

Benefits of the study : Increased levels of granulysin will help to predict the increased severity of the disease.Till date there are only few tests to predict disease severity in alopecia areata.

Confidentiality: Your name and address will not be revealed at any point of time. All information that is collected about you during the course of the research will be kept strictly confidential unless we are required by law to share any information.

Participation:It is up to you to decide whether to take part or not in thestudy. You are still free to withdraw from the study at any time, without giving any reason. A decision not to take part, will not affect the standard of care you receive.

अध्ययन शीर्षक (जनता के लिए):

अल्पाशिया अरेटा के रोगियों में गेनुल।ईसिन के रक्त स्तर का अध्ययन और बीमारी की गंभीरता के साथ गेनुल।ईसिन स्तर के संगति।

हम आपको एक शोध अध्ययन में भाग लेने के लिए आमंत्रित कर रहे हैं। कृपया निम्नलिखित पढ़ें जानकारी सावधानी से और यदि आप चाहें तो दूसरों के साथ चर्चा करें। हमसे पूछें कि क्या है
कुछ भी जो स्पष्ट नहीं है या यदि आप अधिक जानकारी चाहते हैं।

शोध का उद्देश्य:

हम देखना चाहते हैं कि मरीजों के खून में गेनुल।ईसिन के स्तर में वृद्धि हुई है या नहीं अल्पाशिया अरेटा के साथ। हम यह देखने के लिए अध्ययन करेंगे कि बढ़े स्तर हैं या नहीं बीमारी की गंभीर गंभीरता से जुड़ा हुआ है विषय की भागीदारी की अपेक्षित अवधि: आप की जांच की जाएगी
अध्ययन अवधि में केवल एक बार डॉक्टर।

प्रक्रियाओं का विवरण

अल्पाशिया अरेटा के साथ मरीज़ अध्ययन में भाग लेंगे। सभी रोगी जनसांख्यिकीय विवरण लिया जाएगा और पैटर्न की नैदानिक परीक्षा और बालों के झड़ने की सीमा पूरी की जाएगी जो एक विशेष रूप में दर्ज की जाएगी। दो रक्त का एमएल एकत्र किया जाएगा और नैदानिक तस्वीर ली जाएगी और बाल आवर्धन के तहत परीक्षा एक उपकरण का उपयोग करके किया जाएगा डरमोसकोप।

विषय के लिए जोखिम या असुविधा: शून्य

अध्ययन के लाभ: गेनुल।ईसिन के बढ़े स्तर की भविष्यवाणी करने में मदद मिलेगी बीमारी की गंभीरता में वृद्धि हुई और प्रारंभ में बीमारी के संभावित परिणाम प्रस्तुति। आज तक अलगाव में बीमारी की गंभीरता की भविष्यवाणी करने के लिए कोई परीक्षण नहीं है।

गोपनीयता:

आपका नाम और पता किसी भी बिंदु पर प्रकट नहीं किया जाएगा पहर। पाठ्यक्रम के दौरान आपके बारे में एकत्र की गई सभी जानकारी जब तक हमें कानून द्वारा आवश्यकता न हो, तब तक अनुसंधान को सख्ती से गोपनीय रखा जाएगा किसी भी जानकारी साझा करें।

भागीदारी: यह तय करने के लिए आप पर निर्भर है कि अध्ययन में भाग लेना है या नहीं।
आप किसी भी समय बिना किसी दिए बिना अध्ययन से वापस लेने के लिए स्वतंत्र हैं कारण। भाग लेने का कोई निर्णय नहीं, आपको देखभाल के मानक को प्रभावित नहीं करेगा प्राप्त करना।

நோயாளியின் தகவல் தாள்

ஆய்வின் தலைப்பு (பொதுமக்களுக்கு): அலோபிசியா எரேட்டா நோயினால் இரத்தத்தில் உள்ள Granulysin என்னும் நச்சுத்தன்மையின் அளவு மற்றும் இந்த நோயின் தீவிரத்தன்மையுடன் Granulysin என்னும் நச்சுத்தன்மையினான தொடர்பு பற்றிய ஆய்வு.

இந்த ஆராய்ச்சியில் தங்களை பங்குகொள்ளுமாறு கேட்டுக்கொள்கின்றேன். ஆகவே கிழே கொடுக்கப்பட்ட தகவல்களை நன்கு படித்து புறிந்துகொண்டு, தாங்கள் விரும்பினால் மற்ற நபருடன் கலந்தாலோசித்து பிறகு ஆய்வில் பங்குகொள்ளலாம். ஆய்வு பற்றிய மேலும் சந்தேகங்களுக்கும் மற்றும் விவரங்களுக்கும் ஆய்வாளரை அணுகவும்.

ஆய்வின் நோக்கம் :

இந்த ஆய்வில் அலோபிசியா எரேட்டா நோயாளிகலின் இரத்தத்தில் Granulysin என்னும் நச்சுத்தன்மையின் அளவு அதிகரிக்கின்றனவா என்பதனையும், மேலும் இந்த நோயின் தீவிரத்தன்மைக்கு Granulysin என்னும் நச்சுத்தன்மையின் அளவு அதிகரிப்பு தொடர்பு கொண்டுள்ளனவா என்பதையும் அறிய விரும்புகின்றோம்.

நோயாளி பங்குகொள்ள எதிர்பாற்கும் கால அளவு :

இந்த ஆய்வு காலத்தில் மருத்துவர் பங்கேற்பாளரை ஒரு முறை மட்டுமே பறிசோதனையை செய்வார்.

செயல்முறை விளக்கம்:

இந்த ஆய்வில் அலோபிசியா எரேட்டா நோயாளிகளை பங்குகொள்ள கேட்டுக்கொள்கின்றேன். மருத்துவர் பங்கேற்பாளரின் பொதுதகவல் மற்றும் மருத்துவ பரிசோதனை செய்து அதன் மூலமாக முடி உதிர்வின் அளவு மற்றும் அத்தகவலை பாதுகாப்பான தகவல் படிவத்தில் பதிவுசெய்துகொள்வார். பங்கேற்பாளரின் 2ml இரத்தம் ஆய்விர்க்காக எடுக்கப்படும் மற்றும் மருத்துவ புகைப்படம் எடுக்கப்படும் மற்றும் வீடியோ டெர்மொஸ்கோப் என்னும் கருவியினைக்கொண்டு முடியின் உருப்பெருக்கத்தினை மருத்துவ பரிசோதனை செய்யப்படும்.

ஆய்வில் கலந்து கொண்டால் நீங்கள் எதிர்கொள்ளும் ஆபத்து மற்றும் உபாதைகள்:

எதுவும் இல்லை

ஆய்வின் பயன்கள்:

நோயின் தீவித்தன்மையை கணிக்க Granulysin என்னும் நச்சுத்தன்மையின் அளவு அதிகரிப்பு உதவிகரமாக இருக்கும். அலோபிசியா எரேட்டா நோயின் தீவிரத்தன்மையை கணிக்க இன்றுவரை சில பரிசோதனைகள் மட்டுமே உள்ளது.

ANNEXURE 2 – INFORMED CONSENT FORM

Informed consent form to participate in a clinical trial

Study Title: Study of granulysin levels in alopecia areata

Subject's Initials: _____ Subject's Name: _____

Date of Birth / Age: _____

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s) [ ]

(v)I agree for a clinical photography of scalp and or other affected areas

(vi) I agree to take part in the above study. [ ]

(Vii)I understand that a part of blood sample taken from me will be processed for the present study and remaining part would be stored for further research study in future

Signature (or Thumb impression of the Subject/Legally Acceptable Representative):

Signatory's Name: _____ Date: ____/____/_____

Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name: _____

Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

नैदानिक परीक्षण में भाग लेने के लिए सूचित सहमति फॉर्म

<u>अध्ययन शीर्षक:</u> अल्पाशिया अरेटा में गेनुल।ईसिन के स्तर का अध्ययन

<u>विषय के प्रारंभिक:</u>

<u>विषय का नाम:</u>

<u>उम्र:</u>

(i) मैं पुष्टि करता हूं कि मैंने उपर्युक्त के लिए _____ की सूचना पत्र पढ़ और समझ ली है

अध्ययन और प्रश्न पूछने का अवसर मिला है।

ii) मैं समझता हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और मैं किसी भी को वापस लेने के लिए स्वतंत्र हूं

समय, बिना किसी कारण के, मेरी चिकित्सा देखभाल या कानूनी अधिकारों को प्रभावित किए बिना।

(iii) मैं समझता हूं कि नैतिकता समिति और नियामक प्राधिकरणों को मेरी आवश्यकता नहीं होगी

वर्तमान अध्ययन और किसी भी आगे के संबंध में मेरे स्वास्थ्य रिकॉर्ड देखने की अनुमति

शोध जो इसके संबंध में आयोजित किया जा सकता है, भले ही मैं परीक्षण से वापस ले जाऊं। मैं इससे सहमत हूं पहुंच। हालांकि, मैं समझता हूं कि मेरी

पहचान को जारी की गई किसी भी जानकारी में प्रकट नहीं किया जाएगा तीसरे पक्ष या प्रकाशित।

(iv) मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणामों के उपयोग को प्रतिबंधित नहीं करने के लिए सहमत हूं

केवल वैज्ञानिक उद्देश्य के लिए है

(v) मैं खोपड़ी और अन्य प्रभावित क्षेत्रों की नैदानिक फोटोग्राफी के लिए सहमत हूं

(vi) मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूं।

(Vii) मैं समझता हूं कि मेरे द्वारा लिया गया रक्त नमूना का एक हिस्सा वर्तमान अध्ययन के लिए संसाधित किया जाएगा

और शेष भाग भविष्य में आगे के शोध अध्ययन के लिए संग्रहीत किया जाएगा

हस्ताक्षर (या विषय / कानूनी स्वीकार्य प्रतिनिधि के अंगूठे की छाप):

हस्ताक्षरकर्ता का नाम:

दिनांक:

जांचकर्ता का हस्ताक्षर:

अध्ययन जांचकर्ता का नाम:

दिनांक:

ஆராய்ச்சியில் பங்கேற்பதற்கான ஒப்புதல் படிவம்

ஆய்வின் தலைப்பு : அலோபிசியா எரேட்டா நோயில் Granulysin என்னும் நச்சுத்தன்மையின் அளவு பற்றிய ஆய்வு.

பங்கேற்பாளரின் எண்: _____

பங்கேற்பாளரின் பெயர் :_____

பிறந்த தேதி / வயது :_____/_____

1. இந்த ஆராய்ச்சியில் நான் _____ தேதியில் மேற்கண்ட தகவல்படிவத்திலுள்ள அனைத்துதகவல்களையும் நன்கு படித்து புறிந்துகொண்டேன், மேலும் கேள்விகல் கேட்க வாய்பளிக்கப்பட்டது.

2. இந்த ஆராய்ச்சியில் பங்குகொள்வது என் விருப்பம்சார்ந்தது என்பதனையும், இவ்வாராய்ச்சியில் இருந்து எப்போது வேண்டுமானாலும் எக்காரணமும் இன்றி விலகிக்கொள்ளலாம் என்பதையும் புறிந்துகொண்டேன். என்னுடைய விலகல் என் மருத்துவ சிகிச்சைக்கான எந்த ஒரு உரிமையையும் பாதிக்காது என்பதையும் புறிந்துகொண்டேன்.

3. இந்த ஆராய்ச்சி சம்பந்தமான பொறுப்பில் உள்ளவர்கள் சட்டபூர்வமான குழுவைச்சார்ந்தவர்கள் மற்றும் ஒழுங்குமுறை குழுவைசார்ந்தவர்கள் என்பதையும் எனுடைய மருத்துவ பதிவேடுகளை என் அனுமதியின்றி பயன்படுத்தலாம் என்பதற்கு முழு சம்மதத்தைதெரிவித்துகொள்கிறேன். நான் இந்த ஆய்வில் இருந்து விலகினாலும் பதிவேடுகளை பயன்படுத்தலாம். மேலும் ஆய்வின் முடிவுகளை வெளியிடும் பொழுது என்னை குறித்த தகவல்கள் வெளியிடப்படாது என்பதையும் புறிந்துகொண்டேன்.

4. இந்த ஆராய்ச்சியில் பெறப்படும் தகவல்கள் மற்றும் முடிவுகள் அறிவியல் சம்மந்தமாக பயன்படுத்துவதில் எனக்கு எந்த மறுப்பும் இல்லை.

5. நான் என்னுடைய தலைப்பகுதி மற்றும் மற்ற இடங்களில் ஏற்பட்டுள்ள பாதிப்புக்களை மருத்துவ புகைப்படங்கள் எடுக்க சம்மதிக்கிறேன்.

6. நான் இந்த ஆராய்ச்சியில் பங்குகொள்ள முழு மனதுடன் சம்மதிக்கிறேன்.

7. இந்த ஆராய்ச்சிக்காக எடுக்கப்பட்ட இரத்தமாதிரியில் சிரிதளவு இரத்தத்தை எதிர்கால ஆய்வுக்காக பயன்படுத்த முழு மனதுடன் சம்மதிக்கிறேன்.

நோயாளியின் கையொப்பம்/ கைரேகை:.....

தேதி:...../...../.....

நோயாளியின் பெயர்: .....

சாட்சியாளரின் கையொப்பம்/ கைரேகை:..... தேதி:...../...../.....

சாட்சியாளரின் பெயர் மற்றும்

முகவரி:_____

_____

ஆய்வாளரின் கையொப்பம்/ கைரேகை:.....

தேதி:...../...../.....

ஆய்வாளரின் பெயர்: .....

ANNEXURE 3 – CLINICAL RESEARCH PROFORMA

CMCH No:

1.Serial No :

2. Name:

3. Date of visit :

4. Age : Date of Birth :

5. Sex: □ Male □Female

6. Address:

7. Contact No :

8. Duration of hair loss(months) :

9. Family history of alopecia areata □Yes □No

10. History of medical comorbidities

- Thyroid disease □Yes □No

If Yes

□ Hypothyroidism
□ Hyperthyroidism
□ Autoimmune thyroiditis

-Anemia □Yes □ No

- Diabetes □Yes □No

- Hypertension □Yes □No

- Celiac disease □Yes □No

- Rheumatoid arthritis □Yes □No

-Other autoimmune diseases □Yes □No

-Others □Yes □No

11.History of atopy □Yes □No

If Yes

□Bronchial asthma

□Allergic rhinitis

□Atopic eczema

12.History of previous episodes of hair loss □Yes □No

13.**Prior treatments for hair loss**.- □Yes □No

If Yes

i)Topical steroids : □Yes □No

If yes Treatment duration

ii)Intralesional steroids □Yes □No

If Yes ,treatment duration

iii)Systemic immunosuppressants

□ Yes
□ No

if Yes –a**)Steroids**

Yes Treatment duration

□ No

b)Methotrexate

□ Yes Treatment Duration :
□ No

c)Azathioprine

□ Yes Treatment duration
□ No

d)Others

14.Response to prior therapy □ Improved

□Status quo

□Worse

15.Current treatment for hair loss □Yes □No

If Yes

i)Topical steroids : □Yes □No

If yes Treatment duration

ii)Intralesional steroids □Yes □No

If yes Treatment duration

iii)Systemic immunosuppressants

□ Yes □ No

if Yes –a**)steroids**

□ Yes Treatment duration
□ No

b)Methotrexate

□ Yes Treatment Duration :
□ No

c)Azathioprine

□ Yes Treatment duration
□ No

d)Others

16.Response to current therapy Improved

Status quo

Worse

PHYSICAL EXAMINATION

VITAL SIGNS :

Blood pressure - /mm of Hg

Cutaneous examination

Alopecia areata

Site- Scalp

Eye brows

Beard

Body

1.No of patches

- Single
- Multiple
-

2.Extent

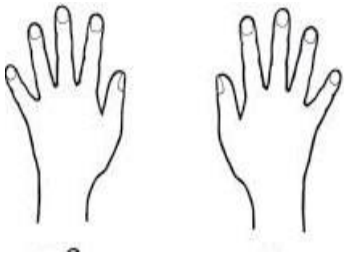
- Patchy
- Totalis
- Universalis
-

3.Pattern

- Reticular
- Sisaipho
- Ophiasis
- Others

4.Nail changes

- Yes
- No



If Yes

- Pits
- Trachyonycia
- Punctate leukonychia
- Beau's lines
- Others

Salt score =

AAPI score Trichoscopy findings

	Black dots	Exclamation mark hairs	Broken hairs	Dystrophic vellus hairs
Left side (L)				
Right side(R)				
Top(T)				
Back (B)				

AAPI score =

TRICHOSCOPY

Yellow dots	☐ Yes ☐ No
Black dots	☐ Yes ☐ No
Broken hairs	☐ Yes ☐ No
Exclamation mark hairs	☐ Yes ☐ No
Coudability hairs	☐ Yes ☐ No
Circle or pig tail hairs	☐ Yes ☐ No
Short vellus hairs	☐ Yes ☐ No
Upright regrowing hairs	☐ Yes ☐ No
Pseudomonilethrix	☐ Yes ☐ No
Pohl Pinkus constriction	☐ Yes ☐ No

Serum granulysin levels:

Annexure 4 - DLQI Questionnaire

Hospital No: ..... **Date:** .....

Name: ..... **Score:** .....

Address: ..... **Diagnosis:** .....

.....

The aim of this questionnaire is to measure how much your skin problem has affected your life OVER THE LAST WEEK. Please tick (✓) one box for each question.

1.	Over the last week, how **itchy**, **sore**, **painful** or **stinging** has your skin been?	Very much A lot A little Not at all	❒ ❒ ❒ ❒		
2.	Over the last week, how **embarrassed** or **self conscious** have you been because of your skin?	Very much A lot A little Not at all	❒ ❒ ❒ ❒		
3.	Over the last week, how much has your skin interfered with you going **shopping** or looking after your **home** or **garden**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒
4.	Over the last week, how much has your skin influenced the **clothes** you wear?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒
5.	Over the last week, how much has your skin affected any **social** or **leisure** activities?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒
6.	Over the last week, how much has your skin made it difficult for you to do any **sport**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒
7.	Over the last week, has your skin prevented you from **working** or **studying**?	Yes No	❒ ❒	Not relevant	❒
	If "No", over the last week how much has your skin been a problem at **work** or **studying**?	A lot A little Not at all	❒ ❒ ❒		
8.	Over the last week, how much has your skin created problems with your **partner** or any of your **close friends** or **relatives**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒
9.	Over the last week, how much has your skin caused any **sexual difficulties**?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒
10.	Over the last week, how much of a problem has the **treatment** for your skin been, for example by making your home messy, or by taking up time?	Very much A lot A little Not at all	❒ ❒ ❒ ❒	Not relevant	❒

Annexure 5 - Institutional Review Board Approval Letter

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

January 25, 2019

Dr. Anju Liya Thomas,
Department of dermatology - 2,
Christian Medical College,
Vellore – 632 002.

Sub: **Fluid Research Grant: New Proposal:**
A case control study on serum granulysin in patients with alopecia areata at a tertiary hospital in South India
Dr. Anju Liya Thomas, DVL II, Dr Susanne Pulimood, DVL II, Dr Dincy Peter DVL II, Dr. Leni George, DVL II, Dr.Geethanjali, Biochemistry, Dr. Gauri Mahabal, DVL II, Dr. Venkataraman, DVL II, Dr. Priya Kuryan DVL II, Mrs. Janai, Clinical Biochemistry, Dr. Jayaseelan, Biostatistics.

Ref: IRB Min. No. 11716 [OBSERVE] dated 03.12.2018

Dear Dr. Anju Liya Thomas,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr Susanne Pulimood, Dept. of dermatology - 2, CMC, Vellore

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

OFFICE OF RESEARCH
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Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

January 25, 2019

Dr. Anju Liya Thomas,
Department of dermatology - 2,
Christian Medical College,
Vellore – 632 002.

Sub: **Fluid Research Grant: New Proposal:**
A case control study on serum granulysin in patients with alopecia areata at a tertiary hospital in South India
Dr. Anju Liya Thomas, DVL II, Dr Susanne Pulimood, DVL II, Dr Dincy Peter DVL II, Dr. Leni George, DVL II, Dr.Geethanjali, Biochemistry, Dr. Gauri Mahabal, DVL II, Dr. Venkataraman, DVL II, Dr. Priya Kuryan DVL II, Mrs. Janai, Clinical Biochemistry, Dr. Jayaseelan, Biostatistics.

Ref: IRB Min. No. 11716 [OBSERVE] dated 03.12.2018

Dear Dr. Anju Liya Thomas,

The Institutional Review Board (**Blue**, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "A case control study on serum granulysin in patients with alopecia areata at a tertiary hospital in South India" on December 03rd 2018.

The Committee reviewed the following documents:

1. IRB application format
2. Patient Information Sheet and Consent Form (English, Tamil, Hindi, Malayalam)
3. Proforma
4. Cvs of Drs. Venkataraman Mani, Anju, Leni George, Dincy, Gouri, Susanne Pulimood, Priya Sara, Ms. Janani.
5. No. of documents 1- 4.

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on December 03rd 2018 in the New IRB Room, Bagayam, Christian Medical College, Vellore 632 004.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Anna Benjamin Pulimood, M.B.B.S., MD., Ph.D.,
Chairperson, Research Committee & Principal

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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Name	Qualification	Designation	Affiliation
Dr. Biju George	MBBS, MD, DM	Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC, Vellore	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Ratna Prabha	MBBS, MD (Pharma)	Associate Professor, Clinical Pharmacology, CMC, Vellore	Internal, Pharmacologist
Mr. Samuel Abraham	MA, PGDBA, PGDPM, M. Phil, BL.	Sr. Legal Officer, CMC, Vellore	Internal, Legal Expert
Dr. John Jude Prakash	MBBS, MD,	Professor, Clinical Virology, CMC, Vellore	Internal, Clinician
Dr. Rekha Pai	BSc, MSc, PhD	Associate Professor, Pathology, CMC, Vellore	Internal, Basic Medical Scientist
Mrs. Sophia V	M.Sc Nursing	Addl. Deputy Dean CMC, Vellore	Internal, Nurse
Dr. Ekta Rai	MBBS, MD MRCA	Professor, Department of Anaesthesia, CMC, Vellore	Internal, Clinician
Rev. Joseph Devaraj	BSc, BD	Chaplaincy Department, CMC, Vellore	Internal, Social Scientist
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC, Vellore	Internal, Clinician

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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Jayaprakash Muliyil	BSC, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist & Epidemiologist
Mrs. Nirmala Margaret	MSc Nursing	Addl. Deputy Nursing Superintendent, College of Nursing, CMC, Vellore	Internal, Nurse
Dr. Asha Solomon	MSc Nursing	Associate Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Santhanam Sridhar	MBBS, DCH, DNB	Professor, Neonatology, CMC, Vellore	Internal, Clinician
Dr. Ajith Sivadasan	MD, DM	Professor, Neurological Sciences, CMC, Vellore	Internal, Clinician
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Dr. Winsely Rose	Dr. Winsely Rose	Dr. Winsely Rose	Dr. Winsely Rose
MBBS, MD (Paed)	MBBS, MD (Paed)	MBBS, MD (Paed)	MBBS, MD (Paed)
Dr. Shyam Kumar NK	MBBS, DMRD, DNB, FRCR, FRANZCR	Professor, Radiology, CMC, Vellore	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) DM (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC, Vellore	Internal, Clinician
Ms. Grace Rebekah	M.Sc., (Biostatistics)	Lecturer, Biostatistics, CMC, Vellore	Internal, Statistician
Dr. Barney Isaac	MBBS, DNB (Respiratory Diseases)	Associate Professor, Pulmonary Medicine, CMC, Vellore	Internal, Clinician

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OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

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Chairperson, Research Committee & Principal

Dr. Biju George, M.B.B.S., MD., DM.,
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of Withdrawals for the study entitled: "A case control study on serum granulysin in patients with alopecia areata at a tertiary hospital in South India" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

Fluid Grant Allocation:

A sum of 50,000/- INR (Rupees fifty Thousand Only) will be granted for 12 Months.

Yours sincerely,

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

IRB Min. No. 11716 [OBSERVE] dated 03.12.2018

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ANNEXURE 6 – DATA SHEET

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE
1	slno	name	cmchno	visit	age	dob	sex	address	contactn	control	hairloss	alopecia	thyroid	yesthyro	anemia	diabetes	hyperter	celiac	arthritis	autoimm	others	othcomc	atopy	yesatopy	prehair	yeshair	treathair	topical	yestopic	intralesi	yesintra
2	1	Prathima	670282(	######	27	######	2	Andhra F	7E+09	2	24	2	2		2	2	2	2	2	2	2		2		1	2	1	2		1	12
3	2	Elizabeth	228193(	######	49	######	2	Chittoor	9E+09	2	24	2	2		2	2	2	2	2	2	2		2		2		1	1	4	1	4
4	3	Meena	412267F	######	24	######	2	Vellore	9E+09	2	12	1	2		2	2	2	2	2	2	2		2		2		1	1	6	2	
5	4	Nagma E	578533(	######	22	######	2	Orissa	9E+09	2	24	1	2		2	2	2	2	2	2	2		2		1	2	1	1	2	1	1
6	5	Chandan	685207(	######	33	######	1	Jharkhan	9E+09	2	2	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
7	6	Md Sadil	550584(	######	28	######	1	Banglad	8E+09	2	84	1	2		2	2	2	2	2	2	2		2		2		1	1	6	2	6
8	7	Jiban Sh	692364(	######	61	######	1	Assam	1E+10	2	12	2	2		2	2	2	2	2	2	2		2		1	10	1	2		1	5
9	8	Gourav S	335745(	######	24	######	1	Jharkhan	8E+09	2	12	2	2		2	2	2	2	2	2	2		2		1	3	1	2		2	
10	9	Elangov	525791(	######	30	######	1	Thiruvar	7E+09	2	6	2	2		2	2	2	2	2	2	2		2		2		1	1	1	2	
11	10	Poonam	678798(	######	20	######	2	Jharkhan	1E+10	2	24	2	2		2	2	2	2	2	2	2		2		2		1	2		1	3
12	11	Laxman	680564(	######	23	######	1	West Be	1E+10	2	6	1	2		2	2	2	2	2	2	2		2		2		1	2		2	
13	12	Jitendra	298929(	######	40	######	1	Chattisg	7E+09	2	3	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
14	13	Kurban S	523399(	######	44	######	1	West Be	8E+09	2	5	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
15	14	Mohd.Gi	601778(	######	29	######	1	Banglad	8E+09	2	3	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
16	15	Begam F	667123(	######	39	######	2	West Be	6E+09	2	1	2	2		1	2	2	2	2	2	2		2		1	1	1	2		2	
17	16	Nandita	506664(	######	46	######	2	Banglad	8E+09	2	1	2	2		2	2	2	2	2	2	2		2		2		1	2		2	
18	17	Tapasi H	116359(	######	22	######	2	West Be	1E+10	2	1	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
19	18	Khadeja	688918(	######	32	######	2	Banglad	8E+09	2	3	2	1	1	2	2	2	2	2	2	2		2		1	10	2	2		2	
20	19	Sandip (	526239(	######	28	######	1	West Be	1E+10	2	3	2	2		2	2	2	2	2	1	2		2		1	10	1	2		1	1
21	20	Rathna	559327(	######	32	######	2	Vellore	9E+09	2	1	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
22	21	Shilpi Sir	857626(	######	33	######	2	Jharkhn	8E+09	2	2	1	2		2	2	2	2	2	2	2		2		2		1	1	3	1	1
23	22	Ravi Sha	433416(	######	50	######	1	Vellore		2	1	2	2		2	2	2	2	2	2	2		1	1	2		2	2		2	
24	23	Carol	178861(	######	35	######	2	Vellore		2	1	2	2		2	2	2	2	2	2	2		1	3	2		2	2		2	
25	24	Shaikh M	603975(	######	18	######	2	Andhra F	1E+10	2	3	2	2		2	2	2	2	2	2	2		2		2		1	1	1	1	1
26	25	Sankar	652636(	######	23	######	1	Karur,Ta	1E+10	2	2	2	2		2	2	2	2	2	2	2		2		2		1	2		1	1
27	26	Ammu	744410(	######	46	######	2	Vellore		2	1	2	2		2	2	2	2	2	2	2		1	1	1	2	2	2		2	
28	27	Chesta	918006(	######	25	######	2	Vellore	9E+09	2	2	2	2		1	2	2	2	2	2	2		2		1	1	1	1	1	2	
29	28	Prakash	663605(	######	38	######	1	Chengal	1E+10	2	3	2	2		2	2	2	2	2	2	2		2		1	1	1	2		1	1
30	29	Rupam	673032(	######	23	######	1	West Be	7E+09	2	2	2	2		2	2	2	2	2	2	2		2		1	1	1	1	3	2	
31	30	Anusha	660325(	######	26	######	2	Gudiyatt	9E+09	2	3	2	2		1	2	2	2	2	2	2		2		2		1	1	1	2	
32	31	Jayakan	584625(	######	48	######	1	Vellore	1E+10	2	4	2	2		2	2	1	2	2	2	2		2		1	3	1	2		1	1
33	32	Soumen	599851(	######	33	######	1	Calcutta	8E+09	2	1	2	2		2	2	2	2	2	2	2		2		1	1	2	2		2	
34	33	Thabithu	595030(	######	49	######	2	Vellore,T	9E+09	2	24	2	1	1	2	2	1	2	2	2	2		2		1	1	1	1	2	1	2
35	34	Jannatu	639483(	######	18	######	2	Banglad	1E+10	2	60	2	2		2	2	2	2	2	2			2		2		2	2		2	
36	35	Amudha	216804(	######	32	######	2	Villipuran	8E+09	2	48	1	2		2	2	2	2	2	2	2	2	2		1	10	1	1	48	1	48
37	36	Lokesh	630579(	######	27	######	1	Arakkon	1E+10	2	8	2	2		2	2	2	2	2	1	1	Vitiligo	2		1	6	1	1	1	1	2
38	37	Getsy Si	100311(	######	29	######	2	Chennai	1E+10	2	6	2	1	1	2	2	2	2	2	2	2		1	1	2		1	1	4	1	4
39	38	Deepa	061994(	######	35	######	2	Thiruvall	1E+10	2	6	2	2		2	2	2	2	2	2	2		1	2	2		2	2		2	
40	39	Alok Pra	607035(	######	41	######	1	Banglad	9E+09	2	120	2	2		2	2	2	2	2	1	2	Vitiligo	2		2		1	2		2	
41	40	Sk Niyan	224431(	######	29	######	1	West Be	8E+09	2	18	2	2		2	2	2	2	2	2	2		2		2		1	1	8	2	
42	41	Mst Salm	388585(	######	27	######	2	Banglad	9E+09	2	120	2	1	1	1	2	2	2	2	2	2		2		2		1	1	12	2	
43	42	Judson	592569(	######	38	######	1	Ramesh	1E+10	2	6	2	2		2	2	2	2	2	2	2		2		2		2	2		2	

	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ
1	systemic	steroids	yesteroi	methotre	yesmeth	azathiop	yesazath	others1	therapy	curntreat	curtopic	yesstero	curintra	yeslesioi	immunos	currsterc	steroyes	currmeth	methoye	currazat	currthera	bpsys	bpdias	scalp	eyebrow	beard	body	patches	extent	pattern	nailchan
2	1	2		2		1	12		2	1	2		1		2						2	112	60	1	2	2	2	2	1	4	1
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4	2	2		2		2			3	1	1	6	1	6	2	2		2		2	2	100	62	1	2	2	2	2	1	4	2
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9	2	2		2		2			1	1	1	6	1	6	1	1	6	1	6	2	1	128	72	1	2	1	2	2	1	4	1
10	2	2		2		2			3	1	1	3	2		2	2		2		2	2	120	90	1	1	1	2	2	1	4	2
11	2	2		2		2			2	1	1	1	2		2	2		2		2	2	100	60	1	2	2	2	2	1	4	2
12	2	2		2		2		homeop	1	2	2		2		2	2		2		2	2	120	80	1	2	1	2	2	1	4	2
13	2	2		2		2			3	1	1	1	2		2	2		2		2	2	110	74	1	2	1	2	2	1	4	2
14	2	2		2		2			2	2	2		2		2	2		2		2	2	138	100	1	2	2	2	2	1	4	2
15	2	2		2		2			2	1	1	1	1	1	2	2		2		2	2	120	70	1	2	2	2	2	1	4	1
16	2	2		2		2		native m	1	2	2		2		2	2		2		2	2	100	60	1	2	2	2	1	1	4	2
17	1	1	1	2		2			2	2	2		2		2	2		2		2	2	110	80	1	2	2	2	1	1	4	2
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19	2	2		2		2		homeop	3	1	2		1	1	2	2		2		2	2	90	60	1	1	2	2	2	1	4	2
20	2	2		2		2			2	1	1	1	2		1	1	1	1	1	2	2	110	84	1	1	1	1	2	3	4	1
21	2	2		2		2		2	3	2	2		2		2	2		2		2	2	100	60	1	2	2	2	1	1	1	1
22	2	2		2		2			1	2	2		2		2	2		2		2	3	112	70	1	2	2	2	1	1	4	2
23	2	2		2		2		2	2	2	2		2		2	2		2		2	2	130	70	1	2	2	2	1	1	4	2
24	2	2		2		2		2	2	2	2		2		2	2		2		2	2	110	60	1	2	2	2	1	1	4	1
25	2	2		1	3	2		2	1	1	1	1	1	1	1	1	1	1	1	2	1	100	60	1	2	2	2	2	1	4	2
26	2	2		2		2			2	2	2		2		2	2		2		2	3	124	60	1	2	2	2	1	1	4	2
27	2	2		2		2			2	2	2		2		2	2		2		2	2	94	60	1	2	2	2	1	1	4	1
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31	2	2		2		2		2	3	2	2		2		2	2		2		2	2	100	60	1	2	2	2	2	1	4	2
32	2	2		2		2			2	2	2		2		2	2		2		2	2	130	90	1	2	2	2	2	1	4	2
33	2	2		2		2			1	2	2		2		2	2		2		2	2	118	80	1	2	2	2	2	1	4	2
34	2	2		2		2			1	1	2		1	2	2	2		2		2	1	90	60	1	2	2	2	1	1	4	2
35	2	2		2		2			3	2	2		2		2	2		2		2	2	100	60	1	2	2	1	2	3	4	1
36	2	2		2		2			3	2	2		2		2	2		2		2	2	110	70	1	2	2	2	2	1	4	2
37	2	2		2		2			1	1	1	2	1	2	2	2		2		2	1	120	74	1	2	1	1	2	3	4	2
38	2	2		2		2			1	2	2		2		2	2		2		2	2	100	60	1	1	2	2	2	1	4	2
39	2	2		2		2			2	1	1	1	1	1	2	2		2		2	2	90	64	1	2	2	2	1	1	4	2
40	1	1	6	2		2			1	2	2		2		2	2		2		2	2	120	78	1	2	2	2	2	1	4	2
41	2	2		2		2			3	1	1	1	1	1	2	2		2		2	1	120	84	1	2	1	2	1	1	4	2
42	1	1	6	2		2			1	2	2		2		2	2		2		2	3	100	62	1	1	2	1	2	3	4	2
43	2	2		2		2			3	1	1	1	2		2	2		2		2	1	120	60	1	2	2	2	2	1	4	2

	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO
1	pits	trachyor	punctate	beauslin	others2	othyes	saltscor	aapiscor	leftblack	leftexcla	leftbroke	leftdyst	rtblack	rtexclam	rtbroken	rtdyst	topblack	topexcla	topbroke	topdyst	backbla	backexc	backbro	backdys	yellowdc	blackdot	brokeha	markhair	coudaha	tailhairs	shorthair
2					1				2	2	2	2	2	2	2	2	1	1	1	2	1	1	1	2	2	1	1	1	2	2	2
3	2	2	2	2	2		6.88	13.76	2	2	2	1	2	2	2	1	2	2	2	2	2	2	2	2	1	1	2	2	2	1	1
4	2	2	2	2	2		10.3	22.04	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1	1	1	1	2	2	2	1
5	2	2	2	2	2		4.68	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
6	2	2	2	2	1		4.32	8.96	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1	2	2	2	2	2	2	1
7	2	2	2	2	2		28.24	24.46	2	2	1	2	2	2	1	2	2	2	2	2	2	2	1	2	1	2	1	2	2	2	2
8	2	2	2	2	1	Longituc	21	22.8	2	2	1	2	2	2	1	2	2	2	2	2	2	2	2	2	1	1	1	2	2	1	2
9	1	2	2	2	2		72.56	110	1	2	1	2	2	2	2	2	1	2	1	2	1	2	1	2	1	1	1	1	2	2	1
10	2	2	2	2	2		1.74	2.4	1	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	1	1	2	2	1	1
11	2	2	2	2	2		15.78	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
12	2	2	2	2	2		2.88	5.76	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	1	2	2	1	2	1	2
13	2	2	2	2	2		0.9	1.8	1	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	1	2
14	2	2	2	2	2		2.52	5.04	2	1	1	2	2	2	1	2	2	2	2	2	2	2	2	2	2	1	1	2	2	1	1
15	2	2	2	2	1	longitudi	8	16	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2	2	1	2	2	2	1
16	2	2	2	2	2		0.72	1.44	1	2	1	1	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	2	2	2	1
17	2	2	2	2	2		1.08	3.24	2	2	2	2	1	1	1	1	2	2	2	2	2	2	2	2	1	1	1	1	2	2	1
18	1	2	2	2	1		1.44	2.88	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	1
19	2	2	2	2	2		6.42	12.84	1	2	1	2	2	2	2	2	2	2	2	2	1	2	2	2	1	1	1	1	2	1	2
20	1	2	2	2	2		30.8	87.36	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	1	1	2	2	2
21	1	2	2	2	2		0.54	1.08	2	2	2	2	1	1	1	2	2	2	2	2	2	2	2	2	1	1	1	2	2	2	1
22	2	2	2	2	2		1.44	2.88	2	2	2	2	1	2	1	2	2	2	2	2	2	2	2	2	2	1	1	2	2	1	1
23	2	2	2	2	2		4	3.6	2	2	2	2	2	2	2	2	1	2	1	2	2	2	2	2	2	1	1	2	2	1	2
24	2	2	2	2	1	longitudi	1.44	2.88	1	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	2	1	2
25	2	2	2	2	2		13.38	40.14	1	2	2	1	1	2	2	1	2	2	2	2	1	2	2	1	2	1	2	2	2	2	1
26	2	2	2	2	2		0.72	1.44	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	1	2	2	2	2	1
27	2	2	2	2	1	longitudi	0.72	1.44	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	1	2	1	1	2	2	2
28	2	2	2	2	2		2.4	4.8	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	1
29	2	2	2	2	2		29.1	68.76	2	2	1	2	2	2	1	2	2	2	1	2	2	2	1	2	1	2	1	2	2	2	2
30	2	2	2	2	2		5.26	8.58	2	1	1	2	2	2	2	2	2	2	2	2	2	2	1	1	2	1	1	1	2	2	2
31	2	2	2	2	2		44.6	109	2	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	1	1	2	2	1	2
32	2	2	2	2	2		14.4	2.88	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	2	2	2
33	2	2	2	2	2		1.8	3.6	1	2	1	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	1
34	2	2	2	2	2		1.2	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2	1
35	1	2	2	2	2		102	0	1	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2
36	2	2	2	2	2		47.2	98	1	2	2	2	1	2	2	2	1	2	1	2	1	2	2	2	2	1	1	2	2	2	2
37	2	2	2	2	2		6	4.32	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	1	2	2	1	2	2	2	1
38	2	2	2	2	2		4.88	9.76	2	2	2	2	2	2	2	1	1	2	2	1	2	2	2	1	2	1	2	2	2	2	1
39	2	2	2	2	2		2.16	4.32	2	2	2	2	1	2	2	1	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2
40	2	2	2	2	2		57.82	110	2	2	1	2	1	2	2	1	1	2	2	1	2	2	2	1	1	1	2	2	2	2	1
41	2	2	2	2	2		6	12	2	2	2	2	2	2	2	2	1	2	2	2	1	2	2	2	2	2	1	2	2	2	1
42	2	2	2	2	2		58.56	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
43	2	2	2	2	2		5.12	6.4	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2

	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO	CP	CQ	CR	CS	CT	CU	CV	CW
1	topdyst	backbla	backexc	backbro	backdys	yellowdc	blackdot	brokeha	markhair	coudaha	tailhairs	shorthair	regrowin	pseudor	pohlpink	flamehai	serumlev	dlqiscr		
2	2	1	1	1	2	2	1	1	1	2	2	2	2	2	1	1	0.18	7		
3	2	2	2	2	2	1	1	2	2	2	1	1	1	2	2	1	0.32	3		
4	1	2	2	2	1	1	1	1	2	2	2	1	2	2	2	2	0.17	0		
5	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.29	11		
6	1	2	2	2	1	2	2	2	2	2	2	1	2	2	2	2	0.24	9		
7	2	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2	0.19	0		
8	2	2	2	2	2	1	1	1	2	2	1	2	2	2	2	2	0.17	12		
9	2	1	2	1	2	1	1	1	1	2	2	1	2	2	2	2	0.17	8		
10	2	1	2	2	2	2	1	1	2	2	1	1	2	2	2	2	0.27	0		
11	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.1	12		
12	2	2	1	2	2	1	2	2	1	2	1	2	2	2	2	2	0.23	4		
13	2	2	2	2	2	2	1	2	2	2	1	2	2	2	2	2	0.17	2		
14	2	2	2	2	2	2	1	1	2	2	1	1	2	2	2	2	0.62	3		
15	1	2	2	2	2	2	2	1	2	2	2	1	1	2	2	2	0.24	0		
16	2	2	2	2	2	1	1	1	2	2	2	1	2	2	2	2	0.14	9		
17	2	2	2	2	2	1	1	1	1	2	2	1	2	2	2	2	0.18	3		
18	2	1	1	1	1	1	1	1	1	2	2	1	2	2	2	2	0.1	2		
19	2	1	2	2	2	1	1	1	1	2	1	2	1	2	2	2	0.27	4		
20	2	1	2	1	2	1	1	1	2	2	2	2	2	2	2	2	0.21	3		
21	2	2	2	2	2	1	1	1	2	2	2	1	2	2	2	2	0.36	1		
22	2	2	2	2	2	2	1	1	2	2	1	1	2	2	2	2	0.19	1		
23	2	2	2	2	2	2	1	1	2	2	1	2	1	2	2	2	0.28	0		
24	2	2	2	2	2	1	1	1	1	2	1	2	1	2	2	2	0.16	0		
25	2	1	2	2	1	2	1	2	2	2	2	1	2	2	2	2	0.69	15		
26	2	2	2	1	1	2	1	2	2	2	2	1	2	2	2	2	0.19	2		
27	2	2	1	1	2	1	2	1	1	2	2	2	2	2	2	2	0.27	3		
28	1	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	0.49	6		
29	2	2	2	1	2	1	2	1	2	2	2	2	2	2	2	2	0.37	0		
30	2	2	2	1	1	2	1	1	1	2	2	2	1	2	2	2	0.21	5		
31	2	1	2	1	2	1	1	1	2	2	1	2	2	2	2	2	0.3	13		
32	2	1	1	1	1	1	1	1	1	2	2	2	1	2	2	2	0.22	2		
33	2	2	2	2	2	2	1	1	2	2	2	1	1	2	2	2	0.26	3		
34	2	2	2	2	1	1	2	2	2	2	2	1	2	2	2	2	0.25	0		
35	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2	2	0.36	14		
36	2	1	2	2	2	2	1	1	2	2	2	2	2	2	2	2	0.14	6		
37	2	2	2	2	1	2	2	1	2	2	2	1	1	2	2	2	0.18	8		
38	1	2	2	2	1	2	1	2	2	2	2	1	2	2	2	2	0.31	9		
39	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2	2	0.21	2		
40	1	2	2	2	1	1	1	2	2	2	2	1	1	2	2	2	0.21	13		
41	2	1	2	2	2	2	1	2	2	2	1	2	2	2	2	2	0.16	21		
42	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.4	1		
43	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	0.27	3		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE
42	41	Mst Salm	386585(	######	27	######	2	Banglad	9E+09	2	120	2	1	1	1	2	2	2	2	2	2		2		2		1	1	12	2	
43	42	Judson	592569(	######	38	######	1	Ramesh	1E+10	2	6	2	2		2	2	2	2	2	2	2		2		2		2	2		2	
44	43	Sukuma	327170(	######	23	######	1	West Be	9E+09	2	7	1	2		2	2	2	2	2	2	2		2		1	10	1	2		1	4
45	44	Birendra	704272(	######	30	######	1	West Be	1E+10	2	7	2	2		2	2	2	2	2	2	2		2		2		1	1	2	1	2
46	45	Jesmin A	613445(	######	25	######	2	Banglad	9E+09	2	12	1	2		2	2	2	2	2	2	2		2		1	1	1	2		1	1
47	46	Md Aslar	613449(	######	29	######	1	Banglad	9E+09	2	84	1	2		2	2	2	2	2	2	2		2		1	6	1	2		1	6
48	47	Sathish I	403870(	######	30	######	1	Vellore		1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
49	48	Shantha	120952(	######	60	######	2	Vellore,T	Tamil Nad	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
50	49	Mohamn	813759(	######	19	######	1	Vellore,1	1E+10	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
51	50	Bhuvana	873005(	######	64	######	2	Vellore,1	9E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
52	51	Gowri	529789(	######	49	######	2	Tamil Nadu		1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
53	52	Ruma Bh	742571(	######	49	######	2	West Be	1E+10	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
54	53	Kazi Jon	681647(	######	40	######	1	Bangladesh		1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
55	54	Balaram	724757(	######	46	######	1	West Be	8E+09	1		2	2		2	2	2	2	2	2	2				2		2	2		2	
56	55	Fayaz Al	658312(	######	41	######	1	Chittoor,	9E+09	1		2	2		2	2	2	2	2	2			2		2		2	2		2	
57	56	Labbi Fa	658308(	######	34	######	2	Chittoor,	9E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
58	57	Anandai	372120(	######	55	######	1	Walajapa	1E+10	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
59	58	Sangeet	189978(	######	21	######	2	Vellore,1	8E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
60	59	Balaram	712289(	######	67	######	1	Vellore,1	9E+08	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
61	60	Velu	710060(	######	30	######	1	Pallikond	8E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
62	61	Nirmal M	949765(	######	46	######	1	Medinipu	1E+10	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
63	62	Seuli Ma	625151g	######	46	######	2	west Ber	1E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
64	63	Moshare	698531(	######	26	######	1	Banglad	7E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
65	64	Smritikar	048872(	######	34	######	2	Raipur,W	9E+09	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
66	65	Alamelu	496571(	######	60	######	2	Vellore,T	Tamil Nad	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
67	66	Haribabu	555744(	######	23	######	1	Chittoor,	1E+10	1		2	2		2	2	2	2	2	2	2		2		2		2	2		2	
68																															
69																															

	AE	AF	AG	AH	AI	AJ	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI
42		1	1	6	2		2			1	2	2		2		2	2		2		2	3	100	62	1	1	2	1	2	3	4
43		2	2		2		2			3	1	1	1	2		2	2		2		2	1	120	60	1	2	2	2	2	1	4
44	4	2	2		2		2			1	1	2		1	2	2	2		2		2	1	110	80	1	2	1	1	2	3	4
45	2	2	2		2		2			3	2	2		2		2	2		2		2	2	120	70	1	2	1	2	2	1	4
46	1	2	2		2		2			3	1	1	2	1	2	2	2		2		2	1	100	60	1	2	2	2	2	1	3
47	6	1	1	3	2		2			3	1	2		2		1	2		1	1	2	2	110	80	1	1	1	1	2	3	4
48		2	2		2		2				2	2		2		2	2		2		2		110	70	2	2	2	2			
49		2	2		2		2				2	2		2		2	2		2		2		120	70	2	2	2	2			
50		2	2		2		2			2	2	2		2		2	2		2		2		110	74	2	2	2	2			
51		2	2		2		2				2	2		2		2	2		2		2		120	84	2	2	2	2			
52		2	2		2		2				2	2		2		2	2		2		2		100	60	2	2	2	2			
53		2	2		2		2				2	2		2		2	2		2		2		100	64	2	2	2	2			
54		2	2		2		2				2	2		2		2	2		2		2		120	78	2	2	2	2			
55		2	2		2		2				2	2		2		2	2		2		2		110	80	2	2	2	2			
56		2	2		2		2				2	2		2		2	2		2		2		120	70	2	2	2	2			
57		2	2		2		2				2	2		2		2	2		2		2		90	64	2	2	2	2			
58		2	2		2		2				2	2		2		2	2		2		2		120	90	2	2	2	2			
59		2	2		2		2				2	2		2		2	2		2		2		90	66	2	2	2	2			
60		2	2		2		2				2	2		2		2	2		2		2		130	88	2	2	2	2			
61		2	2		2		2				2	2		2		2	2		2		2		120	70	2	2	2	2			
62		2	2		2		2				2	2		2		2	2		2		2		130	80	2	2	2	2			
63		2	2		2		2				2	2		2		2	2		2		2		112	80	2	2	2	2			
64		2	2		2		2				2	2		2		2	2		2		2		120	74	2	2	2	2			
65		2	2		2		2				2	2		2		2	2		2		2		110	80	2	2	2	2			
66		2	2		2		2				2	2		2		2	2		2		2		130	80	2	2	2	2			
67		2	2		2		2				2	2		2		2	2		2		2		110	70	2	2	2	2			
68																															
69																															
70																															
71																															

	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN	CO	CP	CQ	CR	CS	CT	CU
42	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.4	1
43	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	0.27	3
44	1	2	1	1	1	2	2	2	2	2	1	1	1	2	1	1	1	1	2	2	2	2	2	2	2	0.23	13
45	2	2	1	2	1	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2	2	2	2	2	2	0.26	1
46	1	2	2	2	2	2	2	2	2	2	2	1	2	2	1	1	1	1	2	2	2	2	2	2	2	0.1	4
47	2	2	2	2	2	2	2	2	2	2	1	2	2	2	1	1	2	2	2	2	2	2	2	2	2	0.18	10
48	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.1	
49	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.26	
50	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.15	
51	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.1	
52	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.1	
53	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.18	
54	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.19	
55	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.16	
56	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.12	
57	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.13	
58	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.13	
59	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.17	
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61	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.28	
62	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.27	
63	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.25	
64	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.22	
65	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.25	
66	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.21	
67	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	0.18	
68																											
69																											
70																											

	BJ	BK	BL	BM	BN	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE	CF	CG	CH	CI	CJ	CK	CL	CM	CN
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44	2	2	2	2	2	2		6.96	30.48	1	2	1	2	1	1	1	2	2	2	2	2	1	1	1	2	1	1	1	1	2	2
45	2	2	2	2	2	2		1.08	2.16	2	2	2	2	1	2	1	2	2	2	2	2	2	2	2	2	2	1	1	2	2	2
46	1	2	2	2	2	2	longitudi	14.76	29.52	1	2	1	2	2	2	2	2	2	2	2	2	2	1	2	2	1	1	1	1	2	2
47	1	2	2	2	2	2	Longitud	99	48	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	1	1	2	2	2	2
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68																															
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