

Mast cell count in Alopecia Areata and Scarring alopecia in patients referred to Imam Khomeini Hospital in Ahvaz (2014-2016)

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Abstract

Alopecia is a Latin word meaning hair loss or baldness and includes many types of hair loss. The underlying cause of AA is unknown, but recent evidence suggests that it is a chronic inflammatory disease of the hair follicles. Based on the histological evidence of peripheral follicular fibrosis and an increase in the number of mast cells - which can increase the synthesis of elastic fibers - it appears that there may be a link between mast cells and alopecia. The present cross-sectional descriptive-analytical study was performed on formalin and paraffin-embedded tissues of patients diagnosed with alopecia, as well as 20 paraffin-embedded scalp samples of "healthy" patients without reporting Alopecia. In order to evaluate the number of mast cells, Giemsa cytochemical staining was performed. The results show that the mean number of mast cells in AA and AS patients was 18 and 19, respectively, which did not show a significant difference. However, this rate was 18.75 in patients with Alopecia and 6.5 in the control group, which shows a statistically significant difference between the two. Also, the number of mast cells in the biopsy of patients with alopecia was significantly higher than normal individuals, which indicates the role of these cells in the pathogenesis of alopecia.

Keywords: Alopecia, mast cell, areata, scarring

1-Introduction

Hair plays an important role in a person's appearance and self-image. Sudden hair loss is a psychologically painful and unpleasant event that has a profound effect on a person's life to the extent that it may limit his or her relationships and social freedoms. Patients consider partial hair loss to be equivalent to baldness and are afraid of total hair loss (1).

Alopecia is a Latin word meaning hair loss or baldness and includes many types of hair loss. One type of hair loss is Scarring Alopecia or Cicatricial Alopecia, in which hair follicles are destroyed and replaced by scar tissue. Scarring Alopecia is caused by any inflammatory process such as burns, bacterial infections, fungal infections, injuries and autoimmune diseases that may lead to permanent damage to the hair follicles (3).

Alopecia areata (AA) is one of the most common autoimmune diseases of hair follicles (HF) associated with CD8 + T cells, which presents clinically with sudden hair loss and usually in the form of patchy hair loss on the surface of the body in hairy areas. Does (4).

The underlying cause of AA is unknown, but recent evidence suggests that it is a chronic inflammatory disease of the hair follicles that is associated with the accumulation of self-reactive lymphocytes. Impaired immune score (IP) hair follicles play a key role in the development of AA (5). Studies have shown that the susceptibility to Alopecia is polygenic, but environmental factors initiate episodes of the

disease (6). Inflammation of the hair follicle with a T cell-dependent autoimmune mechanism and the presence of environmental factors in genetically predisposed individuals can predispose to disease (7).

The disease process is caused by lymphocytic infiltration around the hair follicle. Severe involvement causes total scalp hair loss (Alopecia Totalis). Alopecia Universalis whole body hair loss, diffuse scalp hair loss is called Diffuse Alopecia areata and localized hair loss on the scalp margin is called Ophiasis (2).

The pathogenesis of alopecia has not yet been determined. Some studies have reported an association between alopecia and mast cells. Some studies have reported an increase in the number of mast cells in AA skin lesions (8). One study also reported a high frequency of mast cells in Scarring Alopecia (10).

Mast cells are found in the skin and subcutaneous tissue around blood vessels, smooth muscle cells, nerve terminals, and especially in the connective tissue plate of hair follicles (11). They can be identified by hematoxylin-eosin staining, specific staining such as Giemsa and toluidine blue, and immunohistochemistry (c-kit) techniques (12).

Mast cells (MCs) are vital and important immunomodulatory cells that are involved in the regulation of immune, IP and hair growth-dependent T cells (9). Mast cells release mediators such as histamine, proteases, growth factors, prostaglandins, and cytokines, which are involved in various processes including scar formation and tissue remodeling, resulting in mast cells improving Normal wounds are also involved in the pathogenesis of fibrotic diseases such as scleroderma (13, 14). They also act as fibroblast growth factor receptors and vascular endothelial growth factor receptors, thereby promoting the process of angiogenesis, which is involved in the development of hair follicles (14).

Based on the histological evidence of peripheral follicular fibrosis and an increase in the number of mast cells - which can increase the synthesis of elastic fibers - it seems that there may be a relationship between mast cells and alopecia (15).

The main reason for paying attention to mast cells in the pathogenesis of alopecia is the recognition of mast cells as modifiers of hair growth (16), and also that HF mesenchyme in humans and mice contains mast cell progenitor cells. , From which fully functional adult skin mast cells can be derived (17).

Perifollicular inflammatory cells invade AA-damaged hair follicles containing lymphocytes (CD8 + and CD4 + T cells), natural killer cells, and some Langerhans cells, increasing the number of histologically identifiable mature mast cells. Gives (24). While AA studies have long focused on T cells, particularly CD8 + lymphocytes, few studies have been performed on mast cells in alopecia areata (9).

Due to the wide range of causes of the disease and its association with many diseases, further studies in the field of diagnosis of this disease seem necessary. Therefore, considering the possible role of mast cells in the pathogenesis of Alopecia and considering that no study has been done in Iran on the number of mast cells in infected people, the present study aims to count mast cells in Scarring Alopecia and Alopecia areata in Iranian patients will be performed. If the role of yogurt is confirmed, it can be used for new therapeutic purposes in patient management.

2. About Alopecia

Alopecia areata (AA) is one of the most common disorders of the hair follicle, affecting about 1% of the population. کردن (18). The incidence of alopecia areata can vary between men and women and also affects most Caucasians and Asians. Alopecia areata can occur at any age but is more common at two ages: before puberty and in some cases between 20 and 40 years old. Despite the fact that this disease is considered a benign clinical condition, alopecia areata can have devastating effects on the quality of life of a person and those around him, especially in the case of children and women. It is usually characterized by asymptomatic hair loss and scarring patches that are usually round and vary in number and size and can spread to all parts of the head and other hairy areas of the body. The scalp may sometimes look a little

red and inflamed. In 10% of cases, nail lesions also occur. Spontaneous recovery periods are common, especially in the early and mild cases. However, it is estimated that about 10-30% of cases develop chronic, recurrent, and progressive forms of alopecia areata, and less than 1% develop the global type.

1-2- Clinical features:

The most common form of alopecia areata is the onset of primary lesions in the form of a patch without round hair and completely hairless and smooth. The surface of this patch is usually white and sometimes pink without scars. Some "broken hair" and blackheads can appear around and around it. "Broken hair" Short hair with a distal part is thicker and more colorful than the proximal part. "Black dots" are small black dots that surround the hair follicle orifice. These are due to the accumulation of creatine, fat and melanin in the funnel-shaped base of the hair follicle. Both "broken hair" and "blackheads" are known to be signs of acute alopecia areata, although the former is actually a hair rooted in Far Telogen, due to the process that caused it. , Was created a few weeks ago, and in some cases, the phenomenon of "revitalization" of these broken hair has been seen. In fact, we see this phenomenon as a spontaneous improvement or under steroid treatment (19). In these cases, the distal part is usually longer than before, and the hair follicle is normal or slightly dystrophic and in the anagen phase. This finding, which can be a positive predictor, is very important because it confirms that in cases of alopecia areata in the catagen phase, such as chemotherapy or radiotherapy, these changes are reversible (20). The spread of alopecia on the scalp eliminates ophiosis, and as the patches grow, they tend to spread or band around the center of the scalp. Based on the prevalence of alopecia areata, we can classify patch-like alopecia areata based on severity depending on the percentage of involvement. Alopecia areata Kelly (AT), which loses all hair, and Alopecia areata all over (AU), which loses all hair and body hair. Body hair can fall out in a patch-like form or in general, such as hair loss on the beard, eyelashes or eyebrows. Along the margins of the patches, the hair can be easily removed or broken by the roots in the telogen state, or in the acute phase of alopecia areata in the dystrophic phase of the anagen, and these histopathological issues are due to the spread of lymph mononuclears such as "masses". "Bees" that involve the root part of the follicle (21). The initial patch can improve in a few months or new patches can appear in the next 3-8 weeks, even when the initial patch is improving. If the "hair pull test" around the lesion is positive, this could indicate a final assessment of the cause of the initial lesion. Separate patches can attach quickly or slowly and spread through the remaining hair, although in some cases, the initial hair loss is severe and general alopecia occurs in less than 48 hours. Therefore, it is not possible to determine the rate of spread of the disease based on the location of the initial patch, but it is important to distinguish between anagen effluvium (hair with broken and damaged roots) - which is the true reactivation of the disease - with alopecia areata. Know the occurrence of telogen phase in the hair follicles. These are important to determine the healing process and treatment goals because, for example, the treatment of the true acute phase may not be very useful for someone who has had a few weeks or months of anagen spots and symptoms. Internal symptoms usually do not occur, although sometimes some patients experience itching, tenderness, or pain and numbness immediately before the lesions appear.

2-2- Dermatoscopic features

"Black grains" or "yellow grains" when they contain a small amount of pigment) can remain until new hair grows, so they can not be considered a sign of disease activity (22). Chronic alopecia areata with hairless scalp manifests itself in dermoscopy with yellow grains, which is associated with dilation of the follicle funnel and the absence of a thin or annular hair shaft or stem. In long-term or persistent cases of the disease, white spots can be seen without any intra-follicular structure. When hair begins to grow again, it is usually thin and without pigment. Hair pigmentation may not occur, and sometimes even hair

regrowth in alopecia areata causes permanent white hair. In fact, white hair is safe from the disease, and when alopecia areata occurs in a person with gray hair, they feel that their hair has suddenly turned white. The phenomenon was named when Queen Marie Antoinette was infected, and other historical events have led to the beheadings of Marie Stewart and Francis Bacon, and it has been suggested that melanocyte involvement - and obviously stress - is involved in the pathogenesis of the disease (23).

3-2- Abnormal states of Alopecia areata

Less commonly, alopecia areata can occur in abnormal conditions that can lead to diagnostic errors. The rarest form is androgenic hair loss, which is generally seen in patients whose hair grows back and has feminine or masculine patterns. These problems are easily diagnosed in the service of children, but can also occur in adults. Another rare form of alopecia is "Sisaipho", for which there are various definitions, and some dermatologists prefer to call it "reverse ophiasis". Its definition makes us realize that this form is a type of alopecia areata that starts in the central part of the head and extends to the outer edges of the head.

A case of "Sisaipho" with a specific pattern of hair regrowth is called "target shape", which is due to the accumulation of regrowth hair in the target shape. It is said that it may occur as the opposite aspect of this spread, which is called the "pathological centrifugal wave" and is due to the mechanism of expansion of alopecia patches (24). It has recently been reported that general and acute alopecia areata in women (ADTAFS) has a good recovery. Also in the last half century, the question has been raised whether general alopecia is really a benign form of global alopecia or not? In severe destructive activity with a shorter time can protect areas that have a higher percentage of telogen phase hair, such as eyelashes and body hair and make it resistant to damage (25). The forms of alopecia areata that cause the most diagnostic and classification problems, as well as the pathological problems of the prevalent type of alopecia, are still unknown. These types are created with extensive thinning of the hair and acute condition and involve the whole head without creating noticeable patches. Dermatoscopy usually shows yellow spots and short hair. Histopathological examination also showed broken hair and exudation of inflammatory cells around the hair follicles. It is important to remember that inflammation may be present in the telogen phase, which is based on the recent view that the body's own lethal lymphocytes may invade the hair follicles and cause hair loss in healthy mice. Hairless mice have also been shown this (26)

4-2- Related clinical changes

1-4-2- Nails

Alopecia areata is commonly associated with nail changes, and this suggests that pathological lesions that affect the hair can affect other keratin structures, such as the nail. Nail involvement is common in all plums and all plums. Geometric piercings are usually the most common type of change and occur with tiny dots on the nail plate in the form of geometric shapes. Other nail symptoms include whitening of the nails as a specific mark or "biz" lines or redness of the nail bed that can be seen in other diseases. About 3% of patients with alopecia areata develop thrush or complete loss of 20 nails, which are spread in longitudinal lines and scaling on the nail plate that turns the nail as rough as sandpaper. Traction is more common in children and patients with severe Alopecia areata and can occur before or after Alopecia areata (27).

2-4-2- Eyes

An association between severe types of alopecia areata and cataracts has been reported in some cases, and in two out of five cases in adults, rapid vision loss coincided with a sudden and widespread attack of alopecia areata. In a recent study, corneal and retinal changes were more common in alopecia areata

patients, and especially in atopic patients, different types of autoimmune cases were reported than in the control group. Although this is still debated, if a person has alopecia areata, they should consult an ophthalmologist.

3-4-2- Other related items

The association between alopecia areata and atopia (25-40% of cases), vitiligo (5% of cases) and various autoimmune diseases, especially thyroid involvement (25% of cases) should be investigated in the patient and his family. Alopecia areata is a component of VogtKoyanagi-Arada syndrome. Other components of the syndrome include vitiligo, uveitis, hearing loss, and meningeal involvement. Clearly, this syndrome is a cell-associated autoimmune pathology, which targets melanocytes in all parts of the body and is manifested by involvement of tyrosinase-related proteins (TRP1 and TRP2) (28). It is important to remember that melanocyte antigens (Gp100 / G9-154, Gp100 / G9-280, Gp100 / G9-209 MC1R 291, MART-1 27-35) are the main targets of cellular immunity in their diseases. Immunity in alopecia is yeast (29). Also, one of the interesting recent studies showed that in the most common type of Alopecia areata, the occurrence of hearing loss has been associated with the loss of melanocytes in the inner ear (30). Alopecia areata also occurs in 30% of cases early and severely in association with autoimmune diseases such as thyroiditis, insulin-dependent diabetes, pernicious anemia, chronic active hepatitis, vitiligo and biliary cirrhosis, and several autoimmune syndromes. Glands (PAS-1) that are inherited autosomal recessively and cause chronic cutaneous mucosal candidiasis, parathyroid hypothyroidism, and Addison's disease. Its genetic basis is in a mutation in the autoimmune control gene (AIRE) on chromosome 21. Loss of function of the AIRE gene impairs the elimination of self-reacting T cells as well as dysfunction of regulatory T cells. In addition, Treg cells activated in these patients produce lower levels of the F3XP3 protein (FOXP3) than the control group because of genes that are located on the X chromosome and activate DNA-binding proteins. Disrupts their environmental activation (31). Immune regulation disorders, multiconvulsant involvement, and gastrointestinal involvement and X-linked syndromes (IPEX) are rare types of skin gene disorders associated with dermatitis, severe alopecia, intestinal involvement, type 1 diabetes, thyroiditis, hemolytic anemia, and thrombocytopenia. Are in touch. IPEX is caused by a mutation in the FOXP3 gene (32). Interestingly, a recent study found that two nucleotide changes in the FOXP3 gene in the promoter region and ligand regulatory region (ICOSLG) are associated with common types of alopecia areata and regulatory T cell size (33). It is important to note that endocrinologists have identified syndromic syndrome as a type 3 multinocular autoimmune involvement syndrome (PAS-3C) in which Aloe vera is associated with autoimmune thyroid inflammation (34). Therefore, about 25% of Alopecia areata patients should be evaluated for the most common association of alopecia areata with autoimmune diseases, especially in thyroid and vitiligo diseases that have been confirmed to be associated with anti-thyroid antibodies. It is now known that thyroglobulin and thyroperoxidase antigens are also expressed in the hair follicles. Other associations have been associated with pernicious anemia, chronic atrophic gastritis, rheumatoid arthritis, lichen planus, systemic lupus, celiac disease, severe myasthenia gravis, ulcerative colitis, and gastritis with *Helicobacter pylori* (35). An 8.8% incidence of alopecia areata in Down syndrome is associated with the involvement of the MX1 gene on chromosome 21, which, in addition to the AIRE gene, controls the production of stimulated gamma interferon (36). In addition, it is important to remember that other types of inflammatory alopecia, such as lichen planus pilaris or chronic lupus, can be associated with alopecia areata, and it is interesting to note that alopecia areata is sometimes found in the study of ulcerative alopecia (37).

5-2- Investigation of immunological and pathology causes

The belief that alopecia areata is an autoimmune disease is based on numerous evidences. In the last decade, the role of the lymphocyte population has become more important, and as shown, there is a difference between the total population of lymphocytes and the subgroups that are in the peripheral blood. It has been observed that subgroups of lymphocytes and cellular cytokines are different in different stages of the disease and are known as a sign of autoimmune pathogenic activity. Activated CD8 lymphocytes are known to be the main killers of cell death and damage to the follicles and are also aided by CD4 lymphocytes, all of which stimulate the autoimmune process. In addition, activated T cells release cytokines such as interferon-gamma, which can inhibit keratinocyte proliferation and trigger the expression of incompatible antigens in follicle cells, which are detected by lethal T cells. Are (38).

CD4-CD25 (regulatory T cells) can stop the autoimmune process by producing inhibitory cytokines (TGF bet, IL10) and are responsible for the response to topical immunotherapy with SADBE or Diphenciprone. Elevated serum levels of IL-2, IFN-, IL-13, and IL-17 indicate altered T-helper cell activity, and decreased serum levels of TGF-1 indicate dysfunction of regulatory T cells in alopecia areata patients. . An essential factor that plays an important role in the pathogenesis of alopecia areata is called "immune privilege" (IP). It says that the immune system is not able to recognize all the hair follicle antigens because they are "kept" in a place where the inflammatory cells cannot see them, and because of this secretion under normal conditions the expression of antigens Class 1 and 2 tissue adaptable as well as dendritic cells are unable to present new hair follicle antigens (39).

In the acute phase of alopecia areata, large numbers of dendritic cells surround the hair follicles, especially in the root area, which is the target area for the disease. This cell infiltration is greatly reduced by successful treatment (40). Recently, the role of natural killer lymphocytes (NK) in the development of alopecia areata has been hypothesized: due to the lack of expression of major histocompatibility antigens, NK cells attack to identify factors they do not know. But it is also possible that the existing "safety superiority" is also circumvented under stress, due to changes in its supporting factors and increased expression of adhesive molecules on the surface of NK cells (NKG2D) as well as the production of small amounts. Inhibitory cytokines, such as macrophage inhibitory factor (MIF).

The first damage to the hair follicle by killer lymphocyte cells is caused by the production of interferon-gamma, which causes the expression of HLA antigens on the hair follicle, destroying the "immune superiority" mechanism and leaving the hair follicle as a Target for killer T cells. An important role in this system is played by interleukin-15, which can inhibit the killing of cell-killing lymphocytes by intermittent expression of HLA antigens on target cells. This cytokine is physiologically present in the skin and skin disorders such as alopecia areata increase.

Because the hemorrhagic immune system in alopecia areata lesions includes B lymphocytes, plasma cells, and non-permanent and complementary immunoglobulins, they can destroy the connective tissue and sometimes the outer roots of the hair. In disease-prone humans and animals, Tobin showed that IgG antibodies against 45- and 60-kDa proteins - one of which was "trichohyalin" and the other "cytokeratin 16" - could not directly cause alopecia lesions but could grow. Stop the hair in the affected area (41).

6-2- Histopathology

The histopathology of alopecia areata depends on the stage of the disease or the area being sampled. In the acute phase, when the hair follicles fall out for the first time, we can see a large number of hair follicles in the catagen-telogen phase, usually below the hair follicle, where it is active at the time of anagen, there is also a lymphocyte infiltration. .

In the acute phase of alopecia areata, damage to the funnel part of the hair follicle is an important part of the early changes in alopecia areata lesions and may be caused by the infiltration of T lymphocytes with

mast cells and eosinophils. Finding a hair follicle funnel that contains creatine accumulations may be equivalent to accumulating lost hair lesions or yellow spots. If the follicle is seen in the anagen phase, it can be of normal size - where the root is located in the hypodermis - if it is small - the root is located on the surface and a dark accumulation is usually CD8 + cells and T lymphocytes and macrophages. There are those who tend to surround the hair follicles and destroy the keratinocytes and melanocytes of the hair follicle matrix, destroying them. Langerhans cells and dendritic cells can invade the upper part of the hair and play an important role in stimulating and continuing the disease. The matrix and keratinocytes around the nucleus express HLA class 1 and 2 antigens and the ICAM-1 adhesive molecule; The adhesive molecule ICAM-1, along with VCAM-1 and ELAM-1, are expressed in the endothelium of the hair follicles, and together they facilitate the migration of inflammatory cells to the target follicle. In patients with general alopecia or general alopecia or long-lasting alopecia patches, the most common finding is to see hair follicles in the anagen phase (phases III and IV) as well as more lymphocytic infiltration around the hair follicles. In addition, we can see small follicles in the telogen phase that are usually without hair follicles. Follicles usually have a normal density. In areas where hair growth has resumed, we can see large hair follicles that are in the anagen phase and have a thin, brainless stem. In rare cases, a significant reduction in hair follicle density is seen with progressive destruction of hair follicles due to replacement with fibrous tissue (42).

2-7-Prognosis

The prognosis of alopecia areata is unpredictable. Most patients who present with one or more areas of alopecia usually recover spontaneously within a year. It is estimated that 10-30% develop severe cases and less than 1% develop global alopecia. The recurrence rate is about 50% for up to 5 years, about 80% for up to ten years, and about 100% for up to twenty years. Recurrences are usually more severe than before. An old clinical classification by Ikeda in 1965 linked the prognosis to related prognostic factors: atopia, hypertension, combination cases, and prevalence. Most recent observations have revealed disorders associated with this disease. Alopecia areata has a poor prognosis in atopic cases, and if it occurs before puberty, final regrowth is usually unlikely. Alopecia areata has a good prognosis at any age and in non-atopic cases, especially if it persists for more than 6 months. Alopecia areata does not have a good prognosis. Pregnancy is sometimes associated with recovery and progression to severe cases of alopecia areata over a long period of time, although their treatment is usually temporary. There is no case of alopecia areata that certainly has a good prognosis, especially in children. We have already talked about the important role of innate cases in the development of alopecia areata. On the other hand, although cases of alopecia areata in monozygotic twins usually occur similarly and at the same age, their correlation is only 42%, indicating the importance of environmental factors. We suggest adding DHEA-S and vitamin D deficiency and personality disorders such as "avoidance of attachment" to this list (29).

8-2-Treatments for alopecia areata

Although alopecia areata is a benign disease, it can cause stress and mental disorders for the patient and the surrounding family, and this can have negative effects on a person's quality of life. In these cases, psychological support and pharmacotherapy-psychology are recommended. Since the case of alopecia areata is unpredictable, with changes such as spontaneous recovery periods, recurrences, and hair regrowth intervals, the direct effect of a particular treatment among the many suggestions between the normal course of the disease or the placebo effect cannot be determined. Check out. All of these can make it difficult to evaluate effective treatment based on medical evidence (EBM). Thus, to date, the treatment option for alopecia areata has been based more precisely on different experimental cases, which are based

on the patient's age, disease phase (acute or chronic), and the percentage of head involvement (less than or greater than 50%).

There is currently no specific FDA-approved treatment for alopecia areata in the United States, and there is no universally accepted guideline in Europe, with only recommendations from the FVDJHKDH Association of Dermatologists. The Japan Dermatologist Association has also suggested that taking anti-allergy medications may be especially effective in improving the effectiveness of immunotherapy in severe cases.

Evidence-based medical criteria have confirmed that good effect and acceptable side effects can be beneficial for topical immunotherapy with diphencyprone or squaric acid or intralesional or topical steroid injections in severe cases.

Treatment failure is usually more common in cases where alopecia areata is severe, such as the rate of recurrence in successful cases. At this point we need to remember that the iatrogenic issue that Koebner raised is similar to what happened with the classification of psoriasis, and that severe topical treatments (such as Dithranol, PUVA) cause - especially if used in the acute phase of the disease. Worsening skin problems. This may explain the lack of response or the spread of alopecia, which has been observed by many of us in the treatment of immunotherapy or severe use of steroids in the lesion, especially in children, and on the other hand the best treatment for cases of limited forms with Specify the limited patch. This allows us to determine the appropriate proposal for the treatment algorithm based on the well-known algorithm of the University of Vancouver, British Columbia, but extending the criteria for prescribing the drug not only on the basis of age (above or under 10 years) and severe alopecia (above). Or below 50%) but has spread based on the symptoms of the disease and the complications of the disease. The method of assessing the degree of alopecia can be pull-test wash-test, dermatoscopy, histopathology, but our experience in examining hair can sometimes be inaccurate due to rapid information about destructive changes and induce the effect of Koebner phenomenon. Clearly, the issue of disease activity can cause problems in the global form, and in these cases, the lack of hair can lead to white spots in histopathology, and perhaps the level of MIG - a monokine stimulated by IFN - in the acute phase of alopecia areata. , And serum granulolysin - released by cytotoxic lymphocytes around the lesion - can also be considered a sign of disease severity.

All treatments for alopecia areata should be continued for at least 7-12 months to achieve their beneficial effect. So far, topical immunotherapy is a concern, and some doctors say we should wait up to 32 months for maximum results (78%) in patients. The following are some of the cases that have been proven to be effective treatments for severe cases, as well as the latest treatment recommendations. It is important to remember that most experts recommend the use of combination therapies such as steroids and topical minoxidil and detranol, etc. (44).

3- Research method

3-1- Summary of the implementation method of the plan and the techniques used:

The present descriptive cross-sectional study will be performed on formalin and paraffin-embedded tissues of patients with alopecia diagnosis in the pathology archive of Imam Khomeini Hospital in Ahvaz from 2014 to 2016.

After obtaining permission from the Research Council and the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, 30 scalp biopsy specimens of patients with Scarring Alopecia and Alopecia Areata will be extracted from the archive of paraffin blocks in the pathology of Imam Khomeini Hospital in Ahvaz.

Also, 20 paraffin samples of the scalp of "healthy" patients without Alopecia report of people who had referred for cosmetic surgery are also considered as a control group.

A checklist containing information about the patient, including age, sex, duration of the disease, extent of alopecia and the presence of the underlying disease is extracted from the records of these patients and recorded in the data collection checklist. Finally, the relationship between these variables and the number of mast cells in alopecia will be examined.

In order to observe the mast cells, first 4 micrometer thick slices are prepared on the samples by microtome device. The sections are then paraffinized and dehydrated, and Giemsa cytochemical staining is performed to identify mast cells. Finally, mast cells, which are dense, dark blue cells, are examined by light microscopy at magnifications of 100 and 400.

To count the density of mast cells, first microscopic slides with a magnification of 100 are observed and then the areas with the highest number of mast cells (hot spot) are selected and viewed with a magnification of 400. The count is performed on at least 4 microscopic fields and confirmed by another pathologist. Finally, the density of mast cells is reported as the average number in 4 fields (Mean \pm SD) for each sample.

Finally, the relationship between the number of mast cells and Scarrin Alopecia and Alopecia areata g is investigated.

3-2- Sample calculation method and sampling method:

This study will be performed on all individuals with a definitive diagnosis of alopecia referred to Imam Khomeini Hospital in Ahvaz between 2014-2015.

3-3- Statistical methods of analyzing the results:

Used for statistical analysis of SPSS software version 22. The obtained data are analyzed by descriptive statistics including frequency, mean, standard deviation, frequency percentage. The normality of the data is checked by Kolmogorov-Smirnov test. One-way analysis of variance and Kruskal-Wallis analysis are used to investigate the relationship between the number of mast cells in alopecia lesions. In this study, the significance level in the tests is considered less than 5%.

Table (1): Research variables

Scale	Practical definition	qualitative		Quantitative		Dependent	Independent	Variable specifications
		Ratings	Nominal	divided	Continuously			
Relative	Based on the patient's record			*			*	Age
Nominal	Based on patient record (male / female)		*				*	Gender
Nominal	Based on file information (does not have / has)		*				*	Existence of underlying disease

Scale	Practical definition	qualitative		Quantitative		Dependent	Independent	Variable specifications
		Ratings	Nominal	divided	Continuously			
Relative	Duration of diagnosis until biopsy				*		*	Duration of the disease
Relative	Extent of disease involvement (percentage)			*			*	Extent of conflict
Relative	Number of stained cells			*		*		Number of mast cells

4-Findings

In this study, 30 patients including 10 patients with Alopecia areata and 20 patients with Alopecia scarrin were studied. The mean age of patients was 43.62 with a standard deviation of 15.64. Also, the mean age and standard deviation in the control group were 39.95, respectively. This difference was statistically insignificant based on T-test. Also, sex distribution in patients and controls did not show a statistically significant difference. The incidence of autoimmune diseases in AA and AS patients was equal to 2 and 1 patients, respectively (Chart and Table 1).

The mean number of mast cells in AA and AS patients was 18 and 19, respectively, which did not show a significant difference. However, this rate was 18.75 in patients with Alopecia and 6.5 in the control group, which shows a statistically significant difference between the two

Table (2): Patient characteristics

Factor		Alopecia			Control	P Value
		Areata	Scarrin	Areata+Scarrin		
Age		46.12±15	46.12±15	43.62±15.64	39.95±14.53	P=0.199
Gender	Female	3(37.5%)	8(36.4%)	11(37.9%)	8(38.1%)	p=0.61
	Male	5(62.5%)	14(63.6%)	18(62.1%)	13(61.9%)	
Mast cells		18±4.72	19±4.47	18.75±4.55	6.5±3.4	P<0.0001

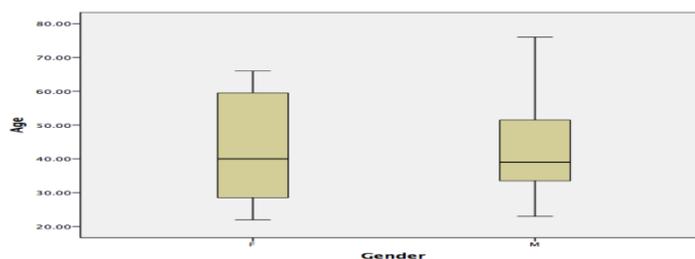


Diagram 1: Comparison of mean age by gender

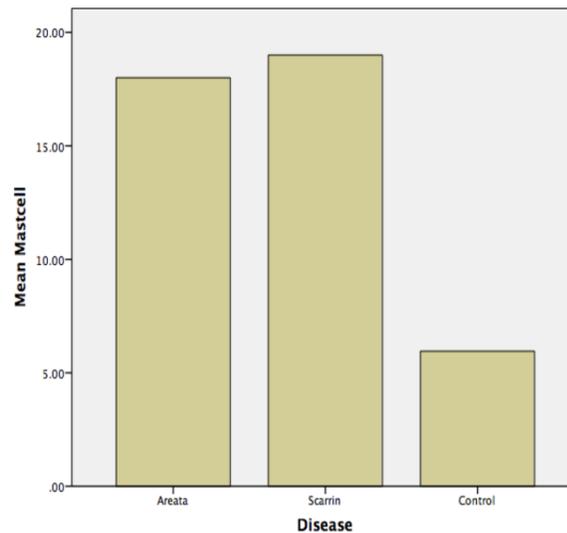


Diagram 2: Comparison of the average of mast cells in all three groups

5-Summary and conclusion

Mast cell cells are naturally present in the skin and lining of the airways as well as in the gastrointestinal tract. The specific granules of these cells contain inflammatory mediators that are normally released only in the presence of the appropriate stimulus. About 10% of mast cell granule proteins contain proteolytic enzymes kimase and tryptase. Mast cells also contain inflammatory mediators of prostaglandins and histamine. These inflammatory mediators are thought to play an important role in allergic reactions, fibrosis, arthritis, angiogenesis, and wound healing. The majority of patients with urticaria pigmentosa have mast cell granulomas in the bone marrow. Most patients with silent skin mastosis also have mast cell secretions out of the skin.

Past studies have partially proven the presence of mast cells in the skin. Keratinocytes produce mast cell growth factors, known as stem cell growth factor or C-kit ligand, and by binding to its receptor, the C-kit, on mast cells are likely to proliferate and accumulate. The etiology of most types of Alopecia remains unknown. Mast cells are involved in the control cycle of hair follicles as well as wound healing and tissue remodeling. Therefore, it is possible that mast cell infiltration into the skull could be the cause of Alopecia. Few studies have been reported on the role of mast cells in Alopecia patients. Mastocytosis was first reported on a case-by-case basis, with two newborns with alopecia and mastocytosis reported in France. However, in the next study evaluating mastostosis in patients with alopecia aerata, they did not observe any abnormal mastocytosis (48). But another study showed that degranulated mast cells abound around lymphocytes in alopecia patients. The results of our study in line with these findings showed that although the amount of mast cells in patients with AA and AS is not significantly different, but this rate in patients with alopecia in general shows a significant increase compared to the control group. A recent study by Kim et al. Also reported a 3-year-old patient with alopecia areata with cutaneous mastocytosis (35).

Zhang et al. In another study on scalp biopsy specimens from 55 patients with AA infiltration examined mast cells, T lymphocytes, and Langerhans cells and showed that mast cell proliferation, especially in the

perifollicular regions and Perivascular was observed and there was a positive and significant relationship with the number of CD8 + T lymphocytes in the deep perifollicular sections (45). In another study, the team looked at the pathological changes in the early stages of AA to identify the role of possible factors in the pathogenesis of the disease in 88 patients. The results showed that mast cells were abundant in the upper dermis, especially around blood vessels, and had a significant relationship with the presence of eosinophils. (32) .Yamakoshi et al. Also histologically examined the skin biopsies of 2 pruritic AA patients, 2 patients with pruritic androgenic alopecia and 2 healthy controls. Toluene staining of skin sections showed a high number of mast cells (total and degranular) around the hair follicles and hair follicles of patients with pruritic AA. As a result, an increase in mast cells and lymphocytes around the hair follicles can play a role in the development of pruritus in AA patients (47). In another study, Cetin et al. Used lesion and non-lesion tissues of 24 patients with AA (including 48 lesion and non-lesion skin specimens) to find a possible link between inflammatory mechanisms, neuropeptide expression, and clinical profile of patients using Immunohistochemical staining was examined. The results showed that CD3 (+), CD8 (+) and CD57 (+) lymphocytes, mast cells, Langerhans cells, NGFR and P (SP) in skin samples with lesion compared to samples without lesion were significantly You had more (10). In a study, Xu et al. Examined the number of mast cells in scalp biopsy specimens using Giemsa staining and tryptase staining. The results showed the presence of mastocytosis in scarring alopecia specimens. Therefore, the high density of mast cells in scarring alopecia biopsy specimens is a key factor in the diagnosis of mild systemic mastocytosis and indicates the involvement of mast cells in the pathogenesis of alopecia (10).

The findings of the present study, in addition to previous studies, show that the number of mast cells in the biopsy of patients with alopecia was significantly higher than normal individuals, which indicates the role of these cells in the pathogenesis of alopecia. Lack of evaluation of immunohistochemical markers of mast cells was one of the limitations of this study

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