UNIVERSIDAD DE GRANADA

TESIS DOCTORAL

FRONTAL FIBROSING ALOPECIA.

CLINICAL, QUALITY OF LIFE AND HISTOPATHOLOGIC ANALYSES.

María Librada Porriño Bustamante

Facultad de Medicina

Programa de Doctorado en Medicina Clínica y Salud Pública

Área de Dermatología

Director: Salvador Arias Santiago

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FRONTAL FIBROSING ALOPECIA.

CLINICAL, QUALITY OF LIFE AND HISTOPATHOLOGICAL ANALYSES.

Frontal fibrosing alopecia.

Clinical, quality of life and histopathological analyses.

Tesis Doctoral que presenta **María Librada Porriño Bustamante** para aspirar al Título de Doctor con Mención Internacional.

Granada, 12 de mayo de 2022

Director de la Tesis Doctoral

Dr. Salvador Arias Santiago

Facultativo Especialista de Área de Dermatología y Venereología

Jefe de Servicio de Área de Dermatología y Venereología en Hospital Universitario Virgen de las Nieves, Granada

Profesor Titular de Dermatología en el Departamento de Medicina de la Universidad de Granada

“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less”.

Marie Curie (1867-1934)

Agradecimientos

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SCIENTIFIC CONTRIBUTIONS

Scientific publications

1. Porriño-Bustamante ML, López-Nevot MÁ, Aneiros-Fernández J, García-Lora E, Fernández-Pugnaire MA, Arias-Santiago S. Familial frontal fibrosing alopecia: A cross-sectional study of 20 cases from nine families. Australas J Dermatol. 2019; 60(2): e113-e118. doi: 10.1111/ajd.12951. Impact factor: 1.78. Dermatology – SCIE Q3 (42/68).

2. Porriño-Bustamante ML, López-Nevot MÁ, Aneiros-Fernández J, Casado-Ruiz J, García-Linares S, Pedrinacci-Rodríguez S, García-Lora E, Martín-Casares MA, Fernández-Pugnaire MA, Arias-Santiago S. Study of Human Leukocyte Antigen (HLA) in 13 cases of familial frontal fibrosing alopecia: CYP21A2 gene p.V281L mutation from congenital adrenal hyperplasia linked to HLA class I haplotype HLA A-*33:01; B*14:02; C*08:02 as a genetic marker. Australas J Dermatol. 2019; 60(3): e195-e200. doi: 10.1111/ajd.12985. Impact factor: 1.78. Dermatology – SCIE Q3 (42/68).

3. Porriño-Bustamante ML, Fernández-Pugnaire MA, Arias-Santiago S. A Cross sectional Study of Rosacea and Risk Factors in Women with Frontal Fibrosing Alopecia. Acta Derm Venereol. 2019; 99(12): 1099-1104. doi: 10.2340/00015555-3286. Impact factor: 4.01. Dermatology – SCIE Q1 (7/68).

4. Porriño-Bustamante ML, Montero-Vílchez T, Pinedo-Moraleda FJ, Fernández-Flores Á, Fernández-Pugnaire MA, Arias-Santiago S. Frontal fibrosing alopecia and sunscreen use: a cross-sectional study of actinic damage. Acta Derm Venereol. 2022. [In press] Impact factor: 4.43. Dermatology – SCIE Q1 (15/69).

5. Porriño-Bustamante ML, Fernández-Pugnaire MA, Castellote-Caballero L, Arias-Santiago S. Colour Doppler Ultrasound study in patients with frontal fibrosing alopecia. Skin Res Technol. 2021; 27(5): 709-714. doi: 10.1111/srt.13004. Impact factor: 2.36. Dermatology – SCIE Q3 (42/69).

6. Porriño-Bustamante ML, Fernández-Pugnaire MA, Arias-Santiago S. Frontal Fibrosing Alopecia: A Review. J Clin Med. 2021; 10(9): 1805. doi: 10.3390/jcm10091805. Impact factor: 4.24. Internal Medicine – SCIE Q1 (39/167).

Oral communications and posters in Conferences

1. "Alopecia frontal fibrosante: a propósito de 9 casos". ML Porriño-Bustamante, S Arias-Santiago, MA López-Nevot, MA Martín-Casares, MA Fernández-Pugnaire, J Aneiros-Fernández. XXIII. Reunión Clínica international de Dermatología, Barcelona. April, 2016. Oral communication.

2. Familial frontal fibrosing alopecia: reporting of 13 cases. Human Leukocyte Antigen (HLA) profile and new histopathological findings. ML Porriño-Bustamante, S Arias-Santiago, E García-Lora, MA López-Nevot, MA Martín-Casares, MA Fernández-Pugnaire, J Aneiros-Fernández. 25th EADV Congress. Vienna. September-October, 2016. Poster.

3. Frontal fibrosing alopecia and rosacea: is there any link? ML Porriño-Bustamante, J Aneiros-Fernández, S Arias-Santiago. 2017 Annual meeting of the American Academy of Dermatology. Orlando. March, 2017. Poster.

4. Study of Human Leukocyte Antigen (HLA) in frontal fibrosing alopecia. ML Porriño-Bustamante, MA López-Nevot, J Aneiros-Fernández, E García-Lora, MA Martín-Casares, MA Fernández-Pugnaire, S Arias-Santiago. 1st European Dermato-epidemiology network (EDEN) forum. Madrid. March, 2017. Poster.

5. "Alopecia frontal fibrosante y rosácea: ¿existe algún vínculo?". ML Porriño-Bustamante, J Aneiros-Fernández, S Arias-Santiago. 45 Congreso Nacional de Dermatología y Venereología. Madrid. May, 2017. Poster.

6. "Ecografía Doppler Color en Alopecia frontal fibrosante". ML Porriño-Bustamante, MA Fernández-Pugnaire, S Arias-Santiago. XXI Reunión Nacional del grupo español de tricología de la AEDV. Madrid, 2019. Poster. Awarded as best poster.

7. "Alopecia frontal fibrosante y uso de fotoprotectores: un estudio transversal sobre daño actínico". ML Porriño Bustamante, FJ Pinedo Moraleda, Á Fernández Flores, T Montero Vílchez, MA Fernández Pugnaire, S Arias Santiago. XXII Reunión Nacional del grupo español de tricología de la AEDV. Barcelona, 2021. Poster.

Placements abroad supporting this doctoral thesis

1. International Clinical Fellow for 1 month and a half at the Department of Dermatology and Venereology and Pediatric Dermatology, of Hospital Ramos Mejía and Hospital Alemán, Buenos Aires (Argentine).

2. International Clinical Fellow for 1 month at the Dermatopathology Department of the University of California, San Francisco, a reference center for dermatopathology.

3. International Clinical Fellow for 1 month at the Dermatology Department, of the "Centro Hospitalar e Universitário de Coimbra" (Portugal), a reference care center for Oncological Surgery.

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ABBREVIATIONS

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AA: Alopecia Areata

AGA: Androgenetic Alopecia

AH: Ancestral Haplotype(s)

AhR: Aryl hydrocarbon Receptor

AhR/KP: Aryl hydrocarbon Receptor-Kyneurine Pathway

ANAs: Antinuclear Antibodies

aOR: adjusted Odds Ratio

BMI: Body Mass Index

CEH: Conserved Extended Haplotypes

CFM: Colour Flow Mode

cGRP: calcitonin Gene-Related Peptide

CI: Confidence Interval

cm: centimetres

CVID: Common Variable ImmunoDeficiency

CYP: Cytochrome

DHEAS: Dehydroepiandrosterone sulfate

DLQI: Dermatology Life Quality Index

DNA: Deoxyribonucleic Acid

e.g.: exempli gratia – abbreviation from Latin - (= for example)

ENA: Extractable Nuclear Antigen

FAPD: Fibrosing Alopecia in a Pattern Distribution

FFA-QLI: Frontal Fibrosing Alopecia Quality of Life Index

FFA: Frontal Fibrosing Alopecia

FFASI: FFA Severity Index

FFASS: FFA Severity Score

FSH: Follicle Stimulating Hormone

GLPLS: Graham-Little-Piccardi-Lasseur Syndrome

GWAS: Genome-Wide Association Study

HADS-A: HADS-Anxiety sub-scale

HADS-D: HADS-Depression sub-scale

HADS: Hospital Anxiety and Depression Scale

HDL: High-Density Lipoprotein

HIV: Human Immunodeficiency Virus

HLA: Human Leukocyte Antigen

i.e.: id est – abbreviation from Latin (= that is)

ICC: Intraclass Correlation Coefficient

IFFACG: International FFA Cooperative Group

IFN: Interferon

IPQ-Q: Revised Illness Perception Questionnaire

JAK: Janus Kinase

kHz: kilohertz

LDL: Low-Density Lipoprotein

LH: Luteinizing Hormone

LLPigm: Lichen Planus Pigmentosus

LPP: Lichen PlanoPilaris

LPPAI: Lichen Planopilaris Activity Index

m/s: metres/second

MHC: Major Histocompatibility Complex

MHz: MegaHertz

mTOR: mammalian Target Of Rapamycin

NRS: National Rosacea Society

OR: Odds Ratio

PPAR: Peroxisome Proliferator-Activated Receptor

PRF: Pulse Repetition Frequency

PRL: Prolactin

PRP: Platelet-Rich Plasma

PSA: Prostate-Specific Antigen

QoL: Quality of Life

ROC: Receiver Operating Characteristic

SD: Standard Deviation

SHBG: Sex Hormone Binding Globulin

T3: Triiodothyronine

T4: Thyroxine

TA: Tractional Alopecia

TGF-β: Transforming Growth Factor-β

Th: T helper

TSH: Thyroid Stimulating Hormone

US: Ultrasound

vs: versus

WAA-QOL: Woman's Androgenetic Alopecia Quality of Life Questionnaire

***Spanish abbreviations**

AFF-ICV: Alopecia Frontal Fibrosante Índice de Calidad de Vida

AFF: Alopecia Frontal Fibrosante

STRUCTURED ABSTRACT

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Introduction

Frontal fibrosing alopecia (FFA) is a scarring alopecia first described in 1994. Since then, its prevalence has been steadily increasing. Nowadays, it is one of the most common types of cicatricial alopecia. FFA is characterized by a frontal and/or temporoparietal hairline recession, leading to a cicatricial alopecic band. Eyebrow alopecia is commonly associated, and eyelash, limb, axillae and pubis alopecia can also be found. Facial papules are noted in some patients, especially over the temples. Some patients may have pruritus and/or trichodynia.

The aetiology of FFA is still unknown, although hormonal and genetic factors are thought to play an important role. The main reasons for considering the involvement of hormones in the pathogenesis are that women, and mostly of menopausal age, are affected more frequently than men, and there is a favourable outcome using 5-alpha reductase inhibitors as a treatment. Familial cases have been reported, although they represent a minority; in fact, familial history of FFA has been reported in up to 8% of patients with FFA. Autoimmunity is also thought to be involved in the aetiopathogenesis of FFA, so different autoimmune conditions have been described together with FFA, especially hypothyroidism. On the other hand, some external substances have also been considered as possible triggers in FFA, especially the use of sunscreens, since a report found a higher use of sunscreens in patients with FFA compared to control subjects, although its real involvement in the development of FFA remains still unclear and controversial.

The main trichoscopic signs in FFA are perifollicular erythema and follicular hyperkeratosis. The absence of follicular openings is a key sign for the diagnosis of scarring alopecia, including FFA. Lonely hair sign is also a common finding. The loss of vellus hair in the hairline implantation is considered an early sign of FFA. The use of other image apparatus to evaluate FFA, such as optical coherence tomography, confocal microscopy, and sonography has been scarce. Sonography is a non-invasive tool which may be helpful in evaluating different skin conditions, although its use in alopecia, especially in FFA, is really limited, and the sonographic signs of FFA are not well known.

Quality of life (QoL) is known to be affected in patients with alopecia, including some scarring types. Women with alopecia can consider themselves to be less feminine, as hair is an

important element in identity and self-image. Questionnaires which are used to assess QoL in alopecia are usually the same ones used with general skin diseases, such as the Dermatology Quality Life Index (DLQI). No specific questionnaire has been developed for the assessment of QoL impairment in FFA.

Histopathologically, FFA is characterized by a lichenoid lymphocytic infiltrate, located mainly around the upper follicle, that is isthmus and infundibulum, including the bulge area – where stem cells are placed -, as well as concentric perifollicular lamellar fibrosis. The loss of sebaceous glands is considered an early finding in FFA. Moreover, the inflammatory infiltrate, and also sebaceous gland atrophy and perifollicular fibrosis, have also been observed in normal-appearing scalp in patients with FFA.

Despite the increasing interest from the dermatologists to unravel the enigmas around FFA, many questions are still waiting to be answered. Is FFA associated with another skin condition? If FFA is considered to be a disease which occurs when predisposed people are exposed to an unknown trigger, is there any genetic profile which may be a marker of a higher risk of developing FFA? Do the familial cases of FFA share the clinical features of non-familial cases of FFA? And what about sunscreens? Could the higher use of sunscreens found in FFA patients be due to another reason? If FFA patients are habitual users of sunscreens, do they have less actinic damage? In relation to the use of sonography to assess FFA, are there sonographic differences in the varied areas of a scalp in a patient with FFA? And regarding QoL disorders in FFA, have patients with FFA an impairment in their QoL? Could a specific questionnaire for the assessment of the QoL in FFA be more precise than the pre-existing ones? Finally, the presence of inflammatory infiltrate and perifollicular fibrosis in normal-appearing scalp in FFA patients have been detected, but are there more histopathological alterations in normal-appearing scalp areas in patients with FFA? Are there any differences with the histopathological features in the hairline implantation and the normal-appearing scalp?

Material and methods

Different studies were performed regarding FFA. For these studies, patients with FFA and a group of healthy control subjects were recruited from the University Hospital San Cecilio of Granada. First, a wide range of information was collected from all of the participants, in order

to perform investigations in several areas related to FFA. Demographic information about the participants, such as age, ethnicity, hormonal status and age of menopause, were recorded. In addition, the use of sunscreens and the presence of peripheral alopecia were assessed in both groups. In FFA patients, data regarding the alopecia, including the age of onset, the severity grade, the presence of facial papules, the existence of symptoms – pruritus and trichodynia -, or the current treatments for FFA, were registered. The coexistence of FFA with other types of alopecia, such as androgenetic alopecia (AGA) and lichen planopilaris (LPP), was also recorded. The presence of trichoscopic signs of FFA was evaluated, that is perifollicular erythema, follicular hyperkeratosis, white dots, red dots, absence of vellus hair in the hairline implantation, lonely hair sign, ivory white background and prominent and branched vessels. Specific variables were collected for some of the studies and are listed below.

1) Clinical study about familial FFA: a cross-sectional study was conducted. The participants had FFA and had at least one first-degree relative who was also affected with FFA.

2) Human Leukocyte Antigen (HLA) study in familial FFA: a case-control study was performed. All participants were given blood tests and their HLA class I and II alleles were determined and compared to the HLA profile of healthy control individuals. Investigation of mutations of CYP21A2, the gene encoding the enzyme 21-hydroxylase, was also performed on all participants.

3) Association with rosacea and hormonal disturbances in FFA patients: a cross-sectional study, including a control group, was carried out. The body mass index (BMI) was calculated and the presence of comorbidities such as diabetes, arterial hypertension or dyslipidaemia was registered. The coexistence with clinical signs of rosacea, in any of its forms, was recorded. Hormonal blood test, including follicle-hormone stimulating hormone (FSH), luteinizing-hormone (LH), oestradiol, progesterone, testosterone, sex hormone binding globulin (SHBG), prolactin (PRL), 17-hydroxy-progresterone, dehydroepiandrosterone sulfate (DHEAS), androstenedione and dihydrotestosterone, were conducted on all patients.

4) FFA and sunscreens. Assessment of the actinic damage in FFA patients: a cross-sectional study, including a control group, was performed. The skin phototype was assessed in both groups. The presence of cutaneous signs of actinic damage, such as solar lentigines, actinic keratoses, and basal cell or squamous cell carcinomas were recorded in both groups. A punch biopsy of the progression hairline was performed on 52 FFA patients.

5) Sonography study in FFA: a cross-sectional study, including a control group, was carried out. Ultrasound equipment with a lineal probe of 18 MHz was used for the evaluation of the participants, with the colour flow mode of the colour Doppler. Three areas were assessed in FFA patients: the alopecic band, the hairline implantation and the normal-appearing area behind the hairline ("healthy area", which was chosen on the basis of the absence of trichoscopic signs of FFA). In control participants, hairline implantation and an area behind the hairline were evaluated. In each area, vessel diameter and flow of the two most significant vessels were evaluated, and the mean values were calculated.

6) Development of a specific and validated QoL questionnaire for FFA: a cross-sectional study, including a control group, was performed. A specific questionnaire was designed based on specific clinical features of FFA and was named Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI). Three questionnaires were administered to both groups, the Dermatology Life Quality Index (DLQI), the Hospital Anxiety and Depression Scale (HADS) and the newly designed FFA-QLI. FFA-QLI consisted of 20 questions, and the responses were scored from 0 (not affected at all) to 3 (highly affected), so the total score ranged from 0 (best QoL) to 60 (worst QoL). The psychometric validation was based on the DLQI.

7) Histopathologic analysis in FFA: a cross-sectional study was designed. Two dermoscopy-guided 4-mm punch scalp biopsies were performed on all patients: the first one from the frontal hairline implantation, in the most clinically affected area (B1); and the second from a "normal-appearing" parietal area, based on the absence of trichoscopic signs of FFA (B2). The specimens were processed and stained with hematoxylin and eosin, and cut into vertical sections. Orcein stain was used to assess elastic fibres.

Results

For the different studies, 101 patients with FFA and 40 healthy control subjects were included. All of them were women. Moreover, 13 patients, including 2 men, were added for the familial studies.

1) Clinical study about familial FFA: 20 patients with FFA, belonging to nine different families, were included in the familial study. The most frequent connection between participants was found to be siblings. Eighteen out of twenty patients were women. Regarding hormonal

status, 66.7% of women were postmenopausal at the onset of the alopecia, and one of the male patients had a primary panhypopituitarism. In relation to the two mother-daughter families, the debut of the alopecia was earlier in the daughters than in their mothers, and the mothers had a larger size of the recession band than their daughters. The brother with the hormonal impairment had had an earlier onset of the alopecia, compared to his brother. All patients had eyebrow alopecia (partial or total) and 70% of them had body hair loss. Regarding trichoscopic signs, perifollicular erythema and follicular hyperkeratosis were both found in 85% of patients, ivory white background in 73.7%, white dots in 63%, vellus absence in the hairline in 79%, lonely hair sign in 68% and red dots in 5% of them. Facial papules were noted in 25% of patients.

2) HLA study in familial FFA: 13 patients with FFA from six families were included. Their HLA profiles were compared to the HLA profile of 636 healthy control individuals. All patients, except for the two brothers, were female. HLA class I haplotype HLA-A*33:01; B*14:02; C*08:02 was shared by 61.5% (8/13) of patients, from four different families, whereas only 3.3% of control subjects had it (p_c <0.000001). Moreover, all of these patients were heterozygous for the p.V281L mutation of the CYP21A2 gene. In addition, the five patients from three different families who did not share the previous haplotype (5/13, 38.5%), shared HLA-B*07:02; C*07:02; four of them, from two families, with the allele HLA-A*03:01, and the other one, from another family, with the allele HLA-A*24:02.

3) Association with rosacea and hormonal disturbances in FFA patients: 99 women with FFA and 40 control subjects were included. No significant differences regarding the presence of arterial hypertension, diabetes or dyslipidaemia were observed between patients and control participants. However, FFA patients had a significantly higher BMI than the control subjects (28.7 vs 26.4 kg/m^2, p = 0.013). Regarding the use of sunscreens, 83.2% of FFA patients and 62.5% of control individuals used them (p = 0.014). No significant differences regarding sex hormone values were noted between patients and control subjects, except in DHEAS, which was lower in patients (66.1 vs 91.9 μg/dl, p = 0.047). Clinical signs of rosacea were present in 61.6% of the patients compared to 30% of the control individuals (p = 0.001), the erythematotelangiectatic form being the most frequent one. Moreover, patients with a more severe grade of FFA were more likely to have rosacea than those with mild grades of alopecia (77.8% in grade V vs 33.3% in grade I, p = 0.02). The presence of pruritus or trichodynia were

not related to a higher prevalence of rosacea. Regarding trichoscopy, only the presence of perifollicular erythema correlated significantly with rosacea (64.7% in patients with rosacea vs 32.6% in patients without rosacea, p = 0.002). Patients with rosacea presented a higher BMI than patients without rosacea (29.5 vs 27.3 kg/mg^2, p = 0.016). No significant hormonal differences were noted between patients with or without rosacea, except in levels of LH, progesterone and dihydrotestosterone, which were significantly lower in patients with rosacea and FFA. After binary logistic regression, perifollicular erythema, BMI and progesterone levels were associated with the presence of rosacea in patients with FFA.

4) FFA and sunscreens. Assessment of the actinic damage in FFA patients: 101 women with FFA and 40 control women were included. Signs of actinic damage were noted in 69.3% of patients compared to 50% of the control participants (p = 0.031), and this difference was also observed in the presence of solar lentigines. Moreover, after multivariate analysis, independently of the skin phototype, FFA was associated with the presence of actinic damage (p = 0.045), especially in the form of solar lentigines (p = 0.029). Concerning the use of sunscreens, 83.2% of FFA patients used them, compared to 62.5% of the control participants (p = 0.008). No differences between FFA patients who used sunscreens and those who did not use them were found regarding the presence of peripheral alopecia, the presence of inflammatory trichoscopic signs or the presence of histopathologic anomalies (such as sebaceous gland involvement and the presence of inflammatory infiltrate). In addition, after conducting a logistic regression model, it was observed that the use of sunscreens and the presence of actinic damage were independent factors related to FFA.

5) Sonography study in FFA: 99 FFA women with FFA and 40 control subjects were included. Patients showed a significantly higher vessel diameter and flow in the hairline implantation than control subjects (0.127 cm vs 0.103 cm, p = 0.03; and 8.183 m/s vs 7.670 m/s, p = 0.05, respectively), and also a significantly higher vessel diameter in the healthy scalp (0.088 cm vs 0.078 cm, p = 0.03). Comparing different areas in FFA patients, a significantly higher vessel diameter was found in the hairline implantation compared to the healthy scalp (0.127 cm vs 0.088 cm, p = 0.01) and also compared to the alopecic band (0.127 cm vs 0.110 cm, p = 0.01). The presence of prominent and branched vessels on trichoscopy was noted in 36.4% of FFA patients, and there were no statistical differences in the presence of them in patients who used topical corticosteroids compared to those who did not use them (p = 0.45). Patients with

prominent and branched vessels in dermoscopy presented a significantly higher flow in the hairline implantation area (8.52 m/s vs 7.98 m/s, p = 0.05) and also a significantly higher vessel diameter in the healthy scalp area (0.096 cm vs 0.084 cm, p = 0.03), compared to those without this trichoscopic sign. No differences in vessel diameter or flow were observed in patients who were under treatment with topical corticosteroids compared to the non-users.

6) Development of a specific QoL questionnaire for FFA: 101 women with FFA and a control group of 40 women were included. The mean FFA-QLI score in patients was 17.11, whereas in control subjects it was 0.98 (p <0.001). Regarding the validation of FFA-QLI, the value for internal consistency (Cronbach α) was 0.685 and the intraclass correlation coefficient between all the items in the questionnaire was 0.870. FFA-QLI correlated positively with DLQI (r=0.729, p<0.001). A test-retest was performed in 30 FFA patients. Patients with more severe grades of FFA showed a higher FFA-QLI score compared to those with mild disease (19.72 vs 14.11, respectively, p = 0.002) and the area under the curve for identifying severe disease was greater in FFA-QLI than in DLQI (area under curve = 0.704, p < 0.001 vs area under curve = 0.603, p = 0.076, respectively). To select patients with mild, moderate and severe impairment in QoL, the following cut-off points were selected: a score <21 in FFA-QLI corresponded with a low impact on QoL (sensitivity 88.9%, specificity 80.7%), values >35 matched with greater QoL impairment (sensitivity 75%, specificity 95.9%) and values ranging from 21 to 35 corresponded to moderate QoL alteration (sensitivity 85.7%, specificity 80.7%).

7) Histopathologic analysis in FFA: 52 women with FFA were included, so 104 scalp biopsies were processed. Sebaceous glands were reduced or absent in 80.8% (42/52) of the B1 samples compared to 42.3% (22/52) of the B2 biopsies (p = 0.001). Inflammatory infiltrate was found in both areas with no significant differences, although it was more severe in B1 (p = 0.013). The inflammatory infiltrate involved the dermis in 88.5% (46/52) of the B1 biopsies compared to 71.2% (37/52) of the B2 samples (p = 0.035). Follicular epithelium changes were noted in 70.6% (36/51) of the B1 samples, whereas they were seen in only 48.1% (25/52) of the B2 biopsies (p = 0.012). Vacuolar degeneration of the basal layer, necrosis of keratinocytes and increased apoptotic activity in the outer root sheath, were all more significantly more common in B1 than in B2 (p = 0.001). Perifollicular fibrosis was observed in 71.2% (37/52) and 30.8% (16/52) of B1 and B2 biopsies, respectively (p<0.001).

Conclusions

1) The most common familial connection between patients in the familial FFA study was found to be between sisters. Clinical and dermoscopic features were concordant with those reported in non-familial cases of FFA. HLA class I haplotype HLA-A*33:01; B*14:01; C:08:02 may predispose familial FFA. This haplotype was linked to CYP21A2 gene p.V281L mutation, since all patients who shared that haplotype were heterozygous to this mutation. FFA may be an antigen-driven disease in these susceptible patients.

2) Rosacea was more frequent in patients with FFA, and even more in severe grades of alopecia. After multivariate analysis, perifollicular erythema, higher body mass index and lower progesterone levels, were associated to rosacea in FFA patients.

3) Patients with FFA had a higher use of sunscreens than control group individuals, but they also had greater actinic damage, especially in the form of solar lentigines. No differences regarding the presence of trichoscopic inflammatory signs, histopathologic anomalies or the presence of peripheral alopecia were noted between FFA patients who used sunscreens and those who did not.

4) An assessment with sonography showed that patients with FFA had higher vessel diameter and flow in the hairline implantation compared to control subjects. They also had higher vessel diameter in the healthy scalp. The presence of branched vessels on trichoscopy images of FFA patients was related to a higher vessel flow in the hairline implantation and higher vessel diameter in the healthy scalp.

5) A specific and validated QoL questionnaire for FFA is presented, called FFA-QLI, with a higher power than the DLQI to select severe cases of FFA. Around a third of FFA patients showed at least a moderate impairment of their QoL after evaluating it with the FFA-QLI.

6) All the histopathologic alterations described in the hairline implantation in FFA were found in the normal-appearing scalp in those patients. The inflammatory infiltrate was more severe in the hairline implantation. The presence of sebaceous gland involvement (reduction or absence), follicular epithelium changes and perifollicular fibrosis, were significantly more frequent in the hairline implantation.

RESUMEN ESTRUCTURADO

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Introducción

La alopecia frontal fibrosante (AFF) fue descrita en el año 1994, y su prevalencia ha aumentado progresivamente desde entonces. Actualmente es uno de los tipos más frecuentes de alopecia cicatricial. La AFF se caracteriza por un retroceso de la línea de implantación frontal y/o temporoparietal, que conduce a una banda de alopecia cicatricial. Comúnmente se asocia con alopecia de cejas, pero también puede aparecer conjuntamente con alopecia de pestañas, miembros, axilas y pubis. En algunos pacientes pueden apreciarse pápulas faciales, especialmente en las sienes. Aunque puede ser asintomática, algunos pacientes con AFF refieren prurito o tricodinia. La etiología de la AFF permanece aún desconocida, pero se piensa que los factores hormonales y genéticos desempeñarían un papel relevante. Las principales razones para considerar que las hormonas están implicadas en la patogenia de la enfermedad son la mayor prevalencia de AFF en mujeres, sobre todo en edad postmenopáusica, y la buena respuesta al tratamiento con inhibidores de la 5-alfa reductasa. Los casos familiares de AFF son una minoría, pero se ha encontrado que hasta el 8% de pacientes con AFF tienen antecedentes familiares de la misma.

La autoinmunidad también se cree que está implicada en la etiopatogenia de la AFF, ya que se han descrito diferentes trastornos autoinmunes asociados a la AFF, especialmente hipotiroidismo. Por otro lado, es probable que haya alguna sustancia exógena que actúe como desencadenante del proceso, y los fotoprotectores son los principales agentes externos que han sido señalados en diversos estudios, desde que se publicó un mayor uso de los mismos en pacientes con AFF en comparación con un grupo control. No obstante, la real implicación de los fotoprotectores en el desarrollo de la AFF es aún controvertida.

Los principales signos tricoscópicos en AFF son el eritema perifolicular y la hiperqueratosis folicular. La ausencia de orificios foliculares es un dato clave para el diagnóstico de las alopecias cicatriciales, incluyendo la AFF. El signo del pelo solitario, en la línea original de implantación, es otro hallazgo común. La pérdida de pelo velloso en la línea de implantación se considera un signo precoz de AFF. Otros dispositivos de imagen han sido empleados para evaluar pacientes con AFF, como la tomografía de coherencia óptica, la microscopía confocal y la ecografía. La ecografía es una herramienta no invasiva que puede ser de ayuda en

diferentes entidades cutáneas, aunque su uso en alopecias, especialmente en AFF, es limitado, por lo que los hallazgos ecográficos de la AFF no han sido bien definidos.

Los pacientes con alopecia pueden tener afectada la calidad de vida, y esto sucede especialmente en alopecias cicatriciales. Las mujeres con alopecia pueden sentirse menos femeninas, ya que el cabello es un elemento físico clave en la identidad personal y la auto-imagen. Los cuestionarios que se usan habitualmente para evaluar la calidad de vida en pacientes con alopecia son generalmente los que se emplean en otras patologías cutáneas, como el Índice de Calidad de Vida en Dermatología ("Dermatology Life Quality Index" – DLQI). No hay actualmente ningún cuestionario específico para valorar la afectación de la calidad de vida en pacientes con AFF.

Las principales características histopatológicas de la AFF son la presencia de un infiltrado linfocítico liquenoide ubicado sobre todo alrededor de la parte superior del folículo, es decir, istmo e infundíbulo, incluyendo el área del "bulge" (donde se encuentran las células madre foliculares), así como la presencia de fibrosis lamelar perifolicular concéntrica. La pérdida de las glándulas sebáceas es considerada un signo precoz de AFF. Además, el infiltrado inflamatorio, así como atrofia de glándulas sebáceas y fibrosis perifolicular, también han sido detectados en zonas aparentemente normales del cuero cabelludo en pacientes con AFF.

A pesar del gran interés de la comunidad dermatológica por descifrar los enigmas que existen en torno a la AFF, existen todavía numerosas preguntas pendientes de responder. ¿Está la AFF asociada a alguna otra patología cutánea? Si la AFF se considera una enfermedad que ocurre cuando personas predispuestas se exponen a un desencadenante aún desconocido, ¿hay algún perfil genético que pueda ser marcador de riesgo para el desarrollo de AFF? ¿Tienen los pacientes con AFF familiar las mismas características clínicas que los casos no familiares? ¿Y qué sucede con los fotoprotectores? ¿Puede ser que el mayor uso de fotoprotectores en pacientes con AFF se deba a otro motivo? Si los pacientes con AFF utilizan habitualmente fotoprotección, ¿tienen un menor daño actínico? En relación a la evaluación de pacientes con AFF mediante ecografía cutánea, ¿hay diferencias ecográficas en diferentes áreas del cuero cabelludo de un paciente con AFF? Y en relación a la calidad de vida en pacientes con AFF, ¿tienen las pacientes con AFF una alteración en la calidad de vida? ¿Podría un cuestionario desarrollado específicamente para la AFF ser más preciso y detectar más casos de alteración de la calidad de vida que los cuestionarios generales ya existentes? Finalmente, se ha

observado la presencia de infiltrado inflamatorio y fibrosis perifolicular en zonas de cuero cabelludo aparentemente sanas en pacientes con AFF, pero ¿existen más alteraciones histopatológicas en zonas de cuero cabelludo aparentemente normales en pacientes con AFF? ¿Hay diferencias en los hallazgos histológicos en comparación con los que se encuentran en la línea de implantación?

Material y métodos

Se han realizado diferentes estudios en relación a la AFF. Para ellos, se incluyó un grupo de pacientes y otro de controles sanos, recogidos del Hospital Universitario San Cecilio de Granada. Una amplia cantidad de información fue recogida de todos los participantes para poder realizar las investigaciones pertinentes en relación a diferentes aspectos estudiados de la AFF. Se registró información demográfica de todos los participantes, como edad, etnia, estado hormonal y edad de la menopausia. El uso de fotoprotectores y la presencia de alopecia periférica fue registrado en ambos grupos. En los pacientes se recogió la edad de debut de la alopecia, el grado de severidad, la presencia de pápulas faciales, la existencia de síntomas (prurito, tricodinia) y los tratamientos actuales para la AFF. La coexistencia de la AFF con otros tipos de alopecia, como alopecia androgénica o liquen plano folicular, también fue registrada. Se valoró la presencia de signos tricoscópicos de AFF, como eritema perifolicular, hiperqueratosis folicular, puntos blancos, puntos rojos, ausencia de vello en la línea de implantación, signo del pelo solitario, fondo blanco y vasos ramificados. Algunas variables específicas que se evaluaron para estudios concretos se especifican a continuación.

1) Estudio clínico sobre AFF familiar: se realizó un estudio transversal, en el que los participantes tenían AFF y al menos un familiar de primer grado que también tenía AFF.

2) Estudio de antígeno leucocitario humano (HLA por sus siglas en inglés, Human Leukocyte Antigen) en AFF familiar: se realizó un estudio de casos y controles. Se hizo un análisis de sangre de los participantes para determinar los alelos HLA clase I y clase II, que posteriormente fueron comparados con el perfil HLA de controles sanos. Además, se determinó en los pacientes la presencia de mutaciones en el gen CYP21A2, que codifica la enzima 21-hidroxilasa.

3) Asociación con rosácea y alteraciones hormonales en pacientes con AFF: se realizó un estudio transversal, que incluyó un grupo control. Se calculó el índice de masa corporal y se registró la presencia de comorbilidades como diabetes, hipertensión arterial o dislipemia. Se evaluó la coexistencia de la AFF con signos clínicos de rosácea, en cualquiera de sus formas. El estudio hormonal incluyó la determinación de la hormona folículo-estimulante, hormona luteinizante, estradiol, progesterona, testosterona, globulina transportadora de hormonas sexuales, prolactina, 17-hidroxi-progesterona, dehidroepiandrosterona sulfato, androstendiona y dihidrotestosterona.

4) AFF y fotoprotectores. Evaluación del daño actínico en pacientes con AFF: se realizó un estudio transversal, que incluyó un grupo control. En ambos grupos se registró el fototipo cutáneo. La presencia de signos cutáneos de daño actínico, como lentigos solares, queratosis actínicas y carcinomas basocelular y epidermoide fue evaluada en ambos grupos. Una biopsia punch de la línea de progresión fue realizada en 52 pacientes con AFF.

5) Estudio ecográfico en AFF: se realizó un estudio transversal, que incluyó un grupo control. Un equipo de ecografía con una sonda lineal de 18 MHz se utilizó para evaluar las variables mediante el modo Doppler color. Tres zonas fueron analizadas mediante el ecógrafo: la banda alopécica, la línea de implantación, y un área de cuero cabelludo aparentemente normal posterior a la línea de implantación que fue seleccionada en base a la ausencia de signos tricoscópicos de AFF. En el grupo control sólo se evaluó la línea de implantación y una zona posterior a dicha línea. En cada área se estudió el diámetro vascular y el flujo de los dos vasos más significativos y se calcularon sus valores medios.

6) Desarrollo de un cuestionario específico y validado para evaluar la calidad de vida en pacientes con AFF: se realizó un estudio transversal, que incluyó un grupo control. Se diseñó un cuestionario específico basado en características clínicas específicas de AFF, llamado en inglés “Frontal Fibrosing Alopecia-Quality of Life Index” (FFA-QLI). Se administraron tres cuestionarios a los dos grupos, el DLQI, el HADS (“Hospital Anxiety and Depression Scale”) y el nuevo FFA-QLI. El FFA-QLI consistía en 20 preguntas, y las respuestas fueron valoradas desde 0 (no afectado en absoluto) a 3 (muy afectado), de manera que la puntuación total se extendía de 0 (mejor calidad de vida) a 60 (peor calidad de vida). La validación psicométrica se basó en el DLQI.

7) Análisis histopatológico en AFF: se realizó un estudio transversal. Dos biopsias guiadas por dermatoscopia fueron realizadas en todos los pacientes.: la primera de la línea frontal de implantación (B1), de la zona con más signos clínicos de afectación; y la segunda de una zona de cuero cabelludo aparentemente normal (B2), en la región parietal, seleccionada en base a la ausencia de signos tricoscópicos de AFF. Las biopsias fuero procesadas y teñidas con hematoxilina y eosina, y divididas en secciones verticales. La tinción de orceína se empleó para valorar las fibras elásticas.

Resultados

Para los diferentes estudios se incluyeron 101 pacientes con AFF y 40 controles sanos. Todos los participantes eran mujeres. Además, 13 pacientes más, incluidos 2 varones, fueron añadidos para los estudios familiares.

1) Estudio clínico sobre AFF familiar: se incluyeron 20 pacientes pertenecientes a 9 familias. La relación de parentesco más habitual fue la hermandad. Dieciocho de los veinte pacientes eran mujeres. En relación al estado hormonal, el 66,7% de las mujeres eran postmenopáusicas al inicio de la alopecia, y uno de los varones tenía un panhipopituitarismo primario. En las dos familias compuestas por madre e hija, el debut de la alopecia fue más precoz en las hijas, y las madres tenían una mayor banda de recesión. El varón con la alteración hormonal había tenido un debut más precoz de la alopecia que su hermano. Todos los pacientes tenían algún grado de alopecia de cejas (parcial o total) y el 70% de ellos tenían pérdida del pelo corporal. En relación a los signos tricoscópicos, se apreció eritema perifolicular e hiperqueratosis folicular en el 85% de pacientes, fondo blanco en el 73,7%, puntos blancos en el 63%, ausencia de pelo velloso en la línea de implantación en el 79%, signo del pelo solitario en el 68% y puntos rojos en el 55% de ellos. La presencia de pápulas faciales fue observada en el 25% de los pacientes.

2) Estudio de HLA en AFF familiar: se incluyeron 13 pacientes con AFF pertenecientes a seis familias. Sus perfiles HLA fueron comparados con los de 636 controles sanos. Todos los pacientes eran mujeres, salvo dos hermanos. El haplotipo HLA clase I, HLA-A*33:01; B*14:02; C*08:02, lo compartían el 61,5% (8/13) de los pacientes, de cuatro familias diferentes, mientras que sólo el 3,3% de los controles lo tenían (pc <0,000001). Además, todos estos pacientes eran heterocigotos para la mutación p.V281L del gen CYP21A2. Por otro lado, los

cinco pacientes restantes, de tres familias diferentes, que no compartían el referido haplotipo (5/13, 38,5%), tenían en común el HLA-B*07:02; C*07:02; cuatro de ellos, de dos familias, con el alelo HLA-A*33:01, y el otro, de otra familia, con el alelo HLA-A*24:02.

3) Asociación con rosácea y alteraciones hormonales en pacientes con AFF: se incluyeron 99 mujeres con AFF y 40 controles sanos. No se observaron diferencias significativas entre los grupos en relación a la presencia de hipertensión arterial, diabetes o dislipemia. Sin embargo, las pacientes tenían un índice de masa corporal significativamente mayor que el grupo control (28,7 vs 26,4 kg/m2, p = 0,013). En relación al uso de fotoprotectores, eran utilizados por el 83,2% de pacientes y el 62,5% de controles (p = 0,014). No se encontraron diferencias significativas en relación al valor de las hormonas sexuales en ambos grupos, salvo en el de la DHEAS, que fue inferior en el grupo de pacientes (66,1 vs 91,9 μg/dl, p = 0,047). Se observaron signos clínicos de rosácea en el 61,6% de las pacientes y en el 30% de controles (p = 0,001), y la forma eritematotelangiectásica fue la más frecuente. Además, la probabilidad de tener rosácea era mayor en las pacientes con grados más severos de AFF respecto a las que tenían una AFF más leve (77,8% para grado V vs 33,3% para grado I, p = 0,02). La presencia de prurito y tricodinia no se relacionó con una mayor prevalencia de rosácea. En relación a los signos tricoscópicos, sólo la presencia de eritema perifolicular se correlacionó significativamente con la presencia de rosácea (64,7% en pacientes con rosácea vs 32,6% en pacientes sin rosácea, p = 0,002). Las pacientes con rosácea tenían un índice de masa corporal mayor que las pacientes sin rosácea (29,5 vs 27,3 kg/m2, p = 0,016). No se apreciaron diferencias hormonales significativas entre pacientes con o sin rosácea, excepto en los niveles de hormona luteinizante, progesterona y dihidrotestosterona, que fueron significativamente menores en pacientes con rosácea y AFF. Tras una regresión logística binaria, el eritema perifolicular, el índice de masa corporal y los niveles de progesterona se mostraron asociados a la presencia de rosácea en pacientes con AFF.

4) AFF y fotoprotectores. Evaluación del daño actínico en pacientes con AFF: se incluyeron 101 mujeres con AFF y 40 controles. Se observaron signos de daño actínico en el 63,9% de las pacientes y el 50% del grupo control (p = 0,031), y esta diferencia se mantuvo en relación a la presencia de lentigos solares. Además, tras realizar un análisis multivariante, la AFF se mostró asociada con la presencia de daño actínico (p = 0,045), especialmente en forma de lentigos solares (p = 0,029), independientemente del fototipo. Sobre el uso de fotoprotectores, el

83,2% de las pacientes los usaban, comparado con el 62,5% del grupo control (p = 0,008). No se apreciaron diferencias entre las pacientes que usaban fotoprotectores y las que no en relación a la presencia de alopecia periférica o de signos tricoscópicos de inflamación, ni tampoco en relación a la existencia de alteraciones histopatológicas (afectación de glándulas sebáceas y presencia de infiltrado inflamatorio). Tras realizar una regresión logística se comprobó que el uso de fotoprotectores y la presencia de daño actínico se comportaban como factores independientes relacionados con la AFF.

5) Estudio ecográfico en AFF: se incluyeron 99 pacientes con AFF y 40 controles. Las pacientes tenían un diámetro y flujo vascular significativamente mayor en la línea de implantación que el grupo control (0,127 cm vs 0,103 cm, p = 0,03; y 8,183 m/s vs 7,670 m/s, p = 0.05, respectivamente), y también un diámetro vascular significativamente mayor en el cuero cabelludo sano (0,088 cm vs 0,078 cm, p = 0,03). Al comparar diferentes áreas en pacientes con AFF, se observó un diámetro vascular significativamente mayor en la línea de implantación que en el cuero cabelludo sano (0,127 cm vs 0,088 cm, p = 0,01) y que en la banda alopécica (0,127 cm vs 0,110 cm, p = 0,01). La presencia de vasos ramificados en la tricoscopia se apreció en el 36,4% de las pacientes, sin diferencias significativas en relación a su presencia en las pacientes que usaban corticoides tópicos y aquellas que no los utilizaban (p = 0,45). Las pacientes con presencia de estos vasos tenían un flujo significativamente mayor en la línea de implantación (8,52 m/s vs 7,98 m/s, p = 0,05) y también un diámetro vascular significativamente mayor en el cuero cabelludo sano (0,096 cm vs 0,084 cm, p = 0,03), respecto a aquellas pacientes sin vasos ramificados. No se apreciaron diferencias en el diámetro ni flujo vascular en las pacientes que estaban en tratamiento con corticoides tópicos respecto a las que no lo estaban.

6) Desarrollo de un cuestionario específico y validado para evaluar la calidad de vida en pacientes con AFF: se incluyeron 101 mujeres con AFF y 40 controles. El valor medio del FFA-QLI en pacientes fue de 17,11, mientras que en el grupo control fue de 0,98 (p <0,001). En relación a la validación del cuestionario, el valor de la consistencia interna (Cronbach α) fue de 0,685 y el coeficiente de correlación intraclase entre todos los ítems fue de 0,870. El FFA-QLI se correlacionó positivamente con el DLQI (r=0,729, p<0,001). Un test-retest se hizo en 30 pacientes con AFF. Las pacientes con grados más severos de AFF mostraron una mayor puntuación en el FFA-QLI en comparación con aquellas con enfermedad más leve (19,72 vs

14,11, respectivamente, p = 0,002) y el área bajo la curva para la identificación de enfermedad severa fue mayor en el FFA-QLI que en el DLQI (área bajo la curva = 0,704, p < 0,001 vs área bajo la curva = 0,603, p = 0,076, respectivamente). Para seleccionar a las pacientes con afectación de la calidad de vida leve, moderada o severa, se establecieron los siguientes puntos de corte: una puntuación <21 en el FFA-QLI para indicar bajo impacto en la calidad de vida (sensibilidad 88,9%, especificidad 80,7%), valores >35 indicarían gran impacto en la calidad de vida (sensibilidad 75%, especificidad 95,9%), y valores de 21 a 35 se corresponderían con una afectación moderada (sensibilidad 85,7%, especificidad 80,7%).

7) Análisis histopatológico en AFF: se incluyeron 52 mujeres, lo que supuso 104 biopsias. Las glándulas sebáceas estaban reducidas o ausentes en el 80,8% (42/52) de las muestras de B1 respecto al 42,3% (22/52) de las de B2 (p = 0,001). La presencia de infiltrado inflamatorio se observó en ambas áreas sin diferencias significativas, pero con mayor severidad en B1 (p = 0,013). La dermis estaba afectada por el infiltrado inflamatorio en el 88,5% (46/52) de las muestras de B1 en comparación con el 71,2% (37/52) de las de B2 (p = 0,035). Se apreciaron cambios en el epitelio folicular en el 70,6% (36/51) de las biopsias de B1, y sólo en el 48,1% (25/52) de las de B2 (p = 0,012). La presencia de degeneración vacuolar de la capa basal, de necrosis de queratinocitos y de actividad apoptótica aumenta en la vaina radicular externa, fue significativamente más frecuente en B1 que en B2 (p = 0,001). Se encontró fibrosis perifolicular en el 71,2% (37/52) y 30,8% (16/52) de las biopsias de B1 y B2, respectivamente (p<0,001).

Conclusiones

1) La mayoría de los casos familiares de AFF se dieron entre hermanas. Los signos clínicos y tricoscópicos fueron concordantes con los publicados en casos no familiares de AFF. El haplotipo HLA-A*33:01; B*14:01; C:08:02 podría predisponer a la AFF familiar. Este haplotipo estaba ligado a la mutación p.V281L del gen CYP21A2, ya que todos los pacientes que compartían el referido haplotipo eran heterocigotos para dicha mutación. La AFF se comportaría como una enfermedad “antigen-driven” en esos pacientes susceptibles.

2) Se observó mayor prevalencia de rosácea en pacientes con AFF, y aún más en aquellas con grados severos de alopecia. Tras realizar un análisis multivariante, la presencia de eritema

perifolicular, un mayor índice de masa corporal y menores niveles de progesterona, se mostraron asociados a la rosácea en pacientes con AFF.

3) Las pacientes con AFF mostraban un mayor uso de fotoprotectores que el grupo control, pero también tenían un mayor daño actínico, especialmente en forma de lentigos solares. No se encontraron diferencias en relación a la presencia de signos tricoscópicos inflamatorios, existencia de anomalías histológicas ni en la presencia de alopecia periférica en pacientes que usaban foroprotectores respecto a las que no los usaban.

4) Mediante evaluación ecográfica se observó que las pacientes tenían un mayor diámetro y flujo vascular en la línea de implantación que el grupo control. También tenían un mayor diámetro vascular en el cuero cabelludo sano. La presencia de vasos ramificados en la tricoscopia de pacientes con AFF se relacionó con un mayor flujo vascular en la línea de implantación y un mayor diámetro en el cuero cabelludo sano.

5) Se presenta un cuestionario específico y validado para valorar la calidad de vida en pacientes con AFF, llamado FFA-QLI, con un mayor poder que el DLQI para seleccionar casos severos de AFF. Alrededor de un tercio de las pacientes con AFF mostraron al menos una afectación moderada de su calidad de vida al realizar el FFA-QLI.

6) Todas las alteraciones histopatológicas descritas en la línea de implantación de pacientes con AFF fueron encontradas en zonas de cuero cabelludo aparentemente sanas en dichos pacientes. El infiltrado inflamatorio fue más severo en la línea de implantación. La presencia de afectación de las glándulas sebáceas (reducción o ausencia), de cambios en el epitelio folicular y de fibrosis perifolicular fue significativamente más común en la línea de implantación.

INTRODUCTION

1. INTRODUCTION

1.1. History, definition and epidemiology.

Frontal fibrosing alopecia (FFA) was described in 1994 by Kossard as a progressive cicatricial alopecia in postmenopausal women, involving the frontal and temporoparietal hairline, the reason why it was initially called "postmenopausal frontal fibrosing alopecia".[1] Although FFA was first considered as an uncommon frontal variant of lichen planopilaris (LPP),[2] whether this is the case, or if FFA is a specific entity, is still highly controversial.[3]

FFA has been described worldwide, but data about its specific prevalence are not available. Recently, the overall crude prevalence for FFA in New York City has been estimated at about 0.015%.[4] Most cases of FFA have been reported in Europe and North America, mainly among Caucasians and fewer among dark-skinned population.[3,5] However, the incidence in Asia may be lower.[6,7] Nowadays, FFA is the most common type of scarring alopecia.[8]

Women are the group most frequently affected by FFA (95-97%),[3,9] which begins at postmenopausal age in around 83% to 95% of them.[3,9-12] Nevertheless, as FFA is not exclusive to postmenopausal women, the initial name was changed to "frontal fibrosing alopecia". The mean age of onset of FFA ranges from 56 to 63 years,[3,10,13] but some cases have been published about younger patients.[9,14,15] FFA can also occur in male patients,[3,16-19] which seem to be affected with FFA at a younger age than women, with a mean age of onset of 47.3 years.[20]

1.2. Aetiopathogenesis and involved factors.

The aetiopathogenesis of FFA is not well known. Hormonal factors, autoimmunity, genetic susceptibility and some exogenous triggers are thought to play a role in the development of FFA.[12]

1.2.1. Aetiopathogenic basis and molecular pathways.

The process of the scarring alopecia could start with the loss of the immune privilege of the hair follicle, which may be induced by interferon-γ (IFN-γ).[21] In FFA, a T helper (Th) 1-biased

cytotoxic T cell autoimmune reaction against hair follicle in the infundibular and the isthmic region, including the bulge area – where the stem cells are placed –, seems to be the main event (Figure 1).[22] This damage would lead to a loss of the regenerative potential of the hair follicle and, subsequently, its destruction. The melanocyte of the hair follicle has been recently proposed as an antigenic target in FFA,[23] which is supported by the lower melanocyte count found in the upper follicle in affected skin of FFA patients.[22,24]

On the other hand, peroxisome proliferator-activated receptor γ (PPAR- γ) has been found to be downregulated and with abnormal function in LPP, which has been proposed as the initial trigger of the inflammation. PPAR-γ plays a central role in lipid homeostasis and in the differentiation and maturation of sebocytes.[25] Its specific deletion in the follicular stem cells of the bulge in mice has been demonstrated to cause scarring alopecia.[26] Peroxisomals polymorphisms and/or environmental toxins may produce this acquired PPAR-γ dysfunction.[26] Dehydroepiandrosterone is clue for the stimulation of PPAR in the gene transcription, fat metabolism, and in mitochondrial activity.[27] In connection with the molecular pathway of PPAR-γ is the mammalian target of rapamycin (mTOR), whose protein expression has recently been found to be lower in the lesional epidermis in patients with LPP/FFA.[28] Moreover, PPAR-γ is a negative regulator of fibrotic events induced by transforming growth factor-β (TGF-β),[29] which may promote fibrosis in FFA via an epithelial-mesenchymal transition process.[30]

Defects in mitochondrial β oxidation of fatty acids, along with an imbalance between the levels of the antioxidant glutathione and the oxidized glutathione, in favour of the second one (marker of oxidative stress), may also be an early process in the development of FFA.[31]

Moreover, the expression of Janus Kinase (JAK) 1 and 3 are upregulated in dermal inflammatory cells in patients with LPP, which may contribute to the IFN-mediated inflammation.[32]

Two more hypotheses have been proposed for FFA. The first one is about neurogenic inflammation, since the total number of mast cells and the proportion of degranulating ones are increased in the perifollicular bulge region in LPP/FFA, and epidermal nerve fibre density is decreased in FFA.[33-35] The second one proposes that the trigger of the immune collapse may be a disturbance in immunological homeostasis mediated via the aryl hydrocarbon receptor-kyneurine pathway axis (AhR/KP),[36] since the aryl hydrocarbon receptor (AhR) is overexpressed in the epidermis of FFA/LPP patients.[37]

1.2.2. Hormones.

The fact that FFA affects women more frequently and especially postmenopausal ones, along with the response to 5-alpha reductase inhibitors, makes the involvement of hormonal factors in its development quite likely.[38] Moreover, the rate of early menopause (14%) seems to be higher in FFA patients than in the general population (6%).[3] The decrease of oestrogens could alter the control of the hair cycle in susceptible patients and act as a trigger in the inflammatory attack on the hair follicle.

The use of an intrauterine device as a contraceptive may protect against the development of FFA, whereas the intake of tamoxifen with the induction of a low-oestrogen environment around the hair follicle may trigger or maintain the pathogenic process of FFA.[39] Hormonal replacement therapy does not seem to prevent the development of FFA or to alter the course of the disease either.[2,38]

Serum hormonal levels are not consistently altered in women diagnosed with FFA, although hormonal involvement from a local mechanism cannot be discarded.[40] However, FFA has been related to androgen deficiency (32.1% of patients)[41]. Dehydroepiandrosterone sulfate (DHEAS) and androstendione have been found to be lower in women with FFA compared to a control group,[42] as well as the levels of the follicle-stimulating hormone (FSH) in premenopausal women with FFA.[43]

1.2.3. Autoimmunity and associated diseases.

Up to 30% of patients with FFA, especially women, have an associated immune disorder.[11] The most frequent one is hypothyroidism (up to 45% of patients).[44] More autoimmune conditions have been described together with FFA, such as other thyroid disorders, different clinical forms of lichen planus, lichen sclerosus, psoriasis, vitiligo, discoid cutaneous and systemic lupus erythematosus and alopecia areata.[3,11,12,44-46]

Moreover, associated dermatologic diseases have been found in around 66% of patients who have FFA.[47] Regarding lichen planus, oral, vulvar, conjunctival, nail and cutaneous forms, including lichen planus pigmentosus (LPPigm), have been noted in FFA patients.[3,12,14,48-51] However, the coexistence of this alopecia with other forms of lichen planus is less common in FFA than in LPP.[10,11,14,44] The clinical type of lichen planus most commonly associated with FFA

is LPP, in 0.8% to 25% of patients.[2,3,11,13,52] Androgenetic alopecia (AGA) is noted concomitantly with FFA in 16 to 57% of women[3,10,52,53] and in 67 to 83% of men.[3,54] An overlap of FFA, AGA and frontal fibrosing in a pattern distribution (FAPD) has also been described.[53] Rosacea (15.5-34%) and atopic dermatitis (43.9%) have also been found together with FFA.[44,47]

1.2.4. Genetic factors.

A family history of FFA is reported by 5 to 8% of patients with FFA.[3,5] In cases of mother and daughter both having the disease, the former tends to have an advanced alopecia and at a higher age than the latter.[55] The occurrence of FFA in families could reflect the exposure to a common environmental trigger, although probably enhanced by a genetic predisposition.

A recent genome-wide association study (GWAS) has demonstrated four susceptibility genomic loci for FFA: 2p.22.2, 6p21.1., 8q24.44, and 15q2.1.[56] The association between a presumed casual missense variant in CYP1B1, which encodes a member of the cytochrome P450 family involved in the oxidative metabolism of oestrogens, and the 2p22.2 loci, was revealed. The strongest effect on FFA susceptibility was observed at the 6p21.1 loci, through the Human Leukocyte Antigen (HLA) -B*07:02 allele, which was shown to confer a five-fold increase in the risk of FFA.

The haplotypes HLA-C*17:01:01:02/B*42:01:01:01 and C*07:02:01:03/B*07:02:01:01 have been identified as susceptibility haplotypes in familial FFA cases.[57]

1.2.5. Surgical procedures and hair and skin care products.

Some cases of FFA and LPP developed after hair transplantation for AGA or after face lift surgery; the Köebner phenomenon or the liberation of a follicular antigen during the surgery may offer some explanation.[17,58,59]

The use of sunscreens has been proposed as a trigger for the development of FFA, since the publication of a study which demonstrated a higher use of these products in FFA than in a control group.[60] This association has been supported in subsequent studies,[61,62] although there are also patients who had not used sunscreens and yet still developed FFA.[54] It seems

that there is a true association between the use of sunscreens and the presence of FFA, although this does not imply a causative relationship.[63]

1.2.6. Drugs, medications and other factors.

Although it still remains controversial, some studies have reported a preponderance of non-smokers within FFA patients, or a less severe FFA in smokers, also after smoking cessation.[3,11,64,65] A relationship between occupational exposure to alkylphenolic substances in women with FFA has been described; these compounds have been shown to interact with PPAR-γ.[62]

No clear association has been found between the onset of FFA and specific medications. [11] Some FFA patients refer to a stressful event as a possible trigger of the development of FFA.[11,66]

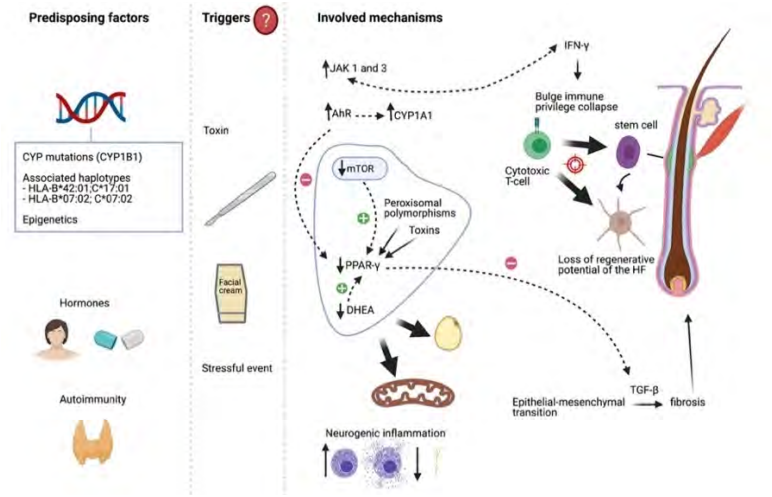

Figure 1. Different unknown triggers, such as facial creams/sunscreens, environmental toxins, surgery, or a stressful situation, may lead to FFA in genetically susceptible individuals. Autoimmunity may also contribute to the predisposition, so people with one autoimmune disease are more likely to have another one. Hormonal factors probably play a role in the development of FFA. Discontinuous lines indicate a regular relationship between two elements. JAK: Janus kinase; AhR: aryl hydrocarbon receptor-kynurenine; mTOR: mammalian target of rapamycin; PPAR-γ: peroxisome proliferator-activated receptor; DHEA: dehydroepiandrosterone; HF: hair follicle; IFN: interferon; TGF: transforming growth factor; HLA: human leukocyte antigen; CYP: cytochrome.
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1.3. Clinical characteristics, trichoscopy and laboratory.

1.3.1. Clinical characteristics.

FFA is a scarring alopecia characterized by a frontal and temporoparietal hairline recession, leading to a cicatricial, shiny and atrophic band (Figure 2a-b). In men, the only reason of consultation can be the loss of sideburns.[67] The hairline recession tends to be bilateral and symmetric, but some asymmetric forms have also been reported.[10] In advanced cases, total hair loss in the frontoparietal area can lead to a "clown alopecic pattern" (Figure 2c).[10] The named "lonely hair sign" reflects the presence of isolated hairs in the original hairline and is an important clue for the diagnosis of FFA (Figure 3a).[68] The occipital area can also be affected (Figure 3b).[3,11,44] Sometimes, the unusual retention of hair along the frontotemporal rim produces a pseudo "fringe sign" (Figure 3c).[69]

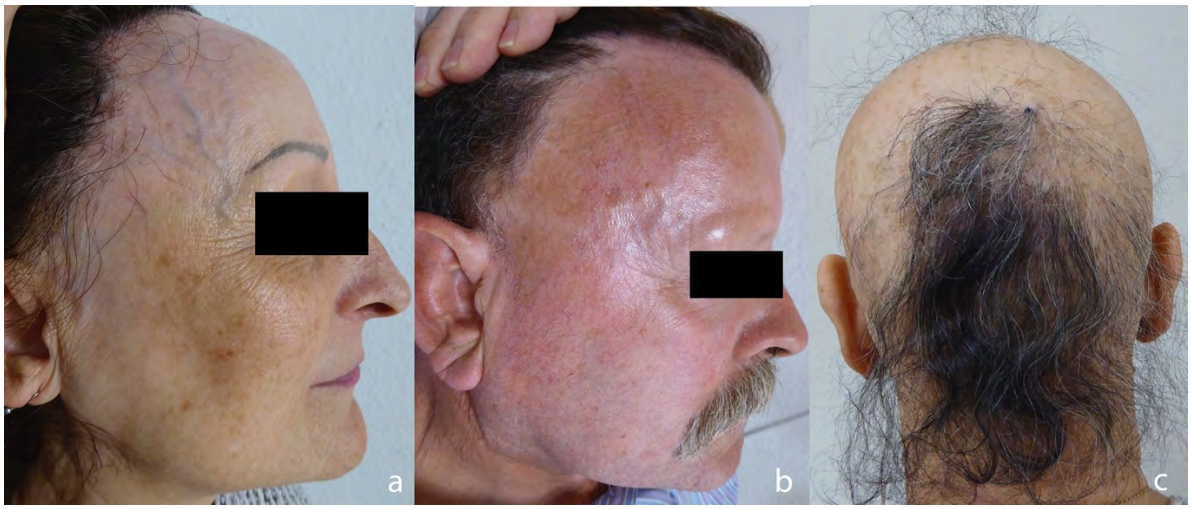

Figure 2. a) Frontal and temporal hairline recession. Note the pale alopecic band, which contrasts with the photoaged skin of the face. Total eyebrow alopecia can also be observed, which is the reason why the patient underwent eyebrow micropigmentation. Some isolated hairs can be noted in the alopecic band. **b)** Male patient who asked for help because of the loss of sideburns and eyebrows. He did not complaint about frontal hairline recession, despite the fact that it was also present. **c)** Advanced alopecia leading to the "clown alopecic pattern".

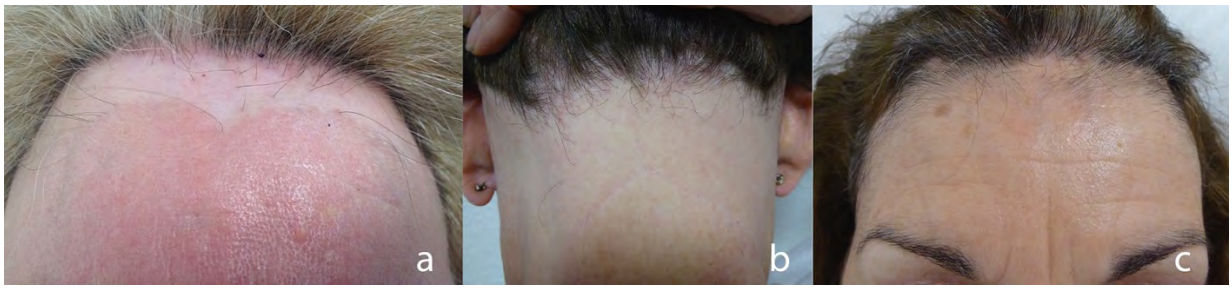

Figure 3. a) Lonely hair sign: note the presence of isolated hairs in the original hairline, which can be easily seen because of the contrast of the pale alopecic band with the skin of the forehead. The absence of vellus hair is also a typical finding. **b)** Involvement of the occipital area in a woman with FFA. Note the presence of some isolated hairs in the original hairline. **c)** Frontal hairline recession, isolated hairs and retention of hair along the frontotemporal rim (pseudo "fringe sign"). Eyebrows remain unaffected.

Partial or total eyebrow alopecia is noted in up to 83% of FFA patients, and in more than a third of cases it precedes the scalp alopecia (Figure 2a-b).[2,3,10-12] Eyelash alopecia can also be observed.[2,3] In men, beard alopecia may also be affected.[3,9] Peripheral hair loss, in limbs, and less commonly in axillae and pubis, can also be present, generally occurring before scalp hair loss.[11,12,49,70]

Facial papules due to vellus hair involvement have also been reported in around 14% of FFA patients (Figure 4a).[3] These tend to be more visible over the temples, but may also appear on the cheeks or chin.[71] Moreover, facial papules are more frequently seen in younger patients, and in Hispanics or Latinos.[52] Follicular red dots are another clinical sign of vellus follicle involvement, and are usually noted in the glabella, forehead, eyebrows and cheeks.[12,71,72]

Other facial lesions have been identified in FFA, such as a diffuse erythema over the eyebrows and cheeks or a reticular-patterned erythema on the face and neck.[71] LPPigm may be associated with FFA, specially in dark phototypes, appearing as brown to grey macular pigmentation on the face and neck, an also in flexural areas (Figure 4b).[52,73] The depression of the frontal veins , the presence of increased pre-auricular wrinkles and increased sweating of the scalp have also been described in patients with FFA.[33,74,75]

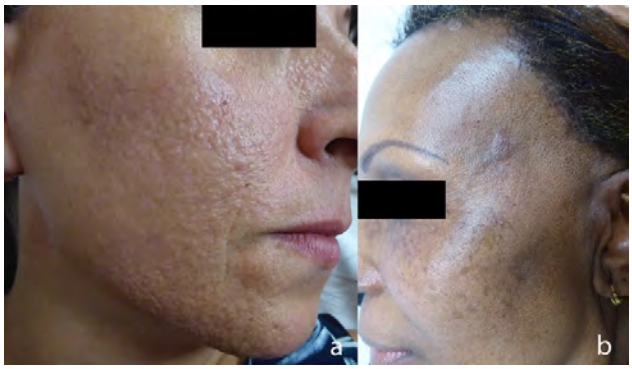

Figure 4. a) Multiple facial papules on the chin and cheeks in a woman with FFA. Note the cobblestone appearance of the skin. **b)** Frontal and temporal hairline recession in a dark-skinned woman. Note the greyish pigmentation on the malar area and on the frontal cheek, compatible with LPPigm.

Regarding symptoms, some patients refer to pruritus and/or trichodynia in the hairline, especially in the frontal area.[3,52,76]

FFA is usually slowly progressive, and tends to stabilize spontaneously after an unpredictable time of progression.[2,3,11] Older patients and patients with a higher age at onset have usually more severe forms.[65] To assess the clinical severity, a five-grade classification has been proposed: I (<1 cm), II (1–2.99 cm), III (3–4.99 cm), IV (5–6.99 cm), and V (>7 cm, also called "clown alopecia"). This size is obtained by measuring the area of cicatricial skin produced by the recession of the frontal and temporal hairline, using the largest measurement to define the grade of severity.[3]

1.3.2. Trichoscopy.

The main trichoscopic findings in FFA are the presence of perifollicular erythema and follicular hyperkeratosis, along with the loss of follicular openings in the affected hairline (Figure 5a-b).[3,6] The presence of follicular ostia with only one hair shaft is another common feature. Follicular hyperkeratosis and perifollicular erythema are rarely seen in the temporal area, where a typical finding is that most of the hair shafts show transparent proximal hair emergence.[77,78] The background in FFA is usually ivory-white, unlike LPP in which is milky-red.[79] Black dots, broken hairs, pili torti and branched capillaries may also be noted, as well as white patches in advanced disease (Figure 5b).[80] In dark-skinned patients, perifollicular hyperpigmentation and pinpoint white dots in the alopecic band can be observed.[5,81]

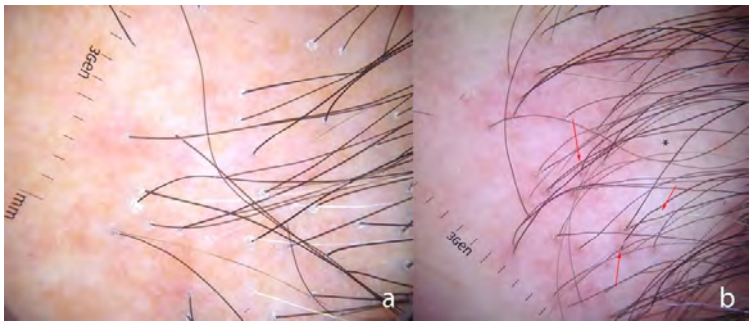

Figure 5. a) Loss of follicular openings, perifollicular erythema and follicular hyperkeratosis. Note the presence of follicles with only one hair shaft, in a whitish background (DermLite II Pro HR, polarized light). **b)** Loss of follicular openings, intense perifollicular erythema with subtle follicular hyperkeratosis, pili torti (arrows), and white background (asterisk) (DermLite II Pro HR, polarized light).

The presence of solitary terminal hairs at the site of the original hairline, as well as the absence of vellus hair in the frontotemporal hairline, are both clinical clues to the diagnosis of FFA, which may also be seen with the naked eye (Figure 3a).[68,82] The second one is the most frequent trichoscopic sign in mild cases of FFA.[83] Another early sign, and therefore more common in mild cases, is the presence of yellow dots, which may be associated with follicles with potential for regrowth.[83,84]

In eyebrows, the presence of a few black dots and dystrophic hairs may suggest the diagnosis of FFA.[85] Red or grey dots may indicate the possibility of local regrowth, whereas loss of follicular openings and pinpoint dots within whitish patches indicate advanced disease.[86] Eyebrow regrowth in different directions and pili torti may be observed (Figure 6).[85-87] In addition, although with a lower frequency than in alopecia areata, tapered hairs, broken hairs and yellow dots may also be seen in FFA eyebrows.[86]

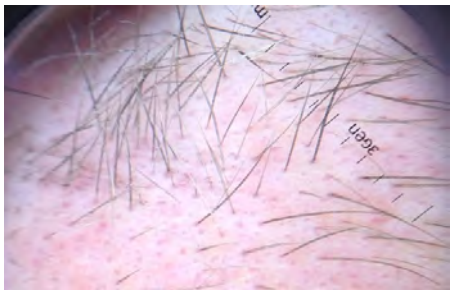

Figure 6. Eyebrow: partial alopecia with follicular red dots and eyebrow regrowth in different directions. (DermLite II Pro HR, polarized light).

1.3.3. Laboratory.

Blood analysis, including hemogram, general biochemical, liver and thyroid function, antinuclear antibodies (ANAs), and sex hormones, is usually normal.[10] Therefore, blood tests seem to be unnecessary in FFA, except for ruling out thyroid disorders.

1.4. Histopathology.

Histopathology of FFA is characterized by a lichenoid lymphocytic infiltrate around the upper follicle, that is, isthmus and infundibulum, including the bulge area, as well as concentric perifollicular lamellar fibrosis.[1] Subsequently, a reduction in the number of hair follicles is observed: a mean of seven terminal hair follicles have been seen per 4 mm punch biopsy in FFA, while in a "normal scalp", around thirty terminal and five vellus hair follicles are seen in Caucasians, and eighteen terminal and three vellus ones in Afro-Caribbeans.[49] All types of hair follicles (terminal, intermediate and vellus) can be affected.[88] Vacuolar degeneration of the basal layer, keratinocyte necrosis, replacement of the pilosebaceous units by fibrous tracts, and loss of elastin fibres are other histologic findings.[89]

The inflammatory infiltrate mainly involves the upper follicle, and classically the lower follicle was considered to remain spared. However, recently, a study comparing FFA and LPP samples has demonstrated that a significant number of patients with FFA may have inflammation extending below the isthmus, or even fibrosis.[90] This has not been supported by other studies,[91] but what is clear is that the maximum degree of inflammation is found at the infundibulum and isthmus.[90] Dermal fat infiltration at the isthmus level and in the arrector pili muscle has been noted in FFA samples.[92]

In early cases, perifollicular fibrosis may not be seen, but the inflammatory involvement of vellus follicles and the atrophy of sebaceous glands would be the histological diagnostic clues in these cases.[93] The follicular triad, consisting of the simultaneous involvement of different types of follicles (terminal, intermediate and vellus) and in a different stage of the cycle (anagen, catagen and telogen) has also been described in early cases of FFA.[94] However, in advanced cases, fibrous tracts and the absence of hair follicles may be the only finding.[10]

The inflammatory infiltrate is characterized by an increase in the percentage of CD8+ T cells, with a reversal of the typical CD4:CD8 ratio (which is normally around 2:1).[30,91] A lower melanocyte count has been recently demonstrated in the upper follicle in FFA patients.[22,24]

The clinically unaffected scalp also has some histological alterations in FFA, mainly infundibular lymphocytic inflammation and early sebaceous gland atrophy.[95-98]

Facial papules are characterized histologically by follicular hyperkeratosis and lichenoid dermatitis involving the infundibulum and isthmus of the vellus hair follicles, or even fibrosis in advanced cases.[71,99,100] However, curiously, sebaceous glands are present in most of cases and are even more prominent.[101,102]

Although FFA histological findings are indistinguishable from LPP according to some authors,[95] others have found differences which makes it more suitable to consider FFA as a specific type of lymphocytic scarring alopecia rather than a type of LPP.[88]

1.5. Quality of life.

Patients with alopecia may have impairments in their quality of life (QoL).[103] Women with scarring alopecia usually show a greater psychological burden and a severely impaired QoL compared with those with non-scarring alopecia, probably depicting the poorer prognosis of the former.[104] A study including patients with LPP and FFA found considerable levels of psychological distress and impaired QoL among these patients.[105] Some women with FFA refer to the fact that alopecia has significant consequences on their lives, and they usually perceive FFA as a chronic disease with an unpredictable course.[106] Moreover, patients with at least one associated non-scalp lesion tend to have a poorer QoL.[107]

1.6. Diagnosis and differential diagnosis.

1.6.1. Diagnosis.

The diagnosis of FFA is mainly clinical, taking into consideration the typical hairline recession with or without the also typical eyebrow alopecia. The presence of alopecia at peripheral body locations and the observation of facial lesions are also signs which may help.

Dermoscopy is really useful, especially in early cases, and for ruling out other differential diagnosis. Biopsy is usually not necessary, but can be performed when the diagnosis is doubtful. Recently, the International FFA Cooperative Group (IFFACG) have published diagnostic criteria for FFA, considering the classic FFA and also probable FFA (Table 1).[108]

Table 1. IFAACG Criteria for FFA.

1. Classic FFA	2. Probable FFA
Frontal hairline recession with loss of follicular ostia* (2 points) plus	Frontal hairline recession without loss of ostia* (1 point)
Positive biopsy of a representative section of affected anterior or temporal scalp or eyebrow consistent with FFA** (2 points)	Positive biopsy of a representative section of affected anterior or temporal scalp or eyebrow consistent with FFA** (2 points)
At least 50% eyebrow loss (in the absence of alopecia areata)*** (1 point)	At least 50% eyebrow loss (in the absence of alopecia areata)*** (1 point)
Perifollicular anterior scalp erythema (1 point)	Perifollicular anterior scalp erythema (1 point)
Perifollicular anterior scalp hyperkeratosis/scale (1 point)	Perifollicular anterior scalp hyperkeratosis/scale (1 point)
	Facial papules (1 point)
	Bilateral preauricular hair loss in a patient who previously had hair in this area (1 point)
	Documented absence of vellus hairs in affected anterior or temporal hairline (1 point)
Classic FFA $\geq$4 points	Probable FFA $\geq$4 points

*Loss of ostia may be confirmed clinically or by trichoscopy. If no loss of ostia can be confirmed by either method, move to Probable FFA criteria, or confirmatory biopsy of FFA from the affected anterior hairline is necessary as well.

**Biopsy of a representative section of the affected scalp or eyebrow that demonstrates a decreased number of hair follicles and sebaceous glands, concentric perifollicular fibrosis and a lymphocytic infiltrate targeting the isthmus and infundibular regions of the hair follicle would be consistent with diagnosis of FFA.

***50% eyebrow loss could be 100% loss of one eyebrow or overall 50% cumulative loss of both eyebrows together.

1.6.2. Differential diagnosis.

The ophiasis type of alopecia areata is probably one of the most important differential diagnosis (Figure 7a-b). Furthermore, in cases of isolated eyebrow alopecia, alopecia areata also needs to be discarded. In these cases, dermoscopy may be an important tool to make a correct diagnosis. Perifollicular erythema and hyperkeratosis are not seen in alopecia areata, in which typical features are yellow dots, dystrophic and broken hairs, black dots, exclamation mark hairs, tapered hairs and regrowing hairs (Figure 7c-d).[79]

AGA with male pattern also needs to be considered (Figure 8). Dermoscopy is again extremely useful to rule out the wrong diagnosis, as hair miniaturization and anisotrichia are present in AGA but not in FFA.

A familial high frontal hairline implantation should also be discarded.

Finally, regarding scarring alopecias, traction alopecia may resemble FFA. The diagnosis may be a challenge especially in dark-skin phototypes. The clinical history, the absence of trichoscopic signs of FFA and the absence of eyebrow alopecia may suggest tractional alopecia.[79]

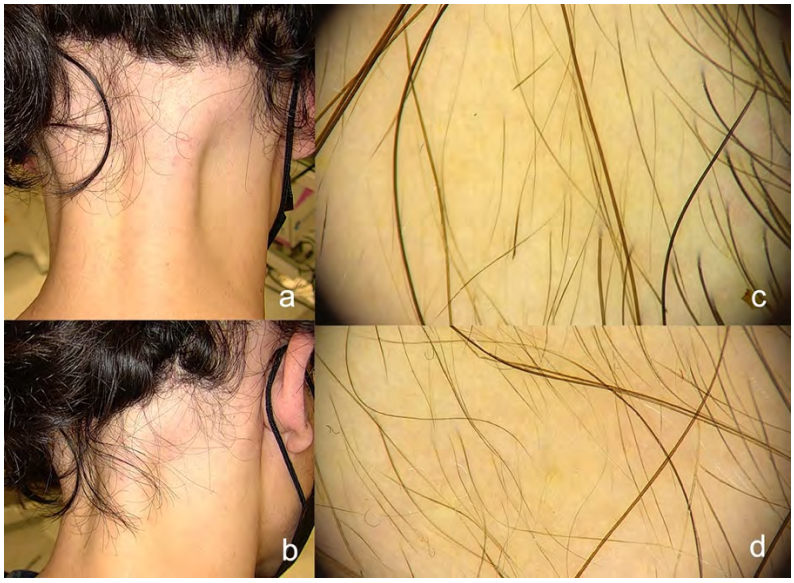

Figure 7. a) Ophiasic alopecia areata affecting the occipital hairline, **b)** and also the retroauricular area. **c)** Exclamation mark hairs and thin hairs can be observed. **d)** Regrowing circular hairs and several yellow dots are also present. **c-d)** Note the absence of perifollicular erythema and follicular hyperkeratosis. (Fotofinder, non-polarized light)

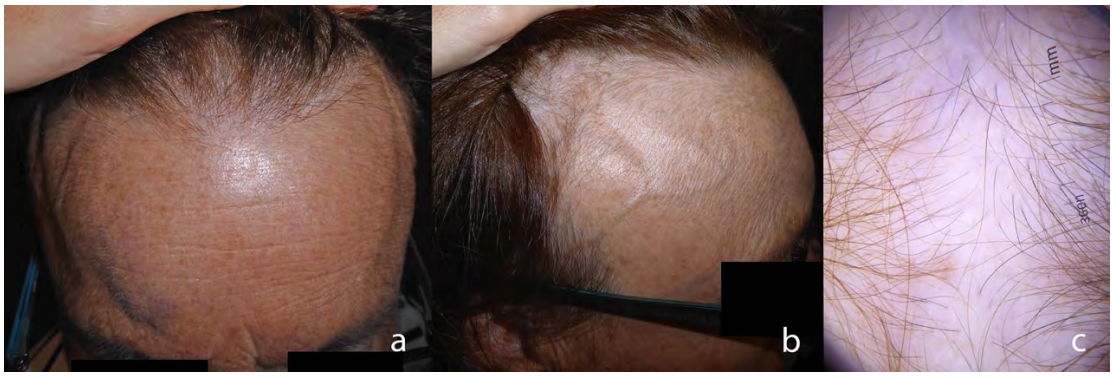

Figure 8. a) Frontal view of a patient with female AGA with male pattern. Note the marked frontotemporal receding hairline, most visible in the lateral view. **b)** Lateral view. The presence of miniaturized and vellus hair can be observed, along with a reduction of the hair density. **c)** Perifollicular erythema and follicular hyperkeratosis are absent, the background does not show differences of colour, and the presence of miniaturized hairs and anisotrichia are the main trichoscopic findings. (DermLite II pro HR, polarized light).

1.7. Treatment.

Nowadays, the objective of the treatment in FFA is to halt the hairline recession, as well as to alleviate the symptoms if present. When the hair follicle is destroyed, hair regrowth cannot be achieved. A combination of both local and systemic treatments may achieve the best outcomes.

1.7.1. Local treatments.

Potent topical corticosteroids and topical calcineurin inhibitors are usually recommended, in monotherapy or combined, for reducing inflammation.[11,15,109] Some reports have found disease stabilization under this treatment,[110] although it is still controversial.

Intralesional corticosteroids (triamcinolone acetonide 10 to 20 mg/ml), injected every 3 to 6 months, into the hairline and also into the eyebrows, may produce stabilization and even improvement in some cases.[3,15,111]

Topical minoxidil has not been shown to slow down the progression of the alopecia.[10] However, it may be useful in cases that are associated with AGA. Bimatoprost 0.03% eye drops may be a therapeutic option in eyebrow and eyelash alopecia.[112]

Regarding hair transplantation, it should only be considered in patients with a minimum of one to five years without activity.[113,114] Despite this, patients tend to lose the hair grafts progressively within years, with a graft survival rate lower than 60% after five years.[115]

1.7.2. Systemic treatments.

Several treatments have been used in FFA, but the oral 5-alpha reductase inhibitors seem to be, for now, the best therapeutic option in achieving stabilization of the alopecia, and even hair regrowth in some patients.[3,116] A report showed that 47% of patients had hair regrowth at the hairline and 53% had stabilization of the alopecia with the use of finasteride (2.5-5 mg/day), whereas 44% of patients displayed improvement and 56% had stabilization of the alopecia with the use of dutasteride (0.5 mg/day).[3] Dutasteride seems to be more effective than finasteride according to some reports.[117] Subsequent studies have shown a stabilization rate of 61.5-64.2% with the use of dutasteride, compared to other systemic treatments and to topical treatment only, and the response was dose-dependent, the most effective dose being five to seven capsules (0.5 mg) per week.[118]

Oral prednisone has been shown to produce a stoppage of hairline recession in more than a third of patients, but relapse is the rule when the treatment is stopped.[10] Hydroxychloroquine has been widely used since the first description of FFA. Although some reports did not find any consistent benefit with its use,[11] others found improvement within the first six months of therapy.[13] One report has shown that oral isotretinoin (20 mg/day) and acitretin (20 mg/day) may produce a stoppage of the progression of the alopecia in more patients than finasteride.[119] Moreover, oral isotretinoin may produce an improvement of erythema and perifollicular hyperkeratosis.[120]

A low dose of oral isotretinoin (10-20 mg/day) seems to be an effective treatment for facial papules,[101,120] although improvement under treatment with oral prednisone and antimalarials has also been reported.[100]

JUSTIFICATION AND HYPOTHESIS

2. JUSTIFICATION AND HYPOTHESIS.

The prevalence of FFA has been increasing gradually worldwide since its description in 1994 by Kossard. Nowadays, FFA is one of the most common types of scarring alopecia, or even the most common. Although one part of this higher prevalence may be due to a better understanding of the clinical features of FFA –therefore reducing the underdiagnosis –, it is thought that an unknown environmental trigger may be responsible for this situation.

During the last seven years, more than three quarters of the publications regarding FFA have been published. Despite this awareness among dermatologists in regard to FFA, several issues are still unclear, and it was even more unclear when we decided to start this research a few years ago. Our objective was to make a detailed study of FFA, including clinical and trichoscopic data, as well as a sonographic and histopathologic study.

Furthermore, as alopecia can reduce the QoL of patients, we performed different general QoL questionnaires and designed a specific one to try to assess how much FFA may affect the patients' QoL. Therefore, we decided not to centre our attention on one specific area of FFA, but to collect a great amount of data to analyse FFA from different view points.

Regarding the clinical study, besides physical examination, data about dermatological and non-dermatological comorbidities, family history of alopecia and autoimmune disorders, the intake of medications and the use of hair and skin care products, were collected.

Some familial cases of FFA have been published. The existence of FFA in different family members may reflect a genetic susceptibility, but as they are not representative of the majority of cases, genetics do not seem to be the only factor involved. In relation to familial cases, do they have any distinct feature? Is there any haplotype shared by familial cases which may predispose people to develop FFA?

On the other hand, is there any other skin condition (different to AGA) which is more frequent in FFA patients? With regards to blood analyses, hormonal factors are supposed to play a role in the development of FFA. No clear blood test alterations have been described. So, we asked ourselves: Is there any hormonal imbalance in patients with FFA?

At the beginning of this research, the first publication about the possible relationship between the use of sunscreens and FFA was published. Therefore, we asked ourselves, can we confirm

a higher use of sunscreens among our patients with FFA compared to a control group? If we assume that patients use more sunscreen, do they have less sun skin damage?

The use of sonography in dermatology is relatively new, but it is expanding into different dermatologic areas, such as hidradenitis suppurativa and other inflammatory diseases, vascular anomalies, nail and skin tumours and psoriasis. As FFA is an inflammatory disease, sonography and especially Colour-Doppler ultrasound, may provide interesting information about FFA scalps. Are there sonographic differences between a healthy scalp and another one with FFA? What about different areas in FFA? Could sonography show differences between the clinically affected hairline in FFA and the apparently healthy scalp?

In addition, alopecia can substantially affect the QoL of patients, especially women. Alopecia in men is socially acceptable, so most of them usually accept it as something "normal" and "related to ageing". However, that is not the case for women, for whom any type of alopecia may make them feel less feminine and produce significant mood changes and feelings of insecurity. Most published studies regarding QoL and alopecia refer to alopecia areata and AGA, although it is also known that severe forms of scarring alopecia can be related to depression. AGA is the most common type of alopecia in women, but effective treatments which can improve hair density are available. Nevertheless, the irreversibility of scarring alopecia is another factor to take into account as it may further frustrate patients who suffer from this. As FFA is a specific type of alopecia, with rather particular clinical features, a specific questionnaire to assess the presence of disturbances in the QoL of patients is needed. How do patients with FFA feel with regards to their alopecia? Does it affect their everyday life? In addition, the loss of eyebrows may significantly change the appearance of both men and women, can this relevant issue be associated with a worse QoL?

With reference to the histopathologic study, the characteristics of the involved hairline area are rather well-known. A lymphocytic T-cell infiltrate, which involves mainly the upper follicle, along with concentric perifollicular lamellar fibrosis, are the main histopathologic findings in FFA. These lead to the subsequent destruction of the hair follicle, and its replacement by fibrous tissue – this final stage is similar to other scarring alopecias-. However, which alterations can be found in the clinically unaffected scalp? Are there differences between this area and the affected hairline?

OBJECTIVES

3. OBJECTIVES.

3.1. General objective.

To describe the clinical and histopathological characteristics of FFA and to assess the QoL of patients with FFA.

3.2. Main objectives.

1. To describe the clinical characteristics of FFA and its association with other dermatological diseases.
2. To assess the QoL of patients with FFA by designing and validating a specific questionnaire.
3. To describe the histopathological characteristics of FFA in clinically unaffected areas.

3.3. Secondary objectives.

Clinical objectives

1. To describe the clinical features and to find a susceptibility HLA haplotype in familial cases of FFA.

2. To describe the association of rosacea and the presence of hormonal disturbances in patients with FFA.

3. To find possible reasons for the higher use of sunscreens in patients with FFA. Assessment of the actinic damage in FFA patients.

4. To find sonographic differences using Colour-Doppler, between an apparently healthy scalp area in FFA patients compared to control group individuals, and also between affected and clinically non-affected scalp in FFA patients.

QoL assessment

1. To introduce and validate a specific questionnaire to assess the QoL in FFA patients.

2. To analyse the power of the new questionnaire to identify patients with more severe alopecia.

Histopathological objectives

1. To analyse the presence of the histopathological features described in the hairline implantation of patients with FFA in a normal-appearing area of the scalp.

2. To compare the histopathological findings in the hairline implantation and the normal-appearing scalp.

PARTICIPANTS AND METHODS

4. PARTICIPANTS AND METHODS.

4.1. Participants: recruitment and inclusion criteria.

The total number of individuals for the study was 101 patients with FFA, and 40 healthy control subjects. All participants were women.

The study participants were selected from the Dermatology Department of the University Hospital San Cecilio in Granada (Spain), from 2015 to 2016. Case subjects were patients with FFA, associated or not with other types of alopecia. To find those patients, a search in the data base of the hospital was performed, with the following key words: “alopecia frontal fibrosante”, “AFF”, “liquen plano pilar” and “liquen plano folicular”. Then, to include the patients in the study, the presence of clinical and/or trichoscopical signs of FFA were indispensable, excluding those cases with only the classical form of LPP. Clinical signs of FFA were the presence of frontal and/or temporoparietal hairline recession, leading to a scarring alopecic band, with or without eyebrow alopecia. Trichoscopic signs of FFA were the absence of follicular openings in the scarring area, with or without perifollicular erythema and follicular hyperkeratosis in the affected hairline. All the patients were called by telephone and were given an appointment in the Dermatology Department in order to collect the data for the study. Finally, after excluding patients who did not fulfil the clinical criteria, 101 patients with FFA were included in the study and all of them signed an informed consent. Moreover, for the familial FFA study, 13 more patients with familial FFA were included, as out of the 101 patients, only 7 were familial ones. In this case, clinical criteria were the same as those for the general sample of patients, but it was necessary for each patient to have another first-degree familial member with FFA. These 13 FFA patients were recruited from the University Hospital Virgen de las Nieves in Granada, and included two men. For the histopathological study, 52 out of 101 patients were included, and two scalp biopsies were taken from each patient.

The control subjects were 40 healthy people without any hair disease, specifically friends of the patients with no familial link to them, and also people seen in the Dermatology Department for different issues, other than hair problems. All of them were women. Exclusion criteria were having any type of alopecia, and being younger than 40 years old. All of the control individuals signed an informed consent to participate in the project.

4.2. General variables and methods.

For all the studies and specifically for the clinical study, a detailed anamnesis was performed, asking for:

- Demographic and general information: sex, age, ethnicity, occupation, civil status, number of children.
- Hormonal status: age of menarche, regularity of menstruation, menopause and age of menopause, hysterectomy +/- double adnexectomy and age at the time of this surgery.
- Subjective data about FFA: age of onset of the alopecia, loss of eyebrows as initial sign - with or without previous erythema-, perception of alopecia development as acute or progressive, time between the perception of hair loss until noticing the alopecia, presence of symptoms like pruritus or trichodynia.
- Previous history of skin diseases: lichen planus, vitiligo, alopecia areata, skin cancer, others.
- Habitual use of sunscreens.
- Comorbidities:
    - Metabolic syndrome: arterial hypertension, diabetes, dyslipidaemia. Weight and height to calculate body mass index (BMI).
    - Autoimmunity: rheumatoid arthritis, thyroid disorder and type, inflammatory bowel disease, celiac disease, Sjögren syndrome, others.
- Toxic habits: current or previous consumption of tobacco, alcohol, other drugs.
- Treatments: hypolipidemic drugs, antihypertensives, antidiabetics, non-steroidal anti-inflammatory drugs, hormonal replacement therapy (current or past), tamoxifen (current or past), oral contraceptives (current or past), others.
- Aesthetic surgical and non-surgical procedures: face-lift, rhinoplasty, dermal fillers, botulinum toxin, hair transplant, others.
- Treatments for FFA and duration: none, minoxidil, topical corticosteroids, topical calcineurin inhibitors, intralesional corticosteroids, oral corticosteroids, hydroxychloroquine, 5-alpha reductase inhibitors, others.

- Hair cosmetics and procedures, and frequency: hairdresser appointments, permanent wave, hair dye, tight hairstyles, hair extensions, hair straightening, heat treatments, hairsprays.
- Family history of: alopecia (type and family member affected), autoimmunity, others.

A meticulous physical examination was performed, collecting data regarding the following issues:

- Clinical signs of FFA: characteristics of clinical hairline recession regarding symmetry, occipital involvement, sideburn loss (isolated or not), facial papules and location, prominent vessels on the forehead. Photographs were taken of all patients.
- Trichoscopic signs of FFA: loss of follicular openings, follicular hyperkeratosis, perifollicular erythema, lonely hair sign, interfollicular erythema, follicular ostium with one hair shaft, ivory white background, red dots, white scarring patches, white dots, absence of vellus hair in the hairline implantation, pili torti, vascular loops and branched vessels. A handheld dermoscope, DermLite II pro HR was used for the trichoscopic examination of the participants. Photographs were taken of all patients.
- Peripheral alopecia: partial or total alopecia of eyebrows, eyelashes, superior limbs, inferior limbs, axillae, pubis and beard (men).
- Size in centimetres of the alopecic band, frontal and temporal, and grade of alopecia (I-V). The grade of FFA was assessed following the previously referred to five-grade classification described by Vañó-Galván et al.[3]
- Hair pull test.
- Clinical perception of status: stable or in progression. It was based on the clinical history registered at the hospital.
- Association with other types of alopecia: AGA and grade (Ludwig/Hamilton), alopecia areata, LPP, discoid lupus, others.
- Association with other dermatosis: rosacea, vitiligo, psoriasis, others.
- Association with other forms of lichen: lichen planus (cutaneous, mucous, LLPigm), lichen sclerosus and atrophicus.

- Fitzpatrick skin phototype (I-VI).[121]
- Cutaneous signs of actinic damage: lentigines, actinic keratoses, basal cell carcinoma, squamous cell carcinoma. Contrast of alopecic band with photoaged skin.
- Trichoscopic signs of other types of alopecia: LPP, alopecia areata, AGA, discoid lupus, others.

Moreover, a full blood test was performed on all patients, including:

- Hemogram and general biochemical profile.
- Lipids: total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides.
- Iron profile: iron, ferritin and transferrin.
- B12 vitamin, folic acid, D vitamin.
- Sex hormones: FSH, LH, oestradiol, progesterone, testosterone, SHBG, PRL, 17-hydrohyprogesterone, androstenedione, DHEAS, dihydrotestosterone.
- Thyroid profile: thyroid-stimulating hormone (TSH), thyroxine (T4), triiodothyronine (T3), thyroglobulin.
- C-Reactive Protein, globular sedimentation rate.
- Prostate-specific antigen (PSA).
- Autoimmunity: Rheumatoid factor, ANAs, extractable nuclear antigen (ENA), citrullinated peptide, anti-transglutaminase and anti-gliadin antibodies, anti-thyroid peroxidase and anti-thyroglobulin antibodies.
- Serology: hepatitis A, B and C viruses, human immunodeficiency virus (HIV).

4.3. Specific variables and methods.

1) Familial study: the relationship between affected relatives was recorded. A blood test was performed. After isolating the deoxyribonucleic acid (DNA), HLA class I and class II alleles were typed by sequence-specific oligonucleotide. Furthermore, eleven mutations of CYP21A2 were analysed: P30L, I2 splice (I2G), Del8 bpE3 (G110del8nt), I172N, Cluster E6 (I236N, V237E, M239K), V281L, L307 frameshift (F306 + T), Q318X, R356W, P453S and R483P.

2) Sonographic study: The US equipment employed was Esaote MyLabTM, with a lineal probe of 18 MHz, using the colour flow mode (CFM) of the colour Doppler. Three sites were analysed in patients: the alopecic band, the hairline implantation and the healthy area of the scalp behind the hairline – the last one was chosen on the basis of the absence of dermoscopic criteria of FFA -. In the control group, the frontal hairline implantation and the area of the scalp behind the hairline, at 3 cm approximately, were assessed. In each area, the diameter (centimetres, cm) and the flow (metres/second, m/s) of the two most significant vessels, were recorded. Mean values were calculated in each area. The study was performed using the following parameters: Doppler frequency of 6.6 MHz and a pulse repetition frequency (PRF) of 1 kHz. Gain was fixed just below the level at which noise artefact from the skull was noted.

3) QoL study: Three questionnaires were given to each participant, both patients and control group individuals. The first one was the DLQI.[122] The second one was the Hospital Anxiety and Depression Scale (HADS).[123] And the third one was an original questionnaire created for this project to assess the QoL decline in FFA patients.

The new specific questionnaire for FFA was called Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI). The questions were created based on the bibliography available and focusing on some of the most typical features of this type of alopecia. For organizational convenience, the questionnaire was divided into three sections: emotional, social and functional. The first one was about personal feelings (self-esteem, self-image, concerns) and symptoms. The second part was about the patients' feelings when socializing (e.g. going to the hairdresser's, giving up activities, friendships and partnerships). Finally, the third part was related to the necessity of trying to hide the alopecia and the time spent during the day thinking about the disease. The other two questionnaires, DLQI and HADS, were used to validated the FFA-QLI. The FFA-QLI included 20 questions and the responses were scored from 0 (not affected at all) to 3 (highly affected). The original questionnaire was developed in Spanish and was validated in Spanish, although an English version is also provided but needs to be validated. The total score of FFA-QLI ranged from 0 (best QoL) to 6 (worst QoL). The questions of the questionnaire were refined after interviewing 25 FFA patients, with the aim of making it clearer and more understandable to the patients. Finally, a test-retest was performed on 30 FFA patients.

4) Histopathological study: 4 mm-punch skin biopsies were performed in two areas: the progression frontal hairline and the "apparently normal" scalp behind the affected hairline.

Trichoscopy was used to select both areas: the first one was the most clinically affected, and the second one was based on the absence of trichoscopic signs of FFA. The total number of skin biopsies was divided into two groups and analysed by two expert dermatopathologists. Vertical sections were used to process the samples, which were stained with hematoxylin and eosin. Orcein stain was used to assess elastic fibres. The following variables were studied:

- Follicular count: total, terminal, intermediate and vellus hairs, anagen and telogen hairs.
- Presence of follicles in the different phases of the hair cycle (yes/no): anagen, catagen, telogen.
- Skin annexes involvement (rather than hair follicle) (yes/no): Sebaceous glands (normal, reduced, absent), erector pili muscle (normal, reduced, absent), presence of spared hair follicles (yes/no).
- Inflammatory infiltrate (yes/no): severity (mild, moderate, severe), types of hair follicle involved (absent, terminal, intermediate, vellus), phase of hair follicles involved (anagen, catagen, telogen), parts of upper hair follicle involved (infundibulum, isthmus), involvement of sebaceous glands (absent, gland, ducts, both, not assessable), involvement of dermis (absent, papillary, reticular, both), interfollicular involvement (absent, interstitial, perifollicular, perivascular, subcutaneous), perivascular superficial lymphohistiocytic infiltrate (yes/no), lichenoid infiltrate in the interfollicular epidermis (yes/no).
- Epithelial changes (yes/no): corneum stratum (normal, hyperkeratosis, follicular plugs), epidermal changes (normal, spongiosis, hyperplasia, lichenoid changes, vacuolar changes, atrophy), follicular epithelium (normal, spongiosis, lichenoid changes, vacuolar changes, tufted hairs), lichenoid interphase dermatitis in the upper follicle (yes/no), vacuolar degeneration of the basal layer of the outer root sheath (yes/no), keratinocyte necrosis of the outer root sheath (yes/no), increased apoptotic activity in the outer root sheath (yes/no), infundibular dilatation and hypergranulosis (yes/no), colloid bodies in the dermoepidermal junction (yes/no).
- Fibrous tissular changes (yes/no): perifollicular fibrosis (absent, terminal hairs, intermediate hairs, vellus hairs), dermal fibrosis (absent, upper dermis, lower

dermis), type of fibrosis (lamellar concentric, fibroplasia, mucinous fibroplasia, hyalinization).

- Interstitial tissue changes (yes/no): interfollicular mucinosis (yes/no), follicular mucinosis (yes/no), elastic fibres alteration (no, perifollicular scar, superficial perifollicular scar, wedge-shaped scar, diffuse scar).

4.4. Statistical analyses.

All data were introduced in SPSS Statistical Software (SPSS Inc, Chicago, IL) for their interpretation and statistical analyses. Differences were considered significant at $p \leq 0.05$.

Continuous data were presented as mean (standard deviation) and categoric data as relative (absolute) frequency. The Student's t test was applied to compare the mean values of quantitative variables. Qualitative variables were analysed with $\chi 2$ test, or Fisher exact test when the size of the sample required it. The McNemar test was used to analyse qualitative variables of both areas in the histopathological study.

For the HLA study, the two-tailed Fisher exact test with 2 x 2 contingency tables (in R program) was used to compare allelic, genotypical and haplotypical distributions among patients and control subjects.

Pearson's correlation coefficient was used to examine the linear correlation between quantitative variables. In the actinic damage study, multivariate logistic regression analyses were performed to explore the variables associated with FFA, use of sunscreens and actinic damage.

For the validation of the specific FFA QoL questionnaire, reliability was evaluated using internal consistency analysis with the Cronbach α and reproducibility analysis with the intraclass correlation coefficient (ICC). To determine the test–retest reliability, the ICC for the global value of the questionnaire and a Cohen's kappa of the items were calculated from the original FFA-QLI. Convergent validity, examining the degree to which two measures of constructs are related, was assessed by calculating the extent of correlation between raw scores from the FFA-QLI and DLQI using Pearson correlation coefficient. The cut-off points to select patients with mild, moderate and severe QoL impairment were calculated using

receiver operating characteristic (ROC) curve analysis and comparison with the DLQI categories.

4.5. Ethics.

This project was approved by the Local Ethical Committee.

RESULTS

5. RESULTS.

The different published reports which constitute the current doctoral thesis are exposed in this section. The last one is a narrative review about FFA.

5.1. Publication 1: Clinical study about familial frontal fibrosing alopecia.

Reference: Porriño-Bustamante ML, López-Nevot MÁ, Aneiros-Fernández J, García-Lora E, Fernández-Pugnaire MA, Arias-Santiago S. Familial frontal fibrosing alopecia: A cross-sectional study of 20 cases from nine families. Australas J Dermatol. 2019; 60(2):e113-e118.

This article, which has been published in the Australasian Journal of Dermatology, is a cross-sectional study, that included twenty patients with FFA, belonging to nine different families. In families composed of affected mother and daughter, mothers had a more advanced alopecia than their daughters and the age of onset of the alopecia was earlier in daughters. The clinical and dermoscopic features of familial cases of FFA seemed to be consistent with those of non-familial cases.

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Familial frontal fibrosing alopecia: A cross-sectional study of 20 cases from nine families.

Original research.

María Librada Porriño-Bustamante,[1] Miguel Ángel López-Nevot,[2,3] José Aneiros-Fernández,[4] Elena García-Lora,[5] María Antonia Fernández-Pugnaire,[6] Salvador Arias-Santiago.[5,7]

[1]Servicio de Dermatología, Hospital Universitario La Zarzuela, Madrid,

[2]Laboratorio Clínico, Unidad de Histocompatibilidad, Hospital Universitario Virgen de las Nieves,

[3]Departamento de Bioquímica, Biología Molecular III e Inmunología, Universidad de Granada,

[4]Servicio de Anatomía Patológica, Parque Tecnológico de la Salud,

[5]Servicio de Dermatología, Hospital Universitario Virgen de las Nieves,

[6]Servicio de Dermatología, Parque Tecnológico de la Salud, and

[7]Facultad de Medicina, Universidad de Granada, Granada, Spain

Correspondence: María Librada Porriño-Bustamante, Hospital Universitario La Zarzuela, Calle de Pleyades, 25. 28023 Madrid, Spain. Email: mporrinobustamante@gmail.com

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ABSTRACT

Background/Objectives: Frontal fibrosing alopecia (FFA) is a scarring alopecia whose prevalence is increasing. The pathogenesis of this disease is not well known. Genetic, environmental, hormonal and autoimmunity related factors have been considered; however, only a few cases of familial frontal fibrosing alopecia have been reported.

Material and methods: A cross-sectional study was performed at University Hospital in Granada (Spain). Twenty patients with frontal fibrosing alopecia belonging to nine different families were included, and clinical and dermoscopic features were analysed.

Results: Overall, 90% of the patients studied were women (mean age 61.4 years). About 50% of the patients had grade II frontal fibrosing alopecia at the time of diagnosis, whilst 35% had grades III or V. Mean recession was 2.83 cm in the frontal area and 1.99 cm in the temporoparietal area. Daughters presented a shorter recession area and earlier debut of the disease than mothers. Androgenetic alopecia was found in only two patients (10%). The dermoscopic signs most commonly found were perifollicular erythema (85%), hyperkeratosis (85%), and absence of vellus hair in the hairline (78.9%).

Conclusion: This study adds to the growing evidence that there is a genetic component to frontal fibrosing alopecia. The clinical pattern of frontal fibrosing alopecia was not different from that found in non-familial cases, but the debut of the disease in daughters of mothers with frontal fibrosing alopecia may be earlier.

Keywords: cicatricial alopecia, familial, frontal fibrosing alopecia, hair, scarring alopecia.

WHAT THIS RESEARCH ADDS

• Few familial cases of FFA have been reported and clinical features are not well known.

• We present a series of cases of familial FFA. Clinical features do not differ from those which are not familial, but the debut of the disease in daughters of mothers with FFA may be earlier. Further studies are needed to clarify any possible genetic susceptibility.

INTRODUCTION

Frontal fibrosing alopecia (FFA) is a scarring alopecia first described in 1994 by Kossard.[1] Since then, its prevalence has been increasing. FFA was initially described as affecting postmenopausal women, but premenopausal women and men may also be affected.[2] FFA is characterised by the recession of the frontal and temporoparietal hairline, leading to a cicatricial alopecic band with no follicular openings. The band may progress laterally and behind the ears, even towards the occipital area.[3,4] Partial or total eyebrow alopecia is often observed (73%), generally preceding hairline recession, and sometimes along with eyelash alopecia (3%) or body hair loss (25%).[2,4-6] The involvement of facial vellus hairs in this process may occur, presenting as facial papules.[4] Patients usually report pruritus, and less frequently trichodynia or a burning sensation, although FFA can be asymptomatic.[4]

Dermoscopy of the hairline usually shows perifollicular hyperkeratosis and erythema. Facial papules are thought to be the consequence of facial vellus hair involvement.[4] Lonely hair sign is another sign of FFA.

Histopathologically, FFA belongs to the lymphocytic primary scarring alopecia type, showing a perifollicular lymphoid cell infiltrate and perifollicular fibrosis with hair follicle destruction. These are similar features to those of lichen planopilaris (LPP).[2] FFA is thought to be a variant of LPP,[1-3,6] although this suggestion is currently controversial.[4]

The aetiology of FFA is unknown. However, hormonal factors are supposed to play a role, and some autoimmune diseases such as vitiligo, discoid lupus erythematosus and thyroid disorders, have been reported to occur concurrently with FFA. Although a genetic component has not yet been established, FFA has been reported in a few families.[7-10] We herein report 20 new cases of FFA from nine families. The aim of this study is to analyse the clinical and dermoscopic features in familial FFA.

PATIENTS AND METHODS

Twenty patients with FFA, belonging to nine different families, were included in this cross-sectional study, which was performed in the University Hospital of Granada in Spain. The inclusion criterion was familial cases of FFA, with clinical and dermoscopic signs of FFA, that is hairline recession with follicular opening loss, with or without eyebrows alopecia, and characterised dermoscopically by perifollicular erythema and/or hyperkeratosis. Patients with no hairline recession but eyebrow loss and dermoscopic signs of FFA were also included. Dermoscopy was performed with a handheld DermLite II PRO HR. A 4 mm punch biopsy was performed in all cases to confirm the diagnosis of FFA.

Patient history including gender, age, time of menopause and personal or familial history of alopecia were registered. Furthermore, data about the FFA such as duration, size of the scarring band, grade, hair loss in other locations of the body and coexistence with other types of alopecia were recorded. The severity of FFA was classified according to the area of cicatricial skin produced by the recession of the frontal and temporal hairline, and includes five grades as follows: I (<1 cm), II (1–2.99 cm), III (3–4.99 cm), IV (5–6.99 cm) and V (>7 cm).[4] The dermoscopic data evaluated included perifollicular erythema and hyperkeratosis, ivory white background, hairline vellus absence and the presence of red dots. In addition, dermoscopic signs of other types of alopecia were recorded, as was evidence of facial papules and lonely hair sign.

The software SPSS 22.0 (IBM Corp, Armonk, NY, USA) was used in order to perform the descriptive analysis of data.

RESULTS

Sample description

Twenty Caucasian patients belonging to nine different families were studied (Table 1). All patients were female except for the third family, which included two brothers (male to female ratio 1:10). All families were composed of sisters or brothers, with the exceptions of the second and the ninth families which included, respectively: a mother and her daughter; a mother, her daughter and the first cousin of the mother. Interestingly, in the second family both women were cousins due to their respective fathers' and mothers' relationship and, curiously, in this case the mother and daughter reported a sudden onset and rapid progression of the alopecia. The fifth family included two sisters and one niece.

Table 1. General data of the patients included in the study

Family number	Patient number	Gender	Age	Menopause (age)	Age of onset of FFA	Familial background of other types of alopecia
1	1 (sister)	Female	62	Yes (50)	59	AGA* (father and one son)
	2 (sister)	Female	66	Yes (54)	63	AGA (father and one of two daughters)
2	1 (mother)	Female	78	Yes (55)	74	None
	2 (first cousin of patient 1)	Female	68	Yes (52)	64	AGA (mother and two sons)
	3 (daughter)	Female	50	No	47	None
3	1 (brother)	Male	44	–	40	None
	2 (brother)	Male	46	–	45	None
4	1 (sister)	Female	39	No	33	None
	2 (sister)	Female	42	No	40	None
5	1 (sister)	Female	88	Yes (50)	86	None
	2 (sister)	Female	72	Yes (50)	71	None
	3 (niece)	Female	64	Yes (50)	60	None
6	1 (sister)	Female	53	Yes (49)	45	None
	2 (sister)	Female	46	No	46	None
7	1 (sister)	Female	74	Yes (39)	50	AGA (father, one of three brothers, one of three sons), Total AA** (one of three sons)
	2 (sister)	Female	72	Yes (51)	52	AGA (father and one of three brothers)
8	1 (sister)	Female	77	Yes (52)	60	None
	2 (sister)	Female	74	Yes (50)	70	None
9	1 (mother)	Female	70	Yes (51)	62	None
	2 (daughter)	Female	44	No	42	None

*AGA, androgenetic alopecia; **AA, areata alopecia.

Clinical features

The mean age of all examined patients was 61.4 years (45 years for males and 63.3 years for females), with a range between 39 and 88 years. Thirteen out of 18 women (72%) were postmenopausal at the moment of the clinical evaluation, and 12 of them (92%) were postmenopausal at the debut of the alopecia. The mean age of menopause was 50.2 (range 39–55). Early menopause (≤45 years) was observed in one patient (5%).

The mean age of the onset of FFA was 55.4 years (range 33–86). The mean age of the debut of symptoms in mothers was 68 years, whereas in daughters it was 44.5 years. Patient No. 1 of the brothers' family had a primary panhypopituitarism due to a surgical removal of a pituitary macroadenoma. Five patients had a family history of androgenetic alopecia, and one also had it of areata alopecia.

The average time for the development of FFA in the sample was 6 years (Table 2). Grade II was the most frequently observed (50%) type, followed by grade III (25%; Fig. 1a,b), grade V (10%; Fig. 1c,d), and equally by grades 0 (5%), I (5%) and IV (5%). The mean recession was 2.83 cm in the frontal area and 1.99 cm in the temporoparietal area. In family No. 2, the mother had 2.8 cm recession in both frontal and temporoparietal areas, whereas her daughter had 2.0 and 2.2 cm, respectively. In fact, although both women had grade II FFA, the size of the alopecic band was higher in the mother, who also had total eyebrows alopecia. Regarding family No. 9, the mother had a grade III FFA with a recession of 3.0 cm in frontal hairline, while the daughter had grade II FFA with a 1.2 cm recession. One patient (second patient of family No. 4) had eyebrow alopecia with dystrophic hairs and dermoscopic signs of FFA in frontal hairline, but no scalp alopecia.

All patients also demonstrated some degree of eyebrow alopecia, and 70% of patients had body hair loss. Coexistence with androgenetic alopecia was found in only two patients (10%), both of whom had a decrease in hair density and the presence of miniaturized hairs (grade I of the Ludwig classification). One patient had patches of scarring alopecia due to lichen planopilaris (LPP; second patient of family No. 3), and his brother had dermoscopic signs of LPP with no scarring alopecia, but occipital involvement because of the FFA.

Table 2. Physical examination features of the patients included in the study

Family number-Patient number	Duration of alopecia (years)	Frontal/temporo-parietal hairline recession (size in cm)	FFA grade	Eyebrows loss	Hair loss in other sites of the body	Other types of alopecia
F1-P1	3	1.2/0	II	Yes (partial)	Upper and lower limbs (partial)	No
F1-P2	3	3/2	III	Yes (partial)	Lower eyelids eyelashes (partial) Upper and lower limbs (total) Axillae (partial)	AGA (Ludwig grade I)
F2-P1	4	2.8/2.8	II	Yes (total)	Upper limbs (total) Lower limbs (partial)	No
F2-P2	4	2.4/2.4	II	Yes (partial)	Upper and lower limbs (partial) Pubis (total)	AGA (Ludwig grade I)
F2-P3	3	2/2.2	II	Yes (partial)	Axillae and pubis (partial) Upper and lower limbs (total) Axillae and pubis (total, removed by laser)	No
F3-P1	4	2/0	II	Yes (partial)	Beard, lower limbs and forearms (total) Axillae and pubis (partial)	Lichen planopilaris
F3-P2	1	1.3/0	II	Yes (partial)	Beard, upper and lower limbs (partial)	Lichen planopilaris with scarring alopecia
F4-P1	6	0.3/0	I	Yes (partial)	Upper limbs (partial)	No
F4-P2	1.5	0/0	0	Yes (partial)	No	No
F5-P1	2	3/3	III	Yes (total)	Upper and lower limbs (total)	No
F5-P2	1.2	1.5/1	II	Yes (total)	No	No
F5-P3	4	1.5/1	II	Yes (total)	Upper and lower limbs and axillae (total)	Occipital involvement
F6-P1	8	11/5	V	Yes (total)	No	No
F6-P2	0,2	1/1	II	Yes (partial)	No	No
F7-P1	24	3.4/3.4	III	Yes (total)	Upper and lower limbs (total) Eyelashes (partial) Axillae (partial)	No
F7-P2	20	8/10	V	Yes (total)	Eyelashes (partial) Upper and lower limbs (partial)	No
F8-P1	17	5/3	IV	Yes (total)	Upper and lower limbs (total) Eyelashes, axillae and pubis (partial)	No
F8-P2	4	3/3	III	Yes (total)	Axillae, lower and upper limbs (partial)	No
F9-P1	8	3/0	III	Yes (total)	No	No
F9-P2	2	1.2/0	II	Yes (total)	No	No

AGA, androgenetic alopecia; F, family; P, patient.

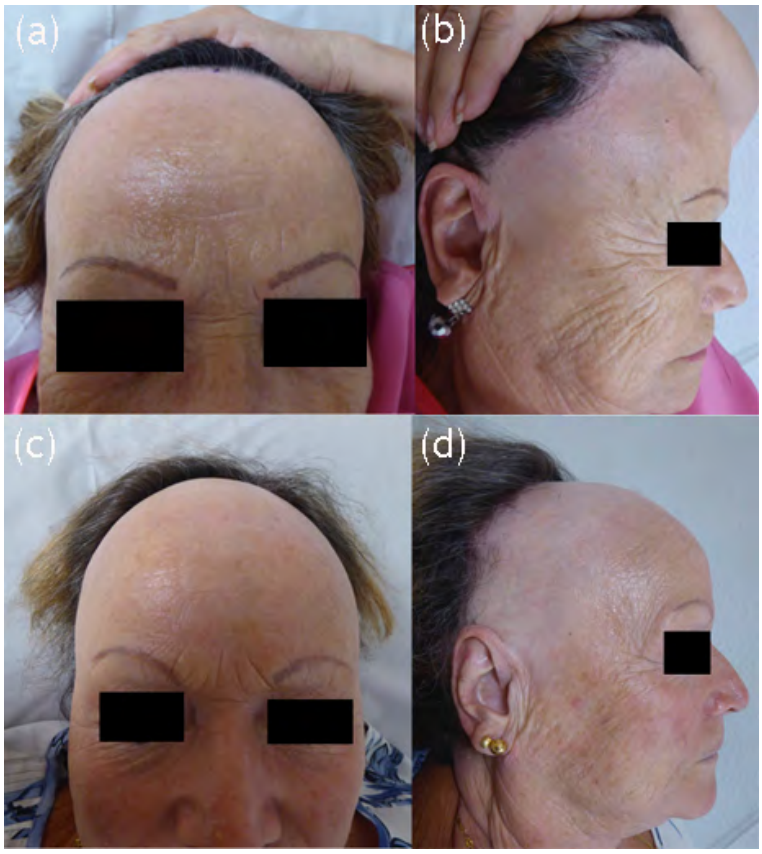

Figure 1. Patients from family No. 7: **(a)** Frontal and **(b)** lateral pictures of patient No. 1, with grade III FFA. **(c)** Frontal and **(d)** lateral pictures of patient No. 2, with grade V FFA. Both women had total alopecia of eyebrows and underwent eyebrows micropigmentation.

Dermoscopic features

Referring to the dermoscopic signs (Table 3), perifollicular erythema and hyperkeratosis were both found in 85% (17/ 20) of patients. Fourteen of nineteen patients had an ivory white background and 63% had white dots. Vellus absence in the hairline and lonely hair sign were both prominently observed features, these found in 79% and 68% of cases, respectively. However, red dots and facial papules were less frequently seen, with a prevalence of 5% and 25%, respectively.

Table 3. Dermoscopic findings of the patients included in the study

Family number-Patient number	Perifollicular erythema/hyperkeratosis	Ivory white background	White dots	Hairline vellus hair absence	Red dots	Facial papules	Lonely hair sign
F1-P1	+/+	+	+	+	–	–	+
F1-P2	+/+	+	+	+	–	–	+
F2-P1	+/+	+	+	+	–	–	+
F2-P2	+/+	+	+	+	–	–	+
F2-P3	+/+	+	+	+	–	–	+
F3-P1	+/+	+	+	+	–	+	+
F3-P2	+/+	+	+	+	–	+	+
F4-P1	+/+	–	–	+	+	+	+
F4-P2	+/+ (frontal)	–	–	–	–	+	–
F5-P1	–/–	+	–	+	–	–	+
F5-P2	+/+	+	–	+	–	–	+
F5-P3	+/+	No data	No data	No data	No data	–	No data
F6-P1	+/+	+	+	+	–	–	+
F6-P2	–/–	–	–	–	–	–	+
F7-P1	+/+	+	+	+	–	–	–
F7-P2	+/+ (intense, in the entire scalp)	+	+	+	–	–	–
F8-P1	+/+	+	+	+	–	–	–
F8-P2	–/+	+	+	+	–	–	–
F9-P1	+/+	–	–	–	–	–	+
F9-P2	+/–	–	–	–	–	+	–
Total	85%/85%	74%	63%	79%	5%	25%	68%

F, family; P, patient.

Histopathological features

The main features found in the biopsies were the presence of a perifollicular lymphoid cell infiltrate, interface dermatitis and concentric perifollicular fibrosis along with follicular destruction. Loss or atrophy of sebaceous glands was also noted.

DISCUSSION

The aetiology of FFA is not well known and is postulated by some authors to be a type of lichen planopilaris (LPP). Hormonal factors are thought to play a role in the pathogenesis of FFA as it affects predominantly postmenopausal women and due to the early onset in women with premature menopause.[4,13] The response of some patients to finasteride or dutasteride also supports this hypothesis.[4] Autoimmune diseases, such as vitiligo, discoid lupus, thyroid dysfunction and Sjögren syndrome, have been previously reported to occur concurrently with FFA in up to 30% of patients.[4,6,12,14,15] Twelve patients out of 18 (66.67%) reported herein were postmenopausal at the onset of the alopecia, and additionally one of them had had an early menopause (patient No. 1 of family No. 7). Interestingly, one of the brothers had a hypogonadotropic hypogonadism in the context of an iatrogenic primary panhypopituitarism.

The genetic basis of this disease has not yet been established. The genetic hypothesis is supported by the coexistence of autoimmune diseases in familial cases.[11] Nevertheless, we have not found autoimmune disease in our cohort. Since the first familial case was reported by Junqueira Ribeiro Pereira and colleagues[7] in two sisters, only a few more familial cases have been published.[8-12.16] Most reports of familial cases of FFA refer to siblings.[8-12] In the largest case study of familial FFA to date, four families (mother and daughter) with FFA were reported.[11] The biggest multicentre study conducted so far regarding FFA found a family history of FFA in 8% of patients.[4] We herein report 20 new patients with FFA belonging to nine families: six composed of sisters (one of them plus a niece); one of brothers; one of a mother and daughter; and another of a mother and daughter plus the mother's first cousin.

Previous authors have described an interesting finding in familial cases of FFA: all mothers were postmenopausal at the time of diagnosis, while all daughters were premenopausal.[11] Moreover, mothers attended the consultation at an advanced stage of the disease, but daughters did so at an earlier stage, allowing the authors to make an early diagnosis. Our two families who were composed of mothers and daughters exhibited the same behaviour. The daughters may have checked their hair carefully due to their mothers' condition and sought medical help earlier, which could explain the early grade of alopecia found in them. In addition, in the second family both mother and daughter curiously had an abrupt onset and rapid progress of the alopecia, which may be related to genetic factors within members of the same family. On the other hand, it seems interesting that the age of onset of the alopecia was

lower in both daughters than in their mothers, since the mean age of debut was 68 years in mothers (all postmenopausal) and 44.5 in daughters (all premenopausal).

The largest study of FFA so far was a multicentre review, in which the authors described the epidemiology and clinical presentation of 355 patients with FFA.[4] Findings of classic LPP elsewhere in the scalp were found in only two patients. Coexistence with androgenetic alopecia was only seen in two patients, a lower rate than in the multicentre study (10% vs 40%).[4] Lonely hair sign and facial papules were found in more patients in our sample than in the mentioned study (68% and 25% in our sample vs 50% and 14%, respectively).[4] However, another study including 249 patients also reported a higher frequency of lonely hair sign (89%).[17] All of our patients had eyebrow alopecia, and body hair was affected in 70% of cases; both higher rates than the reported in the multicentre study[4] (80% and 24%, respectively). The majority of patients had grades II and III of FFA, being altogether 75% of the sample.

Dermoscopy can be useful to achieve an early diagnosis in patients with no clinical alopecia, as happened in our patient No. 2 of family No. 4. For that patient, the frontal hairline showed perifollicular hyperkeratosis and erythema, but no hairline recession. Moreover, dystrophic hairs were the main dermoscopic sign in their alopecic eyebrows. Isolated eyebrow involvement due to FFA should be considered in women eyebrows loss, especially in those cases with few black dots and dystrophic hairs.[18]

Regarding family No. 3 – composed of two brothers – the onset of the alopecia was earlier in the first brother, who was the youngest but had a pituitary macroadenoma, with subsequent iatrogenic primary panhypopituitarism. Both brothers had grade II FFA, but the younger one had a higher hairline recession (2.0 cm vs 1.3 cm). Hormonal disturbance may be related to the earlier development of the alopecia in his case.

Referring to the histopathological findings, our data did not show any differences with respect to the features of non-familial cases. Lymphocytic infiltrate and follicular destruction were the main signs in both groups.[2]

We propose that this study has one main limitation, this being the likelihood recall bias in some epidemiologic data.

In conclusion, we report 20 new cases of familial FFA. The clinical and dermoscopic features in our study are consistent with those of non-familial cases as reported in other studies.

Moreover, the debut of the disease in daughters of mothers with FFA may be earlier. Further studies are needed to clarify the possibility of genetic causality in patients with FFA.

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5.2. Publication 2: Study of the Human Leukocyte Antigen profile in familial frontal fibrosing alopecia.

Porriño-Bustamante ML, López-Nevot MÁ, Aneiros-Fernández J, Casado-Ruiz J, García-Linares S, Pedrinacci-Rodríguez S, García-Lora E, Martín-Casares MA, Fernández-Pugnaire MA, Arias-Santiago S. Study of Human Leukocyte Antigen (HLA) in 13 cases of familial frontal fibrosing alopecia: CYP21A2 gene p.V281L mutation from congenital adrenal hyperplasia linked to HLA class I haplotype HLA A-*33:01; B*14:02; C*08:02 as a genetic marker. Australas J Dermatol. 2019; 60(3):e195-e200.

This article, which has been published in the Australasian Journal of Dermatology, is a case-control study, in which HLA profile was determined in 13 cases of familial FFA. The study showed that all of the patients shared conserved HLA class I haplotypes (F16A, Y16F and S26A) and that CYP21A2 gene p.V281L mutation linked to F16A could be a genetic marker of susceptibility to familial FFA. An antigen-driven mechanism could be the starting point in these patients for the development of the alopecia.

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Study of Human Leukocyte Antigen (HLA) in 13 cases of familial frontal fibrosing alopecia: CYP21A2 gene p.V281L mutation from congenital adrenal hyperplasia linked to HLA class I haplotype HLA-A*33:01; B*14:02; C*08:02 as a genetic marker.

Original research.

María Librada Porriño-Bustamante,[1,2] Miguel Ángel López-Nevot,[3,4] José Aneiros-Fernández,[5] Jorge Casado-Ruiz,[4] Susana García-Linares,[6] Susana Pedrinacci-Rodríguez,[6] Elena García-Lora,[7] María Antonia Martín-Casares,[3] María Antonia Fernández-Pugnaire,[8] Salvador Arias-Santiago.[7,9]

[1]Servicio de Dermatología, Hospital Universitario La Zarzuela, Madrid,

[2]Universidad de Granada.

[3]Laboratorio Clínico, Unidad de Histocompatibilidad, Hospital Universitario Virgen de las Nieves, Granada

[4]Departamento de Bioquímica, Biología Molecular III e Inmunología, Univ. de Granada,

[5]Servicio de Anatomía Patológica, Parque Tecnológico de la Salud, Granada

[6]Unidad de Genética Clínica, Laboratorio Clínico, Hospital Universitario Virgen de las Nieves, Granada

[7]Servicio de Dermatología, Hospital Universitario Virgen de las Nieves, Granada

[8]Servicio de Dermatología, Parque Tecnológico de la Salud, Granada and

[7]Facultad de Medicina, Universidad de Granada

Correspondence: María Librada Porriño-Bustamante, Hospital Universitario La Zarzuela, Calle de Pleyades, 25. 28023 Madrid, Spain. Email: mporrinobustamante@gmail.com

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Conflict of interest: None declared.

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ABSTRACT

Background/Objectives: The aetiology of frontal fibrosing alopecia is unknown, and its genetic aspect remains uncharacterised. The aim of this report is to elucidate if major histocompatibility complex is associated with familial frontal fibrosing alopecia.

Methods: A case–control study was performed of 13 patients with frontal fibrosing alopecia belonging to six families. Their human leukocyte antigen profiles were compared to the data of 636 healthy controls without frontal fibrosing alopecia. Patients underwent high-resolution genomic typing for human leukocyte antigen class I and II loci by PCR-SSO for Luminex. In addition, CYP21A2 gene (major histocompatibility complex class III) mutations were detected by PCR-SSO on strips.

Results: 61.5% of patients shared CYP21A2 gene p.V281L linked to the F16A human leukocyte antigen class I haplotype (HLA-A*33:01; B*14:02; C*08:02; Pc < 0.000001). The patients F16A-negative shared other human leukocyte antigen class I haplotypes: Y16A (3/13) and S26 (2/13).

Conclusion: CYP21A2 gene p.V281L mutation can be used as a genetic marker for susceptibility to familial frontal fibrosing alopecia. Both the linkage of the mutation to F16A and the fact that F16A-negative patients share other human leukocyte antigen class I haplotype, point to an antigen-driven mechanism in susceptible patients with these haplotypes.

Keywords: adrenal congenital hyperplasia, ancestral haplotypes, antigen driven, familial, frontal fibrosing alopecia, haplotype, human leukocyte antigen, scarring alopecia.

INTRODUCTION

Frontal fibrosing alopecia (FFA) is a scarring alopecia characterised by the recession of the frontal and temporoparietal hairline, leading to an alopecic band without follicular openings. Its aetiology is unknown, although hormonal factors are thought to be involved.[1] Autoimmune diseases such as vitiligo, discoid lupus, thyroid disorders and Sjögren syndrome have been reported to occur concurrently with FFA. FFA is considered by some authors as a type of lichen planopilaris (LPP), similar to Lassueur-Graham-Little-Piccardi syndrome.[2]

FFA has been reported in a few families,[1,3-6] with a possible autosomal dominant inheritance with reduced penetrance.[7]

In scarring alopecias, the expression of human leukocyte antigen (HLA) class I and II molecules on the epithelial stem cells of the bulge may break the immune privilege of this site.[8] This may be followed by the apoptosis of these cells induced by a CD4+ and CD8+ lymphocytic infiltrate.

The human major histocompatibility complex (MHC) is a large gene complex, located in the short arm of chromosome 6 (6p21.3).[9] MHC includes HLA genes, which have a high level of polymorphism, and non-HLA genes. The extended version of the human MHC includes 421 loci. HLA class II genes (DRB1, DQB1, DPB1) are the most centromeric in human MHC classical class II subregion, whereas HLA class I genes (A, B, C) are the most telomeric in the human MHC classical class I subregion.[10] HLA class II and class I genes are separated by MHC classical class III subregion, which contains non-HLA genes including CYP21A2 gene codifying for 21-β hydroxylase.[10] MHC genes are inherited in block forming haplotypes. Conserved HLA haplotypes with the same combination of HLA class I and II alleles are named HLA ancestral haplotypes (AH). They have withstood the meiotic recombination throughout evolution and are thought to be conserved due to a selective advantage against infections.[11] These haplotypes are named conserved extended MHC haplotypes (CEH) if allelic variants for MHC non-HLA genes are also considered.

HLA class I proteins are expressed on the surface of all nucleated cells, while HLA class II is limited to immune active cells. The former presents intracellular peptides to the T-cell receptor on CD8+ T cells and to killer inhibitory receptors of the natural killer cells. The latter presents extracellular peptides to the T-cell receptor on CD4+ T cells (often helpers).[9] HLA

polymorphisms have been associated with susceptibility or protection to infectious, inflammatory and autoimmune diseases.[12]

Only two articles have been published about HLA class II polymorphisms in familial FFA.[3,4] These did not find the association with HLA-DR1 previously described in Lassueur-Graham-Little-Piccardi syndrome[13] and in familial[14] and sporadic lichen planus.[15]

The objective of this study is to analyse the HLA class I (A, B, C), II (DRB1, DQB1) and III (CYP21A2 gene) in patients with familial FFA.

MATERIAL AND METHODS

Thirteen patients with FFA, belonging to six different families (Table 1), were included in this case-control study. Inclusion criteria were patients older than 18-year-old with a first degree relative with FFA, fulfilling clinical and dermoscopical FFA's criteria (hairline recession without follicular openings, with perifollicular erythema and/or hyperkeratosis in dermoscopy). Clinical diagnosis was confirmed by a skin biopsy.

All patients were from Spain, and there was no consanguinity between different families. Age, gender, age at onset and coexistence of androgenetic alopecia were analysed. DNA was isolated from anticoagulant-treated peripheral blood using standard methods from Qiagen. HLA class I and class II alleles were typed by sequence-specific oligonucleotide with the LifecodesVR HLA-SSO typing kit (Immucor, Norcross, GA, USA), according to the manufacturer's protocol. Sequence-specific oligonucleotid products were read by Luminex and results interpreted with Match it! DNA version 11 (Lifecodes, Immucor). Furthermore, investigation of mutations of CYP21A2, which is the gene encoding the enzyme 21-hydroxylase, related to congenital adrenal hyperplasia, was performed on all patients. Eleven mutations were analysed: P30L, I2 splice (I2G), Del8 bpE3 (G110del8nt), I172N, Cluster E6 (I236N, V237E, M239K), V281L, L307 frameshift (F306 + T), Q318X, R356W, P453S and R483P, using congenital adrenal hyperplasia StripAssay 4–380 by multiplex PCR amplification, simultaneous biotin labelling, directly on the StrpAssay teststrips for hybridisation, and labelled products detected by streptavidin-alkaline phosphatase for identification.

The HLA profiles of the patients were compared to the data of 636 healthy controls without FFA from Virgen de las Nieves University Hospital.

Statistical analysis was performed to compare allelic, genotypical and haplotypical distributions among patients and controls using the two-tailed Fisher exact test with 2 x 2 contingency tables using R program. Bonferroni correction was applied for AH multiplying the P-value for 37 and also for locus B multiplying the P-value for 42 (corrected P-value or Pc).

RESULTS

Families are referred in this report by the letter 'F' followed by the family number and patients by a 'P' followed by the patient number (Table 1).

All patients were females, excluding two brothers in F2. Both brothers had dermoscopic signs of lichen planopilaris, and one of them (F2-P2) had also scarring patches of alopecia. Coexistence with androgenetic alopecia was found in two patients (11.1%). Epidemiological data are summarised in Table 1.

Table 1. Families and family relationship

Family number	Patient number	Age
1	1 (sister)	88
	2 (sister)	72
2	1 (brother)	44
	2 (brother)	46
3	1 (mother)	78
	2 (first female cousin of patient 1)	68
	3 (daughter)	50
4	1 (sister)	62
	2 (sister)	66
5	1 (sister)	39
	2 (sister)	42
6	1 (sister)	53
	2 (sister)	46

HLA class I haplotype HLA-A*33:01; B*14:02; C*08:02 (F16A) and HLA AH 65.1 are associated with familial FFA

F16A HLA class I haplotype was shared by eight (61.5%) members of F1, F2 and F3 and also by F4-P1 (Table 2), whilst only 21 out of 636 (3.3%) controls were F16A positive ($Pc < 0.000001$; Table 3).

F16A is included in the extended HLA AH 65.1 (HLA- A*33:01; B*14:02; C*08:02; DRB1*01:02, DQB1*05:01), which was present in siblings of F1 and F2 (4/13 patients, 30.8%; Table 2) vs 13 out of 636 (2.0%) in control group ($Pc = 0.007$; Table 3).

F16A was linked to the HLA class II haplotypes HLA- DRB1*04:02; DQB1*03:02 and to HLA-DRB1*03:01; DQB1*02:01 in F3 and F4-P1, respectively. Members from F1 and F3 were HLA class I and II identical in each family (Table 2). Sisters from F2 were haploidentical for AH 65.1, while sisters from F4 shared no HLA haplotypes.

Six of the eight (75%) F16A-positive patients (F1, F3 and F2-P2) had in the other haplotype the HLA-B*44 allele (44:02 or 44:03; Table 2) vs four out of 21 (15.4%) in control group (P = 0.008, Pc = 0.32; Table 3). The remaining two F16A-positive patients (F2-P1 y F4-P1) had HLA-B*35:01 in the other haplotype (Table 2).

HLA class I haplotype F16A is linked to CYP21A2 p.V281L mutation in patients with FFA

CYP21A2 mutations are linked to HLA haplotypes because this gene is located in the class III subregion of human MHC. All of the patients with F16A haplotype (8) were heterozygous for the p.V281L mutation (Table 2).

F16A-negative families

F5 and F6 were F16A-negatives. However, the two sisters of F5 shared another ancestral HLA haplotype, AH7.1: HLA- A*03:01; B*07:02; C*07:02; DRB1*15:01; DQB1*06:02, whereas the two sisters from F6 shared HLA class I haplotype S26A HLA-A*11:01; B*35:01; C*04:01, included in the HLA AH 35.2. F6-P2 had also in the other HLA haplotype the Y16G HLA class I haplotype HLA-A*03:01; B*07:02; C*07:02 that is included in the HLA AH7.1. F4-P2 had also the Y16G HLA class I haplotype HLA-A*24:02; B*07:02; C*07:02 but in this case was related to the HLA AH7.2 (Table 2).

Table 2. Human leukocyte antigen (HLA) ancestral haplotype (AH) typing in familial frontal fibrosing alopecia

Family number-Patient number	AH HLA-B in the other haplotype	HLA class I and II typing by haplotypes	*CYP21A2*
F1-P1	AH 65.1 (F16A)	HLA-A*33:01; B*14:02; C*08:02; DRB1*01:02; DQB1*05:01	V281L
	B*44+	HLA-A*30:02; B*44:02; C*02:02; DRB1*16:01; DQB1*05:02	N
F1-P2	AH 65.1 (F16A)	HLA-A*33:01; B*14:02; C*08:02; DRB1*01:02; DQB1*05:01	V281L
	B*44+	HLA-A*30:02; B*44:02; C*02:02; DRB1*16:01; DQB1*05:02	N
F2-P1	AH 65.1 (F16A)	HLA-A*33:01; B*14:02; C*08:02; DRB1*01:02; DQB1*05:01	V281L
	B*35 +	HLA-A*29:02; B*35:01; C*04:01; DRB1*13:05; DQB1*03:01	N
F2-P2	AH 65.1 (F16A)	HLA-A*33:01; B*14:02; C*08:02; DRB1*01:02; DQB1*05:01	V281L
	B*44+ (D24G)	HLA-A*02:01; B*44:02; C*05:01; DRB1*13:02; DQB1*06:03	N
F3-P1	F16A (AH65.1)	HLA-A*33:01; B*14:02; C*08:02; DRB1*04:02; DQB1*03:02	V281L
	B*44+ (D14G)	HLA-A*29:02; B*44:03; C*16:01; DRB1*13:01; DQB1*06:03	N
F3-P2	F16A (AH65.1)	HLA-A*33:01; B*14:02; C*08:02; DRB1*04:02; DQB1*03:02	V281L
	B*44+ (D14G)	HLA-A*29:02; B*44:03; C*16:01; DRB1*13:01; DQB1*06:03	N
F3-P3	F16A (AH65.1)	HLA-A*33:01; B*14:02; C*08:02; DRB1*04:02; DQB1*03:02	V281L
	B*44+ (AH44.2v)	HLA-A*26:01; B*44:03; C*04:01; DRB1*07:01; DQB1*02:02	N
F4-P1	F16A (AH65.1)	HLA-A*33:01; B*14:02; C*08:02; DRB1*03:01; DQB1*02:01	V281L
	B*35+ (AH35.5v)	HLA-A*24:02; B*35:02; C*04:01; DRB1*11:04; DQB1*03:01	N
F4-P2	Y16G (AH 7.2)	HLA-A*24:02; B*07:02; C*07:02; DRB1*10:01; DQB1*05:01	N
	B*44+ AH 44.2	HLA-A*29:02; B*44:03; C*16:01; DRB1*07:01; DQB1*02:02	N
F5-P1	AH 7.1 (Y16G)	HLA-A*03:01; B*07:02; C*07:02; DRB1*15:01; DQB1*06:02	N
	AH 60.1 (v)	HLA-A*11:01; B*40:01; C*03:04; DRB1*04:04; DQB1*03:02	N
F5-P2	AH 7.1 (Y16G)	HLA-A*03:01; B*07:02; C*07:02; DRB1*15:01; DQB1*06:02	N
	B*44+ AH 44.2 (v)	HLA-A*02:01; B*44:03; C*16:01; DRB1*07:01; DQB1*02:02	N
F6-P1		HLA-A*02:01; B*41:01; C*17:01; DRB1*07:01; DQB1*02:02	N
	S26A/AH 35.2	HLA-A*11:01; B*35:01; C*04:01; DRB1*11:04; DQB1*05:01	N
F6-P2	Y16G (AH 7.1)	HLA-A*03:01; B*07:02; C*07:02; DRB1*11:04; DQB1*03:01	N
	S26A/AH 35.2	HLA-A*11:01; B*35:01; C*04:01; DRB1*11:04; DQB1*03:01	N

F, family, followed by the number; N, Normal *CYP21A2* allele; P, patient, followed by the number.

Table 3. Frequency of the HLA class I extended haplotype in patients and controls

HLA class I haplotype	Patients (n = 13)	Controls (n = 636)	P	P_c^*
Group 1				
AH65.1	4 (30.8%)	13 (2.0%)	0.0002	0.007
F16A (AH 65.1)	8 (61.5%)	21 (3.3%)	<0.00000001	<0.000001
F16A (AH 65.1)/B44+	6/8 (75%)	4/21 (15.4%)	0.008	0.32
F16A (AH 65.1)/B44+ or B35+	8/8 (100%)	4/21 (15.4%)	0.0001	0.004
Group 2				
AH 7.1	2 (15.4%)	19 (1.5%)	0.06	
Y16G (AH 7.1)	3 (23.1%)	45 (7.1%)	0.06	
S26A (AH 35.2)	2 (15.4%)	13 (2.0%)	0.03	>0.99

*P_c, corrected P-value (correction for the number of ancestral haplotype (AH), which are 37 and 42 for HLB locus). Group 1 or F16A positives. Group 2 or F16A negatives.

DISCUSSION

Most of the patients in our cohort shared F16A HLA class I haplotype (HLA-A*33:01; B*14:02; C*08:02), suggesting this may predispose to familial FFA (Pc < 0.000001). F16A-positive patients may be divided into two subgroups depending on the HLA class II haplotypes. Subgroup 1, including patients from F1 and F2, have complete HLA AH 65.1, that is, F16A haplotype linked to HLA-DRB1*01:02; DQB1*05:01; associated with familial FFA (Pc = 0.007). The second subgroup includes patients who are AH 65.1 negative, as happened in F3, in which F16A haplotype is linked to DRB1*04:02; DQB1*03:02; and also in F4-P1, linked to DRB1*03:01; DQB1*02:01.

There are a few published case reports regarding FFA and HLA. HLA-DR1 (serological specificity) was found in a mother and her daughter with Lassueur-Graham-Little-Piccardi syndrome.[13] HLA-DR1 has also been found in lichen planus.[15] However, this allele had not been studied in FFA until the report of two sisters, who were negative for it.[3] HLA-DRB*01:01, which codifies for HLA-DR1, is closely related to DRB1*01:02 allele, included in AH 65.1. In previous reports, the four cases of familial FFA typed for HLA were HLA-DRB1*01 negatives,[3,4] but they had in common the HLA-DRB1*04:02-DQB1*03:02 haplotype, which was found in all members of F3, linked to F16A HLA class I haplotype. There is no report about HLA class I typing in FFA.

F16A HLA class I haplotype showed a stronger association with familial FFA than the complete AH 65.1 haplotype. It has not been associated in block with any autoimmune disease; however, HLA-A*33, B*14 and C*08 was related to lower 6-month CD4+ T-cell count in patients with symptomatic primary human immunodeficiency virus infection (HIV).[16]

Regarding the alleles of the F16A haplotype, HLA-A*33:01 has been associated with drug-induced liver injury, persistent hepatitis B virus infection and vitiligo.[17] HLA-B14 is found in severe aplastic anaemia and associated negatively with progression to autoantibodies generation in patients with diabetes type I.[18] Finally, HLA-C*08:02 is associated with the HIV-1-infected long-term survivor group in Zimbabwe population[19] and with low cytotoxic T lymphocytes immune response in Iranian patients having Human T-cell lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis.[20] There are no data about HLA-C*08:02 association with autoimmune diseases.

The highest frequency of AH 65.1 is found in Hispanics (0.81%, position 4 in a ranking of HLA haplotype frequency), followed by Caucasians (0.49%, position 18).[21] In Europe, the highest frequency is found in Italy (1.41%) and Murcia (Spain; 1.5%).[22] In our cohort of controls, it has a prevalence of 2.04%. In addition, AH 65.1 has been considered as a Jewish HLA haplotype (position 2 in a ranking of 14 Pan-Jewish HLA haplotypes).[23] AH 65.1 has been associated with protection for chronic Chagas disease and with IgA deficiency,[24] but there are no data relating it with autoimmune diseases.

The main finding of this work is the linking in our patients of F16A HLA class I haplotype with CYP21A2 gene p.V281L mutation, since all F16A-positive patients were heterozygous to this mutation. Because of CYP21A2 gene is located in the MHC class III region, the genomic fragment that may transmit the susceptibility to familial FFA could be mapped, from HLA-A*33:01 allele in the F16A HLA class I haplotype (the most telomeric) to the CYP21A2 gene. CYP21A2 gene p.V281L mutation (more prevalent in Ashkenazi Jews) in homozygosis is responsible for the nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency, because of an enzyme with lower function.

The linkage between AH 65.1 and CYP21A2 gene p.V281L, observed in F1 and F2, is included in the conserved extended MHC haplotypes 65.1 described by Dorak and colleagues[25] together with other 36 conserved extended MHC haplotypes. This linkage has been noted in Mediterraneans.[26] In F4-P1, F16A HLA class I haplotype is linked to CYP21A2 gene p.V281L mutation and also HLA class II haplotype DRB1*03:01-DQB1*02:01, which is a MHC extended haplotype recently described for the first time in Croatians.[26] No previous data have been found about the linkage of F16A, V281L mutation and HLA-DRB1*04:02- DQB1*03:02, which is present in F3. These data suggest that these two last extended HLA haplotypes (F4-P1 and F3) have been derived from conserved extended MHC haplotypes 65.1 by interchange of HLA class II region, retaining F16A HLA class I haplotype linked to CYP21A2 gene p.V281L mutation. Implicating the V281L heterozygotic mutation in the pathogenesis of FFA is complicated; however, it may be an adjuvant factor.

Because HLA class I is related to the recognition of intracellular bacteria and viruses, and most of the patients shared F16A HLA class I haplotype, suggests that FFA may be an antigen-driven disease in these susceptible patients.[27,28] The F16A haplotype would confer a higher susceptibility of response of HLA class I molecules to an immunodominant self-antigen

expressed in the follicular bulge during an infection, due to a process of cross-reaction between self-follicular and external antigens. Then, patients having those haplotypes may have an antigen-driven origin for oligoclonal expansion of autoreactive cytotoxic CD8+ T cells, which would produce an inflammatory reaction and lead to the destruction of the hair follicle. Conserved allelic variants of innate immunity genes located between F16 HLA class I haplotype and CYP21A2 gene p.V281L mutation could also play a role; they may help to break the immunoprivilege advantage of the follicle by inducing the expression of HLA class I molecules on the cells of the bulge and then promoting local inflammation. Reports relating hepatitis C virus infection and cutaneous and oral lichen planus support this hypothesis.[29] The determination of the presence of oligoclonal expansion of CD8+ T cells with a narrow T cell receptor repertoire in the inflammatory infiltrate of FFA would be another supporting data. The high frequency of HLA-B44 in the complementary haplotype to F16A suggests that this CD8+ oligoclonal expansion in the lymphocytic infiltrate would be restricted mainly to HLA-B locus in its antigenic peptide recognition. Alternatively, a mutated autosomic dominant gene located between F16A and CYP21A2 genes may be responsible for familial FFA; sequencing of this genomic MHC fragment may confirm this possibility. In this regard, Dlova and colleagues[1] found that their patients lived close by, suggesting that familial cases may be due to the exposure to the same environmental trigger. These triggers could develop the disease in genetically predisposed people so that genetic traits codetermine the susceptibility of an individual to toxics, such as dioxin-like chemicals of fatty animal foods.

In our study, patients from F5, F6 and F4-P2 were F16A HLA class I haplotype negative. F5 shared AH 7.1, which is another HLA AH that include HLA-B*07:02. F4-P2 was also HLA-B7:02 positive, belonging to a related HLA class I haplotype (Y16G) in the HLA AH 7.2. HLA-B7 has already been described associated with familial lichen planopilaris,[30] but not previously in familial FFA. However, the most important finding here is not the presence of an isolated allele, but the existence of an HLA class I haplotype Y16G that includes HLA-B7, which may be related to the FFA development. Curiously, both brothers had small plaques on the scalp with dermoscopic signs of lichen planopilaris, but no one shared HLA-B7. The two sisters from F6 shared HLA class I haplotype S26A, included in the AH 35.2. HLA-B35 was also present in the complementary haplotype in F2-P1 and F4-P1.

Despite the small number of patients in this study, the fact that a very uncommon MHC extended haplotype is shared by most patients is very significant. However, these data should be confirmed in FFA sporadic cases.

In conclusion, all patients in our familial cohort shared conserved HLA class I haplotypes (F16A, Y16G and S26A). The most relevant finding is the linkage of F16A with CYP21A2 gene p.V281L mutation, which could be a genetic marker for susceptibility to familial FFA. Both the linkage of the mutation to F16A and the fact that F16A-negative patients share other HLA class I haplotypes, point to an antigen-driven mechanism in susceptible patients with these haplotypes. The oligoclonal T-cell expansion that destroys the hair follicle cells could be characterised and even used as a future target of treatment. These results may be applied to genetic diagnosis and could help to elucidate the immunopathogenic mechanism of FFA.

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5.3. Publication 3: Study of the prevalence of rosacea and other associated factors in patients with frontal fibrosing alopecia.

Porriño-Bustamante ML, Fernández-Pugnaire MA, Arias-Santiago S. A Cross sectional Study of Rosacea and Risk Factors in Women with Frontal Fibrosing Alopecia. Acta Derm Venereol. 2019;99(12):1099-1104.

This article, which has been published in the Acta Dermato-Venereologica Journal, is a cross-sectional study, that includes 99 patients with FFA and 40 control subjects. The study showed that women with FFA present a higher prevalence of rosacea than a control group. Furthermore, in the group of patients, perifollicular erythema, higher body mass index and lower progesterone levels were associated with a greater risk of rosacea.

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A Cross-sectional Study of Rosacea and Risk Factors in Women with Frontal Fibrosing Alopecia

Orignal research. Clinical Report.

María Librada Porriño-Bustamante,[1,2] María Antonia Fernández-Pugnaire,[3] and Salvador Arias-Santiago.[4,5]

[1]Department of Dermatology, University Hospital La Zarzuela, Madrid,

[2]Faculty of Medicine, University of Granada,

[3]Department of Dermatology, University Hospital San Cecilio, Granada,

[4]Department of Dermatology, University Hospital Virgen de las Nieves, Granada and

[5]Institute of Biosanitary Investigation ibs. Granada, Spain

Corr: Ma Librada Porriño-Bustamante, Department of Dermatology, University Hospital La Zarzuela, Calle de Pleyades, 25, ES-28023 Madrid, Spain. E-mail: mporrinobustamante@gmail.com

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ABSTRACT

Frontal fibrosing alopecia has been related to some autoimmune diseases, but the association with rosacea is not clear. The objective of this study was to analyse the prevalence of rosacea in a group of patients with frontal fibrosing alopecia. A cross-sectional study, including 99 women with frontal fibrosing alopecia and 40 control subjects, was performed, in which clinical, dermoscopic and hormonal data were analysed. Women with frontal fibrosing alopecia presented a higher prevalence of rosacea than the controls did (61.6% vs. 30%, p=0.001), especially those with severe grades of alopecia (77.8% in grade V vs. 33.3% in grade I, p=0.02). Binary logistic multivariate analysis showed that perifollicular erythema (odds ratio (OR) 8.5; 95% confidence interval (95% CI) 1.73–42.30), higher body mass index (OR 1.16; 95% CI 1.01– 1.34) and lower progesterone levels (OR 0.15; 95% CI 0.028–0.89) were associated with a higher risk of rosacea in patients with frontal fibrosing alopecia. In conclusion, patients with frontal fibrosing alopecia presented a higher prevalence of rosacea than the control group did. Perifollicular erythema, higher body mass index and lower progesterone levels were associated with a higher risk of rosacea in the group with frontal fibrosing alopecia.

Keywords: frontal fibrosing alopecia; scarring alopecia; cicatricial alopecia; rosacea; hormones; comorbidity.

SIGNIFICANCE

The association between frontal fibrosing alopecia and rosacea is not clear. In this cross-sectional study, including 99 women with frontal fibrosing alopecia and 40 controls, women with frontal fibrosing alopecia presented a higher prevalence of rosacea than the controls did (61.6% vs. 30%), especially those with severe grades of alopecia (77.8% in grade V vs. 33.3% in grade I). Moreover, perifollicular erythema, higher body mass index and lower progesterone levels were associated with a higher risk of rosacea in the group with frontal fibrosing alopecia.

INTRODUCTION

Frontal fibrosing alopecia (FFA) is a lymphocytic scarring alopecia characterized by progressive recession of the frontal and temporoparietal hairline with loss of follicular openings. The eyebrows are often affected, and sometimes also the eyelashes and body hair.[1] Typical dermoscopic findings include perifollicular erythema and hyperkeratosis.[2] Lonely hair sign occurs in some patients.[3]

Despite the initial description of FFA in postmenopausal women, an increasing number of cases in premenopausal women have been described.[4] Moreover, some men with FFA and familial cases have also been reported.[2]

The pathogenesis of this disease is not well known and autoimmunity, genetic, hormonal, and environmental factors may play a role. Lichen pigmentosus, discoid lupus or vitiligo have been associated with FFA.[5-7] Recently, a cross-sectional study without a control group found that 34% of patients with FFA presented rosacea. In this study, the erythematotelangiectatic rosacea was the most frequent subtype, followed by papulopustular rosacea.[8]

Rosacea is an inflammatory skin condition characterized by recurrent or persistent episodes of centrofacial erythema.[9] Rosacea is more frequent in women (female: male ratio 2–3:1), typically aged between 30 and 50 years, and is considerably more common in light-skinned people. The lesions predominate on the cheeks and chin in women, and on the nose in men. The National Rosacea Society (NRS) Expert Committee recognizes 4 subtypes, which frequently overlap (i.e. erythematotelangiectatic, inflammatory papulopustular, phymatous and ocular rosacea).[10,11] Moreover, a single variant, namely granulomatous or lupoid rosacea, is also recognized.[10] This classification was proposed in 2002 and has been used worldwide to compare data about rosacea, although recently the NRS has proposed a new classification based on phenotypes.[12]

The association between FFA and rosacea is not clear, although rosacea-like lesions have been reported on the cheeks in patients with FFA (i.e. perifollicular erythema, sometimes with follicular keratosis, such as keratosis pilaris-like papules).[13,14]

The objectives of this study were to analyse the prevalence of rosacea in a group of women with FFA compared with a control group, and to explore associated factors.

MATERIALS AND METHODS

A cross-sectional study with a control group was performed in the University Hospital of Granada, Spain. Patients with a clinical diagnosis of FFA and controls were included. Inclusion criteria for patients were: age over 18 years, absence of active hormonal therapies, and recession of the frontal and/or temporoparietal hairline, with typical dermoscopic features of FFA, i.e. loss of follicular openings with or without perifollicular erythema and scaling. Eyebrow loss may sometimes be present. A control group with women consulting the Dermatology Department for other reasons (naevi, seborrhoeic keratosis, etc.) was included. The exclusion criteria for controls were the presence of any primary scarring alopecia. All patients and controls signed an informed consent and the project was approved by the local ethics committee.

Age, menarche and age of menopause, and time of evolution of FFA were obtained. The weight and height of participants were measured, and their body mass index (BMI) (kg/m^2) was calculated. Personal history of diabetes, hypertension or dyslipidaemia was recorded. Moreover, the use of sunscreens by the participants was also registered. The severity of the alopecia was assessed using a classification that includes 5 grades of severity and is determined by measuring the area of cicatricial skin between the initial hairline and the recessed one, in the frontal and temporal region. The largest of these areas was used to define severity, with the following grades: I (< 1 cm), II (1–2.99 cm), III (3–4.99 cm), IV (5–6.99 cm) and V (≥ 7 cm, also called “clown alopecia”) (15). Also, typical signs of FFA, such as perifollicular hyperkeratosis/ erythema, lonely hair sign and facial papules, were collected. The presence of pruritus of the scalp or trichodynia were also registered. Diagnosis of rosacea was based on clinical history and physical examination (fixed facial erythema and telangiectasias, facial flushing, papules, pustules or phymatous lesions). The subtypes of rosacea considered were erythematotelangiectatic, inflammatory papulopustular, and phymatous. Clinical information regarding other skin diseases, such as vitiligo, lichen planus or psoriasis, was also obtained.

Blood tests searching for hormonal anomalies were performed in both groups, including follicle-stimulating hormone (FSH), luteinizing hormone (LH), oestradiol, progesterone, testosterone, sex hormone binding globulin (SHBG), prolactin (PRL), 17-hydroxy-progesterone, dehydroepiandrosterone sulfate (DHEAS), androstenedione and dihydrotestosterone.

Student's t-test was applied to compare mean values of quantitative variables, the Shapiro–Wilk test to examine the normality of their distribution, and the Levene's test to study the variance. Qualitative variables were analysed with $\chi 2$ test or, when conditions for this test were not fulfilled, with Fisher's exact test. Binary logistic regression model was performed to analysed factors associated with rosacea in patients with FFA. Differences were considered significant at $p \leq 0.05$ and nearly significant at $p \leq 0.1$. SPSS software (SPSS 20.0, SPSS Inc., Chicago, IL, USA) was used for data analyses.

RESULTS

This study included 99 women with FFA and 40 controls. No significant differences between patients and controls were detected regarding age (63.3 vs. 61.7 years, p = 0.26), age of menarche (12.6 vs. 13 years, p = 0.17), age of menopause (50.4 vs. 49.9 years, p = 0.49) for patients and controls, respectively. Ten percent of patients with FFA and 10.1% of controls were premenopausal (p = 0.98). Patients with FFA presented significantly higher weight and BMI than controls (68.9 vs. 63.6 kg, p = 0.012; 28.7 vs. 26.4 kg/m2, p = 0.013) without differences in height (155 vs. 156.6 cm, p=0.23). Regarding personal history of hypertension (45.5% vs. 40%), diabetes (14.1% vs. 12.5%) or dyslipidaemia (44.4% vs. 42.5%), no significant differences were observed between patients and controls. Four percent of patients were active smokers vs. 15% of controls (p = 0.07) and, regarding alcohol intake, 39.4% of patients and 45% of controls drank less than 40 g/day and 60.6% of patients and 55% of controls did not drink any alcohol (p=0.69). Regarding the use of sunscreens, 83.2% of patients with FFA and 62.5% of controls used them (p = 0.014).

Sex hormone values are summarized in Table I, and no significant differences were observed between patients and controls, except in dehydroepiandrosterone sulfate (66.1 vs. 91.9 µg/dl, p = 0.047, for patients with FFA and controls, respectively). Of the patients with FFA, 30.3% presented androgenetic alopecia and no significant differences in prevalence of psoriasis, vitiligo or lichen planus were observed between patients and controls.

Table I. Serum hormonal levels in patients with frontal fibrosing alopecia and controls

	Patients with FFA Mean	Control Mean	*p*-value
Follicle-stimulating hormone, mUI/ml	62.8	60.3	0.65
Luteinizing hormone, mUI/ml	26.9	25.1	0.49
Oestradiol, pg/ml	27.1	53.4	0.20
Progesterone, ng/ml	0.37	0.90	0.15
Testosterone, ng/dl	25.3	24.6	0.89
Sex hormone binding globulin, nmol/l	57.9	61.6	0.49
Prolactin, ng/ml	7.9	8.2	0.74
17 OH progesterone, ng/ml	0.60	1.1	0.28
Dehydroepiandrosterone sulphate, µg/dl	66.1	91.1	0.047
Androstenedione ng/ml	1.9	2.0	0.91
Dihydrotestosterone, ng/ml	0.12	0.13	0.37

p-values of the Student's *t*-test.

The mean age of onset of FFA was 58.7 years and the mean duration of the disease was 58.8 months. The severity of FFA in the sample of patients was: 3% grade I, 42.4% grade II, 34.3% grade III, 11.1% grade IV and 9.1% grade V. Of patients with FFA, 74.7% had pruritus and 18.2% had trichodynia. Erythema, follicular hyperkeratosis, facial papules and lonely hair sign were observed in 86.9%, 92.9%, 16.2% and 70.7%, respectively. Eyebrow alopecia presented in 83.8% and eyelash alopecia in 27.3%. Alopecia at other body sites presented as follows: 13.1% occipital, 88.9% arms, 92.9% legs, 56.6% axilla, and 42.4% pubis.

Clinical signs of rosacea presented in 61.6% of patients compared with 30% in the control group (p=0.001). Erythematotelangiectatic rosacea was the most frequent form (88.5% [54/61]) (Fig. 1a, b), followed by the papulopustular form (11.5% [7/61]) (Figs 2a, b and 3a, b). Patients with more severe FFA were more likely to have rosacea than those with mild grades of alopecia (prevalence of rosacea 77.8% in grade V vs. 33.3% in grade I, p = 0.02) (Table II). No statistically significant differences regarding rosacea were encountered in patients related to menopause; the percentage of rosacea in menopausal patients being 61.8% vs. 60% in non-menopausal patients (p = 0.91). No significant differences were observed in age of menarche (12.6 vs. 12.6 years, p = 0.98) or menopause (50 vs. 50.2 years, p = 0.68) regarding the presence of rosacea. Pruritus or trichodynia were not related to a higher prevalence of rosacea. Regarding clinical signs of FFA, the presence of perifollicular erythema (Figs 1c, 2c and 3c) correlated significantly with rosacea (64.7% in patients with rosacea vs. 32.6% in patients without rosacea; p = 0.002), whereas perifollicular hyperkeratosis (Figs 2c and 3c) did not show that correlation (60.9% in patients with rosacea vs. 39.1% in patients without rosacea; p = 0.58). Neither facial papules nor lonely hair sign match the presence of rosacea. No correlation was found between duration of FFA and rosacea, or between presence of rosacea and alopecia of the eyelashes, eyebrows or occipital area.

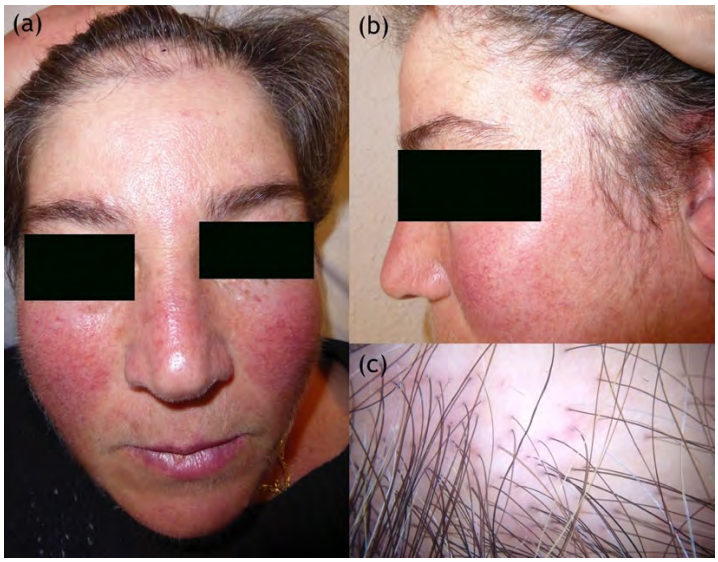

Fig. 1. (a) Frontal side: intense centrofacial fixed erythema with telangiectasias in a patient with grade II frontal fibrosing alopecia. **(b)** Lateral view: erythema and telangiectasias on the cheek. **(c)** Dermoscopy showing loss of follicular openings and marked perifollicular erythema. Permission from the patient is given to publish these photos.

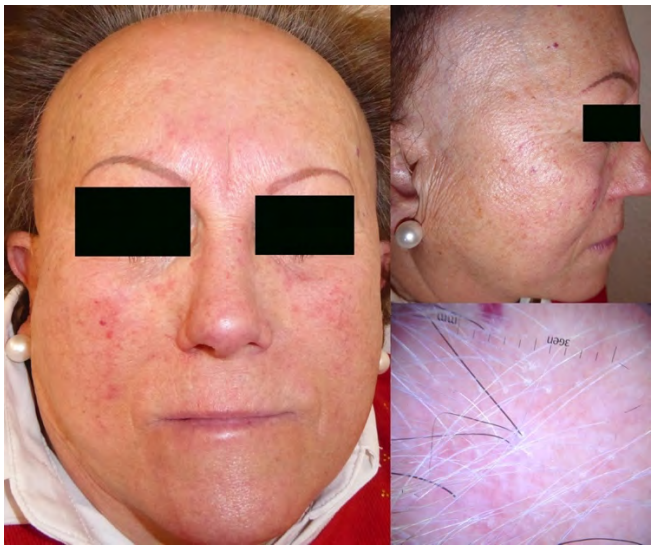

Fig. 2. (a) Frontal side: mild centrofacial fixed erythema with telangiectasias and small papules in the glabellar area and on the cheeks and nose, in a patient with grade III frontal fibrosing alopecia. **(b)** Lateral view: erythema and telangiectasias on the cheek, and few small papules. **(c)** Dermoscopy showing loss of follicular openings and perifollicular hyperkeratosis and mild erythema. Permission from the patient is given to publish these photos.

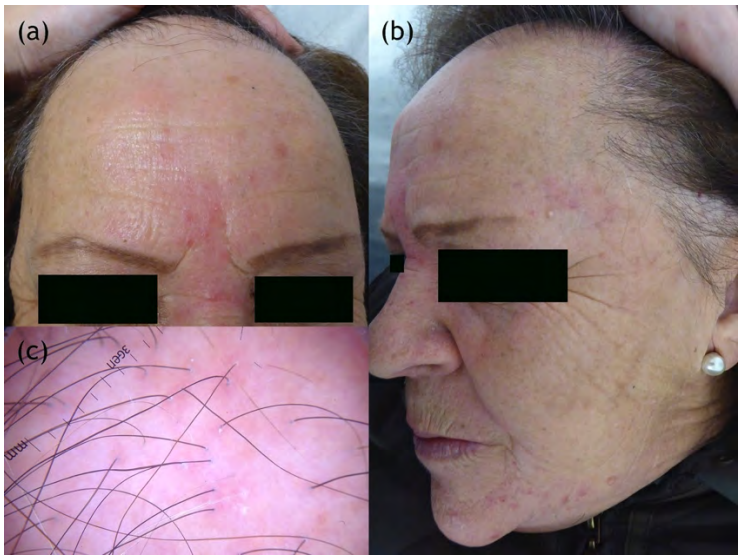

Fig. 3. (a) Frontal side: small papules and pustules in the glabellar area surrounded by mild erythema, in a patient with grade IV frontal fibrosing alopecia. **(b)** Lateral side: small papules and pustules also in the chin and temple. **(c)** Dermoscopy showing loss of follicular openings and intense perifollicular erythema with hyperkeratosis. Permission from the patient is given to publish these photos.

Table II. Prevalence of rosacea according to the grades of frontal fibrosing alopecia (FFA)

Grades of FFA	I	II	III	IV	V
Prevalence of rosacea, %	33.3	61.9	58.8	63.6	77.8

Alcohol and tobacco were not associated with the presence of rosacea in patients with FFA. However, patients with FFA and rosacea presented higher means weight (71.1 vs. 65.4 kg, p = 0.016) and BMI (29.5 vs. 27.3 kg/m2, p=0.016) than patients without rosacea. Patients with FFA and rosacea did not present a higher prevalence of diabetes, hypertension or dyslipidaemia. There were no statistically significant differences in the use of sunscreens in patients with FFA and rosacea and those with FFA but no rosacea (82.5% vs. 84.2%, respectively; p = 0.82).

Data regarding hormones in patients with FFA with or without rosacea are summarized in Table III. No significant differences were observed between groups, except in levels of luteinizing hormone (LH), progesterone and dihydrotestosterone, which were lower in patients with rosacea and FFA.

Binary logistic regression analysis in patients with FFA is shown in Table IV. Perifollicular erythema, BMI and progesterone levels were associated with presence of rosacea in patients with FFA.

Table III. Serum hormonal levels in patients with frontal fibrosing alopecia (FFA) and rosacea and patients with FFA without rosacea

Serum hormones	Patients with FFA and rosacea Mean	Patients with FFA and without rosacea Mean	*p*-value
Follicle-stimulating hormone, mUI/ml	58.6	69.5	0.06
Luteinizing hormone, mUI/ml	23.9	31.7	0.008
Oestradiol, pg/ml	25.7	29.6	0.61
Progesterone, ng/ml	0.31	0.48	0.029
Testosterone, ng/dl	25	25.8	0.89
Sex hormone binding globulin, nmol/l	55.4	61.9	0.25
Prolactin, ng/ml	7.9	8	0.83
17-OH progesterone, ng/ml	0.5	0.7	0.09
Dehydroepiandrosterone sulphate, µg/dl	61.1	73.8	0.12
Androstenedione, ng/ml	1.9	2.0	0.54
Dihydrotestosterone, ng/ml	0.10	0.14	0.02

p-values of Student's *t*-test.

Table IV. Binary logistic regression analysis in patients with frontal fibrosing alopecia (FFA) and rosacea

	OR	95% CI	*p*-value
Age, years	0.95	0.89–1.02	0.16
BMI, kg/m^2	1.16	1.01–1.34	0.035
Perifollicular erythema	8.57	1.73–42.30	0.008
Severity of FFA (per grade)	1.01	0.57–1.81	0.90
Progesterone, ng/ml	0.15	0.028–0.89	0.036
Luteinizing hormone, mUI/ml	0.97	0.93–1.01	0.23
Dihydrotestosterone, ng/ml	0.002	0.001–2.02	0.079

OR: odds ratio; 95% CI: 95% confidence interval.

DISCUSSION

Women with FFA presented a higher prevalence of rosacea than the controls did. The most frequent subtype was erythematotelangiectatic rosacea. Severe grades of alopecia were associated with a higher prevalence of rosacea. Multivariate analysis has shown that perifollicular erythema, higher BMI and lower progesterone levels were associated with a higher risk of rosacea in patients with FFA. Menopause or diseases such as diabetes, hypertension or dyslipidaemia were not associated with a higher risk of rosacea.

The prevalence of FFA is currently increasing gradually. Continuous characterization of this disease has been achieved since its description in 1994 by Kossard,[16] but several enigmas remain unresolved. Most patients with FFA are women, and the mean age of patients and the mean age of onset of FFA in this study were similar to those in previous reports.[15] The majority of patients presented grades II and III alopecia, representing more severe disease than in previous series.[15] Typical facial findings of patients with FFA include facial papules, which are thought to be due to involvement of vellus hair, and red dots in the glabella and eyebrows, representing perifollicular inflammation.[17,18]

FFA has been reported to occur concurrently with androgenetic alopecia.[2] Autoimmune diseases, such as vitiligo, discoid lupus erythematous, thyroid dysfunction and Sjögren syndrome, have been previously reported to occur simultaneously with FFA in up to 30% of cases.[7,19-21] Moreover, reports of both FFA and lichen planus or lichen planus pigmentosus have been published.[5,22,23] The association between FFA and rosacea has not been reported clearly, although a recent cross-sectional study without a control group reported a prevalence of 34% for rosacea in patients with FFA. In the current study the prevalence of rosacea was significantly higher than in the control group.[8]

Rosacea is a common chronic cutaneous inflammatory disease, mainly affecting the facial area, characterized by flares of centrofacial erythema (flushing or transient erythema), causing a characteristic centrofacial fixed erythema, often with telangiectasias. Papules and pustules or less frequently, phymas, may also appear. The cause of rosacea is unknown and probably multifactorial.[24] The National Rosacea Society Expert Committee differentiates 4 main subtypes, which frequently overlap, i.e. erythematotelangiectatic, inflammatory papulopustular, phymatous and ocular rosacea.[10,11,24] The prevalence of this disease is

estimated as 22% in the general population, although in our study the prevalence in controls was slightly higher, perhaps because it referred to a specific sex and age group.[25]

Rosacea has been associated with migraine (suggesting a vascular abnormality), depression, hypertension, dyslipidaemia, coronary artery disease, and other chronic systemic illnesses, although it may be explained by shared environmental or lifestyle factors rather than by a common genetic predisposition.[9,26-29] An increased prevalence of Helicobacter pylori infection has also been found.[30] Recently, rosacea has been linked to a cluster of autoimmune diseases, such as type 1 diabetes mellitus, coeliac disease, multiple sclerosis and rheumatoid arthritis. All of these were significantly associated with rosacea in women; whereas the association in men only reached statistical significance for rheumatoid arthritis.[9] The genetic component of rosacea could be stronger than assumed so far, and autoimmune inflammatory pathways could contribute to the disease course.[9] In this study multivariate analysis revealed that rosacea was significantly associated with higher BMI. A recent study has shown that the risk of rosacea was elevated for those with increased BMI and greater waist and hip circumference in a 14-year follow-up study.[31]

Keratosis pilaris-like papules over the forehead and cheeks and follicular erythema on the cheeks have been described in a few patients with FFA.[13] Moreover, a recent study found diffuse erythema on the cheeks, forehead or eyebrows, sometimes with a reticular pattern, more visible over the zygomatic area.[14] These findings matched with follicular and interfollicular lichenoid infiltrate. Some women in this study also reported episodes of flushing linked to thermal or emotional changes.[14] In our study, perifollicular erythema, but not perifollicular hyperkeratosis, was significantly associated with the presence of rosacea after multivariate analysis. A common inflammation of the pilosebaceous unit may be involved in the pathogenesis of rosacea and FFA. Prostaglandin D2 has been reported to inhibit hair growth,[32] and has been involved in the development of rosacea.[33]

Hormonal factors have been suggested to play an important role in the pathogenesis of FFA due to the higher prevalence of this type of alopecia in postmenopausal women and the response to anti-androgenic drugs. Androgen deficiency was identified in 30% of women with FFA in a recent study.[34] However, hormonal levels are not altered in premenopausal women diagnosed with FFA.[35] In our study no differences were found in hormonal levels between patients with FFA and controls, with the exception of dehydroepiandrosterone sulfate

(DHEAS), a mainly adrenal hormone, which was lower in patients with FFA. This hormone is elevated in hyperandrogenism and its deficiency in FFA women could be related to the androgen deficiency described previously. Dehydroepiandrosterone (DHEA) is an immunomodulatory hormone essential for peroxisome proliferator-activated receptor (PPAR) functions, and is reduced in some processes characterized by fibrosis, such as idiopathic pulmonary fibrosis.[36,37] PPARγ is the main regulator of lipid cell metabolism and sebocyte development, and is indispensable for the maintenance of stem cells of functional epithelium in hair follicles.[38] Deletion of the PPARγ gene in the follicular bulge resulted in a process similar to lichen planopilaris.[38,39] In addition, PPAR is a negative regulator of transforming growth factor-beta 1 (TGFβ1), which promotes fibrotic events.[36] Therefore, the reduced activity of this hormone may be related to the fibrogenic inflammatory process of FFA. However, it is important to note that the evaluation of the hormone in the blood does not necessarily reflect the degree of local action at the hormone receptors, therefore patients with normal values may have an impairment in the sensibility or the integrity of the target receptor, as occurs in androgenetic alopecia.[40] No relationship between DHEAS and rosacea has been reported.

In patients with FFA and rosacea, significantly lower serum levels of progesterone were detected by multivariate analysis. Progesterone has not been clearly implicated in the pathogenesis of rosacea; there is only one case report of an association between a progesterone-releasing intrauterine contraceptive device and rosacea.[41]

Since the study of Aldoori et al.,[42] suggesting a possible association between the use of sunscreens and moisturizers and the development of FFA, an increasing number of publications considering them as a possible trigger in the development of FFA have been reported.[43,44] In accordance with previous reports, the use of sunscreens was higher in the group of patients with FFA than in controls. However, despite the fact that people with rosacea are likely to use more sunscreens than people with no skin disease, there were no statistically significant differences in the use of sunscreens in patients with FFA and rosacea and those with FFA but no rosacea. On the other hand, it is not known whether the higher use of sunscreens in patients with FFA is a cause or consequence of the alopecia.[45]

In conclusion, women with FFA presented a higher prevalence of rosacea, and severe grades of alopecia were associated with a higher prevalence of rosacea. Perifollicular erythema, higher BMI and lower progesterone levels were associated with a higher risk of rosacea in

patients with FFA. Lower levels of dehydroepiandrosterone sulfate may be implicated in the pathogenesis of FFA. Further studies are required to confirm this association and the pathogenic implications of the risk factors.

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5.4. Publication 4: A study about the use of sunscreens and the prevalence of actinic damage in patients with frontal fibrosing alopecia.

Porriño-Bustamante ML, Montero-Vílchez T, Pinedo-Moraleda FJ, Fernández-Flores Á, Fernández-Pugnaire MA, Arias-Santiago S. Frontal Fibrosing alopecia and sunscreen use: a cross-sectional study of actinic damage. Acta Derm Venereol. 2022. [In press]

This article, which has been published in the Acta Dermato-Venereologica, is a cross-sectional study, which includes 101 women with FFA and 40 control subjects. The study showed that patients with FFA had higher actinic damage, especially in form of solar lentigines, compared to a control group. Patients also had a higher use of sunscreens than the control subjects. However, as patients had a more sun-damaged skin, the higher use of sunscreens may be a posterior acquired behaviour rather than an old one which triggers the development of the alopecia.

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Frontal fibrosing alopecia and sunscreen use: a cross-sectional study of actinic damage.

Short title: Actinic damage in frontal fibrosing alopecia.

Original research

María Librada Porriño-Bustamante,[1,2] Trinidad Montero-Vílchez,[3] Fernando Javier Pinedo-Moraleda,[4] Ángel Fernández-Flores,[5] María Antonia Fernández-Pugnaire,[6] Salvador Arias-Santiago.[3,7]

1. Dermatology Department. University Hospital La Zarzuela, Madrid, Spain.

2. University of Granada, Granada, Spain.

3. Dermatology Department, University Hospital Virgen de las Nieves, Granada, Spain

4. Pathology Department. University Hospital Fundación Alcorcón, Alcorcón, Madrid, Spain

5. Pathology Department. University Hospital El Bierzo, Fuentesnuevas, León, Spain

6. Dermatology Department. University Hospital San Cecilio, Granada, Spain

7. School of Medicine, Institute of Biosanitary Investigation ibs, Granada University, Granada, Spain

Corr: Trinidad Montero Vílchez, Department of Dermatology, University Hospital Virgen de las Nieves, Avenida de Madrid, 15, 18012, Granada, Spain. Email: tmonterov@gmail.com

ABSTRACT

Patients with frontal fibrosing alopecia report higher rates of sunscreen use. However, it remains unknown whether the greater use of sunscreens is a cause or a consequence of the alopecia. The aim of this study is to assess the presence of actinic damage in patients with frontal fibrosing alopecia. A cross-sectional study was carried out on 101 patients with frontal fibrosing alopecia and 40 control subjects. The presence of actinic damage, solar lentigines and actinic keratoses, basal and squamous cell carcinomas was recorded in both groups, together with their sunscreen use. Trichoscopy and skin biopsy were performed on patients. Actinic damage was more frequently present in patients (69.3% versus 50% of control individuals, p=0.031). With regards to the use of sunscreens, patients used them more frequently than the control individuals (83.2% versus 62.5%, p=0.008). Nevertheless, the prevalence of trichoscopic inflammatory signs, peripheral alopecia, and inflammatory infiltrate and sebaceous gland involvement in skin biopsy, were similar in patients who used sunscreens compared to the non-sunscreen users. To conclude, patients with frontal fibrosing alopecia had greater actinic damage, which may be the reason for their higher sunscreen use. Thus, the use of sunscreens may not be the trigger of frontal fibrosing alopecia that dermatologists have proposed.

SIGNIFICANCE: Patients with frontal fibrosing alopecia have higher rates of sunscreen use than control subjects. For the first time, it has been observed that frontal fibrosing alopecia patients have greater actinic damage. Therefore, the higher use of sunscreens in these patients could be a consequence of this greater actinic damage rather than a cause of the alopecia.

KEYWORDS: frontal fibrosing alopecia, sunscreen, actinic damage, histopathology, trichoscopy

INTRODUCTION

The possible involvement of sunscreen use in the development of frontal fibrosing alopecia (FFA) was proposed in 2016, when some authors found a higher use of sunscreens in FFA patients (48%) compared to a control group (24%) ($p<0.001$) (1). The use of sunscreens has increased worldwide in the last forty years, along with concerns about skin cancer and photoaging. Subsequently, most publications have reported a higher use of sunscreens in FFA patients (2-4).

However, the hypothesis about sunscreen use as the initial trigger in FFA is highly controversial (5-8) for several reasons:

1. Some FFA patients had not used sunscreens (or, at least, had not consciously used them, as ultraviolet filters are rather ubiquitous in moisturizers and make-up) and yet have still developed FFA (9, 10).

2. The increasing number of FFA cases reported in dark-skinned people, among whom the rates of sunscreen use are generally low (5, 11).

3. There could be various reasons why FFA patients use more sunscreens than the control group individuals. This may just be a new behaviour adopted because of the alopecia, or may even reflect higher economic status, something which has been observed with respect to FFA patients (6, 7, 12).

4. The incidence of FFA remains very low compared to the incidence of sunscreen use (5).

Therefore, it seems clear that patients with FFA have a higher use of sunscreens than the control individuals. Sunscreen use has been shown to prevent actinic damage, that is the development of pigmented lesions, actinic keratoses and skin cancer (13-15). As far as we are aware, actinic damage in patients with FFA has not been assessed. The aim of this publication is to evaluate actinic damage in FFA patients.

MATERIAL AND METHODS

A cross-sectional study was carried out on women with FFA and a control group. The individuals were recruited from the Dermatology Department at the University Hospital San Cecilio and the University Hospital Virgen de las Nieves, both in Granada, Spain. Inclusion criterion for FFA patients was the presence of frontal and/or frontotemporal hairline recession, supported by the presence of typical dermoscopic features (loss of follicular openings, perifollicular erythema and follicular hyperkeratosis). Exclusion criteria for patients were cases with no clear diagnosis of FFA and male patients. Patients were under treatment with 5-alpha reductase inhibitors, topical minoxidil and/or topical corticosteroids. Inclusion criteria for the control group were as follows: women aged between 45 and 95 years old without any hair disease. The control individuals were people who had consulted the Dermatology Department for other reasons (naevi, seborrhoeic keratosis, etc.). Each participant made one visit, wherein all the data were recorded. All participants signed an informed consent form and the project was approved by the local ethics committee in Granada.

Demographic information, such as age and ethnic group, was recorded. In patients, the age of onset of the alopecia, the presence of perifollicular erythema and follicular hyperkeratosis, the severity grade and the existence of pruritus and trichodynia were registered. The severity grade was assessed following the previously described V-grade classification (10), and grouped into mild (I-II) or severe (III-V) FFA. In both groups, the presence of cutaneous signs of actinic damage on the face, that is, solar lentigines, actinic keratoses, and basal cell or squamous cell carcinomas, were recorded, by physical examination and reviewing clinical reports. Regarding sunscreen use, individuals were asked for “habitual use of sunscreens” (considering habitual use as using them at least 5 days per week) for a long time (more than five years). Skin phototype was evaluated using Fitzpatrick Skin Phototype Classification (16). The presence of peripheral alopecia (in eyebrows, eyelashes, limbs, axillae and the pubis) was also recorded. Furthermore, a 4 mm-punch skin biopsy of the hairline progression was taken in 52FFA patients.

The student’s t-test was applied to compare the mean values of quantitative variables. Qualitative variables were analysed with the $\chi 2$ test. Differences were considered significant at $p \leq 0.05$ and nearly significant at $p \leq 0.1$. Multivariate logistic regression analyses were

performed to explore the variables associated with FFA, sunscreen use and actinic damage. SPSS software (SPSS 20.0, SPSS Inc., Chicago, IL, USA) was used for data analysis.

RESULTS

A total of 101 women with FFA and a control group of 40 women were included. Case and control groups were comparable regarding age and ethnic group, although FFA patients had lower skin phototype than control subjects (p=0.012) (Table I). The mean age of onset of the alopecia was 58.53 years and the severity of FFA in the sample of patients was as follows: 4% grade I, 42.6% grade II, 33.7% grade III, 10.9% grade IV and 8.9% grade V. Regarding symptoms, 75.2% and 18.8% of patients had pruritus and trichodynia, respectively. The presence of eyebrow, eyelash and limb alopecia was found more frequently in FFA patients than in control subjects, and this difference was statistically significant (Table II). However, no differences were noted between the groups regarding axillary and pubic alopecia.

Table I. General data about patients and the control group

	FFA group	**Control group**	**P value**
n	101	40	
Female sex	100% (101/101)	100% (40/40)	
Age (mean)	63.45 (SD 9.32)	63.05 (SD 10.01)	0.824
Caucasian ethnicity	95% (96/101)	100% (40/40)	0.561
Fitzpatrick's skin phototype I-II III-IV V-VI	 36.6% (37/101) 59.4% (60/101) 4% (4/101)	 15% (6/40) 85% (34/40) 0%	0.012

Student's t-test was applied to compare mean values of quantitative variables. Qualitative variables were analysed with the χ2 test.

FFA: Frontal fibrosing alopecia; SD: standard deviation

Table II. Peripheral alopecia in patients and the control group

	FFA group (n=101)	**Control group (n=40)**	**P value***	**P value****
Eyebrow alopecia	84.2% (85/101)	2.5% (1/40)	<0.001	<0.001
Eyelash alopecia	26.7% (27/101)	7.5% (3/40)	0.012	0.030
Upper limb alopecia	88.1% (89/101)	40% (16/40)	<0.001	<0.001
Lower limb alopecia	92.1% (93/101)	62.5% (25/40)	<0.001	<0.001
Axillary alopecia	56.4% (57/101)	50% (20/40)	0.521	0.678
Pubic alopecia	42.6% (43/101)	32.5% (13/40)	0.543	0.333

*The χ2 test was used to analyse these qualitative variables.

** Logistic regression model adjusted by phototype (I-II vs. III-IV vs. V-VI)

FFA: frontal fibrosing alopecia

Regarding the presence of actinic damage, 69.3% of patients had actinic damage vs 50% of control individuals (p=0.031) (Table III). This difference was also observed in the presence of solar lentigines, which were noted in 68.3% of patients vs 47.5% of control subjects (p=0.021). Regardless of the skin phototype, FFA was associated with the presence of actinic damage (p=0.045) after performing multivariate analysis, especially in form of solar lentigines (p=0.029) (Table III).

Concerning the use of sunscreens, 83.2% of FFA patients used them, compared to 62.5% of the control subjects (p=0.008). The use of sunscreens was not associated with disease severity.

Table III. Actinic damage and sunscreen use in FFA and the control group

	FFA group	Control group	P value*	P value**
Actinic damage (including all of the subcategories below)	69.3% (70/101)	50.0% (20/40)	0.031	0.045
Lentigines	68.3% (69/101)	47.5% (19/40)	0.021	0.029
Actinic keratoses	16.8% (17/101)	7.5% (3/40)	0.152	0.458
Basal cell carcinoma	9.9% (10/101)	7.5% (3/40)	0.657	0.703
Squamous cell carcinoma	0%	0%	-	-

*The $\chi 2$ test was used to analyse these qualitative variables.

** Logistic regression model adjusted by phototype (I-II vs. III-IV vs. V-VI).

FFA: frontal fibrosing alopecia

No differences regarding skin phototype were found between FFA patients who used sunscreens and those who did not use them, or in the presence of peripheral alopecia (Table IV).

With reference to trichoscopic signs, perifollicular erythema and follicular hyperkeratosis were noted in 85.1% and 93.1% of patients, respectively (Table IV). No differences were found regarding the presence of these signs in patients who used sunscreens compared to those who did not use them.

Regarding histopathology, sebaceous gland involvement (reduction or absence) was found in 80.8% of patients, the presence of inflammatory infiltrate was observed in 92.3%, and the presence of inflammatory infiltrate involving follicular infundibulum or isthmus was noted in 51% and 60.8% of patients, respectively (Table IV). No differences were found regarding the presence of these histopathological signs in sunscreen users compared to non-users.

Moreover, after conducting a logistic regression model it was observed that the use of sunscreens (OR=2.80, p=0.019) and the presence of actinic damage (OR=2.85, p=0.084) were independent factors related to FFA (Table V).

Table IV. Clinical, trichoscopic and histopathological differences between sunscreen users and non-users FFA patients.

FFA patients (n= 101)	**Sunscreen users (n=84)**	**Sunscreen Non-users (n=17)**	**Total of patients**	**P value**
Female sex	100%	100%	101	-
Age (years) - 40-63 - 64-84	 47.6% (40/84) 52.4% (44/84)	 29.4% (5/17) 70.6% (12/17)	 44.6% (45/101) 55.4% (56/101)	0.168
Fitzpatrick's skin phototype	I-II 36.9% III-VI 58.3% V-VI 4.8%	I-II 35.3% III-IV 64.7% V-VI 0%	36.6% (37/101) 59.4% (60/101) 4.0% (4/101)	0.632
Clinical data				
Pruritus	73.8% (62/84)	82.4% (14/17)	75.2% (76/101)	0.457
Trichodynia	17.9% (15/84)	23.5% (4/17)	18.8% (19/101)	0.585
Eyebrow alopecia	83.3% (70/84)	88.2% (15/17)	84.2% (85/101)	0.614
Eyelash alopecia	23.8% (20/84)	41.2% (7/17)	26.7% (27/101)	0.140
Upper limb alopecia	86.9% (73/84)	94.1% (16/17)	88.1% (89/101)	0.402
Lower limb alopecia	90.5% (76/84)	100% (17/17)	92.1% (93/101)	0.185
Axillary alopecia	54.8% (46/84)	64.7% (11/17)	56.4% (57/101)	0.451
Pubic alopecia	42.9% (36/84)	41.2% (7/17)	42.6% (43/101)	0.898
FFA grade - Mild (I-II) - Severe (III-V)	 46.4% (39/84) 53.6% (45/84)	 47.1% (8/17) 52.9% (9/17)	 46.5% (47/101) 53.5% (54/101)	0.962
Actinic damage (including all of the subcategories below)	70.2% (59/84)	64.7% (11/17)	69.3% (70/101)	0.652
Lentigines	69.0% (58/84)	64.7% (11/17)	68.3% (59/101)	0.726
Actinic keratoses	15.5% (13/84)	23.5% (4/17)	16.8% (17/101)	0.418
Basal cell carcinoma	9.5% (8/84)	11.8% (2/17)	9.9% (10/101)	0.778
Squamous cell carcinoma	0%	0%	0%	
Trichoscopy				
Perifollicular erythema	83.3% (70/84)	94.1% (16/17)	85.1% (86/101)	0.254
Follicular hyperkeratosis	91.7% (77/84)	100% (17/17)	93.1% (94/101)	0.217
Histopathology				
Sebaceous gland involvement (reduced or absent)	78.2% (36/46)	100% (6/6)	80.8% (42/52)	0.428
Inflammatory infiltrate	91.3% (42/46)	100% (6/6)	92.3% (48/52)	0.452
Inflammatory infiltrate involving infundibulum	50% (23/46)	60% (3/5)	51% (26/51)	0.671
Inflammatory infiltrate involving isthmus	58.7% (27/46)	80% (4/5)	60.8% (31/51)	0.354

The $\chi 2$ test was used to analyse these qualitative variables.

FFA: frontal fibrosing alopecia

Table V. Factors associated with FFA.

Variable	aOR	IC 95%	P value
Sunscreen (yes)	2.80	1.19 – 6.62	0.019
Age (years)	0.999	0.96-1.04	0.949
Actinic damage (yes)	2.085	0.91-4.79	0.084
Fitzpatrick's skin phototype	0.89	0.53-1.49	0.663

A logistic regression model was constructed to determine the variables influencing the presence of FFA (dependent variable) adjusted for age (continuous), phototype, sunscreen use and the presence of actinic damage. Adjusted odds ratios (aOR) and a confidence interval of 95% (CI 95%) are shown.

DISCUSSION

The use of sunscreens and the presence of actinic damage are independent factors related to FFA. It seems to be true that FFA patients have a higher use of sunscreens compared to the control groups. (1-5), as was observed in the present sample of patients. However, whether this higher use is a cause or a consequence of the alopecia is still unknown (6). Patients who consult a dermatologist about a hair problem may have more appearance-related concerns. Indeed, a recent study found a significantly higher rate of facial moisturizer and sunscreen use in both FFA and androgenetic alopecia patients compared to the control subjects, suggesting that the use of facial care products may not be truly associated with FFA (17). This behaviour may be due to a reason other than the alopecia, such as higher economic status (6, 12), more frequent visits to a dermatologist or the presence of another skin alteration. Moreover, daily facial sunscreen application has not been associated with worsening disease progression in treated FFA patients (18). The use of sunscreens in our patients was not associated with the severity of the disease, in line with previous reports (19).

The presence of actinic damage in FFA patients has never been assessed, except for observing the existence of a contrast between the white alopecic band and the photoaged forehead skin. In the current study, a higher prevalence of actinic damage was observed in FFA patients compared to a control group. The most common sign of actinic damage in both groups was the presence of solar lentigines, which were also more common in patients (68.3% in FFA patients vs 47.5% in control subjects). No statistically significant differences were found regarding the presence of actinic keratoses and basal cell carcinoma, despite them being more frequent in FFA patients, which may be due to the small sample of those subgroups. The higher prevalence of actinic damage, in the form of solar lentigines, was also present after carrying out a logistic regression model adjusted by skin phototype. Moreover, it has been demonstrated that FFA patients have higher rates of rosacea compared to healthy individuals (4, 20); therefore, a more sensitive skin and a lighter skin phototype could also be related to this greater actinic damage.

On the other hand, it is not easy to assess individuals' sunscreen use, either in patients or control subjects, and this may have inaccuracies (5). Ultraviolet filters are present not only in sunscreens, but also in other skin and hair-care products. In fact, in a random review of hair care products, 60% of leave-on hair products and 51% of wash-off products contained a

chemical sunscreen (21). None of the studies regarding FFA and sunscreens were able to perform subanalysis on sunscreen type, but chemical sunscreens are the most commonly used by the general population and also by FFA patients (3). The inability to assess specific ingredients in the reported products is also an important limitation in these studies. Recall bias and temporal ambiguity regarding the onset of symptoms in relation to sunscreen use is another relevant limitation. Assessing the exact period of time using sunscreens or moisturizers containing sunscreens is very complicated. However, continuous and prolonged use of sunscreens should be associated with a lower incidence of signs of actinic damage, so if FFA patients had been using sunscreens for a considerable length of time, they should have had a less sun-damaged skin. A likely hypothesis to explain this issue is that FFA patients have sun-damaged skin and may have visited a dermatologist for that reason, for which the main medical prescription is to avoid sun exposure and to use sunscreens; or they may have started using sunscreens by themselves because of the appearance of solar lentigines.

Regarding trichoscopic signs in FFA, perifollicular erythema has been considered to be a marker of FFA activity (22), and many patients with a receding hairline have persistent inflammatory signs (perifollicular erythema and follicular hyperkeratosis). However, there is growing recognition that these inflammatory signs may persist in patients despite there being no progression in hairline recession (12, 23, 24), and others may have hair loss progression without inflammatory signs (24). No differences were found regarding the presence of perifollicular erythema or follicular hyperkeratosis in FFA patients who used sunscreens compared to those who did not use them. Considering those trichoscopic signs as diagnostic clues and to some extent related to the disease activity, if sunscreen use were related to the development of FFA, differences would be expected between sunscreen-users and non-users.

Concerning histopathological signs, the atrophy or loss of sebaceous glands is considered an early sign of FFA, along with the inflammatory infiltrate involving hair, but without perifollicular fibrosis (which is a more advanced sign) (25-27). No differences were observed between FFA patients who used sunscreens compared to those who did not use them in relation to sebaceous gland involvement, the presence of inflammatory infiltrate or the presence of inflammatory infiltrate involving infundibulum or isthmus. Therefore, the use of sunscreens does not seem to be related to a higher prevalence of histopathological alterations.

Alopecia at other body sites has also been noted in FFA patients, especially on the eyebrows (63-83%) (10, 23, 28), but also on eyelashes (3-14%) (10, 12), limbs (17-24%) (10, 23) axillae (21%) and the pubis (18%) (10). Reduction of peripheral hair (mostly in limbs, axillae and pubis) is a common finding in healthy women after menopause. Moreover, no clear association between alopecia and sunscreen use on the rest of the body has been reported (5). In the current cohort, the presence of eyebrow, eyelash and limb alopecia was more prevalent in women with FFA than in the control individuals and this difference reached statistical significance (but not for axillary and pubic alopecia). Nevertheless, no differences were found in relation to the use of sunscreens and the presence of peripheral alopecia in FFA patients.

The main limitation of this study is the presence of recall bias regarding the sunscreen use and the type of sunscreen (physical/chemical). This bias is similar in patients and control subjects, as they were asked the same question about the use of sunscreens, so it is a non-differential bias. There could also be recall bias regarding any previous history of skin tumours among the few participants who did not have any medical history at the hospital. Another limitation may be the fact that patients were under treatment for FFA.

In conclusion, FFA patients have greater actinic damage than control subjects, which could be the reason for their higher use of sunscreens. Furthermore, trichoscopic inflammatory signs, either the presence of sebaceous gland damage or inflammatory infiltrate, are not more frequent in FFA patients who use sunscreens. Moreover, there are no differences regarding the presence of peripheral alopecia in FFA patients who use sunscreens compared to those who do not use them. Therefore, sunscreen use may not be the trigger of FFA that some dermatologists have suggested. More studies are required to confirm this hypothesis and to point to other possible triggers. Indeed, the increase in the prevalence of FFA suggests that an exogenous factor may trigger the onset of the disease, so further research is required to explore this.

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5.5. Publication 5: Sonographic study in patients with frontal fibrosing alopecia.

Porriño-Bustamante ML, Fernández-Pugnaire MA, Castellote-Caballero L, Arias-Santiago S. Colour Doppler Ultrasound study in patients with frontal fibrosing alopecia. Skin Res Technol. 2021; 27(5): 709-714.

This article, which has been published in the Skin Research and Technology Journal, is a cross-sectional study, which includes 99 women with FFA and 40 control subjects. Colour Doppler ultrasound showed a higher vessel diameter and flow in the hairline implantation in patients, and also a greater vessel diameter in the healthy scalp, pointing the presence of active and subclinical inflammation.

Skin Res Technol.

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Dermatology – SCIE Q3 (42/69)

Colour Doppler ultrasound study in patients with frontal fibrosing alopecia

Original article

María Librada Porriño-Bustamante,[1,2] María Antonia Fernández-Pugnaire,[3] Luisa Castellote-Caballero,[4] Salvador Arias-Santiago.[5,6]

[1]Dermatology Department, University Hospital La Zarzuela, Madrid, Spain

[2]University of Granada, Granada, Spain

[3]Dermatology Department, University Hospital San Cecilio, Granada, Spain

[4]Radiology Department, University Hospital San Cecilio, Granada, Spain

[5]Dermatology Department, University Hospital Virgen de las Nieves, Granada, Spain

[6]School of Medicine, Institute of Biosanitary Investigation ibs, Granada University, Granada, Spain

Correspondence:

María Librada Porriño-Bustamante, Hospital Universitario La Zarzuela, Calle de Pleyades, 25. 28023. Madrid, Spain.

Email: mporrinobustamante@gmail.com

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ABSTRACT

Background: The sonographic characteristics of frontal fibrosing alopecia have been rarely studied. The aim of this study was to perform a colour Doppler ultrasound evaluation in frontal fibrosing alopecia.

Materials and methods: A cross-sectional study including 99 women with frontal fibrosing alopecia and 40 control subjects was performed using ultrasound equipment with a lineal 18 MHz probe. Three areas were evaluated per patient: the alopecic area (a), the hairline implantation area (b) and healthy scalp (c). The diameter (cm) and flow (m/s) of the two most significant vessels were recorded.

Results: With regard to the hairline implantation area, patients presented higher vessel diameter (0.127 cm vs 0.103 cm, P = .03) and vessel flow (8.183 m/s vs 7.670 m/s, P = .05) than the control group. Vessel diameter was higher in the healthy scalp area in patients than in the control group (0.088 cm vs 0.078 cm, P = .03).

Conclusion: Patients presented higher vessel diameter and flow in the hairline implantation area compared to the control group.

KEYWORDS

Colour Doppler, frontal fibrosing alopecia, scarring alopecia, sonography, ultrasound

INTRODUCTION

Frontal fibrosing alopecia (FFA) is a primary scarring alopecia, which causes a frontal and/or temporoparietal hairline recession, leading to a cicatricial band. There are no precise epidemiological data regarding its population prevalence. However, today FFA is probably one of the most common scarring alopecias.[1] The aetiology of FFA still remains unknown. The higher frequency in postmenopausal women and the response to 5-alpha-reductase inhibitors point to an involvement of hormonal factors in its pathogenesis.[2] Moreover, several autoimmune diseases such as thyroid disorders, discoid lupus erythematous, alopecia areata, Sjögren syndrome and vitiligo, and other dermatological diseases such as rosacea, androgenetic alopecia and lichen planus, have been reported occurring concurrently with FFA.[3]

Recently, a genetic basis of susceptibility has been proposed, linking FFA to specific conserved human leukocyte antigen (HLA) class I haplotypes,[4] and to the loci HLA-B*07:02.[5]

High frequency ultrasound (US) is a non-invasive image technique which has been used in dermatology to assess skin tumours, vascular anomalies and also inflammatory diseases, such as hidradenitis suppurativa, morphea and psoriasis.[6] In trichology, US is less widely used, but some reports about dissecting cellulitis, acne keloidalis nuchae, folliculitis decalvans and androgenetic alopecia have been published.[7-9] Lichen planopilaris—and specifically FFA—have been rarely studied with US.[10,11] Blood flow can be estimated by using colour Doppler mode. The objective of this study is to evaluate the scalp of patients with FFA via colour Doppler US, comparing these results to a control group without hair disease.

MATERIALS AND METHODS

A cross-sectional study with a control group was performed in the University Hospital of Granada, Spain. A total of 99 women with FFA and 40 control subjects were included. The inclusion criterion for patients was recession of the frontal and/or temporoparietal hairline, with typical dermoscopic features of FFA, that is loss of follicular openings with or without perifollicular erythema and follicular hyperkeratosis. A control group of women consulting the Dermatology Department for other reasons (naevi, seborrhoeic keratosis, etc) was included. The exclusion criterion for the control group was the presence of any primary scarring alopecia or inflammatory condition of the scalp. All patients and control subjects signed an informed con- sent, and the project was approved by the Local Ethics Committee.

The severity of the alopecia was assessed using a classification which includes 5 grades of severity, and is determined by measuring the area of cicatricial skin between the initial hairline and the recessed one in the frontal and temporal region. The largest of these areas was used to define the severity according to the following grades: I (<1 cm), II (1-2.99 cm), III (3-4.99 cm), IV (5-6.99 cm) and V (≥7 cm, also called “clown alopecia”).[2] Data regarding the age of onset of FFA and dermoscopic signs such as perifollicular hyperkeratosis/erythema, and the presence of prominent and branched vessels, were collected. The use of sunscreen was also registered in both groups. Topical and systemic current treatments were recorded.

The US equipment employed in the study was Esaote MyLabTM with a lineal probe of 18 MHz, using the colour flow mode (CFM) of the colour Doppler. Three sites were analysed in patients: (a) the alopecic band, (b) the hairline implantation and (c) the healthy area of the scalp behind the hairline (Figure 1). The latter was chosen on the base of the absence of dermoscopic criteria for FFA. Two sites were analysed in the control group: (b) the hairline implantation and (c) the area of the scalp behind the hairline, at 3 cm approximately. In each area, the diameter (centimetres, cm) and the flow (metres/second, m/s) of the two most significant vessels were recorded (Figure 2). Mean values were calculated in each area. The study was performed using the following parameters: Doppler frequency of 6.6 MHz and a pulse repetition frequency (PRF) of 1 kHz. Gain was fixed just below the level at which noise artefact from the skull was noted. Sonography was performed after manual separation of the hair shafts and application of a considerable amount of gel to the surface of the scalp.

Student's t test was applied to compare the mean values of quantitative variables. Pearson's correlation coefficient was used to ex- amine the linear correlation between those variables. Differences were considered significant at P < .05 and nearly significant at P < .1. SPSS software (SPSS 20.0, SPSS Inc) was used for data analyses.

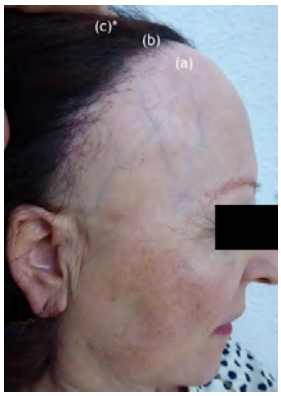

Figure 1. Patient with FFA. Note the different zones, which were measured: **(a)** alopecic band, **(b)** hairline implantation and **(c)** healthy area behind (selected on the base of the absence of trichoscopic signs of FFA)

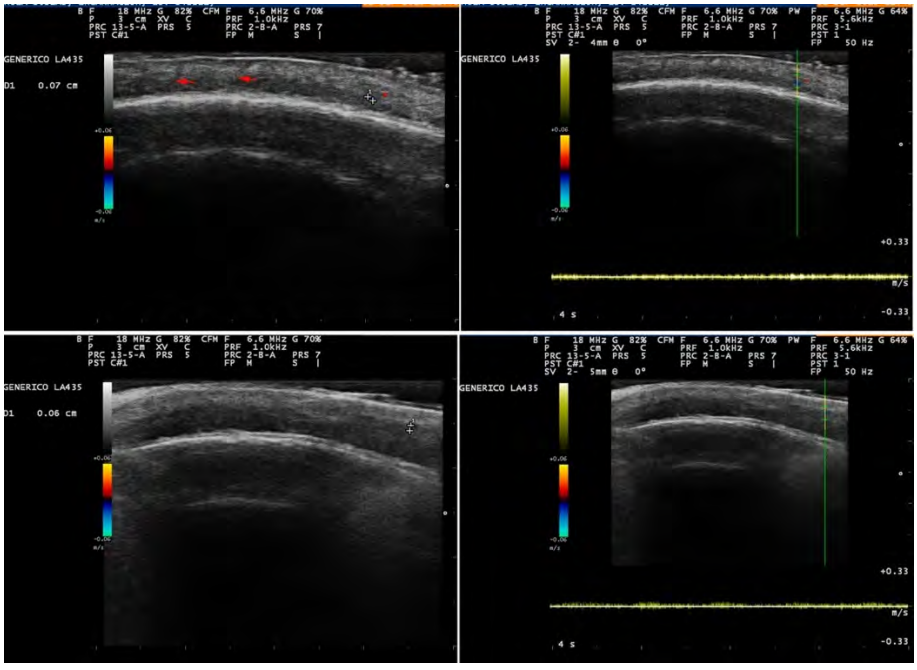

Figure 2. Upper images: CFM, patient, hairline implantation: diameter (left image) and flow (right image). Red arrows in the left image point out widened hair follicles which are found deep down in the skin, reaching the hypodermis (hypoechoic lines). Lower images: CFM, control subject, hairline implantation: diameter (left image) and flow (right image)

RESULTS

This study includes 99 women with FFA and 40 control subjects. No significant differences regarding mean age were observed (63.6 vs 61.7, for patients and control group, respectively, P = .26). The average age of onset of FFA was 58.7 years, and the mean of the duration of the disease was 58.8 months; 11.1% were premenopausal. The severity of FFA in the sample of 99 patients was as follows: 3% with grade I, 42.4% with grade II, 34.3% with grade III, 11.1% with grade IV and 9.1% with grade V. 63.1% of patients were under treatment at the moment of the study. In reference to systemic treatments, 13.6% were taking hydroxychloroquine and 43.7% finasteride. Regarding topical treatments, 67.6% were using corticosteroids and 32.3% were not. Considering trichoscopic features, 86.9% and 92.9% of patients presented perifollicular erythema and follicular hyperkeratosis, respectively, and 36.4% showed prominent and branched vessels (Figure 3). Of the 67 patients who were using topical corticosteroids (67/99), 26 (38.8%) of them had branched vessels. In the group of non-users of topical corticosteroids (32/99), 10 patients (31.2%) showed branched vessels (P = .46). Patients with FFA reported a higher significant use of sunscreens than the control group did (82.8% vs. 62.5%, P = .01).

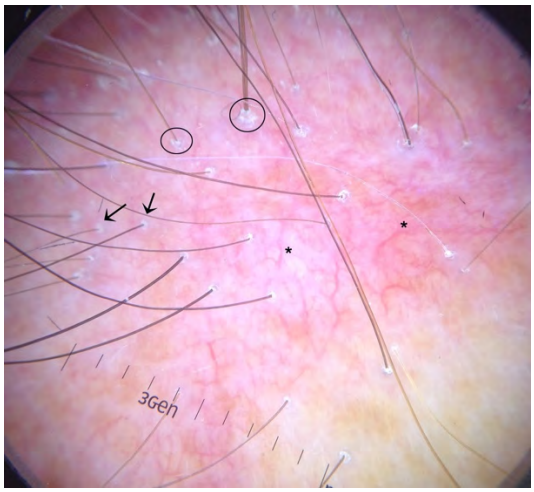

Figure 3. Trichoscopic signs in hairline implantation of a patient with FFA. Loss of follicular openings and a whitish background may be noted. Perifollicular erythema (arrows), marked follicular hyperkeratosis (circles) and branched vessels (asterisks) are also present

With respect to sonographic measurements, patients presented a significantly higher vessel diameter in the hairline implantation area compared to the control group (0.127 cm vs 0.103 cm, P = .03). Patients also had a higher vessel flow in the hairline implantation than the control group (8.183 m/s vs 7.670 m/s, P = .05). In addition, vessel diameter was higher in the healthy scalp area in patients with FFA than it was in the control group (0.088 cm vs 0.078 cm, P = .03) (Table 1).

Table 1. Vascular diameter and flow in different areas in patients and control group

	Vessels	Patients	Control group	*P* value
(a) Alopecic area	Diameter (cm)	0.110	-	-
	Flow (m/s)	7.827	-	-
(b) Hairline implantation area	Diameter (cm)	0.127	0.103	.03
	Flow (m/s)	8.183	7.670	.05
(c) Healthy scalp	Diameter (cm)	0.088	0.078	.03
	Flow (m/s)	7.681	7.220	.10

Regarding patients with FFA, no significant size/flow differences were observed within different severity grades. However, a higher significant vessel diameter was observed in the hairline implantation area than in the alopecic area (0.127 cm vs 0.110 cm, P = .01) and also higher than in the healthy scalp area (0.127 cm vs 0.088 cm, P = .01) (Table 2). In addition, patients with eyebrow alopecia presented a higher significant vessel flow in the hairline implantation area (8.47 m/s vs 7.78 m/s, P = .004). A significant correlation between vessel diameter in the hairline implantation and alopecic areas (r = 0.32, P = .001) and between hairline implantation and healthy scalp area (r = 0.33, P = .001) was found. No significant correlation between flow or vessel diameter and the duration of the disease was found.

Table 2. Comparison of vessel diameter and flow between different areas in patients

Vessels	Area	Mean value (SD)	P value
Diameter (cm)	(b) Hairline implantation area	0.127 (0.05)	.01
	(c) Healthy scalp	0.088 (0.03)	
	(b) Hairline implantation area	0.127 (0.05)	.01
	(a) Alopecic area	0.110 (0.04)	
	(a) Alopecic area	0.110 (0.04)	.99
	(c) Healthy scalp	0.088 (0.03)	
Flow (m/s)	(b) Hairline implantation area	8.183 (1.33)	.10
	(c) Healthy scalp	7.681 (1.41)	
	(b) Hairline implantation area	8.183 (1.33)	.14
	(a) Alopecic area	7.827 (1.22)	
	(a) Alopecic area	7.827 (1.22)	.63
	(c) Healthy scalp	7.681 (1.41)	

Regarding trichoscopic features, no significant differences in vessel diameter or flow were observed in patients with FFA with respect to the presence of perifollicular erythema or follicular hyperkeratosis. However, patients with prominent and branched vessels in dermoscopy presented a higher significant flow in the hairline implantation area (8.52 m/s vs 7.98 m/s, P = .05) and also a higher significant vessel diameter in the healthy scalp area (0.096 cm vs 0.084 cm, P = .03), compared to patients without those prominent and branched vessels. Comparing patients in treatment with topical corticosteroids with patients without them, no significant differences were noted either in vessel diameter or flow. Also, no significant correlation between the length of the use of topical corticosteroids and vessel diameter or flow was found.

Regarding the use of sunscreens, patients with FFA who applied sunscreens presented a nearly significant higher vessel diameter in the alopecic area compared to those patients who did not use them (0.11 vs 0.09 cm P = .073).

DISCUSSION

FFA has been widely described with dermoscopy, but sonographic characterisation of FFA has not been as well documented. Normally, scalp hair follicles are seen on sonography as oblique hypoechoic bands in the dermis, reaching the deep dermis and even the upper hypodermis in the anagen phase, but restricted to the upper dermis in the telogen phase.[8] The scalp has a centripetal blood flow coming from branches of both external and internal carotid arteries, mainly from the temporal and occipital arteries. These branches decrease their diameter from the lateral to the medial area of the scalp.[6]

Active inflammation of the scalp may modify the diameter and/or echogenicity of the hair follicle—appearing increased and hypoechoic—along with a higher blood flow when using colour Doppler US. Dermal and subcutaneous inflammation is revealed in hypoechogenicity in the dermis and hyperechogenicity in the subcutaneous tissue.[9] Therefore, in FFA some areas without adipose tissue along with areas with higher hyperechogenicity in the subcutaneous layer may be observed.[10] A recent study of sonography in FFA including 8 patients found hypoechoic perifollicular thickening in 62.5% of patients and increased dermal vascular flow in 40% of them, along with an increased dermal capillary circulation in the XFlow study.[11]

In the current study, patients had a statistically significant higher vessel diameter and flow in the hairline implantation area compared to the control group, which may be explained because of the presence of active inflammation. On the other hand, no significant differences were observed between severity grades; although curiously, patients with eyebrow alopecia presented a higher significant vessel flow in the hairline implantation area.

According to previous studies, affected areas in FFA are usually hypovascular in colour Doppler US.[10] In the present study, the group of 99 patients' vessel diameters were higher in the hairline implantation area than in the alopecic area, probably due to the presence of both active inflammation in the hairline and fibrosis in the alopecic band. In accordance with this hypothesis, vessel diameter in patients was also higher in the hairline implantation area than in the healthy scalp. Pearson's correlation showed a linear relation between those parameters, demonstrating a significant positive correlation between vessel diameter in the hairline implantation area and in the alopecic area, and also between vessel diameter in the hairline implantation area and in the healthy scalp. The inflammation present in patients with

FFA is the likely explanation for those findings. In that way, patients with active disease—that is with active clinical or subclinical inflammation—may have a higher vessel diameter in the three areas, although it is even higher in the most active one (the hairline implantation). Dilated vessels have been noted with optical coherence tomography in patients with FFA, in both the hairline implantation area and the alopecic area.[12] In addition, an increased vascular flow was observed in inflammatory regions in the referred study; however, this may be due to neo- vascularisation as seen in other ischaemic diseases, or be the result of the inflammatory response.[12]

Curiously, vessel diameter was significantly higher in the healthy scalp area in patients with FFA than it was in the control group. This observation may be explained by the presence of subclinical inflammation in the still unaffected areas in patients, and may be a predictor of future progression. Clinical and trichoscopic signs (perifollicular erythema and follicular hyperkeratosis) are not always related to progression;[13] therefore, sonography may play a role in this way.

Regarding trichoscopic features in patients with FFA, no significant differences were observed in vessel diameter or flow in relation to perifollicular erythema or follicular hyperkeratosis. This finding supports the observation that those trichoscopic signs are not always related to clinical progression. Interestingly, the presence of prominent and branched vessels did match to a higher significant flow in the hairline implantation area, and also with a higher significant vessel diameter in the healthy scalp. A clinically observable local erythema and vascular structures such as arborising vessels, thick arborising vessels and extravasated haemorrhages are more frequent in patients who make chronic use of topical corticosteroids.[14] Topical steroids may modify the results of this study; however, comparing both groups of corticosteroids users and non-users in our cohort of patients, no significant difference was found in the matter of the presence of branched vessels. In addition, no significant differences were noted in the vessel diameter or flow between corticosteroids users and non-users or regarding time of use. Therefore, these branched vessels seen with trichoscopy may be the reflex of active inflammation and may be hypothetically related to progressive disease.

Since the first publication relating FFA to the use of sunscreens,[15] different observational studies have supported this hypothesis, although controversy has been present from the beginning.[16] The greater use of sunscreens by these patients may be a consequence of their

alopecia instead of the cause of it, or may be an associated factor, but probably not the only cause.[17] In the current study, patients presented a greater use of sunscreens than the control group did. Moreover, patients with FFA and greater use of sunscreens presented a nearly significant higher vessel diameter in the alopecic area than those who did not use sunscreens.

All of these findings may be related to the inflammatory process in FFA, which may be reflected in a higher vessel diameter and/or flow in areas with active inflammation, compared to those atrophic (alopecic band) or unaffected areas (healthy scalp).

The main limitation of this report is a lack of follow-up observation regarding the 99 patients because of the cross-sectional design of the study. Prospective studies may be necessary to assess the relationship of those sonographic findings with the progression of the disease.

CONCLUSION

Patients with FFA presented a higher vessel diameter and flow in the hairline implantation area compared to the control group. Moreover, patients with FFA showed a higher vessel diameter in the hairline implantation area than in the alopecic area, probably due to the presence of inflammation in the former and fibrosis in the latter. In addition, comparing both groups, the vessel diameter in the healthy scalp was higher in patients than in control subjects, which may be related to subclinical inflammation. Therefore, sonography may provide additional information regarding the evaluation of patients with FFA, especially in the assessment of the presence of inflammation.

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5.6. Publication 6: A bibliographic review.

Porriño-Bustamante ML, Fernández-Pugnaire MA, Arias-Santiago S. Frontal Fibrosing Alopecia: A Review. J Clin Med. 2021; 10(9):1805.

This article, which has been published in the Journal of Clinical Medicine, is a narrative review about FFA, which includes all the information available up to the date of publication regarding this type of alopecia.

Journal of Clinical Medicine

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Internal Medicine – SCIE Q1 (39/167)

Frontal Fibrosing Alopecia: A Review

Review.

María Librada Porriño-Bustamante,[1,2] María Antonia Fernández-Pugnaire,[3] Salvador Arias-Santiago.[4,5]

[1]Dermatology Department, University Hospital La Zarzuela, Madrid, Spain

[2]Dermatology Department. University of Granada, Granada, Spain

[3]Dermatology Department, University Hospital San Cecilio, Granada, Spain

[4]Dermatology Department, University Hospital Virgen de las Nieves, Granada, Spain

[5]Institute of Biosanitary Investigation ibs, School of Medicine, Granada University, Granada, Spain

* Correspondence: mporrinobustamante@gmail.com; Tel.: +34-915-85-80-00

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Abstract: Frontal fibrosing alopecia is a scarring alopecia, the prevalence of which is increasing worldwide since its first description in 1994. The reason for this emerging epidemic may be a higher exposure to an unknown trigger, although its aethiology and pathogenesis still remain enigmatic. Clinical, trichoscopic, sonographic, and histopathological findings are allowing clinicians to understand more aspects about this type of cicatricial alopecia. Several treatments have been used in frontal fibrosing alopecia, although the 5-alpha reductase inhibitors seem to be the most promising. The aim of this report is to provide a compilation about the published data regarding frontal fibrosing alopecia in a narrative review.

Keywords: frontal fibrosing alopecia; scarring alopecia; bulge; trichoscopy; histopathology; treatment

1. Introduction

1.1. Definition and History

Frontal fibrosing alopecia (FFA) was described in 1994 by Kossard as a progressive scarring alopecia in postmenopausal women, affecting the frontal and temporoparietal hairline, and was initially called postmenopausal frontal fibrosing alopecia.[1] FFA was referred to in 1997 by Kossard as a frontal uncommon variant of LPP.[2] However, this is still flatly controversial, and other authors consider that FFA is a distinct entity from LPP.[3] Nowadays, FFA is probably one of the most common types of scarring alopecia, if not the most common.[4] The gradual increase in publications related to FFA may be due to a higher awareness among clinicians in regard to this alopecia.[5] However, a higher prevalence of a still unknown trigger in recent years may be another relevant factor in this epidemic of FFA.

1.2. Aim and Methods

The aim of this report is to perform an updated and complete review about FFA regarding epidemiology, aetiopathogenesis, clinical characteristics (clinical description, trichoscopy, image techniques), prognostic factors, histopathology, diagnosis, differential diagnosis, and treatment. For that, an exhaustive review of all of the references related to FFA and published in PubMed has been done by searching for “frontal fibrosing alopecia”, including references written in English, Spanish, German, and French, from 1994 to 2021. A total of 487 articles have been reviewed. Articles with a more significant number of patients have been included. In addition, publications with a lower number of patients that provided new information about FFA have also been incorporated.

2. Epidemiology and Demographic Data

There are no specific data about the worldwide prevalence of FFA so far. Recently, the overall crude prevalence for FFA in New York City has been estimated at about 0.015%.[6]

FFA was described initially as affecting almost exclusively postmenopausal women. However, although this group seems to be the most frequently affected, it is not the only one. The first report of a man with FFA dates from 2002,[7] but many more have been published since

then.[3,8,9] Moreover, a fair number of cases of FFA in premenopausal women have been published since its first description.[10,11] In spite of this, FFA begins at postmenopausal age in around 83% to 95% of women (Caucasians and Asians).[3,12-15] Nevertheless, the biggest study of FFA in black-skinned patients reported that 74% of the women were premenopausal.[16] Regardless, it seems that premenopausal cases are also increasing.

The mean age of onset of FFA ranges from 56 to 63 years.[3,12,17] Even so, some cases have been published about younger patients, the youngest one being a 15-year-old female.[18] In a 355 patient cohort, the rate of early menopause (14%) was higher than in the general population (6%); moreover, 13% of patients had undergone a hysterectomy.[3] The mean time to the diagnosis reported in different studies is about 3.4 to 5.3 years.[3,13,19] Assessing the exact duration of the disease can be difficult sometimes, because its slow progression makes it complicated for the patient to detect the real time of onset.

Male patients with FFA seem to be affected with FFA at a younger age than women, with a mean age of onset of 47.3 years.[20] FFA in men is probably underdiagnosed because of its overlap with androgenetic alopecia (AGA); indeed, the main complaint among men having FFA is usually eyebrow loss rather than scalp alopecia.[9]

Regarding the human race, FFA has been described worldwide, although most cases have been reported in European and North American countries, mainly among Caucasians and fewer among black-skinned populations,[16] whereas only a few cases have been reported in Asia, where the incidence may be lower.[21-24] Indeed, a recent study regarding the prevalence of FFA and LPP in New York City observed that the prevalence of the combined group LPP/FFA was highest among non-Hispanic Caucasians (0.091%).[6]

With reference to external factors, a study found that patients with FFA belonged to a more affluent group compared to both a comparable control group with other types of alopecia and to a general control group.[13]

3. Aetiopathogenesis

The aetiopathogenesis of FFA remains unknown, although hormonal factors, autoimmunity, genetic susceptibility, and some exogen factors are thought to play a role (Figure 1).[14]

The loss of the immune privilege of the hair follicle would be the starting point in the development of scarring alopecias.[25] This bulge immune privilege collapse may be induced by IFN-γ.[25] In FFA, a Th1-biased cytotoxic T cell autoimmune reaction against the hair follicle in the infundibular region and, to a more variable degree, the isthmic region, seems to play a major part.[26] This damage would include the bulge area, where stem cells are placed, leading to a loss of the regenerative potential of the hair follicle and its total destruction. A decrease to the absence of labelling with Ki-67, a proliferative marker, and a downregulation of the hair follicle epithelial progenitor cell marker keratin 15, within the bulge area, have already been described in LPP.[27,28] The melanocyte of the hair follicle might be an antigenic target in FFA;[29] this is supported by the lower melanocyte count found in the upper follicle in lesional skin from FFA patients (not seen in LPP).[26,30]

The onset and progression of FFA in a woman with psoriasis, treated with ustekinumab (an anti-IL12/23 p40 monoclonal antibody), suggests that the Th1 and Th17 pathways do not play a major role in FFA.[31]

On the one hand, in LPP, the downregulation and the abnormal function of the peroxisome proliferator-activated receptor γ (PPAR-γ) have been proposed as the initial triggers of the inflammation. PPAR-γ plays a central role in lipid homeostasis and in the differentiation and maturation of sebocytes.[28] Targeted deletion of PPAR-γ in the follicular stem cells of the bulge in mice causes a phenotype resembling scarring alopecia, suggesting that this receptor is essential for healthy pilosebaceous units.[32] Peroxisomal polymorphisms and/or environmental triggers (toxins such as dioxin) may lead to this localized and acquired PPAR-γ dysfunction.[32]

The mammalian target of rapamycin (mTOR) is a pathway that combines signals and acts as a central regulator for metabolism, growth and cell proliferation, and is a major regulator of adiposity due to PPAR-γ activation.[33] A recent study has found that the expression of all mTOR signaling pathway proteins are decreased in the lesional epidermis of patients with LPP/FFA.[34] In addition, dehydroepiandrosterone (DHEA) has an immunomodulatory role, and is essential

for the stimulation of PPAR in the transcription of genes, fat metabolism, and in mitochondrial activity.[35] It is possible that the benefits obtained by the treatment of FFA with 5a-reductase inhibitors are the result of DHEA impediment in reaching its final conversion to dihydrotestosterone.[35]

Moreover, transmission electron microscopy and global metabolomics profiling data have identified defects in mitochondrial β oxidation of fatty acids, leading to the accumulation of medium- and long-chain fatty acids, along with decreased levels of (antioxidant) glutathione and elevated levels of oxidized glutathione (a marker of oxidative stress) in both lesional and non-lesional FFA scalp samples. Therefore, mitochondrial dysfunction may be an early process in the pathogenesis of FFA.[36]

The expression of Snail1 noted in the fibrotic dermis in FFA suggests that the fibroblasts are, in part, derived from the hair follicle via an epithelial-mesenchymal transition process.[23] The transforming growth factor-β (TGF-β) is an inducer of this transition, promoting fibrosis, differentiating epithelial cells and quiescent fibroblasts intro myofibroblasts, and increasing the expression of the extracellular matrix.[23] In this way, increased expression of the Treg marker FOXp3+ has been described in LPP/FFA, and the Treg-mediated TGF-β-signaling appears to drive fibrosis through this transition.[37] Moreover, PPAR-γ is a negative regulator of fibrotic events induced by TGF-β1.[38]

On the other hand, the expression of Janus kinase (JAK) 1 and 3 are significantly upregulated in dermal inflammatory cells in patients with LPP.[39] Therefore, JAK inhibition may reduce IFN-mediated inflammation associated with LPP and prevent further hair follicle destruction.

Neurogenic inflammation is another hypothesis for the pathogenesis of scarring alopecias.[40] The total number of mast cells, along with the proportion of degranulating ones, are increased in the perifollicular bulge region in LPP/FFA.[41] Moreover, decreased epidermal nerve fibre density has been found in FFA, as well as an increased concentration of substance P in the unaffected areas compared to the affected ones, and higher expression of calcitonin gene-related peptide (cGRP) in affected areas of cases with mild inflammation.[42]

A new hypothesis proposes that FFA may arise as a result of excessive facial photoprotection, with a resultant disturbance in immunological homeostasis mediated via the aryl hydrocarbon receptor-kynurenine pathway axis (AhR/KP), leading to the collapse of immune privilege at

the hair bulge.[43] Overexpression of the aryl hydrocarbon receptor (AhR) in the epidermis of FFA and LPP has been newly described in both unaffected and affected scalp.[44] CYP1A1 gene, the expression of which is directly controlled by AhR signaling to metabolize xenobiotics, has been described as being upregulated in affected and unaffected skin in LPP.[32] Multiple chemical substances can inhibit the metabolism of CYP enzymes and thereby indirectly cause AhR activation. Moreover, AhR is involved in the suppression of PPAR-γ.[44]

A new study has identified the presence of circulating microRNAs as being highly predictive of disease status in FFA.[45]

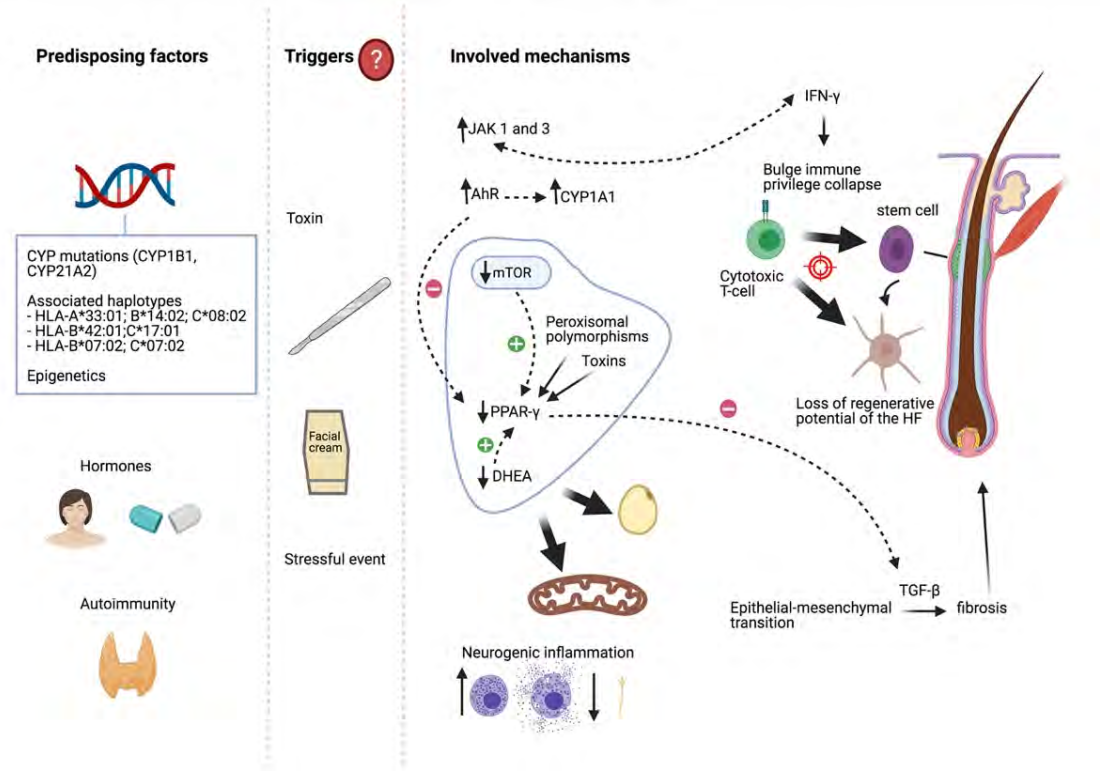

Figure 1. Different unknown triggers, such as facial creams/sunscreens, environmental toxins, surgery, or a stressful situation, may lead to FFA in genetically susceptible individuals. Autoimmunity may also contribute to the predisposition, so people with one autoimmune disease are more likely to have another one. Hormonal factors probably play a role in the development of FFA. Discontinuous lines indicate a regular relationship between two elements. JAK: Janus kinase; AhR: aryl hydrocarbon receptor-kynurenine; mTOR: mammalian target of rapamycin; PPAR-γ: peroxisome proliferator-activated receptor; DHEA: dehydroepiandrosterone; HF: hair follicle; IFN: interferon; TGF: transforming growth factor; HLA: human leukocyte antigen; CYP: cytochrome. Created with BioRender.com (accessed on 1 March 2021)

3.1. Hormones

Hormonal factors are thought to play a role in FFA, due to their higher frequency in women, especially postmenopausal ones, and their response to 5-alpha reductase inhibitors.[5]

Oestrogens produce a decrease in the hair shaft growth and favor the catagen to telogen transition.[46] Therefore, the decrease of oestrogens due to physiological or surgical menopause could alter the control of the hair cycle and be the trigger for the inflammatory attack on the hair follicle in susceptible patients.[3] Moreover, the potential role of oestrogens as an anti-fibrotic and immunomodulatory agent in FFA has been discussed.[47]

In postmenopausal women, the course of the disease appears to be unaltered when hormone replacement therapy is introduced, and it does not seem to prevent the onset of the disease either.[2,5] However, early menopause could be an issue involved in the premature development of FFA, or may imply a higher risk of developing FFA.[2,14,48]

The use of an intrauterine device as a contraceptive may protect against the development of FFA, whereas the intake of tamoxifen with the induction of a low-oestrogen environment around the hair follicle may trigger or maintain the pathogenic process of FFA.[48]

A few male patients have been reported as having FFA and a history of prostate cancer treated with neoadjuvant hormonal therapy (antiandrogens or oestrogens) before the onset of FFA.[49,50] One man was receiving testosterone because of an iatrogenic hypogonatotropic hypogonadism and developed FFA afterwards; moreover, he developed alopecia earlier than his brother, who also had FFA.[9]

Serum hormonal levels are not consistently altered in women diagnosed with FFA, although this does not exclude a potential hormonal involvement by a local mechanism.[51] In fact, a recent study about hormonal dysfunction found that LPP is associated with androgen excess (testosterone or DHEAS), whereas FFA is related to androgen deficiency (32.1% of patients).[52] According to these findings, DHEAS and androstendione have been found to be lower in women with FFA compared to a control group.[19,53] Moreover, abnormal oestrogen and testosterone values have been associated with lesser disease activity.[18] Serum levels of the follicle-stimulating hormone (FSH) in premenopausal women with FFA have been demonstrated to be lower (although within normal ranges) compared to a control group.[54] In the same study, the levels of luteinizing hormone (LH) and FSH were significantly lower in

postmenopausal women with premenopausal onset in comparison to the ones with postmenopausal onset. Progesterone serum levels have also been noted to be lower in patients having both FFA and rosacea.[19]

3.2. Associated Diseases and Autoimmunity

About 9.7% to 30% FFA patients, mostly women, have an associated immune disorder;[13,55] the most frequent are thyroid diseases, especially hypothyroidism (8–44.6%).[3,13,14,56] Other autoimmune conditions, which have been described together with FFA, are referred to in Table 1.[3,13,14,56-60] Several studies have demonstrated that patients with FFA are significantly more likely to have systemic lupus erythematosus, while patients with LPP and FFA are less likely to have diabetes.[61,62] Vitiligo and FFA are sometimes associated, and both diseases may share common pathogenic pathways.[63]

Only one case of a woman with common variable immunodeficiency (CVID) and FFA has been published.[64]

Different forms of lichen planus, such as oral,[3,14,65] vulvar and conjunctival,[14,66] nail,[67] and cutaneous[3,10,14] lichen planus, have been described together with FFA. However, LPP appears along with cutaneous or mucous lichen planus in up to 50% of patients, whereas around 2 to 18% of patients with FFA have lesions of lichen planus in other locations.[10,12,13,56] Actually, the clinical form of lichen planus most commonly linked to FFA is LPP, in 0.8 to 25% of patients.[2,3,11,13,17,68] Vulvar lichen sclerosus[13,56,69] and lichen planus pigmentosus (LPPigm)[70] have also been found concurrently with FFA. One case of actinic lichen planus triggered by drug photosensitivity and preceding the onset of the FFA has been published.[71] Another entity placed in the spectrum of LPP, Graham-Little-Piccardi-Lasseur Syndrome (GLPLS), consisting of keratotic papules on the limbs or trunk, multifocal cicatricial alopecia, and non-atrophic axillary and pubic hair loss, has also been described concomitantly with FFA.[72]

A high prevalence of atopy (43.9%) has been demonstrated in a FFA patients' cohort.[56] However, according to different studies, one of the most frequently associated cutaneous condition seems to be rosacea, with a prevalence of 15 to 61%.[19,55,56] Interestingly, patients with more severe FFA appear to be more likely to have rosacea than those with mild grades of alopecia.[19]

Androgenetic alopecia (AGA) is observed concomitantly with FFA in 16 to 57% of women[3,11,12,19,68,73] and in 67 to 83% of men.[3,74] An overlap of FFA, AGA, and frontal fibrosing in a pattern distribution (FAPD) has also been described.[73] One case of a patient having AGA, FFA, and trichotemnomania has also been published.[75]

A woman with continued hair growth in a vascular nevus in an area otherwise affected by FFA has been recently described; the Renbök phenomenon describes how the emergence of one skin condition inhibits another.[76]

Table 1. Autoimmune diseases described concomitantly with FFA.

Autoimmune Associated Diseases
Thyroid disorders: Hypothyroidism (8–44.6%) Hashimoto thyroiditis (8.1%) Graves disease (1.4%)
Lichen planus (1.7–18.2%): Cutaneous (3–6.5%) Mucosal (3–16.7%) Pilaris (0.8–25.3%)
Psoriasis (7.4%)
Vitiligo (0.6–5.6%)
Inflammatory bowel disease (5.4%)
Lichen sclerosus (0.3–5.4%)
Sjögren syndrome (1.7–4.1%)
Discoid cutaneous lupus erythematosus (-)
Systemic lupus erythematosus (3.4%)
Coeliac disease (1.5–2.0%)
Pernicious anaemia (1.2–1.7%)
Alopecia areata (0.6–1.7%)
Scleroderma (1.4%)
Rheumatoid arthritis (1.4%)
Polymyalgia rheumatic (-)
Primary biliary cirrhosis (-)
Mucous membrane pemphigoid (-)

(-) Data from isolated reports.

3.3. Genetic Factors

Human leukocyte antigen (HLA)-DR1 has been related to familial cases of LPP and LGLPs.[77] Later, an association of HLA-B7 in familial cases of LPP (but not in sporadic cases) was published.[78] Since the first report of familial FFA in 2010,[79] more cases have been published, the largest series so far being one including 20 cases from nine different families.[80] A subsequent report regarding two sisters with FFA found negativity for HLA-DR1.[81] A woman

with FFA and her daughter with LPP, both with the same HLA type (DRB1*04,13; DQB1*03:02,06), have also been reported,[82] as well as a woman with FFA whose mother had FAPD.[83]

A study including 13 cases of familial FFA found that most of the patients of that cohort shared HLA-A*33:01; B*14:02; C*08:02, suggesting that this haplotype may predispose to familial FFA.[84] Moreover, it was found to be linked with the CYP21A2 gene p.V281L mutation (from congenital adrenal hyperplasia). HLA-B*07:02 was also included in the haplotypes of some of the patients.

A recent genome-wide association study (GWAS) demonstrated a significant association with FFA in four genomic loci: 2p22.2, 6p21.1, 8q24.44, and 15q2.1. Fine mapping within the 2p22.2 and 6p21.1 loci revealed associations with a presumed casual missense variant in CYP1B1 (which encodes a member of the cytochrome P450 family involved in the oxidative metabolism of oestrogens) and the HLA-B*07:02 allele, respectively.[85]

A study of HLA profiles in a familial cluster (seven members with FFA and four unaffected) and seven sporadic cases found two susceptibility haplotypes in the familial cases (C*17:01:01:02/B*42:01:01:01 and C*07:02:01:03/B*07:02:01:01), which were also shared by three unaffected family members.[86] This suggests that other genetic or environmental factors may modulate the HLA association. Moreover, five out of seven sporadic cases also shared the referred haplotypes (3/7 the former, 2/7 the latter). Another woman with sporadic FFA presented HLA-A*33:01:01; B*14:02:01; C*08:02:01, which was previously associated to FFA.[84]

A review of eight cases of familial FFA from four different families (mother and daughter) revealed that all of the mothers were postmenopausal at the time of the diagnosis and had an advanced alopecia, whereas all of the daughters were premenopausal and had a mild form of the disease.[87] This has been confirmed in subsequent familial studies.[80]

The occurrence of the disease in families can indicate exposure to a common environmental trigger, probably enhanced by a genetic predisposition. The possibility of a common environmental exposure is supported by a case of connubial FFA in a genetically unrelated couple, although it may have been coincidental.[88]

An autosomal dominant transmission with reduced penetrance has been proposed as a hypothetic inheritance pattern in FFA.[89] Three monocigotic twins have been identified with FFA, which reinforces the fact that epigenetics may play a fundamental role in FFA pathogenesis, in addition to a genetic predisposition.[47]

With regards to a family history of the disease, this is reported by 5 to 8% of patients with FFA.[3,16]

3.4. Surgical Procedures and Hair and Skin Care Products

Some cases of FFA or LPP developed after hair transplantation for AGA, so hair transplants may also be affected.[8,90,91] In addition, one female developed FFA after face lift surgery.[91] Explanations for these situations include the Koebner phenomenon induced by surgical trauma, or an autoimmune attack from a follicle antigen liberated during surgery or induced by a post-surgery pro-inflammatory environment.[91] In that way, the Köebner phenomenon may have also been the cause in a woman with FFA who developed LPP in the areas of wig attachments.[92]

The use of sunscreens has been proposed as a possible trigger for the development of FFA since the publication of a study that found a higher use of these products in FFA patients compared to a control group.[93] This finding has been confirmed in most subsequent reports,[94,95] although there are also some FFA patients who had not used sunscreens and yet still developed FFA.[74] There is an interesting case of a woman with FFA who had hair regrowth following the cessation of sunscreen use on the forehead.[96] However, daily facial sunscreen application has not been associated with worsening disease progression in treated FFA patients.[97] Interestingly, in a random review of hair care products, 60% of leave-on hair products and 51% of wash-off products contained a chemical sunscreen.[98]

Nanoparticles of titanium dioxide (a substance found in physical sunscreens) have been detected along the hair shafts of a patient presenting FFA.[99] It is not clear if titanium dioxide can penetrate the stratum corneum, but it is known that it can deposit itself in the follicular opening. However, these deposits on hair shafts have not been found in subsequent studies,[100] or have been observed in both patients and control subjects,[101] hence this is

meaningless. Furthermore, most of the sunscreens used by patients with FFA are chemical ones.[95]

A higher frequency of positive patch test in FFA patients, mainly for fragrances,[93] benzyl salicylate,[102] cobalt, nickel, and potassium dichromate,[103] have been found in different studies, although others do not suggest any association of FFA with photoallergy to several cosmetic-related substances (including chemical sunscreen filters) or titanium dioxide.[100,104] Moreover, the positive patch test may be a consequence of increased exposure to these substances.

Therefore, the relation of sunscreen use and FFA remains flatly controversial.[105] Concordant results across multiple population-based studies suggest that a true correlation between sunscreen use and FFA may exist, but this does not necessarily imply causation.[105] The higher usage may simply reflect a new behaviour adopted because of the alopecia, or may even reflect higher economic status.[106] Moreover, the increasing number of FFA cases reported in black-skinned patients, among whom the rates of sunscreen use are generally low, is also less consistent with the sunscreen hypothesis of causality.[16,105]

Curiously, the frequency of shampooing has been found to be significantly lower in patients with FFA compared to a control group; this may reflect the common concern that hair washing may worsen hair loss, or suggest the possibility that frequent shampooing reduces the risk of developing FFA by achieving a more efficient removal of exogenous particles that could penetrate the follicular infundibulum and trigger the inflammatory response in patients who are genetically predisposed.[93,107]

3.5. Drugs, Medications, and Other Factors

Some studies have reported a preponderance of non-smokers within FFA patients,[3,13] or a less severe FFA in smokers, also after smoking cessation.[108,109] However, the issue about smoking and FFA is still controversial, and it is not supported by other studies.[93]

With regard to specific medications, no clear association was found in connection with the onset of FFA.[13] However, a higher history of oral contraceptive in a control group compared to FFA patients has been found,[93] although it may be related to a possible selection bias in the control group (hospital staff).[106]

Recently, the case of a woman who received nilotinib (a tyrosine kinase inhibitor) for chronic myeloid leukemia and developed keratosis pilaris, body hair loss, eyebrow alopecia, and frontal hairline recession has been published.[110]

Regarding dietary habits, a greater consumption of buckwheat and millet groats has been reported in FFA patients,[111] although subsequent studies have not found any association between phyto-oestrogens (i.e., soy) or natural PPAR-γ agonists (i.e., grapes) and FFA.[95]

Moreover, a relationship between occupational exposure to alkylphenolic compounds in women with FFA has been described; these substances have been shown to interact with PPAR-γ and inhibit transformation of DHEA to DHEAS.[95]

The occurrence of an intense and stressful event just before the onset of FFA has been referred to by some patients (76%).[13,80,107]

4. Clinical Characteristics

4.1. Clinical Features

FFA is a scarring alopecia characterized by frontal and temporoparietal hairline recession, leading to a cicatricial band, which tends to contrast with the photo-aged skin of the superior forehead. The occipital area may also be involved (15–30.4%).[3,13,56] In men, the loss of sideburns may be the only sign of the disease.[112] The alopecic area appears as a shiny, atrophic, and pale band of incomplete hair loss.[113]

When the original hairline is missing, the maneuver of cocking both eyebrows may help to find it: a sharp muscular demarcation is noted between the forehead and the scalp.[114] Wood's light examination may also help to define the missing hairline.[115]

The hairline recession is usually bilateral and symmetric,[12] but asymmetric forms have also been described. Advanced cases can lead to a "clown alopecic pattern", with total hair loss in the frontoparietal area.[12] Three clinical patterns of FFA, established according to frontal hairline recession, have been described (Table 2, Figure 2).[116] Unusual patterns have also been reported (Table 2, Figure 3).[117,118]

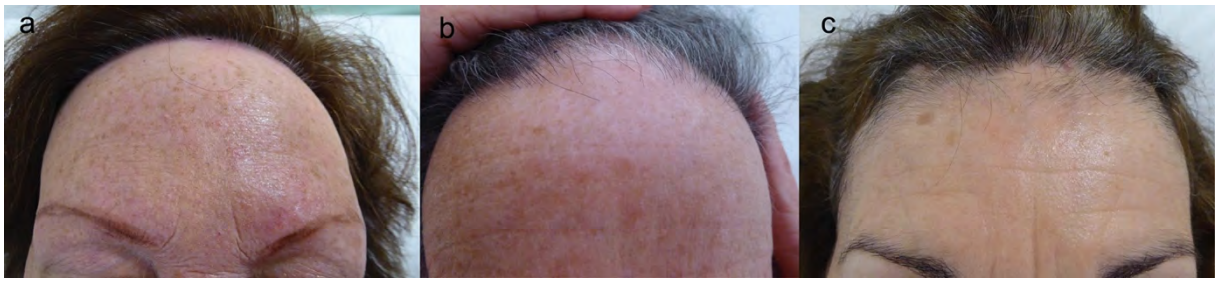

Figure 2. (a) Pattern I: linear and uniform hairline recession, without loss of hair density behind the new hairline. **(b)** Pattern II: diffuse alopecia behind the frontal hairline with loss of hair density behind. **(c)** Pattern III: unaffected primitive frontal hairline followed by an alopecic band, forming the pseudo "fringe sign". Note the absence of eyebrow alopecia.

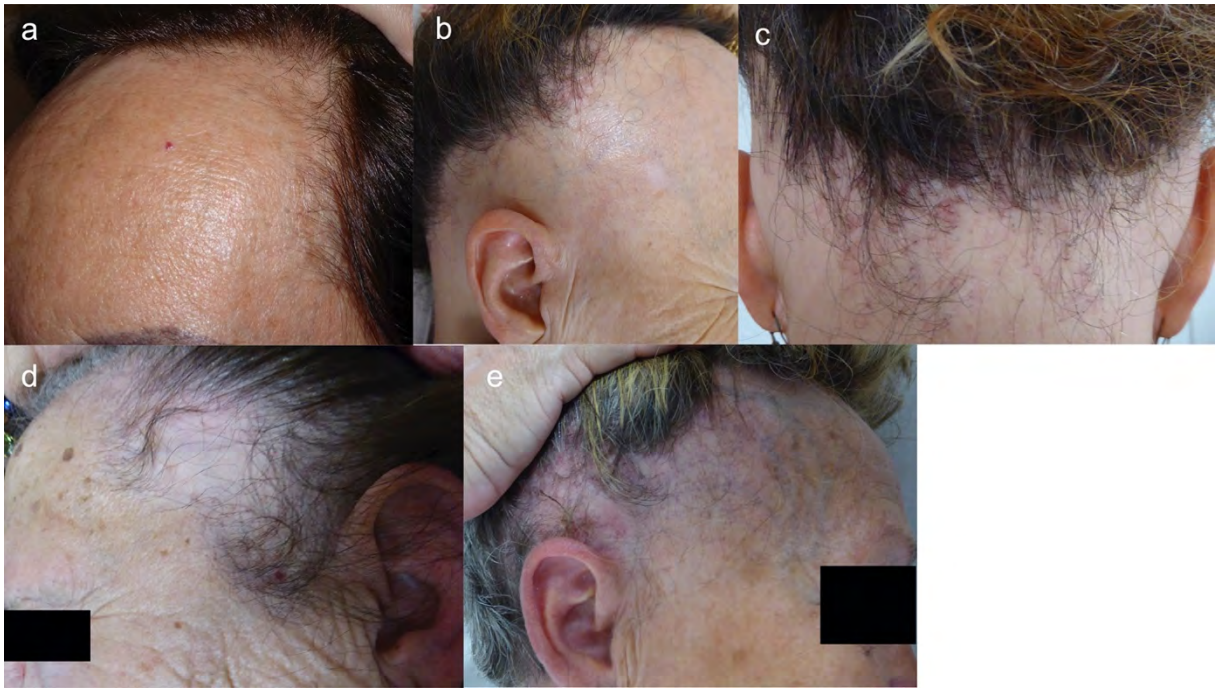

Figure 3. (a) Recession of both fronto-temporal hairlines, mimicking male AGA (AGA-like pattern). **(b, c)** Recession of the whole hairline, from frontal to occipital (ophiasis-like pattern). **(d)** Oval alopecic patches in the temporal region, sparing a thin band of temporal hairline (cockade-like pattern). **(e)** Recession of temporal hairline extending upwards to the parietal scalp (upsilon pattern). In this patient, the frontal hairline is also affected, but not the occipital area, and neither is the retroauricular region.

Table 2. Typical and unusual patterns of FFA.

Pattern Name	Clinical Description
Typical Patterns	
Pattern I (linear)	Uniform band of frontal hairline recession in the absence of loss of hair density behind the hairline
Pattern II (diffuse)	Diffuse or zigzag band-like alopecia affecting the frontal hairline with significant loss of hair density behind the hairline (at least a 50% decrease in normal hair density) with a compatible trichoscopy.
Pattern III (pseudo-fringe-sign)	Unaffected primitive frontal or temporal hairline forming the pseudo "fringe sign."
Unusual Patterns	
AGA-like pattern	Symmetric recession of frontotemporal hairlines, with sparing of the paramedian frontal hairline (mimicking male pattern AGA).
Ophiasis-like pattern	Continuous involvement of the hairline from frontal to occipital regions.
Cockade-like pattern	Presence of oval patches of alopecia in the temporal regions, with sparing of a band of temporal hairlines.
Upsilon pattern	Band-like pattern along the frontotemporal scalp extending into two symmetrical triangles along the parietal scalp.

AGA: androgenetic alopecia.

The presence of isolated hairs in the original hairline is a helpful diagnostic clue (lonely hair sign).[119] Sometimes, the unusual retention of hair along the frontotemporal rim produces a pseudo "fringe sign".[120] Loss of the vellus and intermediate hairs along the primitive hairline gives an appearance of a doll hairline.[121]

Partial or total eyebrow alopecia is noted in around 63–83% of patients.[2,12-14,122] Eyebrow alopecia can start as hair loss in the external third of the eyebrow or as a diffuse thinning, and can occur either before (more than a third of cases) or after the onset of the hairline recession, without clinical inflammation (although diffuse erythema and pruritus may be associated with eyebrow loss).[12,14,66,123,124] Sometimes, eyebrow alopecia may be the only sign of FFA.[123] Eyelash alopecia can also be noted (3–14%).[2,3,13] In men, the beard can also be affected (8–55%).[3,18,74,125]

Clinically non-inflammatory peripheral hair loss, that is, axillary, pubic, and mainly extremity hair loss, is found in 22–77% of patients with FFA,[13,14,66,126] generally occurring before hair scalp loss.[127] Interestingly, a patient with FFA with upper limb alopecia developed hypertrichosis in the forearm after removal of a plaster cast.[128]

Facial papules due to vellus hair involvement are another common finding (6–37%).[3,14,129,130] They are distributed over the facial skin and are more visible over the temples, although they

may also appear on the cheeks or chin.[131] No inflammatory signs are associated with these papules (although they might also be erythematous in patients with light phototypes),[132] and facial vellus tend to be less or absent.[133] Sometimes, these papules may be keratosis pilaris-like, with keratin-filled dilated infundibula.[131] Papules are more prevalent or can be better noted in younger patients (premenopausal),[68] probably because they appear early in the course of the disease, or because they are more easily visible without wrinkles and solar elastosis.[131] In addition, facial papules may disappear over the years, leaving smoother skin without visible follicular openings.[131] They are more frequent in Hispanics/Latinos, similar to other facial lesions in FFA.[68] Some studies suggest that facial papules, as well as eyelash loss and body hair involvement, are associated with severe forms of FFA.[3] Moreover, some authors consider that facial papules (33–50%) and occipital involvement (33%), AGA (67–83%), and body hair loss (42–83%) may be more frequent in men with FFA than in women,[3,74] although they seem to have a lower incidence of eyebrow involvement and hypothyroidism.[18,20] Recently, yellow facial papules have been described.[134]

Follicular red dots are also a clinical sign of vellus follicle involvement.[135] They are sometimes associated with follicular keratosis and may be noted in the glabella, forehead, eyebrows, cheeks,[14,131,135] or even on the body (hip, chest).[136,137]

Other facial lesions have been identified in FFA, such as a more diffuse erythema, especially over the eyebrows and cheeks, or a generalized erythema on the facial skin and neck, adopting a reticular pattern.[131] The erythema tends to disappear progressively, and sometimes lentiginous blue-grey or brown perifollicular macules may gradually appear. This diffuse erythema may be related to the higher prevalence of rosacea described in patients with FFA, especially the erythematotelangiectatic form.[19]

LPPigm may be associated to FFA, mainly in dark skin types, such as Hispanic/Latino and black-skinned patients (44–54%),[24,29] as well as Asians.[24] It appears as brown to grey macular pigmentation mostly on the face and neck, but also in flexural areas.[15,68,138] LPPigm seems to be more frequent in premenopausal women, and precedes the onset of FFA in many cases.[70]

Depression of the frontal veins has been described as another clinical sign of FFA,[139] probably due to atrophy of the overlying skin of the forehead, and has been associated with a worse initial hairline recession and initial and final eyebrow involvement.[116] This finding appears

independently of the use of topical corticosteroids, although their use may worsen the condition.

Other signs described for FFA are the presence of increased pre-auricular lines in patients with FFA[140] and increased sweating of the scalp.[40] Follicular re-pigmentation of the white/grey hair in the frontal, temporal, and occipital hairline in patients with FFA has also been reported.[141,142]

Regarding symptoms, some patients have pruritus (35–53%) and/or trichodynia (20–25%) in the hairline,[3,68] which seem to be less frequent in the occipital area compared to the frontal hairline.[143] Moreover, older patients with FFA seem to be more likely to have anxiety or depression.[144]

4.2. Clinical Course and Prognostic Factors

FFA is usually insidious, but can be rapidly progressive, and may also remain static for periods of time[13] or become stabilized spontaneously after several years of evolution.[2,3] However, the level of progression before stabilization is unpredictable. Without treatment, the hair loss per year, measured by the distance of recession of the hairline, may range from 0.2 to 2.1 cm.[3] In the early disease, eyebrow regrowth may be achieved with some local treatments. The patient's age and age at disease onset are both predictors of FFA severity, with higher age and age at onset being related to more severe forms.[109] A lower educational level might also be associated with severe forms of the disease.[109,145] Higher body mass index has been found to be associated with severe forms,[109] as well as with the presence of rosacea in patients with FFA.[19] Moreover, patients with more severe FFA seem to be more likely to have rosacea.[19] Regarding clinical patterns, pattern III is associated with the best prognosis and pattern II with the worst, whereas pattern I has an intermediate prognosis.[116]

5. Trichoscopy

Perifollicular erythema and follicular hyperkeratosis, along with the loss of follicular openings in the affected hairline, are the main trichoscopic findings in FFA (Figure 4a).[22] The presence of follicular ostia with only one hair shaft is another frequent feature. The background in FFA is ivory-white.[146] Perifollicular hyperpigmentation, as well as pinpoint white dots in the alopecic band, are characteristics of darker-skinned patients with FFA.[15,16,147] Black dots, broken hairs, pili torti, and branching capillaries may also be seen, as well white patches in advanced disease.[148]

Yellow dots may also be found in FFA, and may be an early feature associated with follicles with a potential for regrowth;[149] therefore, they are more frequent in mild cases.[150]

In the temporal area, where follicular hyperkeratosis and perifollicular erythema are rarely seen,[116,151] a characteristic finding is that most of the hair shafts show transparent proximal hair emergence.[151]

The presence of solitary terminal hairs at the site of the original hairline[119] and the absence of vellus hair in the frontotemporal hairline[152] are very helpful clinical clues to the diagnosis of FFA, and also help to rule out other differential diagnosis. Loss of vellus hair in the frontal hairline is the most common trichoscopic sign in mild cases of FFA, although it may be partially or totally preserved in some of them.[150]

Although perifollicular erythema has been considered as a marker of FFA activity[153] and many patients with a receding hairline have persistent inflammatory signs (perifollicular erythema and scaling), there is growing recognition that these inflammatory signs can persist in patients despite there being no progression in hairline recession,[13,14] and others may have hair loss progression without inflammatory signs.[154] Moreover, the presence of peripilar erythema has been correlated with the coexistence of rosacea.[19] On the other hand, patients with pubic hair loss presented more cicatricial white patches, and those have been associated with the severity of FFA.[155]

In the eyebrow area, the presence of a few black dots and dystrophic hairs may suggest the diagnosis of FFA.[123] Red or grey dots (Figure 4b) may indicate a favorable prognostic factor for local regrowth, while loss of follicular openings and pinpoint dots within whitish areas are seen in advanced disease.[124] Eyebrow regrowth in distinct directions and pili torti may also be

noted.[123,124,156] Tapered hair, broken hair, and yellow dots may be observed in FFA eyebrows, but less frequently than in alopecia areata (AA).[124]

Four dermoscopic patterns of LPPigm in patients with FFA have been described: pseudo-network, speckled blue-grey dots, dotted pattern, and blue-grey dots arranged in circles.[138]

A recent study found that telangiectasias, red dots, follicular plugs, and perifollicular erythema are more frequent in phototypes I–III, while peripilar hyperpigmentation, black dots, dystrophic hairs, short thin hair/vellus, peripilar casts, and broken hairs are more frequent in phototypes IV–VI.[157]

Vascular structures (arborizing vessels and extravasated hemorrhages) become more common when there is chronic use of topical corticosteroids, whereas perifollicular erythema and the peripilar cast become less visible.[158]

Ultraviolet light-enhanced trichoscopy may be helpful to predict the efficacy of local treatment, so that positive fluorescence ("starry night sky sign" pattern), which is due to the presence of Propionibacterium acnes, may be a sign of a still-preserved hair follicle.[159]

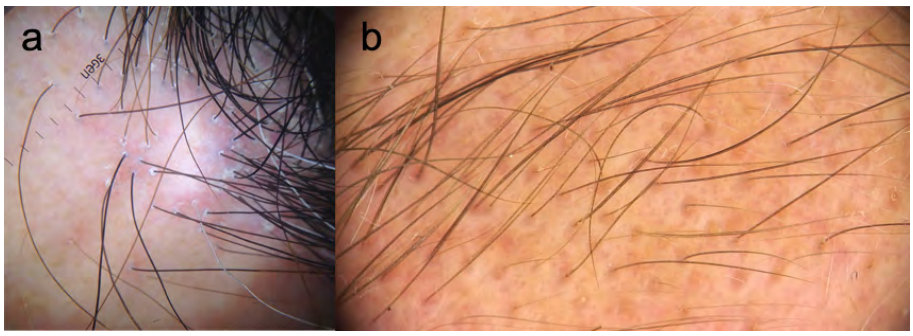

Figure 4. (a) Frontal hairline: perifollicular erythema and hyperkeratosis, follicles with one hair shaft, white patches, and loss of follicular openings. (DermLite Pro II HR, non-polarized light) **(b)** Eyebrows: partial alopecia with red follicular dots (DermLite Pro II HR, non-polarized light).

6. Clinical Classification and Severity Scores

A five-grade classification to assess the clinical severity of FFA has been proposed: I (<1 cm), II (1–2.99 cm), III (3–4.99 cm), IV (5–6.99 cm), and V (>7 cm, also called "clown alopecia"). This size is obtained by measuring the area of cicatricial skin produced by the recession of the frontal and temporal hairline and using the largest measurement to define the grade of severity.[3]

Currently, two validated scoring systems for FFA assessment exist: the FFASI (FFA Severity Index), which gives scores for hairline recession, inflammatory band, non-scalp loss, and associated features,[160] and the FFASS (FFA severity score), which also includes signs of local inflammation and patients' symptoms.[161.]

7. Laboratory

Blood analysis, including hemogram, general biochemical, liver function, thyroid function, antinuclear antibodies, and sex hormones, is usually normal.[12] Low levels of positivity for antinuclear antibodies have been found in some patients with FFA.[2,12] Therefore, blood tests seem to be unnecessary in FFA, except for rejecting thyroid disorders.

8. Image Techniques

Optical coherence tomography in FFA has shown increased epidermal thickness in the inflammatory hairline and decreased thickness in the alopecic band, as well as a lower vascular flow in the alopecic band compared to the inflammatory scalp in the superficial dermis, but increased flow in the deeper plexus.[162]

Reflectance confocal microscopy in LPP and FFA allows visualization of the major key diagnostic features, such as infundibular hyperkeratosis, perifollicular lichenoid inflammatory infiltrate, and extensive perifollicular fibrosis.[163]

Regarding sonography, FFA patients have a higher vessel diameter and flow in the hairline implantation area in comparison with a control group, which may be explained by the presence of active inflammation.[164] Interestingly, the vessel diameter has been demonstrated to be higher in the healthy scalp area in FFA patients than in a control group, which may reflect

the presence of subclinical inflammation in the still unaffected areas. The presence of branched vessels has been related to a higher significant flow in the hairline area in those patients, independently of the use of topical corticosteroids, so these vessels may be the reflex of active inflammation.

9. Histopathology

Histological findings in FFA seem to be indistinguishable from LPP, according to some authors.[165] FFA is characterized by a lichenoid lymphocytic infiltrate around the upper follicle, i.e., isthmus and infundibulum, including the bulge area, as well as concentric perifollicular lamellar fibrosis.[1] A strong correlation between the severity of the peripilar cast and the degree of lymphocytic infiltration has been identified.[166] It is thought that the destruction of the external root sheath at the level of the isthmus is responsible for irreversible alopecia.[167] The lower part of the follicle usually remains spared. A reduction in the number of hair follicles is a consequent finding: a mean of seven terminal hair follicles have been seen per 4 mm punch biopsy in FFA, whereas "normally" around thirty terminal and five vellus hair follicles are seen in Caucasians, and eighteen terminal and three vellus ones in Afro-Caribbeans.[66]

The loss of sebaceous glands is an early finding in FFA.[120] Indeed, in eyebrow samples, sebaceous gland preservation may be the pathological correlation for the reversibility of eyebrow loss.[168] In early cases, the inflammatory involvement of the vellus follicles and atrophy of the sebaceous glands are the histological clues, not including perifollicular fibrosis.[169]

Vacuolar degeneration of the basal layer, keratinocyte necrosis, and replacement of the pilosebaceous units by fibrous tracts, along with loss of elastin fibres, are other FFA signs.[170] Advanced cases may only reveal fibrous tracts and the absence of hair follicles, without any inflammatory infiltrate, similar to other cicatricial alopecias in their later stage.[12] Dilated eccrine glands have been identified in a patient with FFA, together with increased scalp sweating.[40]

Initially, it was considered that intermediate and vellus-like follicles were more commonly affected than terminal follicles by the lymphocytic inflammatory infiltrate and perifollicular fibrosis.[5] Nowadays, it is accepted that terminal follicles are involved in the same way as the

others.[167] The follicular triad has been described in early FFA, and describes the simultaneous involvement of follicles of different types (terminal, intermediate, and vellus) and in a different stage of the cycle (anagen, catagen, and telogen).[171]

Regarding the composition of the inflammatory infiltrate in FFA, this is characterized by an increase in the percentage of CD8+ T cells,[37,172] with a reversal of the typical CD4:CD8 ratio (which is approximately 2:1). However, this ratio is increased (>3:1) in uninvolved follicles in FFA, which may be because of the migration of CD8+ T cells from uninvolved areas to involved ones.[170] A significant increase in perifollicular and interfollicular Langerhans cells has also been described in FFA. Plasmocytoid dendritic cells, the most potent I IFN producers, are increased in LPP and FFA, mainly confined to the upper dermis surrounding the hair infundibulum.[173] Moreover, a reduced number of CD1a+ and CD209+ dendritic cells in the perifollicular mesenchyme adjacent to the infundibulum in both LPP and FFA has been described, whereas increased total numbers and degranulation status of perifollicular mast cells have been found in lesional LPP and FFA hair follicles.[37]

A lower melanocyte count has been demonstrated in the upper follicle in FFA patients compared to LPP, and is associated with the hypopigmentation observed clinically in the alopecic band in FFA.[26,30]

A recent study provides data for significant immune dysregulation in FFA, with increased infiltration of CD8+ T cells, CD11c+ dendritic cells, CD69+ and CD103+ TRM, tryptase+ mast cells, and FOXP3+ Tregs, in addition to significant upregulation of Th1 and JAK-STAT pathways.[172] Moreover, K15 and CD200 expression in the hair follicle bulge has revealed some preservation of stem cells in lesional FFA.[172]

Few histological abnormalities have been described in clinically unaffected scalp in FFA and LPP, such as infundibular lymphocytic inflammation[174-176] and early sebaceous gland atrophy.[165] Perifollicular fibrosis and mucin deposits have also been noted in unaffected scalp.[176] On the other hand, a relative increase of CD4 + FOXP3 + T regulatory cells in the perifollicular infiltrate in both affected and unaffected scalp in FFA has been described.[175]

Recently, dermal fat infiltration at the isthmus level and in the arrector pili muscle has been observed in FFA samples.[177] Interestingly, the arrector pili muscle is thought to play an

important role in protecting the stem cells in the bulge area. Moreover, a dermal displacement of eccrine sweat coils has also been noted in a fair number of patients.

Biopsy of the facial papules also reveals follicular hyperkeratosis and lichenoid dermatitis involving the infundibular and isthmus portions of the vellus hair follicles,[131] or even fibrosis around the vellus hair or complete follicular destruction.[130,133] Interestingly, sebaceous glands are present in the majority of cases, different from the picture one might expect in scalp samples.[178] Indeed, in most cases, prominent sebaceous glands with dilated ducts are seen. The destruction of elastic fibres may be responsible for the "pop-out" of sebaceous glands and the formation of the yellow facial papules.[134] A lymphocytic folliculitis without perifollicular lamellar fibrosis has been detected in some cases of limb biopsies, similar to frontal scalp biopsies in early FFA.[179] The histopathology of LPPigm is characterized by epidermal atrophy, mild vacuolar dermatitis, sparse perivascular lymphocytic infiltrate (in early phases), and pigment incontinence, and can share the pattern of lichenoid folliculitis, also observed in biopsies from the scalp, eyebrows, limbs, and facial lesions of patients with FFA.[180]

With regards to eyelashes, small and narrow bulbs, irregular caliber, and irregular pigment distribution have been observed. Demodex folliculorum infestation was noted in a patient with mild FFA and eyelash loss, which suggests that it might accelerate autoimmune inflammation and produce premature eyelash alopecia.[181]

10. Are LPP and FFA the Same Disease?

Whether FFA is a variant of LPP or a different entity is still unclear.[2,3] Clinically, LPP is usually associated with multifocal areas of scarring alopecia that may coalesce to produce large alopecic areas.[3] The most typical locations are the vertex and parietal areas, although it may extend throughout the scalp in a patchy manner.[167] Moreover, LPP is associated with LP at other sites more often than FFA is.[167] Regarding trichoscopic signs, perifollicular hyperkeratosis in LPP is more intense than in FFA, and the background is typically milky-red.[146]

FFA and LPP share main histological features. However, a few differences have been found between LPP and FFA (Table 3), which makes it more suitable to consider FFA as a specific type of lymphocytic cicatricial alopecia rather than a variant of LPP.[167] The inflammatory infiltrate in FFA is usually milder than in LPP.[167,182] According to one study, most FFA patients exhibit

the maximum degree of inflammation at the isthmus, but a significant number of patients with FFA may have inflammation extending below the isthmus, or even fibrosis, in comparison with LPP;[183] however, this has not been supported by other studies.[172] Moreover, the damage to the basal layer tends to be subtler in FFA than in LPP.[167] The presence of a superficial perivascular lymphohistiocytic inflammatory infiltrate is common in LPP, but not in FFA.[167] Eosinophilic necrosis of keratinocytes of the external root sheath is prominent in FFA, especially at the isthmus, whereas it is not as marked in LPP and, if present, is located at the lower follicle.[167] A foreign body reaction following follicular destruction is usually more intense and frequent in FFA than in LPP.[167] Moreover, the interfollicular epidermis is commonly spared in FFA, but it is affected in 50% of cases of LPP.[167] Furthermore, concentric lamellar fibroplasia seems to be more frequently present in LPP than in FFA, while the presence of terminal catagen-telogen hairs is more frequently found in FFA.[182] The presence of direct immunofluorescence deposits is more frequent in LPP;[167,184] in FFA, it is usually negative, although IgM deposits over the basement membrane and cytoid bodies in the papillary dermis have been described.[133,185] Moreover, the epidermis is thinner in FFA than in LPP;[30] this reduction in the epidermal thickness (and also dermal) has been noted in the alopecic band of FFA naïve patients.[174] A recent study has found that macrophages exist in different functional phenotypes in LPP and FFA; CD86 is downregulated in LPP compared with FFA, whereas CD163 is increased in LPP and decreased in FFA.[37]

A lower melanocyte count has been demonstrated in the upper follicle in FFA patients compared to LPP.[26.]

Table 3. Histopathological differences between FFA and LPP

Histological Features	FFA	LPP
Inflammatory infiltrate degree	+	++
Inflammation/fibrosis below the isthmus	++/−	+/−
Basal layer damage degree	+	++
Superficial perivascular infiltrate	+/−	++
Keratinocyte necrosis in the external root sheath	++	+
Foreign body reaction	++	+/−
Involvement of interfollicular epidermis	−	++
Concentric lamellar fibroplasia	+	++
Presence of terminal catagen-telogen hairs	++	+/−
Direct immunofluorescence deposits	+/−	+
Epidermal thickness reduction	++	+
Macrophage polarization	+CD86, −CD163	−CD86, +CD163
Lower melanocyte count in the upper follicle	+	−

FFA: Frontal fibrosing alopecia. LPP: lichen planopilaris. (−) Absence, (+) Presence. (++) instead of (+) indicate a higher degree or intensity compared to the other (FFA vs LPP).

11. Diagnosis

Diagnostic criteria for FFA are referred to in Table 4.[186,187]

Table 4. Diagnostic criteria for FFA

Major Criteria	Minor Criteria
1. Cicatricial alopecia of the frontal, temporal, or frontotemporal scalp, in the absence of follicular keratotic papules on the body.	1. Typical trichoscopic features (perifollicular erythema and/or follicular hyperkeratosis, lonely hair sign).
	2. Histopathological features of FFA and LPP.
2. Diffuse bilateral eyebrow alopecia.	3. Involvement (hair loss or perifollicular erythema) of additional FFA sites (occipital area, facial hair, sideburns, or body hair).
	4. Non-inflammatory facial papules.
	5. Preceding or concurrent symptoms (pruritus or pain) at the areas of involvement.

The diagnosis of FFA requires two major criteria or one major criterion and two minor criteria.

12. Differential Diagnosis

The ophiasis pattern of AA, which affects the margins of the scalp, may masquerade as FFA. In addition, AA may produce eyebrow alopecia, sometimes as an isolated finding. However, perifollicular erythema and hyperkeratosis are absent in AA, whereas yellow dots, dystrophic and broken hairs, black dots, exclamation mark hairs, tapered hairs, and regrowing hairs are common features.[146]

Traction alopecia may resemble FFA. The clinical background, with a history of use of tight hairstyles and the absence of typical trichoscopic signs of FFA, may be useful. Moreover, traction alopecia is not associated with eyebrow hair loss.

Other scarring alopecias, such as LPP, discoid lupus erythematosus, and pseudopelade of Brocq, tend to produce multifocal alopecic areas.

A familial high frontal hairline should also be discarded.

AGA with male pattern may also be considered, especially when the frontal or temporal hairline is receded. However, hair miniaturization (with an increased proportion of thin and vellus hair) and anisotrichia[146] are not trichoscopic signs in FFA.

13. Treatment

Almost all information about treatment in FFA is based on retrospective cohort studies and cases reports.[12,13,188] The aim of the treatment is to alleviate symptoms and signs and arrest the progression of the hair loss, since hair regrowth is not possible once destruction of the follicles has happened.

13.1. Local Treatments

Topical corticoids are recommended, especially in the early inflammatory stage, but relapse occurs upon their discontinuation.[12.188] Potent topical steroids and calcineurin inhibitors reduce inflammation, but without any clear benefit in slowing the alopecia,[11,13,189] although disease stabilization with a combination of both treatments has been published.[190] However, a study including 92 FFA patients revealed that patients treated with 0.3% tacrolimus were

more likely to stabilize in three months compared to those treated with clobetasol/betamethasone.[191]

With regards to intralesional corticosteroids, 20 mg/mL of triamcinolone acetonide used in the hairline (every 3–6 months) may obtain hair regrowth in some patients;[11] 34% of patients showing improvement, 49% stabilization, and 5% worsening are the outcomes found in a cohort of 130 patients.[3] It is also a useful treatment in eyebrow alopecia, where 10 mg/mL (or even more diluted) every three months, may obtain hair regrowth, especially in cases of partial eyebrow loss.[11,122] Moreover, it seems to be uncommon for patients with FFA and with eyebrow alopecia to experience eyebrow regrowth with systemic therapy alone.[122]

Topical minoxidil has not shown clinical improvement in the slowing down of the progression of the alopecia.[12]

Bimatoprost 0.03% eye drops, a prostaglandin analogue, may be used for eyebrow loss; a study involving three patients who applied it twice daily showed regrowth in two of them after nine months of treatment.[192] It may also be an option in eyelash loss.

Some authors referred to the fact that excimer laser may be effective in reducing inflammation and peripilar casts in patients with active disease.[193] Application of superluminescent diodes as an adjuvant therapy in patients with FFA/LPP showed a

decrease in subjective symptoms and perifollicular hyperkeratosis, and even an increased number of thick hairs within the treated area.[194]

One patient of recalcitrant FFA treated with platelet-rich plasma (PRP) (0.1 mL/cm2, five treatments with a one-month interval) injected into the frontotemporal hairline and eyebrows showed improvement in trichoscopic signs and no further hair loss after five months.[195]

13.2. Systemic Treatments

Oral prednisone (0.5–1 mg/kg/day, three to eighteen months) has been shown to produce a stoppage of hairline recession in almost 43% patients, but with a relapse when the treatment is stopped.[12] Intramuscular triamcinolone acetonide (40 mg every three weeks) has also been used, but with no therapeutic effect observed.[5]

A study including 36 patients revealed an improvement in symptoms and signs of FFA in 73% of patients treated with hydroxychloroquine, though most were partial responses. The maximal benefits were seen within the first six months of therapy. On the other hand, 60% of them produced a response to mycophenolate mofetil, though mainly partial ones.[17] Other reports did not find any consistent benefit with the use of hydroxychloroquine.[13]

A report including 102 patients treated with oral finasteride (2.5–5 mg/day), which inhibits the isoenzime type II of 5-alpha reductase, displayed that 47% of patients showed improvement (regrowth in the hairline) and 53% showed a stabilization of the alopecia.[3] There is even one report of a woman with FFA who experienced frontal hair regrowth and reversal of cutaneous atrophy within three to twelve months of treatment with oral finasteride.[196] Subsequently, favorable outcomes have been described with oral dutasteride, which is about three times as potent as finasteride at inhibiting type II 5-alpha reductase and more than 100 times as effective at inhibiting type I.[188] In 18 patients with FFA treated with oral dutasteride (0.5 mg/week), 44% of patients displayed improvement and 56% of them showed a stabilization of the alopecia.[3] Moreover, a report combining oral dutasteride with topical pimecrolimus revealed a stoppage in hairline recession along with hair regrowth in eyebrows and axillae.[188,189] Other authors have found a stabilization of hair loss in 70% of FFA patients treated with dutasteride and in 33% of patients treated with finasteride.[129] Therefore, oral 5-alpha reductase inhibitors seem to be the most effective therapy in FFA patients, as all patients experienced at least stabilization.[3] This oral therapy should be accompanied by intralesional corticosteroid infiltration in the hairline when inflammatory signs are present.[3]

A retrospective analysis of 54 women with FFA treated with oral isotreinoin (20 mg/day), acitretin (20 mg/day), or finasteride (5 mg/day) showed a stoppage of progression in 76%, 73%, and 43% of patients, respectively.[197] Alitretinoin has also been used in a woman with FFA, who showed improvement after one month with 30 mg/day.[198]

Recently, a study including 224 FFA patients has compared the effectiveness of oral dutasteride against other systemic treatments (finasteride, hydroxychloroquine, doxycycline, isotretinoin) and with a group receiving no systemic therapy (just topical minoxidil 5% and clobetasol propionate 0.05% solution).[199] Authors found significant differences in the percentage of stabilized patients after twelve months of therapy between patients treated with dutasteride versus the other groups, with a stabilization rate of 61.5–64.2%. The

response was dose-dependent, and the most effective dose was five to seven capsules of dutasteride (0.5 mg) per week.

Regarding facial papules, an improvement of the facial skin surface and regrowth of vellus after six months of treatment with oral prednisone and antimalarials have been reported.[133]. A favorable outcome has also been published with oral isotretinoin (10 mg/day) in two to four months.[134,182]

The only randomized controlled trial is in regard to the use of oral isotretinoin (initially 20 mg/day, then 20 mg every other day after one month) combined with topical clobetasol (0.05%) and tacrolimus (0.1%) compared to topical treatment alone in FFA.[200] Authors found improvement of facial papules, no deterioration of the FFASI variables, and improvement of erythema and perifollicular keratosis in the frontal line in the treatment group after six months.

Other treatments, such as griseofulvin, azathioprine, or tetracyclines, have shown no efficacy or inconsistent outcomes so far.[1,2,5,11,17,129] Few patients have achieved stabilization of their FFA during treatment with methotrexate.[129,201]

Pioglitazone hydrochloride (15 mg/day), an oral PPAR-γ agonist, showed improvement of itching and a decrease in the inflammatory infiltrate in a patient with LPP, but no remission;[202] however, studies with more patients have shown a negative outcome in most patients.[203] No successful results have been observed in FFA patients.[129]

Oral minoxidil has been demonstrated to improve the background hair thickness in LPP, especially in patients with diffuse LPP; however, the report excluded patients with FFA.[204] Further studies regarding its efficacy in FFA are needed.

With regards to biological therapies, a study including 10 patients with recalcitrant LPP and FFA (2/10) who were treated with oral tofacitinib, a pan-JAK inhibitor, 10–15 mg/day from two to nineteen months, showed a clinical response in 80% of patients and clinical improvement in both FFA patients.[205] One woman who had refractory FFA and LPP showed improvement after around four to thirteen months of treatment with tildrakizumab (100 mg subcutaneously at week zero, four and subsequently twelve weekly), an anti p19 IL23 monoclonal antibody.[206] One case of a woman who was receiving adalimumab for hidradenitis

suppurativa and rheumatoid arthritis and experienced hair regrowth in the area affected by LPP has been reported.[207]

13.3. Hair Transplant

A minimum of one to five years without activity is recommended before hair transplantation is used in scarring alopecias.[208,209] Most FFA patients who underwent hair transplantation lost the hair grafts in around four years, suggesting that FFA displays recipient dominance.[209,210] Similar results have been observed in eyebrow transplantation.[211] A recent study has shown a decrease in graft survival over time, independently of the period of time since clinical remission, with a graft survival rate lower than 60% after five years.[212] Therefore, a hair transplant should only be offered to selected patients with FFA to improve small areas and after first discussing with the patient the long-term survival rate of the grafts.

According the published data, a simplified algorithm of treatment is represented in Figure 5.

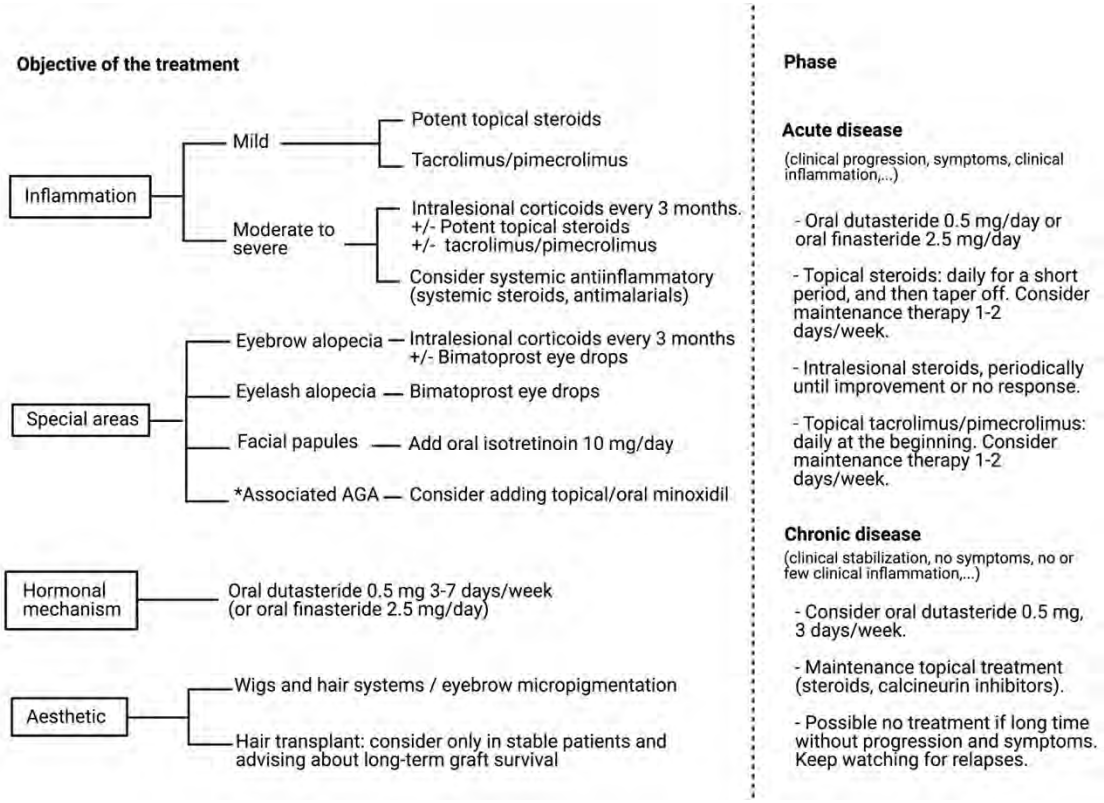

Figure 5. Algorithm of treatment. *Some patients with FFA may associate AGA; in these cases, adding minoxidil may provide an additional therapeutic benefit. Created with BioRender.com (accessed on 1 March 2021).

14. Conclusions

FFA prevalence has increased during the last few years, and so has the interest of the medical community regarding its characterization, pathogenesis, and treatment. However, most studies are observational reports, so further investigations and clinical trials are needed to clarify important issues, such as the possible responsible trigger. In that way, further studies about hair and skin cosmetic routines in patients with FFA may be an interesting research prospect. Moreover, the study of the existence of environmental factors that may explain differences in the prevalence of FFA in different geographic areas could contribute to a better understanding of FFA. The precise knowledge of its ethology and pathogenic mechanisms may expose specific therapeutic targets. Although the use of 5-alpha reductase inhibitors has permitted the stabilization of a considerable number of patients, research for new treatments, such as oral minoxidil or biological therapies, is still lacking.

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OTHER RESULTS

6. OTHER RESULTS.

The QoL and the histopathological studies, which have not been published yet, are exposed in this section. They are also part of this doctoral thesis.

6.1. Quality of life results.

Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI): a validated disease-specific questionnaire

María Librada Porriño-Bustamante,[1,2] Trinidad Montero-Vílchez,[3] María Antonia Fernández-Pugnaire,[4] Salvador Arias-Santiago.[3,5]

1. Dermatology Department. University Hospital La Zarzuela, Madrid, Spain.

2. University of Granada, Granada, Spain.

3. Dermatology Department, University Hospital Virgen de las Nieves, Granada, Spain

4. Dermatology Department. University Hospital San Cecilio, Granada, Spain

5. School of Medicine, Institute of Biosanitary Investigation ibs, Granada University, Granada, Spain

Corresponding author: Trinidad Montero Vílchez. Email: tmonterov@gmail.com

University Hospital Virgen de las Nieves. Avenida de Madrid, 15, 18012, Granada, Spain.

ABSTRACT

Background: Frontal fibrosing alopecia (FFA) is currently the most common type of scarring alopecia. Quality of life (QoL) can be affected in patients with alopecia, especially in women. The few studies that evaluate QoL in FFA use unspecific and general questionnaires, and therefore, there is a need to develop a specific questionnaire to assess QoL in patients with FFA.

Objective: To design and validate a new specific questionnaire to assess the impairment of QoL in patients with FFA.

Methods: A cross-sectional study was performed. A specific questionnaire, called Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI), was designed and validated using the Dermatology Life Quality Index (DQLI).

Results: The study included 101 women with FFA and 40 control subjects. The questionnaire was based on 20 questions about emotional, social and functional disorders. The value for internal consistency was 0.865 (Cronbach α), and the intraclass correlation coefficient between all the items in the questionnaire was 0.870. FFA-QLI correlated positively with DLQI ($r=0.729$, $p<0.001$). A test-retest was performed on 30 FFA patients. Patients with severe FFA showed a higher FFA-QLI score compared to those with a mild disease (19.72 vs 14.11, respectively, $p = 0.002$) and the area under the curve for identifying severe disease was greater in FFA-QLI than in DLQI (area under curve = 0.704, $p < 0.001$). Cut-off points were to select patients with mild, moderate and severe impairment in QoL. A score <21 in FFA-QLI corresponded with a low impact on QoL (sensitivity 88.9%, specificity 80.7%), values >35 matched with greater QoL impairment (sensitivity 75%, specificity 95.9%) and values ranging from 21 to 35 corresponded to moderate QoL alteration (sensitivity 85.7%, specificity 80.7%).

Conclusion: A validated disease-specific questionnaire to assess QoL in FFA patients is here presented, with a greater power to discriminate severe cases of FFA than DLQI.

KEYWORDS: frontal fibrosing alopecia, scarring alopecia, quality of life

INTRODUCTION

Frontal fibrosing alopecia (FFA) is a scarring alopecia characterized by a frontal and/or temporoparietal hairline recession, leading to a cicatricial alopecic band.[1] FFA's prevalence has been increasing progressively since its first description by Kossard in 1994,[2] becoming the most common type of scarring alopecia nowadays.[3]

As hair is an important element in identity and self-image, alopecia can impact negatively on quality of life (QoL). QoL is generally assessed by unspecific self-reporting questionnaires, which should be easy to complete by the patient without medical supervision. It has been demonstrated that alopecia may have substantial psychological consequences, especially in women.[4] Feelings of loss of self-confidence, low self-esteem and heightened self-consciousness have been identified in people affected by alopecia.[5] Furthermore, people with alopecia are more likely to have depression and anxiety.[5,6] Generic questionnaires may not detect all patients with QoL disorders, so for some specific diseases a specific questionnaire may provide a more appropriate assessment, as happens in suppurativa hidradenitis, alopecia areata or androgenetic alopecia (AGA).[7-9]

The psychological impact of alopecia is determined by disease-related factors (e.g. visibility of hair loss), demographic factors (e.g. gender), psychological factors (e.g. beliefs about illness) and behavioural factors (e.g. coping).[4]

Most studies regarding QoL and alopecia are about alopecia areata and AGA.[8,10] Patients with alopecia areata have a higher risk of developing major depression, generalized anxiety disorder, social phobia, and paranoid disorder than healthy individuals.[6] Depression is more frequent in women with AGA, while anxiety and aggressiveness seem to be more prevalent in men.[10] Patients with AGA may even consider their hair loss to be comparable to a physical impairment, such as the loss of a limb.[11] The visibility of the alopecia in women means that they feel less feminine and in men that they feel much older.[11,12] In fact, almost 30% of the women with AGA could not cope with the problems of hair loss and were worried about the negative consequences.[12] These problems may manifest themselves in the efforts taken by patients to hide their alopecia, such as taking care where to sit, avoiding socialising, not going swimming or staying at home when it rains or is windy. Other problems may include a loss of self-confidence, feelings of inferiority and feelings of insecurity in company.[11]

However, there are few reports regarding scarring alopecia and QoL, and even fewer about FFA. All of them used pre-existent general scales, such as Illness Perception Questionnaire, Hospital Anxiety and Depression Scale (HADS) and Dermatology Life Quality Index (DLQI).[13-18] The DLQI is a 10-item self-reported measure of QoL, (scored from 0 to 30), in patients with dermatological conditions, in which scores 0-1 indicate that QoL is not affected, 2-5 identify mild QoL impairment, 6-10 reflect a moderate impact, 11-20 a severe impact and >21 a very severe QoL impairment.[16] HADS is a 14-item self-reported measure of anxiety (HADS-A, 7 items) and depression (HADS-D, 7 items); the sub-scales range from 0 to 21, and scores <7 suggest a non-case, 8-10 a probable case and >11 a definite case.[17]

The main objective of this study was to design and validate a new disease-specific questionnaire for FFA, the Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI) for QoL measurement in patients with FFA.

MATERIAL AND METHODS

A cross-sectional study, including women with FFA and a control group, was performed in the Dermatology Departments of the University Hospital San Cecilio and Virgen de las Nieves, both situated in Granada, Spain. Inclusion criteria for FFA patients were the presence of frontal and/or frontotemporal hairline recession, leading to a scarring band without follicular openings, with or without perifollicular erythema and follicular hyperkeratosis. Inclusion criteria for the control group were as follows: women aged between 45 and 95 years, without any hair disease. Control individuals were people who had consulted the Dermatology Department for other reasons (naevi, seborrhoeic keratosis, etc.). Exclusion criterion for both groups was the male sex. All patients and control subjects signed an informed consent and the project was approved by the local ethics committee.

Demographic and general information was recorded for both patients and control subjects, such as age, ethnic group, marital status, number of children, and age of menopause. In patients, data regarding the alopecia were obtained, such as age of onset, presence of perifollicular erythema and follicular hyperkeratosis, presence of itchiness or trichodynia, severity grade, eyebrow and eyelash alopecia, the existence of facial papules and the coexistence with other types of alopecia. Severity grade was assessed following the V-grade classification described by Vañó et al and grouped into mild (I-II) or severe (III-V) FFA.[19]

Three types of questionnaires were administered to both patients and control subjects: the DLQI, the HADS and the newly designed FFA-QLI (annex 1). A translated English version of the questionnaire is provided in annex 2, although the validated version is the Spanish one (annex 1).

The FFA-QLI consisted of 20 questions divided into three parts: the first part included 7 questions related to emotions (items 1-7); the second part included 7 questions about their social life (items 8-14); and the third part included 6 questions about functional changes (items 15-20). Division into these three parts was purely organizational. The responses were scored from 0 (not affected at all) to 3 (highly affected). As the study was performed in Spain, the original questionnaire was developed in Spanish. The results of FFA-QLI have been presented on a scale varying between 0 (best QoL) and 60 (worst QoL). The questions were developed by dermatologists working in the trichology area. They were selected on the basis of the main clinical signs and symptoms of FFA (eyebrow loss, scalp hair loss, pruritus and trichodynia),

focusing on different areas of life in which patients can feel ashamed or worried and also on behaviours that could be modified because of the alopecia. The questionnaire was refined after interviewing 25 patients, and the questions were modified to improve the patients' understanding of them, as a result of the feedback from the first impressions of the interviewed patients.

The psychometric validation of FFA-QLI was based on the DLQI, and the HADS. Reliability was evaluated using internal consistency analysis with the Cronbach α (acceptable if> 0.7) and reproducibility analysis with the intraclass correlation coefficient (ICC) (adequate if> 0.7). To determine the test–retest reliability, the ICC for the global value of the questionnaire and a Cohen's kappa (acceptable strength of agreement if kappa >0.5) of the items were calculated from the original FFA-QLI.

Convergent validity, examining the degree to which two measures of constructs are related, was assessed by calculating the extent of correlation between raw scores from the FFA-QLI and the DLQI using the Pearson correlation coefficient. The cut-off points to select patients with mild, moderate and severe QoL impairment were calculated using receiver operating characteristic (ROC) curve analysis and comparison with the DLQI categories. A ROC curve was also constructed to assess the discriminative power of the scores to select severe FFA patients according to Vañó et al.[19]

There were no missing data in any of the completed questionnaires. Continuous data were presented as mean (standard deviation) and categoric data as relative (absolute) frequency. Continuous data were tested for normality using the Kolmogorov–Smirnov test. The student's t-test was applied to compare mean values of quantitative variables. Qualitative variables were analysed with $\chi 2$ test. Statistical significance was defined by a two-tailed $p<0.05$. SPSS version 24.0 (SPSS Inc, Chicago, IL) was used for statistical analyses.

Regarding sample size, at least four participants per variable would be necessary to validate a questionnaire.[20] As the FFA-QLI consists of 20 questions, a total of 20 variables were evaluated, requiring at least 80 participants.

RESULTS

A total of 101 women with FFA and 40 healthy women were included. Case and control groups were comparable regarding age (63.45 vs 63.05 years, respectively – p=0.824) and sex. Additional data about patients are listed in Table 1.

Table 1. Additional data about FFA patients

N = 101	**Mean values (SD) and frequencies**
Female sex	100%
Age (years)	63.45 (SD 9.32)
Marital status - Single, divorced, widowed - Married	 31.7% (32/101) 68.3% (69/101)
Occupational status - Active - Inactive (retired, unemployed)	 32.7% (33/101) 67.3% (68/101)
Children (yes)	90.1% (91/101)
Menopause (yes)	89.1% (90/101)
Age of menopause (years)	50.38 (SD 3.87)
Age of onset of FFA (years)	58.53 (SD 9.66)
Pruritus (yes)	75.2% (76/101)
Trichodynia (yes)	18.8% (19/101)
Grade of FFA - Grade I - Grade II - Grade III - Grade IV - Grade V	 4% (4/101) 42.6% (43/101) 33.7% (34/101) 10.9% (11/101) 8.9% (9/101)
Long-lasting alopecia (>36 months) (yes)	48.5% (49/101)
Eyebrow alopecia (yes)	84.2% (85/101) - Partial 49.5% (50/101) - Total 34.7% (35/101)
Eyelash alopecia (yes)	26.2% (27/101) (all partial)
Facial papules (yes)	15.8% (16/101)
Perifollicular erythema (yes)	85.1% (86/101)
Follicular hyperkeratosis (yes)	93.1% (94/101)
Other alopecias (yes)	43.6% (44/101) - AGA 33.7% (34/101) - LPP 7.9% (8/101) - AA 1% (1/101) - TC 1% (1/101)

AGA: androgenetic alopecia; LPP: lichen planopilaris; AA: alopecia areata; TA: tractional alopecia; SD: standard deviation

The mean FFA-QLI score in patients was 17.11 (SD 9.37) (ranging from 3 to 44), whereas in control subjects it was 0.98 (SD 1.31) (ranging from 0 to 5) ($p < 0.001$). The mean DLQI score in patients was 3.42 (SD 3.65), compared to 0.50 (SD 0.99) in control individuals ($p < 0.001$). The mean HADS-A score in patients was 7.91 (SD 4.02) while in control participants it was 5.65 (SD 3.37) ($p = 0.002$). Finally, the mean HADS-D score in patients was 4.6 (SD 3.39), compared to 4.25 (SD 3.24) in control individuals ($p = 0.57$). Regarding FFA-QLI in control individuals, only a few items showed a low grade alteration in some of them, and they were those regarding the presence of pruritus (item 4), and less frequently, the ones regarding loss of self-esteem (item 2), worrying about eyebrow (item 5) or hair loss (item 6) and use of eyebrow make-up (item 19).

After the interview with the small group of 25 patients and the refinement of the 20 items of the questionnaire, a final questionnaire was used for all the participants. The value for internal consistency (Cronbach α) was 0.911 in the overall cohort, 0.865 in patients with FFA and 0.324 in healthy individuals. The intraclass correlation coefficient (ICC) between all the items in the questionnaire was 0.912 in the overall cohort, 0.870 in patients with FFA and 0.324 in healthy individuals. Regarding convergent validity, FFA-QLI was positively correlated with DLQI ($r=0.729$, $p<0.001$) and HADS ($r=0.361$, $p<0.001$) in patients with FFA (Table 2).

Table 2. Convergent validity between FFA-QLI, DLQI and HADS.

	Pearson correlation coefficient				
	FFA-QLI	DLQI	HADS-total	HADS-A	HADS-D
FFA-QLI	-	0.729*	0.361*	0.360*	0.262*
DLQI	-	-	0.286*	0.266*	0.230*
HADS-total	-	-	-	0.893*	0.846*
HADS-A	-	-	-	-	0.516*

*Pearson correlation coefficient, expressed as r value is showed. Statistically significant values ($p \leq 0.05$) are marked.

According to Vañó et al. classification, 46.5% (47/101) had a mild disease and 53.5% (54/101) had a severe disease. Patients with a severe disease showed higher values in FFA-QLI (14.11 vs 19.72, p=0.002) and DLQI (2.47 vs 4.24, p=0.011), without differences in HADS scores. Moreover, it was observed that FFA-QLI had a higher discriminative power to select severe FFA patients (area under curve = 0.704, p < 0.001) than DLQI (area under curve = 0.603, p=0.076) (Fig. 1).

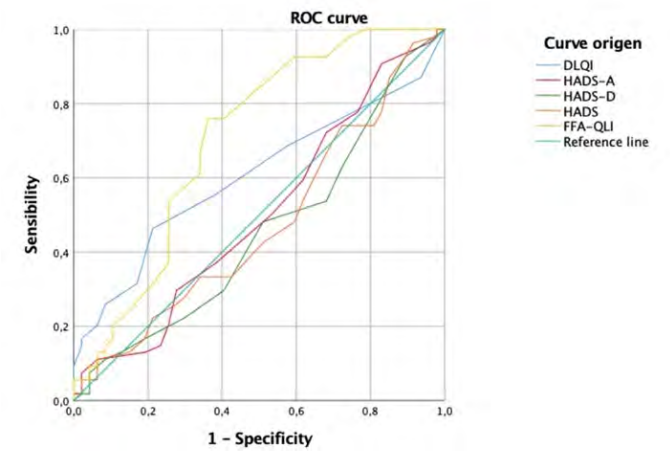

Figure 1. Receiver operating characteristic (ROC) curve to assess discriminative power differences between scales to select severe FFA. FFA-QLI showed higher discriminative power to discriminate patients with severe FFA, compared to all the other questionnaires, DLQI, HADS, HADS-A and HADS-D.

To select patients with mild, moderate and severe QoL impairment, cut-off points were delimited by comparing the values for FFA-QLI and DLQI. The DLQI was used because it yielded the best correlation values. ROC curve analysis showed that values lower than 21 in FFA-QLI correspond to patients with low QoL impairment, with a sensitivity of 88.9% and a specificity of 80.7% (area under the curve= 0.942, p<0.001) (Fig. 2a). To select patients with greater QoL disorder, it was proposed that the cut-off point was 35, with a sensitivity of 75% and a specificity of 95.9% (area under the curve= 0.961, p=0.002) (Fig. 2b). The separation between the categories mild and moderate was proposed at a cut-off point of 21, with a sensitivity of 85.7% and specificity of 80.7% (area under the curve=0.929, p<0.001) (Fig. 2c).

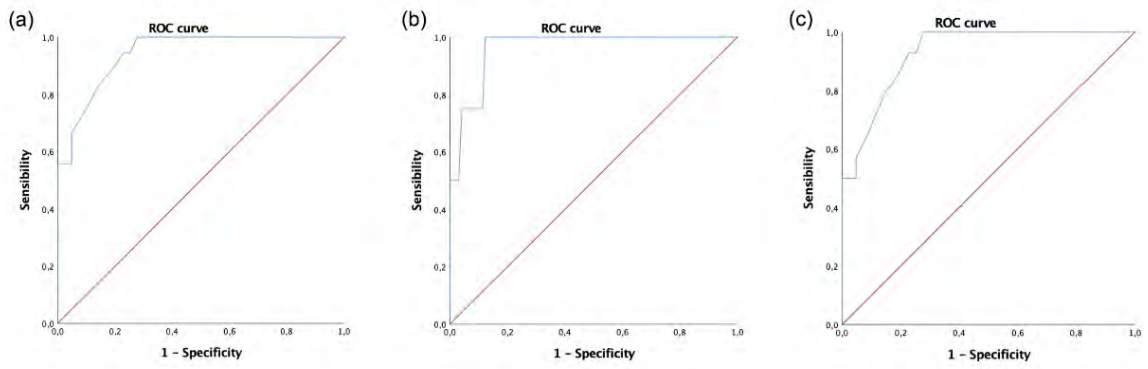

Figure 2. Receiver operating characteristic (ROC) curve to select cut-off points in Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI) to split patients in **a)** mild, **b)** severe or **c)** moderately impaired quality of life.

In the current sample, 68.3% (69/101) of patients had a mild impairment of their QoL (FFA-QLI <21), 24.8% (25/101) had a moderate impairment (FFA-QLI 21-35) and 6.9% (7/101) had a severe impairment (FFA-QLI >35). However, regarding DLQI, 36.6% (37/101) of patients showed no QoL impairment, whereas 45.5% (46/101), 13.9% (14/101), 3% (3/101) and 1% (1/101) had mild, moderate, severe and very severe impairment of their QoL. In relation to HADS-A, 48.5% (49/101) were non-cases, 28.7% (29/101) were probable cases and 22.8% (23/101) were definite cases; while about HADS-D, 82.2 (83/101) were non-cases, 11.9% (12/101) were probable cases and 5.9% (6/101) were definite cases. Comparing items of FFA-QLI in both groups of severity, patients with a more severe disease had significantly higher mean scores in some items, specifically those regarding worrying about eyebrow (item 5, $p = 0.022$) and hair loss (item 6, $p = 0.05$), using a different hairstyle to cover the alopecia (item 16, $p = 0.001$), capacity to forget the alopecia (item 18, $p = 0.005$), use of eyebrow make-up or having the eyebrows micropigmented (item 19, $p = 0.003$) and use of wigs or any type of hair system (item 20, $p = 0.032$).

The test-retest reliability was tested in 30 patients with FFA. The mean FFA-QLI was similar in the first and the second test (17.48 vs 16.86). The time between the test and retest was 30 days. The test-retest reliability was high, showing an ICC of 0.989. The correlation between items in the two tests was also high. Moreover, Cohen's kappa was >0.5 ($p<0.001$) between each item in the first and second questionnaire, showing at least an acceptable strength of agreement.

No differences in FFA-QLI scores were noted between patients who had pruritus or trichodynia and those who did not have it, or about the age of patients. Regarding age of onset of the

alopecia, the mean score FFA-QLI was higher in those with an earlier debut (<58 years) compared to those with later age of onset (>58 years) (mean scores 18.9 vs 15.28, respectively, p = 0.052). Patients with a long-lasting alopecia (>36 months) had higher FFA-QLI scores (mean scores 21.12 vs 13.33, p = 0.001); FFA-QLI had a higher discriminative power to select patients with long-lasting alopecia (area under curve = 0.739, p < 0.001) compared to DLQI (area under curve = 0.696, p=0.001) (Fig. 3a). With regards to extrascalp involvement, patients with facial papules had higher FFA-QLI scores (mean 21.5 vs 16.28, p = 0.04); although the FFA-QLI did not have power enough to select patients with facial papules (area under curve = 0.629, p = 0.104), and neither did the DLQI (area under curve = 0.557, p = 0.468) (Fig. 3b).

Patients with eyebrow alopecia had higher FFA-QLI scores (mean value 18.29 vs 10.81, p < 0.001); FFA-QLI had a higher discriminative power to select patients with eyebrow alopecia (area under curve = 0.766, p = 0.001) compared to DLQI (area under curve = 0.496, p=0.963) (Fig. 3c). No significant differences were found in FFA-QLI scores in patients regarding eyelash alopecia (19.26 in patients with eyelash alopecia vs 16.32 without eyelash alopecia, p = 0.165), or in relation to the presence of other types of alopecia (18.57 with other alopecias vs 15.98 without other alopecias, p = 0.170). Regarding their social life, no differences were noted in FFA-QLI scores regarding the occupational status (active vs inactive), civil status (living alone vs married) and having children (yes vs no).

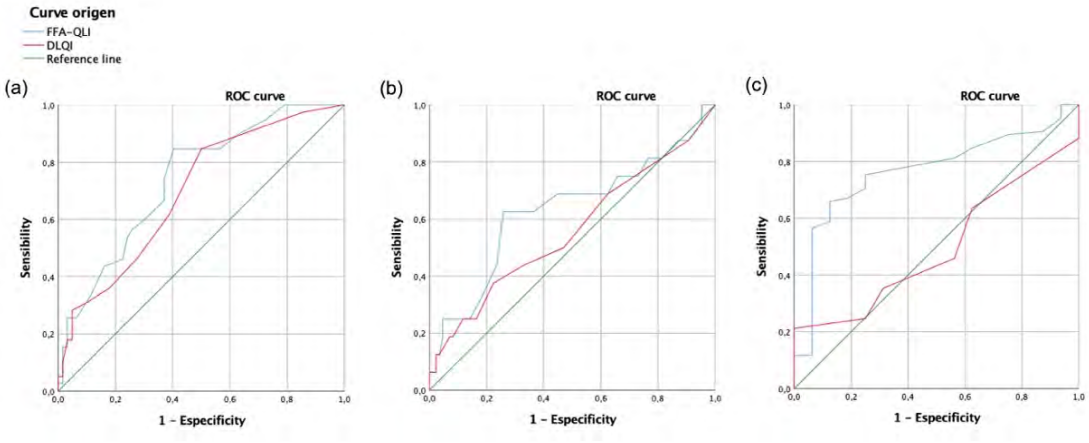

Figure 3. Receiver operating characteristic (ROC) curve to assess discriminative power differences between scales to select long-lasting cases of FFA **(a)**, patients with facial papules **(b)** and patients with eyebrow alopecia **(c)**. FFA-QLI showed higher discriminative power than DLQI to discriminate patients with long-lasting FFA **(a)** and eyebrow alopecia **(c)**. However, although FFA-QLI has more power to select patients with facial papules than the DLQI **(b)**, it does not have enough power to do it.

DISCUSSION

In this study, a specific and validated questionnaire to assess QoL in patients with FFA is proposed, called FFA-QLI. It has demonstrated a higher capacity than DLQI to select patients with severe FFA and also with long-lasting FFA and eyebrow alopecia, features which were related to a worse FFA-QLI score in FFA patients.

Around a third of patients with FFA showed at least a moderate impairment of their QoL after evaluation with FFA-QLI. Scalp hair plays a major role in determining physical attractiveness and is a relevant feature in interpersonal contact. Women's QoL is usually more affected than men's,[4] and this may be related to the fact that male alopecia is socially more acceptable, and even considered normal, than alopecia in women. Regarding scarring alopecias, one study found that patients with primary cicatricial alopecia experienced significant psychological distress and impaired QoL, both of which were associated with key beliefs about illness.[13] In addition, in lichen planopilaris (LPP), higher disease activity was correlated with depression. Illness perceptions and age were correlated with Dermatology Life Quality Index (DLQI) scores.[4,13]

Only three reports regarding assessment of QoL in specifically FFA patients have been published. Saceda-Corralo et al study included 82 FFA patients and used the DLQI, the HADS and the Revised Illness Perception Questionnaire (IPQ-R).[14] They found a negative association between QoL and FFA, without association between QoL and the alopecia severity. Other findings were that older patients had worse scores in HADS – this means being more likely to have anxiety or depression -, that patients with severe alopecia appeared to feel powerless to control their illness, and that trichodynia was related to impaired QoL. Valesky et al study included 12 patients with FFA, and found that QoL of their patients was good (but not excellent) and found no significant correlation between QoL and duration of disease or maximal hairline regression.[15] They concluded that there might be a slightly negative correlation between FFA and QoL. On the other hand, during the validation of the Frontal Fibrosing Alopecia Severity Score (FFASS), Saceda-Corralo et al, found no correlation between the severity index and QoL.[21] In the third article, Doche et al compared the disease activity in LPP (n = 10) and FFA (n = 27) using the Lichen Planopilaris Activity Index (LPPAI) with the score in DLQI, and found no significant association between them in both groups.[18] However, they

noted that patients with LPP and FFA with at least one associated non-scalp lesion, tended to have a higher DLQI score and, consequently, a poor QoL.

All the questionnaires used in these studies were unspecific, as the DLQI is used for different types of skin conditions and HADS is even more general. FFA is nowadays the most common type of scarring alopecia,[3] which has very characteristic features, such as eyebrow alopecia, facial papules and the typical scarring alopecic band, which may specifically affect the QoL of patients. Therefore, to get an accurate assessment of QoL impairment in patients with FFA, a specific questionnaire is necessary. The proposed FFA-QLI showed a close correlation with DLQI and a poor correlation with HADS, meaning that FFA-QLI is more valuable in assessing the global impairment in QoL than evaluating anxiety or depression. Moreover, the discriminative power to assess FFA severity was even higher than with DLQI.

Most of our patients had mild alteration in their QoL, similar to previous reports.[15] However, more than a third of patients showed moderate or severe detriment in their QoL; the higher frequency compared to previous reports may be due to the use of a more specific questionnaire. Furthermore, FFA-QLI scores were higher in patients with severe disease, and FFA-QLI demonstrated a high power to select FFA patients with severe disease, something which DLQI did not show according to previous studies.[14,15] Patients with a more severe disease were more worried about eyebrow and hair loss and about covering the hair defects with make-up, micropigmentation or wigs, and they felt that the disease was more present in their thoughts during the day. The presence of symptoms was not associated with higher total scores in FFA-QLI, neither was the age of patients. Nevertheless, patients with a longer duration of their alopecia showed higher scores in FFA-QLI, which may be due to a greater accumulation of psychological tiredness. Patients with facial papules and with eyebrow alopecia had a greater FFA-QLI score, in accordance with previous reports which found a poor QoL in patients with at least one associated non-scalp lesion.[18] Eyebrows are an important feature of self-image, which may explain the poorer QoL in patients with eyebrow loss and the fact that many of them usually undergo micropigmentation. Interestingly, the social environment of patients, regarding marital status, motherhood or occupational status, was not related to any differences in FFA-QLI.

Limitations to the present study include possible cultural bias related to the monocultural development of the questionnaire. Nevertheless, this is a potential limitation inherent in most questionnaire development processes.

In conclusion, a specific validated questionnaire for FFA is proposed, called FFA-QLI. It also showed a higher power to discriminate patients with a more severe disease than the DLQI. The impact of FFA on QoL could be higher than that which was previously reported using only unspecific questionnaires. This questionnaire may help dermatologists to identify patients with a greater impairment in their QoL and seek help for the patients who need it.

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Annex 1: "Alopecia Frontal Fibrosante Índice de Calidad de Vida" (AFF-ICV). Frontal Fibrosing Alopecia Quality of Life Index (FFA-QLI)

Emocional
1. ¿Siente que no es usted misma al haber perdido las cejas? 0. No. / No las he perdido. 1. Sí, un poco. 2. Sí, bastante. 3. Sí. Mucho.
2. ¿Ha perdido la confianza en usted misma? 0. No. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
3. ¿Preferiría padecer un problema de salud interno en lugar de la alopecia? (más importante pero no visible, como diabetes, hipertensión,…) 0. No. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
4. ¿Tiene picor o dolor en el cuero cabelludo? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
5. ¿Le preocupa la pérdida de las cejas? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
6. ¿Le preocupa la pérdida de cabello? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
7. ¿Piensa que la alopecia altera su imagen corporal? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
Social
8. ¿Piensa que su alopecia interfiere en su vida laboral? 0. No, nada. / No, ya no trabajo. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
9. ¿Ha dejado de ir a la peluquería o disminuido la frecuencia por la alopecia? 0. No. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
10. ¿Siente que la gente la mira debido a su alopecia? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.

11. ¿Ha abandonado actividades que antes realizaba (ocio, deporte,…) por la alopecia? 0. No, nada. 1. Sí, un poco. 2. Si, bastante. 3. Sí, mucho.
12. ¿Siente que la alopecia interfiere en su vida sexual? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
13. ¿Siente que la alopecia interfiere en su relación de pareja? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
14. ¿Siente que la alopecia interfiere en su relación con sus amistades? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
Funcional
15. ¿Pierde tiempo intentando ocultar la alopecia? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
16. ¿Se peina diferente para intentar ocultar la alopecia? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
17. ¿Ha modificado la frecuencia de peinado o lavado de cabello por miedo a incrementar la caída? 0. No, nada. 1. Sí, un poco. 2. Sí, bastante. 3. Sí, mucho.
18. ¿Consigue en algún momento olvidar su alopecia? 0. Sí, muchas veces. 1. Si, bastantes veces. 2. Sí, pero pocas veces. 3. No, en ninguno.
19. ¿Se pinta las cejas para ocultar el defecto o se las ha tatuado? 0. No. / No, no he perdido las cejas. 1. Sí, me las pinto ocasionalmente. 2. Sí, me las pinto bastantes veces. 3. Sí, me las pinto a diario. / Sí, las tengo tatuadas.
20. ¿Utiliza algún tipo de prótesis capilar para disimular su alopecia (peluca, sistema capilar o similar)? 0. No, nunca. 1. Sí, ocasionalmente. 2. Sí, a menudo. 3. Sí, siempre.
Puntuación: afectación leve (<21), afectación moderada (21-35), afectación severa (>35)

Annex 2: English version (non-validated) of the FFA-QLI

Emotional
1. Do you feel like you are not the same person because of having lost your eyebrows? 0. No. / I have not lost my eyebrows. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
2. Have you lost your self-confidence? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
3. Would you prefer to suffer from an internal disease, more serious but not visible, instead of alopecia? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
4. Do you feel itchy or pain in your scalp? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
5. Are you worried about eyebrow loss? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
6. Are you worried about scalp hair loss? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
7. Do you think that your alopecia alters your body image? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
Social
8. Do you feel that your look interferes with your work? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
9. Have you stopped going to the hairdresser's or reduced the frequency you go there because of the alopecia? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
10. Do you feel that people look at you (stare at you) because of your alopecia? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.

11. Have you given up activities that you used to do because of your alopecia (leisure, sport,…)? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
12. Do you feel that your alopecia interferes with your sex life? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
13. Do you feel that your alopecia interferes with your relationship with your partner? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
14. Do you feel that your alopecia interferes with your relationship with your friends? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
Functional
15. Do you waste time trying to hide your alopecia (eyebrows or scalp)? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
16. Do you comb your hair differently than usual to hide your alopecia? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
17. Have you changed the frequency of combing or washing your hair because of fear of increasing your hair loss? 0. Not at all. 1. Yes, a little. 2. Yes, a lot. 3. Yes, very much.
18. Do you forget about your alopecia at any moment? 0. Yes, a lot of the time. 1. Yes, quite often. 2. Yes, but rarely. 3. No, never.
19. Do you make-up your eyebrows to hide the alopecia or have them tattooed? 0. No. / No, I have not lost my eyebrows. 1. Yes, I use make-up on them occasionally. 2. Yes, I use make-up on them quite often. 3. Yes, I use make-up on them everyday. / Yes, I have eyebrow tattoos to cover the eyebrow alopecia.
20. Do you use any type of wig or capilar prothesis to hide your alopecia? 0. No, never. 1. Yes, ocassionaly. 2. Yes, quite often. 3. Yes, always.
Score: mild impairment (<21), moderate impairment (21-35), severe impairment (>35)

6.2. Histopathological results.

Frontal fibrosing alopecia: a histopathological comparison of the hairline implantation with normal-appearing scalp.

Short title: Histopathological features of normal-scalp in frontal fibrosing alopecia.

María Librada Porriño-Bustamante,[1,2] Fernando Javier Pinedo-Moraleda,[3] Ángel Fernández-Flores,[4] Trinidad Montero-Vílchez,[5] María Antonia Fernández-Pugnaire,[6] Salvador Arias-Santiago.[5,7]

1. Dermatology Department. University Hospital La Zarzuela, Madrid, Spain.

2. University of Granada, Granada, Spain.

3. Pathology Department. University Hospital Fundación Alcorcón, Alcorcón, Madrid, Spain

4. Pathology Department. University Hospital El Bierzo, Fuentesnuevas, León, Spain

5. Dermatology Department, University Hospital Virgen de las Nieves, Granada, Spain

6. Dermatology Department. University Hospital San Cecilio, Granada, Spain

7. School of Medicine, Institute of Biosanitary Investigation ibs, Granada University, Granada, Spain

Corresponding author: María Librada Porriño Bustamante. Email: mporrinobustamante@gmail.com

University Hospital La Zarzuela. Calle de Pleyades, 25, 28023, Madrid, Spain.

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ABSTRACT

Frontal fibrosing alopecia is characterised by the presence of a lymphocytic inflammatory infiltrate around the upper follicle and by perifollicular fibrosis, which results in the destruction of the hair follicle. Recent reports have also found the presence of those findings in clinically unaffected areas. The aim of this report is to perform a deeper analysis of the histopathological features of this apparently unaffected scalp. A cross-sectional study including 52 women with frontal fibrosing alopecia was performed. Two areas were biopsied: the hairline implantation and a normal-appearing scalp area. Sebaceous glands were reduced/absent in 80.8% of the hairline implantation samples compared to 42.3% of the "healthy scalp" samples (p=0.001). Inflammatory infiltrate was observed in 92.3% of patients in the hairline implantation and in 86.5% of them in the "healthy scalp" area (p=0.508), although the severity was higher in the former (p=0.013). Follicular epithelium changes were seen in 70.6% of the hairline implantation biopsies compared to 48.1% of the "healthy scalp" biopsies (p=0.012). Fibrous tissular changes were noted in 80.8% and 53.8% of the hairline implantation and "healthy scalp" biopsies, respectively (p=0.003). In conclusion, the histopathological features of frontal fibrosing alopecia are shared by both affected and clinically unaffected areas.

SIGNIFICANCE: Patients with frontal fibrosing alopecia also have histopathologic evidence of disease in the clinically unaffected scalp, in which the same alterations as in the hairline implantation area can be noted. However, the histopathological anomalies in the normal-appearing scalp were less common or with a lower intensity. The reason why the hairline develops scarring alopecia and the parietal area remains spared remains unknown.

KEYWORDS: frontal fibrosing alopecia, scarring alopecia, histopathology

INTRODUCTION

Frontal fibrosing alopecia (FFA) is a lymphocytic scarring alopecia which has become the most common type of cicatricial alopecia (1). Clinically, it is characterized by progressive frontal or temporoparietal, or even occipital, hairline recession, leading an alopecic cicatricial band (2, 3).

The loss of the immune privilege of the hair follicle is considered the starting point in the development of cicatricial alopecias (4, 5). After the exposure of the hair follicle to immune attacks, a cytotoxic T cell autoimmune reaction - induced by an unknown trigger- against the infundibular and isthmic regions, could lead to the damage of the stem cells of the bulge area and then to the irreversible destruction of the hair follicle. Histopathological typical features of FFA include a lichenoid lymphocytic infiltrate around the upper follicle, that is isthmus and infundibulum, as well as concentric perifollicular lamellar fibrosis (6, 7). Deep follicular involvement is less common but possibly to be found in FFA samples, and the interfollicular epidermis is usually spared (7-9).

Dermatologists can assess by the “naked-eye” and trichoscopy the “clinically affected area”, evaluating the size of the scarring area and the presence or absence of inflammatory trichoscopic signs. However, sonographic and histopathological abnormalities have been found in “normal-appearing” areas in FFA patients, suggesting that subclinical inflammation may exist (10-14). The presence of the inflammatory infiltrate, sebaceous gland atrophy and perifollicular fibrosis were the histopathological features found in the normal-appearing scalp.

The aim of this study was to make a broad comparison of the histopathological features of both clinically affected and “normal-appearing” areas in FFA. Up to now, this is the largest study of its kind.

MATERIAL AND METHODS

A cross-sectional study was performed in the University Hospital San Cecilio in Granada (Spain). Inclusion criteria were as follows: women, with clinical and trichoscopic signs of FFA, that is, hairline recession which leads to a scarring band without follicular openings, with or without perifollicular erythema and follicular hyperkeratosis. Exclusion criterion was the male sex. All patients signed an informed consent and the study was approved by the Local Ethical Committee.

Demographic and clinical information were recorded, including age, age of onset of the alopecia, age of menopause, grade of the alopecia – following the 5 grades' classification described by Vañó et al (2), the duration of the alopecia, the presence of symptoms – pruritus, trichodynia -, the presence of facial papules and peripheral alopecia. Current treatment with corticosteroids was also registered. Moreover, the presence of trichoscopic signs, such as perifollicular erythema and follicular hyperkeratosis, was also recorded. A handheld dermoscope DermLite II pro HR was used.

Two dermoscopy-guided 4-mm punch scalp biopsies were performed on all patients. The first one was done in the frontal hairline (B1), in the most clinically affected area. The second one was done in a "normal-appearing" parietal area (B2), based on the absence of trichoscopic signs of FFA.

All specimens were divided and analysed between two dermatopathologists, and then processed and stained with hematoxylin and eosin and cut in vertical sections. Orcein stain was used to assess elastic fibres.

SPSS software (SPSS 25.0, SPSS Inc., Chicago, IL, USA) was used for data analyses. Student's t-test was applied to compare mean values of quantitative variables. Qualitative histologic variables between both areas were analysed with McNemar test. The other qualitative variables were analysed with $\chi 2$ test, or the two-tailed Fisher exact test with 2 x 2 contingency tables in case of small samples. Differences were considered significant at $p \leq 0.05$.

RESULTS

A total of 52 women with FFA were included, which implied 104 scalp biopsies. The mean distance between both areas was 4 cm (1.4-17.6 cm, SD 3.26).

General characteristics of the patients are described in table 1. With regards to current treatment for FFA, 26.9% (14/52) were not taking any, and only 36.5% (19/52) were using topical corticosteroids at the moment of the evaluation (twice a week) and 3.8% (2/52) were receiving intralesional corticosteroids injections every 3 months in addition to the topical therapy.

Table 1. Characteristics of FFA patients.

n: 52	**Mean**
Age (years)	63.04 (SD 9.69)
Age of menopause (years)	50.5 (SD 3.61)
Age of onset of the alopecia (years)	58.42 (SD 10.17)
Duration of the alopecia (months)	55.58 (SD 37.63)
	Frequencies
Menopause	88.5% (46/52)
Pruritus	71.2% (37/52)
Trichodynia	23.1% (12/52)
Occipital involvement	9.6% (5/52)
Eyebrow alopecia	86.5% (45/52)
Facial papules	21.2% (11/52)
FFA severity grade: - Grade I - Grade II - Grade III - Grade IV - Grade V	 5.8% (3/52) 38.5% (20/52) 36.5% (19/52) 11.5% (6/52) 7.7% (4/52)
Perifollicular erythema	84.6% (44/52)
Follicular hyperkeratosis	88.5% (46/52)

The hair count in the two scalp areas is described in table 2. No differences were found regarding the presence of anagen, catagen or telogen hairs in B1 compared to B2. Regarding the involvement of other skin annexes, rather than hair follicles (sebaceous glands and erector pili muscle), it was found in 86.5% (45/52) of B1 biopsies compared to 50% (26/52) of B2 biopsies ($p < 0.001$). In B1, sebaceous glands were normal in 19.2% (10/52), reduced in 30.8% (16/52) and absent in 50% (26/52), whereas in B2, normal sebaceous glands were observed in

57.7% (30/52), reduced in 15.4% (8/52) and absent in 26.9% (14/52) (p = 0.001). In relation to the erector pili muscle, in B1 it was normal in 76.9% (40/52) and reduced in 23.1% (12/52), while in B2 it was normal in 86.5% (45/52), reduced in 11.5% (6/52) and absent in 1.9% (1/52) (p = 0.227). Spared hair follicles were noted in 75% (39/52) of B1 samples compared to 92.3% (48/52) of B2 biopsies (p = 0.035).

Table 2. Follicular count in both areas, hairline implantation (B1) and healthy scalp (B2)

N52	**Mean (SD)**		
Follicular count	**Hairline implantation – B1**	**Healthy scalp – B2**	**P value**
Total	5.10 (2.77)	7.79 (3.87)	<0.001
Terminal hairs	4.25 (2.59)	6.58 (3.48)	<0.001
Intermediate hairs	0.38 (0.91)	0.50 (1.16)	0.479
Vellus hairs	0.46 (0.85)	0.71 (0.98)	0.166
Anagen hairs	4.67 (2.90)	7.35 (3.80)	<0.001
Telogen hairs	0.44 (0.67)	0.44 (0.78)	1.000

The characteristics of the inflammatory infiltrate were presented in table 3. All types of hair follicles – terminal, intermediate and vellus – were affected by the inflammatory infiltrate in both areas (figure 1a), although terminal hair follicles were the most commonly involved (71.15% - 37/52- in B1 vs 36.54% -19/52- in B2). Hair follicles were not affected by inflammatory infiltrate in 25% (13/52) of B1 samples compared to 46.2% (24/52) in B2 (p=0.027). The inflammatory infiltrate also involved hair follicles in all phases of the hair cycle in both areas, although as anagen hairs were the most frequently present, they were also the most frequently infiltrated (55.8% - 29/52 – in B1 vs 51.9% - 27/52 – in B2). Dermal involvement included, in most cases, papillary and reticular dermis (61.5% -32/52- in B1 and 38.5% -20/52- in B2) (table 3). The inflammatory infiltrate also involved the interfollicular space, mainly with a perifollicular and perivascular distribution. A lichenoid infiltrate in the interfollicular epidermis was only found in one patient in B1.

Table 3. Presence of inflammatory infiltrate and its characteristics in both areas

N52	**Hairline implantation– B1**	**Healthy scalp – B2**	**P value**
Inflammatory infiltrate (yes)	92.3% (48/52)	86.5% (45/52)	0.508
Severity - Mild - Moderate - Severe	 35.4% (17/48) 60.4% (29/48) 4.2% (2/48)	 71.1% (32/45) 26.7% (12/45) 2.2% (1/45)	0.013
Sebaceous gland involvement (yes)	13.5% (7/52)	5.8% (3/52)	0.368
Dermal involvement (yes)	88.5% (46/52)	71.2% (37/52)	0.035
Interfollicular involvement (yes)	86.5% (45/52)	71.2% (37/52)	0.057
Superficial perivascular lymphohistiocytic infiltrate	86.4% (44/52)	69.2% (36/52)	0.039

Epithelial changes are shown in table 4. Corneum stratum changes were noted as hyperkeratosis or follicular plugs, whereas epidermal changes were observed as hyperplasia in both areas, and also vacuolar changes and atrophy in B1. Follicular epithelium changes were more commonly seen than the other epithelial changes, especially in B1, and the most frequent type, in both areas, was the vacuolar change (62.7% - 32/51 – in B1 and 40.4% - 21/52- in B2) (figure 1b), followed, at a considerable distance, by lichenoid changes, cysts, spongiosis and tufted hairs. Colloid bodies in the dermoepidermal junction were found in only 5.9% (3/51) of the B1 samples.

Table 4. Epithelial changes and fibrous tissular changes

	Hairline implantation – B1	**Healthy scalp – B2**	**P value**
Epithelial changes			
Corneum stratum changes (yes)	11.5% (6/52)	24.5% (12/49)	0.180
Epidermal changes (yes)	17.3% (9/52)	6.1% (3/49)	0.07
Follicular epithelium changes (yes)	70.6% (36/51)	48.1% (25/52)	0.012
Interphase dermatitis lichenoid in the upper part of the hair follicle	23.5% (12/51)	15.4% (8/52)	0.125
Vacuolar degeneration basal layer outer root sheath	64.7% (33/51)	36.5% (19/52)	0.001
Necrosis of keratinocytes outer root sheath	43.1% (22/51)	15.4% (8/52)	0.001
Increased apoptotic activity in outer root sheath	45.1% (23/51)	19.2% (10/52)	0.001
Infundibular dilatation and infundibular hypergranulosis	13.7% (7/51)	7.7% (4/52)	0.508
Fibrous tissular changes	80.8% (42/52)	53.8% (28/52)	0.003
Perifollicular fibrosis	71.2% (37/52)	30.8% (16/52)	<0.001
Dermal fibrosis	53.8% (28/52)	36.5% (19/52)	0.122

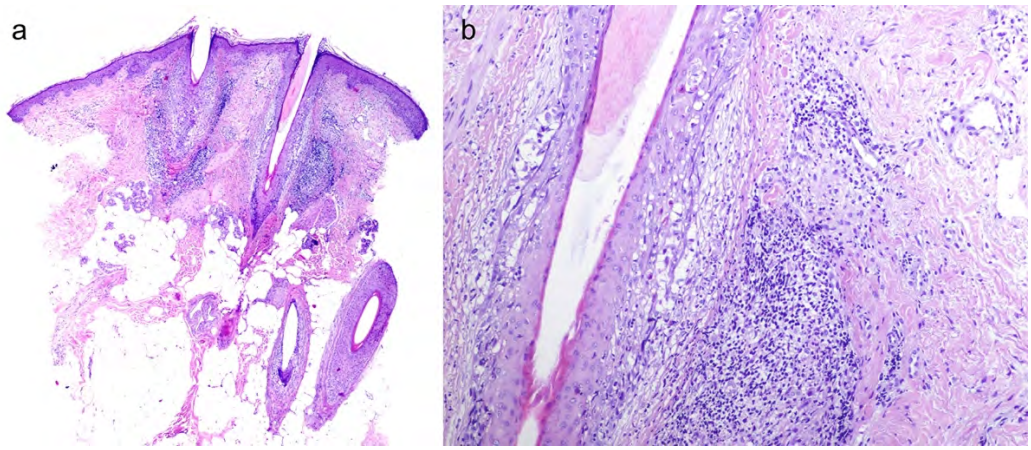

Figure 1: a) Lichenoid inflammatory infiltrate around the isthmus of the hair follicle. The infiltrate erodes and destroys the follicle epithelium (Hematoxylin-Eosin x20). **b)** The infiltrate erodes the follicle which shows vacuolization of the basal layer (Hematoxylin-Eosin x100).

Fibrous tissular changes are also presented in table 4. Terminal, intermediate and vellus hairs were affected by perifollicular fibrosis, although the former type was the most frequently involved (65.4% -34/52- in B1, and 21.2% -11/52- in B2). Dermal fibrosis was located, most commonly, in the upper dermis, and was generally noted as follicular lamellar concentric fibrosis and fibroplasia. Fibrous tracts were noted in both areas, and no significant differences were found between them. Interfollicular mucinosis was noted in only one patient in B2, whereas mild follicular mucinosis was observed in 9.6% (5/52) and 7.7% (4/52) of B1 and B2 samples, respectively. Elastic fibres pattern was generally normal (69.2% -36/52- in B1 and 67.3% -32/52- in B2), although in some they were destructed (figure 2). Foreign reaction granulomas were noted in some patients, in both B1 and B2.

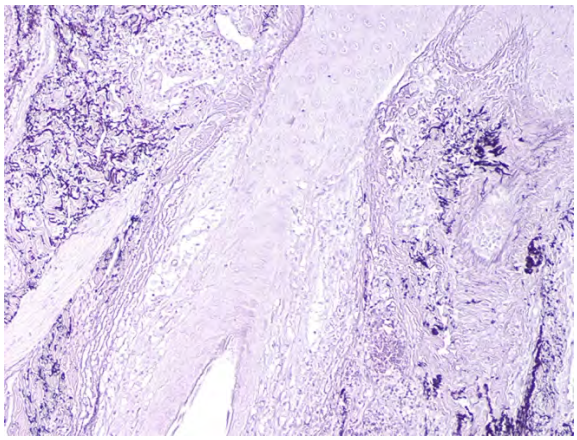

Figure 2: A histochemical stain for elastic fibres demonstrates destruction in the scarring areas around the follicles (Orcein x100).

Patients with follicular hyperkeratosis and perifollicular erythema more frequently had inflammatory infiltrate in both areas, although the difference did not reach statistical significance. No association was noted between the presence of histological inflammation and the presence of symptoms. Involvement of other skin annexes rather than hair follicles (sebaceous glands and erector pili muscle) in B1 were observed in 63.6% (7/11) of patients with facial papules compared to 92.7% (38/41) of patients without facial papules ($p = 0.029$). Vacuolar degeneration of the basal layer of the outer root sheath in B2 was seen in 53.8% (14/26) of the patients with an earlier debut of the alopecia compared to 19.2% (5/26) of patients with a later debut ($p = 0.020$). Fibrous tissular changes in B1 were more frequent in

patients with absence of vellus hair in the hairline implantation (84% -42/50-), compared to those with presence of vellus hairs in which fibrous changes were not observed (p = 0.034). Regarding the use of corticosteroids, no differences were noted in the presence of the inflammatory infiltrate in B1 or in B2, in patients who were having maintenance therapy, compared to those not using corticosteroids.

DISCUSSION

All the histopathological features described in FFA were observed in both the implantation active hairline and the "normal-appearing" scalp, although some of them were less frequent or at a lesser degree in the latter. The largest histopathological study so far about features in normal-appearing scalp in FFA included 28 patients (14).

A lower number of hair follicles would be expected as a consequence of the scarring process: Chew et al found that a mean of seven terminal hair follicles were observed per 4-mm punch biopsy in FFA patients, while around thirty terminal and five vellus hair follicles would be a common finding in healthy Caucasians (15). The mean follicular number found in the implantation hairline in our patients was lower, which may be due to a more severe alopecia among them. Interestingly, the mean follicular count found in the "healthy scalp" was also reduced compared to a truly healthy scalp, although the older age of our patients could be partly responsible for that (16). A significant reduction in total follicular count and in terminal follicular count was observed in the hairline implantation compared to the "healthy scalp".

The loss of sebaceous glands is an early finding in FFA, and their atrophy along with the inflammatory involvement of the vellus follicles may be the histologic clues in these early phases (17). Atrophic sebaceous glands have also been found in normal-appearing scalp in FFA (11). In the present report, sebaceous gland reduction or absence were noted in both areas, but this impairment was significantly more common in the hairline implantation. The erector pili muscle was less often affected.

Perifollicular lymphocytic inflammation around the isthmus/infundibulum in "healthy scalp" was found, in accordance with previous reports (11, 12, 14). Around 86% of patients showed this feature, a higher percentage than that reported by Doche et al (64.3%) (14). As stated by previous studies (13), no significant differences were found between the presence of the inflammatory infiltrate in the "healthy scalp" compared to the hairline implantation area, although there were significant differences regarding the severity of the infiltrate, which was milder in the former.

All type of follicles, in all phases of the hair cycle, were affected in the same way by the inflammatory infiltrate in both areas, in accordance with previous reports (7), although terminal and anagen hairs were the most commonly affected.

Unlike previous reports, which found that the interfollicular epidermis was usually spared by the inflammatory infiltrate (7, 8), around 86% and 71% of the patients in the current study had interfollicular involvement in the hairline implantation and the "healthy scalp", respectively, and it was mainly with a perifollicular and perivascular distribution. Vacuolar degeneration of the basal layer is another FFA sign (7). In our patients, follicular epithelium changes were significantly more frequent in the hairline implantation and the main type was the vacuolar degeneration. Keratinocyte necrosis in the external root sheath is also a prominent feature in FFA, especially at the isthmus (18). This finding was significantly more frequent in the hairline implantation, where an increased apoptotic activity, in the outer root sheath, was also seen more often.

Doche et al found perifollicular fibrosis in normal-appearing areas in FFA in almost 18% of patients (14). In the present study, fibrous tissular changes were commonly seen in both areas, but significantly more often in the hairline implantation. Loss of elastin fibres has also been described as a finding associated with the fibrosis (8), although in our patients, the elastic fibres pattern was generally normal. Doche et al found mucin deposits in the affected hairline in 35.7% of patients and in 7.1% in the "healthy scalp" (14), a higher frequency than that found in the current study, in the hairline implantation, and a similar frequency, in the "healthy scalp".

The presence of inflammatory infiltrate in both areas tended to be more frequent in patients with trichoscopic inflammatory signs, but it did not reach statistical significance. This could be explained by the low number of patients without trichoscopic inflammatory signs or because the association between both may not be real. Involvement of sebaceous glands/erector pili muscle in the hairline implantation was significantly less prevalent in patients with facial papules. One explanation could be that facial papules may appear early in the course of the disease, as was proposed by some authors (19), maybe even earlier than the alteration of these skin annexes appears. Moreover, patients who maintained vellus hairs in the hairline implantation had no fibrous tissular changes in this area, which is compatible with the clinical observation that vellus hair may be clinically present in incipient FFA cases (20).

Although the involvement of "normal-appearing scalp" and the presence of peripheral alopecia may suggest that FFA is a generalized inflammatory process, a recent study has found that FFA has scalp immunity and fibrosis dysregulation but without systemic involvement (21).

The reason why this normal-appearing scalp, with histopathological evidence of the disease, does not develop alopecia like the hairline implantation does, is still unknown; the hairline implantation may be more susceptible to the hair loss than the parietal area.

The limitations of this study included the cross-sectional design, without a follow up of the histopathological changes, and the fact that some patients were receiving treatment with topical corticosteroids. However, they were using them as a low-frequency maintenance therapy, and no differences were found regarding the presence of inflammatory infiltrate in patients who were using corticosteroids compared to the non-users.

In conclusion, all of the histopathological features of FFA were also present in the supposedly healthy scalp. Further studies are needed to clarify why these areas do not develop alopecia despite having the same histopathological features as the affected hairline.

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DISCUSSION

8. DISCUSSION.

The incidence of FFA has been increasing over the last two decades and it has become one of the most common types of scarring alopecia. However, pathophysiology and trigger factors of FFA have not yet been fully understood. A better characterization of FFA could help dermatologists to achieve a closer understanding and a proper management of this type of alopecia.

8.1. Clinical data.

8.1.1. Familial frontal fibrosing alopecia. Genetic background.

Family history of FFA is reported by 5 to 8% of patients with FFA, [3,5] similar to the prevalence found in the present cohort (6.93% - 7/101). The most affected family members are usually women (88%), and the most common familial connection between them is being sisters (56%), followed by mother and daughter (32%) and brother and sister (8%).[124] Familial patients in this cohort were almost all sisters, including the extra 13 cases included for the familial study.[125] When affected patients are mother and daughter, mothers are usually postmenopausal at the time of diagnosis and attend the consultation at an advanced stage of the alopecia, while daughters are usually premenopausal and have an early stage of the alopecia when asking for help.[55] This behaviour was also observed in the current sample of patients. Clinical and dermoscopic features in the current familial cohort were similar to those found in non-familial cases of FFA, as reported in other studies.

Most of the familial cases are reported in women,[55,124,126-129] which seems logical due to the higher prevalence of FFA in females. The cases of FFA reported in men (familial and non-familial) are scarce compared to those reported in women, however they have been increasing over the last few years.[18,130] Although the prevalence of FFA in men is clearly lower, it is probably an underdiagnosed alopecia, because of the misdiagnosis with AGA and a lower attendance at consultation by male patients. Some of them end up accepting the alopecia and only consult dermatologists because of eyebrow, sideburn or beard alopecia.[20,67,130,131] Eyebrow and beard alopecia may be helpful in differentiating FFA from AGA in male patients, since the second one does not affect these areas. Only two cases of familial FFA had been published in men (two reports of brother and sister).[127,132] Two brothers (not included in the

101 cohort) with FFA were recruited for the present study. They attended the consultation because of eyebrow alopecia. Interestingly, one of the brothers had a primary panhypopituitarism because of a surgical excision of a follicle-stimulating hormone-secreting pituitary macroadenoma. This imbalance in sex hormone levels could have played a role in the development of FFA, similar to what happens in postmenopausal women. Indeed, this brother was the youngest one and developed the alopecia earlier than the other.

The study of HLA profile in familial cases of FFA had only been performed in isolated reports with a small number of patients,[128,133,134] until the publication in 2019 of a GWAS.[56] It included a United Kingdom cohort (844 patients and 3760 control subjects) and a Spanish cohort (172 patients and 385 control subjects), and found a significant association with FFA at four genomic loci: 2p22.2, 6p21.1, 8q24.22 and 15q2.1. The strongest effect on FFA susceptibility was observed at 6p21.1, which is located within the Major Histocompatibility Complex (MHC) region. Fine-mapping at this locus indicated that the association was driven by the HLA-B*07:02 allele, which conferred a five-fold increase in risk of FFA. A lead variant at 8q24.22 was located in intron 1 of ST3GAL1, which encodes a membrane bound sialyltransferase, which could produce a T cell dysfunction. The authors also implicated at 2p22.1, a putative causal missense variant in CYP1B1, encoding the Cytochrome P450 1B1 microsomal enzyme, which contributes to xenobiotics and sex hormones metabolism and is also implicated in human immune cell regulation.

The same year, our HLA study, that included 13 patients with FFA and the genetic profiles of 636 control subjects, was published.[135] Most of these patients (61.5% - 8/13 -) shared the haplotype HLA-A*33:01; B*14:02; C*08:02, compared to 3.3% of the control group individuals, suggesting this may predispose them to familial FFA. Furthermore, these 8 patients were heterozygous to CYP21A2 gene p.V282L mutation - this mutation in homozygosis is responsible for the nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency-. The link of this haplotype with this mutation is interesting, and mapping the genomic fragment between HLA-A33*01 allele to the CYP21A2 gene (in the MHC class III region) could help to know more about the susceptibility to familial FFA. In addition, four patients, who did not share the previously referred haplotype, shared HLA-B*07:02, the allele which was found to be associated with FFA in the GWAS. This allele had already been

described as being associated with familial LPP.[136] These findings support the hypothesis of an antigen-driven disease in these susceptible patients, also suggested in the GWAS.

Another study about FFA and HLA was developed in Brazil and included a large familial cluster (6 sisters and 1 daughter) with FFA. They lived in different homes and some resided in different cities. It also included unaffected brothers/sisters and seven sporadic FFA cases.[57] The authors found two different susceptibility haplotypes, HLA-C*17:01:01:02/B*42:01:01:01 and HLA-C*07:02:01:03/B*07:02:01:01; the first one was newly described in FFA, but the second one has been already observed in FFA patients in the GWAS and our familial cohort.[56,135] Some unaffected participants shared one of these haplotypes, indicating that other genetic or environmental factors may modulate the HLA association. Furthermore, 5 out of 7 sporadic cases also shared one of those haplotypes (43% the first one, 29% the second one); and one of the remaining two sporadic cases presented HLA-A*33:01:01; B*14:02:01; C*08:02:01, like the majority of our cohort.[135] Finally, they also observed that two sporadic cases carrying HLA-C*17:01:01:02/B*42:01:01:01 also presented a null HLA-G allele, G*01:05N, which do not produce full-length membrane-bound HLA-G molecules because of a premature stop-codon, which could be associated with FFA.

A common environmental trigger is probably partly responsible for the development of FFA in familial (and nonfamilial) cases, as some family members do live close by.[19,125,127] Additionally, a genetically unrelated married couple, who both had FFA, has been published.[131] However, the recent HLA publications suggest that a genetic susceptibility linked to HLA haplotypes could play an important role in the pathogenesis of FFA. The disease may be a complex immune-inflammatory condition supported by risk alleles in MHC Class I molecule-mediated antigen processing and T cell homeostasis and function. The exposure of vulnerable people to an unidentified exogenous trigger may produce an abnormal response against normally hidden bulge follicular autoantigens, because of a cross-reaction between the external antigens and the follicular ones, leading to follicular destruction. The combination of the genetic charge of susceptibility, which may be higher or lower, and the exposure to the unknown trigger, which may also be higher or lower, could lead to the development of the disease.

8.1.2. Comorbidities in frontal fibrosing alopecia. Association with rosacea.

FFA has been described concomitantly with different conditions. AGA was observed in 40% of women and 67% of men with FFA in a cohort of 355 patients.[3] The prevalence of AGA in our cohort of patients was 30.3%.[137] The association with other autoimmune diseases has been found in up to 30% of FFA patients, the most frequent one being hypothyroidism (23%).[11] However, several autoimmune conditions have been described in connection with FFA.[138]

Moreover, associated dermatologic diseases are a frequent comorbidity in FFA, present in 66% of FFA patients.[47] Lichen planus, in different clinical forms, such as cutaneous, nail, oral, vulvar and conjunctival may appear along with FFA, although the most common form is LPP, and LPPigm in dark phototypes.[3,9,11,13,138,139] However, one of the commonest dermatosis in FFA seems to be rosacea (34%).[47]

On the other hand, patients with FFA had higher significant weight and BMI than control participants. In previous reports, a higher BMI in FFA patients had already been related to a greater severity of the alopecia.[65]

Rosacea is an inflammatory chronic skin condition characterized by recurrent or persistent flares of centrofacial erythema, causing a typical fixed erythema often with telangiectasias.[140] Clinical signs of rosacea were noted in 61.6% of our patients, compared to 30% in the control group.[137] The most frequent form was the erythematotelangiectatic rosacea (88.5%), in accordance with previous reports.[47] Severe grades of alopecia were associated with a higher prevalence of rosacea. This could be one reason to explain the higher prevalence of rosacea in our cohort, since the majority of patients presented grades II (42.4%) and III (34.3%) of alopecia, representing a more severe disease than in previous series.[3]

Interestingly, after multivariate analysis, perifollicular erythema, higher BMI and lower progesterone levels, were associated with a higher risk of rosacea in patients with FFA.[137] In this way, the risk of rosacea has been demonstrated to be greater in patients with increased BMI and greater waist an hip circumference.[141] Regarding inflammatory trichoscopic signs, i.e. follicular hyperkeratosis and perifollicular erythema, only the second one was significantly associated with the presence of rosacea. A common inflammation of the pilosebaceous unit may be involved in the pathogenesis of both rosacea and FFA. Prostaglandin D2 has been reported to inhibit hair growth[142] and has also been involved in the development of rosacea.[143]

Regarding hormonal disturbances in FFA, androgen deficiency was identified in 30% of women with FFA, mainly from the adrenal gland.[41] However, hormonal levels are not consistently altered in premenopausal women diagnosed with FFA.[40] In our cohort, the only hormonal difference between patients and control individuals was the level of DHEAS, a mainly adrenal hormone, which was significantly lower in FFA patients. This is an interesting immunomodulatory hormone, also involved in the functions of PPAR, which is considered to be a negative regulator of fibrotic events and whose dysfunction in the follicular bulge has been shown, in vitro, to cause a scarring alopecia similar to LPP.[25-27,144] Therefore, a reduced activity of DHEAS may be related to the fibrogenic inflammatory process of FFA. Although the serum sex hormone levels are probably not directly implicated in the pathogenesis, a possible local hormonal mechanism cannot be discarded. After multivariate analysis, lower levels of progesterone were associated with rosacea in FFA patients. Lower level of progesterone has not been implicated in the pathogenesis of rosacea. Further studies may be necessary to clarify this finding.

8.1.3. The use of sunscreens in frontal fibrosing alopecia. Assessment of actinic damage.

Since the proposal of a possible involvement of sunscreens in the development of FFA in 2016,[60] several controversies have arisen about the actual relationship between sunscreens and FFA.[66,145-148] However, most reports have found that patients with FFA have a higher use of sunscreens than control subjects,[60-62,149,150] as was noted in the current cohort of patients. Recently, some authors have found that this greater use also happens in AGA patients;[150] therefore, the use of facial sunscreens may not be truly associated with FFA.

Actinic damage in FFA patients had not been previously assessed. In the present cohort, a higher prevalence of actinic damage in patients with FFA compared to a control group was observed, especially in the form of solar lentigines, and this was also present after realizing a logistic regression model adjusted by skin phototype. Other signs of actinic damage, such as actinic keratoses and basal cell carcinoma, were also more common in patients, but the difference did not reach statistical significance, probably because of the small size of the sample in those subgroups. The presence of greater actinic damage in patients with FFA suggests that it is unlikely that they had been using sunscreens during their lifetime, since regular sunscreen use has been shown to prevent actinic damage.[151,152] The use of sunscreen may be a new behaviour adopted following dermatologist recommendations or because of a later awareness of the appearance of their skin. No differences regarding sunscreen use were found in patients with mild grades of alopecia compared to those with severe grades, in accordance with previous reports.[65] The use of sunscreens and the presence of actinic damage were independent factors related to FFA, after conducting a logistic regression model.

Regarding trichoscopic signs, follicular hyperkeratosis and especially perifollicular erythema are considered inflammatory signs, and the latter was considered as a marker of activity in FFA.[153] Nevertheless, they are not always related to disease activity, so sometimes they can be present in patients with no progression in the hairline recession or be absent in patients with progressive hair loss.[11,12,154] No differences in the presence of those trichoscopic signs was found in FFA patients who used sunscreens compared to those who did not use them. If sunscreen use were related to the development of FFA, some differences would be expected between both groups. Similarly, if the hypothetic role of the sunscreens in the pathogenesis of FFA were true, histopathological differences would be expected when comparing

sunscreen-users with non-users. However, no differences regarding sebaceous gland involvement or the presence of inflammatory infiltrate, were noted between those groups.

In relation to the presence of peripheral alopecia, eyebrow, eyelash and limb alopecia were more common in FFA patients than in control subjects, but no differences were observed about the use of sunscreens and the presence of peripheral alopecia in FFA patients.

8.1.4. Sonography in frontal fibrosing alopecia.

High frequency US is a non-invasive image technique which may be a helpful tool in dermatology to assess different conditions, such as skin tumours, vascular lesions and inflammatory diseases (especially psoriasis and hidradenitis suppurativa).[155] The use of US in trichology is less common, but some reports exist about AGA and scarring alopecia (such as dissecting cellulitis, acne keloidalis nuchae and folliculitis decalvans).[156-158] LPP and FFA have been studied much less with US.[159,160]

Colour Doppler mode allows the estimation of blood flow in the scalp, showing higher blood flow in cases of active inflammation.[158] A study of sonography in 8 FFA patients showed hypoechoic perifollicular thickening in 62.5% of patients and increased dermal vascular flow in 40% of them, along with an increased dermal capillary circulation in the XFlow study.[160] There were no other specific reports about sonography in FFA. In our study of 99 patients, our patients had a higher vessel diameter and flow in the hairline implantation area, compared to control individuals; this finding makes sense, since this area is supposed to be the one with more active inflammation.[161] However, there were no differences between severity grades of alopecia, although patients with eyebrow alopecia showed a higher vessel flow in this area, which may also reflect the presence of inflammation in the eyebrow area. Interestingly, a higher vessel diameter was also found in the healthy scalp of patients compared to control participants, which may reveal the existence of subclinical inflammation in apparently healthy areas of the scalp, in accordance with previous studies which found perifollicular inflammation in biopsies of “normal-appearing” scalp areas.[98] Further research is needed to clarify if this subclinical inflammation may be a predictor of future progression of the alopecia.

Moreover, when comparing different areas in FFA patients, these were scarring band, implantation hairline and “healthy scalp”, some differences in vessel diameter were observed in Colour Doppler mode. For example, vessel diameter was higher in the hairline implantation than in the alopecic band. A reasonable explanation for that could be the presence of both active inflammation in the hairline implantation area and the presence of fibrosis in the scarring band. Indeed, previous studies had already found that affected areas in FFA are usually hypovascular in colour Doppler US.[159] Moreover, vessel diameter was also higher in the hairline implantation than in the healthy scalp. The presence of clinical and subclinical active inflammation may be a likely explanation for the higher vessel diameter found in these

areas, especially in the hairline implantation, which is supposed to be the most active one. A study about optical coherent tomography in FFA patients found the presence of dilated vessels in both the hairline implantation area and the alopecic band.[162] A report about sonography in the hairline implantation, which was published later, found a higher echogenicity of the dermis and epidermis, the loss of precision between the exact division of epidermis and dermis, and an hypoechoic enlargement in the follicular bulbs. The authors have proposed that the inflammatory infiltrate may produce changes in the architecture of the skin layers and around the follicular bulbs.[163]

On the other hand, no significant differences were noted in vessel diameter or flow with regards to trichoscopic inflammatory signs, i.e. perifollicular erythema or follicular hyperkeratosis. Perifollicular erythema has been considered as a marker of activity in FFA,[153] and many patients with actively progressive alopecia have persistent inflammatory trichoscopic signs. However, these signs can persist in patients with no progression of their alopecia,[11,12] or can be absent in patients with hair loss progression.[154] Therefore, the lack of differences in sonography regarding the presence of those inflammatory signs could be due to the fact that they are not always related to clinical progression. Nevertheless, the presence of prominent and branched vessels on trichoscopy did match to a higher flow in the hairline implantation area and to a higher vessel diameter in the healthy scalp. Although the use of topical corticosteroids makes the presence of vascular structures more frequent,[164] our findings were not related with the use of topical corticoids, so no differences were found between corticosteroids users and non-users. Therefore, the presence of branched vessels in trichoscopy may be related with active inflammation and could be a marker of progressive disease.

8.1.5. Assessment of quality of life in patients with frontal fibrosing alopecia. The development and validation of a specific questionnaire.

QoL may deteriorate in patients with alopecia, especially in women.[165] Most studies about QoL in patients with alopecia are about AGA and alopecia areata, for which specific questionnaires have been developed.[166,167] Specific questionnaires are more accurate and may detect more patients with affected QoL than the general ones. However, QoL studies about scarring alopecia are scarce and all of them use general questionnaires, mainly DLQI.[122] In fact, there are only three articles regarding QoL assessment in patients with FFA.[106,107,168] Recently, the IFFACG has suggest the utilization of the questionnaire designed for AGA, called Woman's Androgenetic Alopecia Quality of Life Questionnaire (WAA-QOL),[167] they point out that the validation of this questionnaire or of a specific one for FFA is needed.[108]

A specific and validated questionnaire to assess QoL in patients with FFA is presented, named FFA-QLI. It has demonstrated a higher power than DLQI to discriminate patients with severe FFA, and also with long-lasting alopecia and with eyebrow alopecia. Almost 18% of the FFA patients showed at least moderate impairment of their QoL based on DLQI, whereas FFA-QLI detected that almost 32% of patients had at least a moderate impairment. Moreover, FFA-QLI identified around 68% of patients with mild impairment of their QoL compared to around 36% of patients identified by the DLQI. The more specific the questionnaire is, the more capacity it has to detect disturbances in QoL.

One of the previous reports about QoL in FFA patients found a negative association between QoL and FFA, but no association between QoL and the severity of the alopecia, using the DLQI, the HADS and the Revised Illness Perception Questionnaire (IPQ-R).[106] Nevertheless, in the current study, patients with severe alopecia obtained significantly higher scores in FFA-QLI. In fact, FFA-QLI has demonstrated a higher power to discriminate severe cases of FFA compared to the DLQI. Patients with more severe disease showed more concerns about eyebrow and hair loss and about covering the alopecia when combing their hair or even using wigs. They also were more worried about covering up their eyebrow loss, with make-up or with micropigmentation. Moreover, they felt that thoughts about the alopecia were present almost all of the day.

The second previous report about QoL in FFA patients showed good, but not excellent, QoL in FFA patients and found no significant correlation between QoL and duration of disease or

maximal hairline regression.[168] However, in the current study, patients with long-lasting alopecia had higher FFA-QLI scores, and the questionnaire was also more powerful than DLQI to discriminate these patients. The longer duration of the alopecia may lead to an accumulated psychological tiredness from the disease.

The third previous report about QoL in patients with FFA found that patients with at least one associated non-scalp lesion tended to have a higher DLQI score.[107] In this regard, patients with facial papules or eyebrow alopecia got significantly higher FFA-QLI scores. FFA-QLI also showed higher discriminative power than the DLQI to select patients with eyebrow alopecia. Eyebrows are really important in the aesthetics of the face and also for gestures and social relationships. In fact, most of FFA women with eyebrow alopecia cover them with make-up or have them tattooed, and eyebrow loss is a common reason of consultation in male with FFA (even more than the scalp alopecia).[19] Facial papules cause a rougher appearance of facial skin and less face brightness, so they can make patients to feel uncomfortable with their personal image.

FFA is currently one of the most common types of alopecia and the most common form of scarring alopecia.[8] Therefore, a specific questionnaire to assess QoL is necessary, since the use of general questionnaires for a such specific disease as FFA may miss some patients with an impaired QoL. The FFA-QLI could help dermatologist to select patients who have worse QoL due to the alopecia and seek help for them if they need it.

8.2. Histopathological analysis in frontal fibrosing alopecia: a comparison between frontal hairline implantation and a normal-appearing scalp area.

The broad histopathological analysis, which we performed, revealed that few histopathological differences can be noted between the frontal active hairline and an apparently normal area of the scalp in patients with FFA. Most of the histopathological features were shared by both areas, although some of them with a lower frequency or lower intensity in the "healthy scalp". Previous studies had shown that the clinically spared scalp also had histopathological evidence of disease in patients with scarring alopecia, including FFA and LPP.[95] However, the number of FFA patients in the previous reports was lower than in the presented sample (the largest one included 28 FFA patients) and the variables assessed were the presence and severity of inflammatory infiltrate, the existence of perifollicular fibrosis, mucin deposits, and sebaceous gland atrophy.[96-98]

The total and terminal follicular count was significantly lower in the hairline implantation than in the "healthy scalp", which is explained by the loss of hair follicles because of the scarring process.

The atrophy of sebaceous glands, the inflammatory infiltrate involving vellus follicles and the follicular triad are considered early findings in FFA.[93,94] When the disease progresses, the follicular triad is normally absent, probably because the scalp behind the original hairline contains fewer intermediate and vellus follicles.[94] Sebaceous glands were much more commonly affected (reduced or absent) in our patients than the erector pili muscle; they were spared in around 19% of hairline implantation biopsies compared to 58% in the "healthy scalp" samples. Most hair follicles were affected in the hairline implantation, while in the normal-appearing scalp, spared hair follicles were found in almost all patients.

Previous reports found the presence of inflammatory infiltrate in the normal-appearing scalp, which was more frequent there than in the alopecic band,[96] and less common or with the same frequency than in the hairline implantation area – but with less severity-.[97,98] In our cohort, no significant differences regarding the frequency of the inflammatory infiltrate in the two areas were noted, although it was generally milder in the "healthy scalp". The inflammatory infiltrate involved dermis in both areas, although with a significantly higher frequency in the hairline implantation. Unlike previous reports, which found a usually spared

interfollicular epidermis,[88,89] a considerable number of our samples showed interfollicular epidermis involvement in both areas, with no significant differences between them.

The most common epithelial changes were those affecting the follicular epithelium, especially as vacuolar changes, and were significantly more present in the hairline implantation area than in the "healthy scalp". Interestingly, there were also significant differences regarding the presence of necrosis of keratinocytes and an increased apoptotic activity, in the outer root sheath, which were more habitual in the hairline implantation.

Perifollicular fibrosis was significantly more frequently seen in the hairline implantation, although it was also present in a considerable number of the "healthy scalp" biopsies, even more than in previous reports.[98] Dermal fibrosis was present in both areas, but mainly in the hairline implantation and affecting the upper dermis. Fibrous tracts were observed in both areas. Mild follicular mucinosis was found in a small number of patients in both areas, with no differences between them. Elastic fibre pattern was generally normal in both areas.

Patients with follicular hyperkeratosis and perifollicular erythema more frequently showed inflammatory infiltrate in both areas, although the difference did not reach statistical significance. This may be due to the higher number of patients with both inflammatory infiltrate and trichoscopic inflammatory signs or because this relationship does not exist. Indeed, previous reports found no association between the degree of histological inflammation in both areas and the presence of symptoms or scalp signs.[98] No association was found between the presence of histologic inflammation and the presence of symptoms.

Interestingly, the involvement of sebaceous glands/erector pili muscle was significantly less frequent in patients with facial papules. As some authors have suggested, facial papules may be an early finding in FFA, since they are more commonly seen in younger patients.[71] Therefore, facial papules may even be an earlier finding than the involvement of those skin annexes. The presence of vellus hair in the hairline implantation can also be noted in very early cases of FFA.[83] Indeed, patients who had their vellus hair conserved in the hairline implantation, had no fibrous tissular changes in this area. The fibrous process is responsible for the loss of hair follicles, so the presence of vellus hairs in incipient cases of FFA seems to match with a less irreversible histopathological features.

8.3. Limitations of the studies.

The main limitation of the studies is the presence of recall bias regarding some epidemiological data and especially about the use of sunscreens and their type (physical/chemical). However, these biases, and especially the one regarding the use of sunscreens, are similar in patients and control subjects, as they were asked about the variables and the use of sunscreens using the same question, so they are a non-differential bias. In the study about actinic damage, the history of skin tumours in all the participants was collected from the medical history registered in the hospital, but recall bias may be present in the participants (patients and control subjects) whose medical history was not registered at the hospital.

Another limitation for some studies may be the fact that patients were under treatment for FFA. However, being under treatment has been taken into account for the statistical analyses, i.e. topical corticoids use in the sonographic or histopathological study, which could have modified the results.

The lack of follow-up observation in the sonographic and histopathological studies, because of the cross-sectional design of them, is another limitation. A prospective study of the patients could provide information about sonographic and histopathological changes through time.

As the study was performed in Spain, the questionnaire to assess QoL in FFA was designed in Spanish, and this is the version which has been validated. Therefore, possible cultural bias related to the monocultural development of the questionnaire needs to be considered, although this is a potential limitation inherent in most questionnaire development processes.

FUTURE PERSPECTIVES

9. FUTURE PERSPECTIVES.

This Doctoral Thesis is the starting point to develop further research about different areas in FFA. The existence of a genetic background for higher susceptibility for developing FFA, based on different genetic locus and HLA profiles, is now known. We found a possible marker of susceptibility for familial FFA, but it needs to be confirmed in sporadic cases of FFA. Our findings pointed to an antigen-driven mechanism in susceptible patients sharing the vulnerability described haplotype. However, the high prevalence of FFA worldwide and the fact that there is a lower frequency of familial cases suggest that the genetic background alone is not enough to cause the development of FFA, but gives the individual a greater susceptibility when exposed to an unknown trigger. Further genetic studies may help to identify specific genes which may be linked to the development of FFA.

Hormonal factors probably play a role in the aetiopathogenesis of FFA, although their actual implications are still unclear. No clear and constant hormonal imbalances have been detected in blood analysis in FFA patients, although some reports have found a few alterations. We found lower levels of DHEAS in FFA patients, a hormone that also has an immunomodulatory role and is involved in the PPAR function, which may be implicated in the aethiopatogesis of FFA. Further research about the meaning of DHEAS deficiency in some FFA patients is needed.

Sunscreens have been pointed out as possible triggers for the development of FFA in susceptible patients, since patients with FFA have a higher use of sunscreens than control subjects. However, this greater use also happens in patients with AGA. Therefore, sunscreens may not be the trigger, but an associated factor in those dermatologic patients, who are more worried about the health of their skin. We found higher actinic damage in patients with FFA, which should not be the case if they had used sunscreens with regularity during their lifetime. The slow progressive nature of FFA makes a prospective study difficult, but these findings suggest that maybe we should look for another possible trigger rather than sunscreens.

A prospective sonographic study in FFA patients could be interesting to determine if the vascular differences that we found can change after using a specific treatment or because of the natural progression of the disease. In this regard, sonography would be a more helpful tool if it could be used to assess clinical improvement or worsening of the disease. It is not known if the presence of sonographic or even histopathological alterations in the normal-

appearing scalp may be a predictor of progression. Indeed, we found the same histopathological alterations in the normal-appearing scalp as in the hairline implantation area, but with a lower frequency and lower intensity. However, it seems that although all the scalp may show evidence of the disease, both with sonography and in skin biopsy, not all the areas develop the scarring alopecia. The reason why the hairline is clinically affected by the alopecia and not the parietal area still remains unknown. It could be a specific characteristic of the hairline area which makes it more prone to developing the alopecia, or some feature in the rest of the scalp which protects these other areas. Further studies are needed to clarify the meaning of these findings. A deeper evaluation of the relationship between those histopathological findings and the clinical features of the FFA patients will also be performed.

Regarding the QoL questionnaire, the high frequency of FFA makes the need for proper tools to assess the QoL disturbances in these patients a necessity. An English version of the FFA-QLI questionnaire is provided, but it is still pending a transcultural validation which would permit its use in English. Furthermore, as the QoL study was performed only on women, a forthcoming study among male patients with FFA will be carried out.

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CONCLUSIONS

10. CONCLUSIONS.

1) A prevalence of almost 7% of familial cases was found, and the most common familial connection between patients was observed to be siblings. The clinical and trichoscopic features were consistent with those of non-familial cases. HLA class I haplotype HLA-A*33:01; B*14:02; C*08:02 was shared by most of the patients in our cohort, which suggests that this may predispose one to familial FFA. This haplotype was linked to the CYP21A2 gene p.V.281L mutation, since all of these patients were heterozygous to this mutation.

2) The presence of rosacea was significantly more frequent in patients with FFA than in control participants. The BMI was significantly higher in FFA patients than in the control individuals. Lower levels of DHEAS were observed in FFA patients. Moreover, the prevalence of rosacea was higher in patients with a more severe grade of FFA. Perifollicular erythema, higher BMI and lower progesterone levels were associated with higher risk of rosacea in FFA patients.

3) An assessment with sonography showed that patients with FFA had a higher use of sunscreens than control individuals, but also had greater actinic damage, especially in form of solar lentigines. No differences regarding the presence of trichoscopic inflammatory signs, histopahological anomalies and the presence of peripheral alopecia were noted between FFA patients who used sunscreens and those who did not.

4) Patients with FFA had higher vessel diameter and flow in the hairline implantation compared to control subjects. FFA patients also had higher vessel diameter in the healthy scalp. Patients showed higher vessel diameter in the hairline implantation compared to the healthy scalp and also compared to the alopecic area.

5) A specific and validated questionnaire to assess QoL in patients with FFA is presented, called FFA-QLI, which detects more patients with impairment in the QoL than the DLQI. Moreover, FFA-QLI showed a higher power to select patients with more severe disease, with long lasting-alopecia and with eyebrow alopecia than the DLQI.

6) All the histopathological features described in the hairline implantation in FFA were also found in the "healthy scalp". The inflammatory infiltrate was present in both areas, although was milder in the "healthy scalp". Sebaceous glands alterations were significantly more commonly seen in the hairline implantation, as well as perifollicular fibrosis. Changes in the follicular epithelium, such as vacuolar degeneration of the basal layer, necrosis of keratinocytes and increased apoptotic activity in the outer root sheath, were significantly more common in the hairline implantation area.

CONCLUSIONES

10. CONCLUSIONES.

1) Una prevalencia de casi el 7% de casos familiares se encontró en la cohorte de 101 pacientes. La relación de parentesco más frecuente era la hermandad. Las características clínicas y tricoscópicas fueron concordantes con las de los casos no familiares. El haplotipo HLA clase I, HLA-A*33:01; B*14:02; C*08:02, estaba presente en la mayoría de pacientes de la actual cohorte, lo que sugiere que podría predisponer a la AFF familiar. Este haplotipo se mostró ligado a la mutación p.V281L del gen CYP21A2, ya que todos esos pacientes eran heterocigotos para dicha mutación.

2) La prevalencia de rosácea en las pacientes con AFF fue mayor que en el grupo control. El índice de masa corporal fue significativamente mayor en pacientes que en controles. Se observaron niveles menores de DHEAS en pacientes con AFF. Además, la prevalencia de rosácea fue mayor en pacientes con grados más severos de AFF. El eritema perifolicular, un mayor índice de masa corporal y unos niveles menores de progesterona se asociaron a un mayor riesgo de rosácea en pacientes con AFF.

3) Las pacientes con AFF usaban más fotoprotectores que el grupo control, pero también tenían un mayor daño actínico, especialmente en forma de lentigos solares. No se observaron diferencias en relación a la presencia de signos inflamatorios tricoscópicos, anomalías histológicas, ni en la presencia de alopecia periférica entre pacientes con AFF que usaban fotoprotectores y aquellas que no los usaban.

4) Mediante valoración ecográfica se observó que las pacientes con AFF tenían un diámetro y flujo vascular mayor en la línea de implantación que el grupo control. Las pacientes con AFF tenían también un mayor diámetro vascular en el cuero cabelludo sano. Las pacientes mostraron un mayor diámetro vascular en la línea de implantación en comparación con el cuero cabelludo sano y también con la banda alopécica.

5) Se presenta un cuestionario para evaluar de calidad de vida en AFF, específico y validado, llamado FFA-QLI, el cual detecta más pacientes con afectación de la calidad de vida que el DLQI. Además, el FFA-QLI también demostró un mayor poder que el DLQI para seleccionar pacientes con enfermedad severa, alopecia de larga duración y pacientes con alopecia de cejas.

6) Todos los hallazgos histopatológicos descritos en la línea de implantación de pacientes con AFF se encontraron también en el "cuero cabelludo sano". La presencia de infiltrado inflamatorio se observe en ambas áreas, pero fue más ligero en el "cuero cabelludo sano". La alteración de glándulas sebáceas fue significativamente más frecuente en la línea de implantación, así como la fibrosis perifolicular. Los cambios en el epitelio folicular, como la degeneración vacuolar de la capa basal, la necrosis de queratinocitos y la actividad apoptótica aumentada en la vaina radicular externa, fue significativamente más común en la línea de implantación.

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