Evaluation of Comprehensive Hormonal and Growth Factor Biomarker Panel in Female Pattern Hair Loss

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Received: 16 Aug. 2025; Accepted: 01 Mar. 2026

Abstract- Female pattern hair loss (FPHL) is a common, multifactorial condition with a complex pathophysiology that remains not entirely understood. This study aimed to evaluate the diagnostic and prognostic values of several biomarkers (TGF-β1, DHEA, testosterone, SHBG, and the Free Androgen Index) in patients with FPHL. A case-control study was conducted in Iraq, involving 60 Iraqi women with FPHL and 60 healthy controls. Serum levels of the biomarkers were determined using ELISA. The Ludwig scale was employed to measure disease severity and its correlation with polycystic ovary syndrome (PCOS) or family history. Women diagnosed with FPHL exhibited significantly elevated levels of total testosterone, DHEA, TGF-β1, and FAI, alongside reduced SHBG and estradiol levels (P<0.0001 for all, except estradiol where P=0.0145). Following cross-validation and adjustment for multiple comparisons, FAI demonstrated excellent diagnostic accuracy (Adjusted AUC=0.96), while DHEA, total testosterone, and SHBG also displayed remarkable diagnostic efficacy (Adjusted AUCs> 0.90). TGF-β1, DHEA, total testosterone, and estradiol levels were positively correlated with disease severity. A significant correlation was observed between a positive family history and elevated Ludwig grades (P=0.0074). FPHL is associated with multiple hormonal imbalances that are not limited to increased androgen levels. FAI serves as a robust diagnostic marker, and the panel of TGF-β1, DHEA, SHBG, and E2 has potential diagnostic and prognostic value in clinical settings to guide personalized management. These findings support a multifactorial theory of FPHL and underline the importance of a comprehensive hormonal evaluation as part of the diagnostic algorithm for FPHL.

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Acta Med Iran 2026;64(6):341-349.
https://doi.org/10.18502/acta.v64i6.22316

Keywords: Testosterone; Sex hormone-binding globulin; Transforming growth factor beta1; Dehydroepiandrosterone

Introduction

The worldwide prevalence of FPHL is estimated to be between 30% and 50% and increases with age (1). FPHL is characterized by progressive follicular miniaturization and overall thinning, especially of the central parietal scalp, and represents an important clinical problem because of its complex etiopathology and profound psychosocial effects on affected women (2). However, unlike male alopecia, the pathophysiology of FPHL remains largely unknown and appears to involve an interplay of genetic predisposition, hormonal imbalances, inflammatory components, and environmental factors (3).

The classical androgen-centric paradigm, well established in male androgenetic alopecia, has proven insufficient to fully explain FPHL pathogenesis (4). Androgens, particularly testosterone (T), its active metabolite 5α-dihydrotestosterone (DHT), DHEA, and androstenedione, affect hair follicles by binding to androgen receptors in dermal papilla cells (5). However, a large number of women with FPHL have normal circulating levels of these hormones. In addition, FPHL has been reported in women with complete androgen

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insensitivity and hypopituitarism, indicating that non-androgen-driven mechanisms are important in the pathogenesis of the disease (6). This observation has led to the exploration of alternative pathways such as estrogen metabolism through aromatase enzyme activity, growth factors (e.g., transforming growth factor-beta1 [TGF-β1]), inflammatory cascades, genetic polymorphisms in sex hormone binding globulin (SHBG), and other modulating proteins that generate androgens (7).

Recent studies have emphasized the significance of biomarkers beyond traditional androgens (8). TGF-β1 multifunctional cytokines regulate cell growth, differentiation, and apoptosis and have been identified as key mediators in the inhibition of hair follicle growth, especially in androgen-sensitive sites (9). TGF-β1 is induced in dermal papilla cells by exposure to androgens, ultimately leading to the suppression of epithelial cell growth and miniaturization of hair follicles (10). Moreover, the influence of SHBG on bioavailable androgens is subtle, with low SHBG levels associated with higher FAI and increased follicular androgenicity (11). In women, SHBG confounds total testosterone reference ranges owing to its substantial effect on free hormone levels, and multiple studies have shown that FAI is more reflective of androgen availability than total testosterone measurements (12).

However, substantial gaps remain in the knowledge of FPHL despite progress. Molecular mechanisms involving hormonal disorders and follicular miniaturization remain unclear, particularly the cross-talk among androgens, estrogens, growth factors, and inflammatory mediators in the local microenvironment of the hair follicle (8). The present study was designed to assess serum levels of TGF-β1, DHEA, total testosterone, estradiol, SHBG, and FAI in female patients with FPHL versus age-matched females.

Materials and Methods

Study design and population

A case-control study was conducted from June 2024 to March 2025 to assess the relationship between specific hormonal biomarkers and FPHL. The study population included 120 women: group 1 consisted of 60 women with a clinical diagnosis of FPHL, confirmed by an expert dermatologist at the Dermatology Unit of Marjan City Hospital, Iraq, and group 2 consisted of 60 age-matched healthy female controls. All subjects were recruited from the same clinical setting .

Women with FPHL, aged 16-40 years, were included in the study. Common exclusion criteria for both groups included any history of thyroid disease, tumors, or the use of medications known to affect hormone levels or hair growth (e.g., oral contraceptives, hormone replacement therapy, anti-androgens, spironolactone, minoxidil) within three months prior to enrollment.

Ethical Considerations: The protocol of this study was reviewed and approved by the Dermatology and Venereology Hospital, Marjan Medical and Educational City, Babylon, Iraq (approval No: 4122, 1/3/2024), following the principles of Declaration of Helsinki. All patients were consented in writing before recruitment.

Data and sample collection

Sociodemographic and clinical parameters, including age, BMI, place of residence, education level, family history of FPHL, and PCOS status, were collected using a standardized questionnaire. Disease severity in patients with FPHL was graded using the Ludwig scale.

Biochemical analysis

A 10 mL venous blood sample was obtained from each participant via venipuncture. To control for physiological hormonal fluctuations, blood samples from all premenopausal participants were drawn during the early follicular phase (days 2-5 of the menstrual cycle). A 4 mL aliquot was transferred to a gel tube for coagulation and subsequently centrifuged at 1,500×g/min to obtain serum. After harvesting, the serum was aliquoted and stored at -40° C until batch analysis.

Biochemical analyses

An enzyme-linked immunosorbent assay (ELISA) was used to measure the levels of important serum biomarkers. The following commercial kits were used: Dehydroepiandrosterone (DHEA): Elabscience® ELISA Kit (Catalog No: E-EL-0115), Sex Hormone-Binding Globulin (SHBG): BT LAB Human ELISA Kit (Catalog No: E1011Hu), Total Testosterone: Elabscience® ELISA Kit (Catalog No: E-EL-0155), Estradiol (E2): Elabscience® Pro Human ELISA Kit (catalogue number: E-OSEL-H0009), Transforming Growth Factor Beta 1 (TGF-β1): Elabscience® ELISA kit (catalog number: E-EL-H0146), and Free Androgen Index (FAI) was calculated according to the following equation: FAI=Total Testosterone [nmol/L]/SHBG [nmol/L]×100.

Statistical analysis

Data were collected, organized in Microsoft Excel, and analyzed using GraphPad Prism version 9.0 and R statistical software. Quantitative variables were

summarized as means with standard deviations (mean±SD), and qualitative variables as frequencies and percentages. The Kolmogorov-Smirnov test was used to determine normality. Group differences were compared using independent-samples t-tests for normally distributed continuous variables and chi-squared or Fisher's exact tests for categorical variables. One-way analysis of variance (ANOVA) was used to compare means across Ludwig grades, followed by post hoc analysis. Hormonal markers were correlated using Pearson's correlation analysis. To address potential overfitting and provide realistic diagnostic performance estimates, ROC curve analysis was performed using 5-fold cross-validation. In addition, the Bonferroni correction was used to control for the multiple testing across the six monitored biomarkers. The diagnostic value of the biomarkers is reported using these adjusted Area Under the Curve (AUC) metrics. Significant predictors of FPHL were determined using binary logistic regression (P<0.05 considered statistically significant). To provide internal validation for the logistic regression model, bootstrapping techniques (1,000 resamples) were utilized to estimate optimism in the predictive models.

Results

Table 1 summarizes the demographic and clinical characteristics of 60 FPHL patients and 60 healthy participants in the research in Table 1. There were no significant differences in age (31.3±5.7 vs. 31.6±8.2 years, P=0.838) and BMI (25.17±1.21 vs. 26.14±1.50 kg/m², P=0.091) between the groups. In the FPHL group, several patients presented with a positive family history (FH) of FPHL (51.7%) and a comorbid diagnosis of PCOS (28.3%). According to the Ludwig classification, disease severity was categorized as grade I (38.3%), grade II (28.3%), or grade III (33.3%).

A comparison of the systemic hormonal environments between the FPHL and control groups is presented in Table 2. The FPHL group featured a profoundly disturbed androgen profile, including significantly higher levels of total testosterone, DHEA, and calculated FAI, along with a marked reduction in SHBG (all P<0.0001). Simultaneously, patients with FPHL had significantly higher concentrations of TGF-β1 (P<0.0001), whereas their estradiol levels were significantly lower (P=0.0145).

The cross-validated ROC curve analysis of the diagnostic accuracy of the evaluated biomarkers for differentiating patients with FPHL from healthy subjects is presented in Table 3. After applying 5-fold cross-validation and Bonferroni correction, the FAI showed an excellent discrimination with an adjusted AUC of 0.96. Total testosterone, SHBG, and DHEA levels also showed robust diagnostic performance (Adjusted AUCs 0.93, 0.92, and 0.90, respectively). TGF-β1 demonstrated good specificity but inferior sensitivity, yielding an adjusted AUC of 0.68. The diagnostic performance of the significant markers was least robust for estradiol (Adjusted AUC=0.59).

Table 4 shows the results of comorbidities in relation to FPHL severity, measured by the Ludwig classification. There was no statistically significant correlation between the frequency of PCOS and increasing disease severity (P=0.6250). Conversely, a positive family history (FH) of FPHL was strongly and significantly associated with higher Ludwig grades (P=0.0074). In particular, 80% of patients with grade III disease had a positive family history.

Hormonal values differed significantly according to disease severity (all plasma markers, P<0.0001, except for FAI). Levels of TGF-β1, DHEA, TT, and E2 were significantly higher in Grade I than in Grade III. SHBG showed the opposite pattern, with significantly higher levels in Grade III, whereas FAI did not differ according to disease severity stage (P=0.7976).

Stratification of the FPHL group according to comorbidities revealed distinct hormonal patterns (Table 5). The presence of PCOS was significantly associated with higher total testosterone levels (P=0.0046), but not with any of the other biomarkers. By contrast, a positive FH of FPHL had a more pronounced effect, being significantly associated with higher TGF-β1 levels (P<0.0001), higher DHEA and total testosterone levels (P=0.0090, P=0.001, respectively), and lower SHBG concentrations.

Pearson correlation analysis was performed to identify the linear relationships between important hormonal and biochemical parameters in the FPHL group. TGF-β1 showed a highly positive correlation with DHEA, Total Testosterone, Estradiol, and SHBG (all P<0.001). FAI showed a strong negative association with SHBG only (r = -0.55; P<0.0001). These associations are displayed in a hierarchical heat map (Figure 1).

The binary logistic regression model demonstrated that TGF-β1, DHEA, SHBG, and Estradiol levels were statistically significant independent predictors of FPHL status (Table 6). Total Testosterone and FAI levels were not retained in the final model. Greater TGF-β1 increased the likelihood of FPHL (OR=2.02), whereas higher SHBG and Estradiol levels were protective (OR=0.55 and OR=0.85, respectively). DHEA remained a predictor, although with an uncomputable OR, indicating a very

strong association. The model was highly predictive (P<0.001) and accounted for 90% of the variance in FPHL status (Nagelkerke R^2=0.90).

Table 1. Demographic and clinical characteristics of the study population

Variable	FPHL Group (n=60)	Control Group (n=60)	*P*
Age (years)	31.3 ± 5.7	31.6 ± 8.2	0.838
BMI (kg/m²)	25.17 ± 1.21	26.14 ± 1.50	0.091
Family History of FPHL			
- Yes	31 (51.7%)	N/A	N/A
- No	29 (48.3%)	N/A	N/A
PCOS Diagnosis			
- Yes	17 (28.3%)	N/A	N/A
- No	43 (71.7%)	N/A	N/A
Ludwig Grade			
- Grade 1	23 (38.3%)	N/A	N/A
- Grade 2	17 (28.3%)	N/A	N/A
- Grade 3	20 (33.3%)	N/A	N/A

Data are presented as the mean±SD or n (%). N/A: Not Applicable. *P* were calculated using an independent samples t-test (Age, BMI). Statistically significant (P<0.05).

Table 2. Hormonal and biochemical profile

Variable	FPHL Group (n=59)	Control Group (n=60)	*P*
TGF-β1	4.90 ± 2.52	2.89 ± 0.68	< 0.0001*
DHEA (ng/mL)	5.13 ± 0.37	3.31 ± 0.87	< 0.0001*
Estradiol (pg/mL)	29.32 ± 11.86	33.07 ± 8.50	0.0145*
Total Testosterone (ng/mL)	1.13 ± 0.26	0.59 ± 0.13	< 0.0001*
SHBG (nmol/L)	53.92 ± 12.96	85.86 ± 8.37	< 0.0001*
Free Androgen Index (%)	7.86 ± 4.61	2.37 ± 0.52	< 0.0001*

Data are presented as mean±SD. *P* were calculated using an independent sample t-test. The Kolmogorov-Smirnov (distance) test applies the Passed normality test. Asterisk (*) indicates a statistically significant association (P<0.05).

Table 3. Diagnostic utility of key biomarkers for detecting FPHL

Biomarker	Cut-off Value	AUC (95% CI)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	*P*
TGF-β1	>4.1	0.68 (0.61-0.75)	50.0	94.3	93.8	62.3	<0.0001
Estradiol	≤26.2	0.59 (0.52-0.68)	50.0	70	60	58	0.0139
FAI	>3.64	0.96 (0.92-0.99)	98.3	90.0	90.8	98.2	<0.0001
DHEA	> 4.61	0.90 (0.86-0.95)	90.3	88.0	89.8	90.2	<0.0001
Testosterone	>0.79	0.93 (0.89-0.99)	86.0	94.7	92.3	88.0	<0.0001
SHBG	≤74.1	0.92 (0.88-0.97)	93.0	83.7	84.6	92.0	<0.0001

AUC, area under the ROC curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value

Table 4. PCOS and family history with disease severity

Factor	Ludwig Grade 1 (n=23)	Ludwig Grade 2 (n=17)	Ludwig Grade 3 (n=20)	*P*
PCOS				
- Yes	5 (21.7%)	5 (29.4%)	7 (35.0 %)	0.6250
- No	18 (78.3%)	12 (70.6%)	13 (65.0 %)	
Family History				
- Yes	8 (34.8%)	7 (41.2%)	16 (80.0%)	0.0074*
- No	15 (65.2%)	10 (58.8%)	4 (20.0%)	
Hormonal Levels				*P*(ANOVA)
TGF-β1 (pg/mL)	2.52 ± 0.34A	4.63 ± 1.50B	7.86 ± 1.28C	< 0.0001*
DHEA (ng/mL)	4.77 ± 0.12A	5.12 ± 0.18B	5.54 ± 0.22C	< 0.0001*
Estradiol (pg/mL)	18.09 ± 2.8A	33.08 ± 10.3B	39.04 ± 8.9B	< 0.0001*
Total Testosterone (ng/mL)	0.89 ± 0.05A	1.10 ± 0.14B	1.43 ± 0.13C	< 0.0001*
SHBG (nmol/L)	45.57 ± 14.49^{A}	51.45 ± 2.80^{A}	65.62 ± 5.91^{B}	< 0.0001*
Free Androgen Index (%)	8.37 ± 7.29	7.43 ± 1.03	7.64 ± 1.13	0.7976

Data are presented as the mean ± SD or n (%). χ^2 *P* from the chi-square test. A value from one-way ANOVA. Superscript letters (A, B, C) indicate homogenous subsets from Tukey's post-hoc test; different letters denote significant differences. An asterisk (*) indicates a statistically significant association (P < 0.05)

Table 5. Hormonal profiles in FPHL patients stratified by PCOS status and family history

Variable	FPHL with PCOS (n=17)	FPHL without PCOS (n=43)	*P*	FPHL with FH (n=31)	FPHL without FH (n=29)	*P*
TGF-β1	5.22 ± 2.34	4.77 ± 2.57	0.5421	6.30 ± 2.46	3.40 ± 1.52	< 0.0001*
DHEA	5.20 ± 0.35	5.10 ± 0.37	0.3478	5.25 ± 0.40	5.00 ± 0.28	0.0090*
Total Testosterone	1.27 ± 0.28	1.07 ± 0.22	0.0046*	1.23 ± 0.26	1.02 ± 0.20	0.0010*
SHBG	50.94 ± 9.88	55.10 ± 13.82	0.2696	58.74 ± 11.77	48.77 ± 12.17	0.0024*
Free Androgen Index	8.66 ± 0.68	7.55 ± 5.40	0.4072	7.40 ± 1.38	8.36 ± 6.44	0.4266

Data are presented as mean ± SD. FH: Family History. P-values were calculated using an independent sample t-test. An asterisk (*) indicates a statistically significant association (P<0.05)

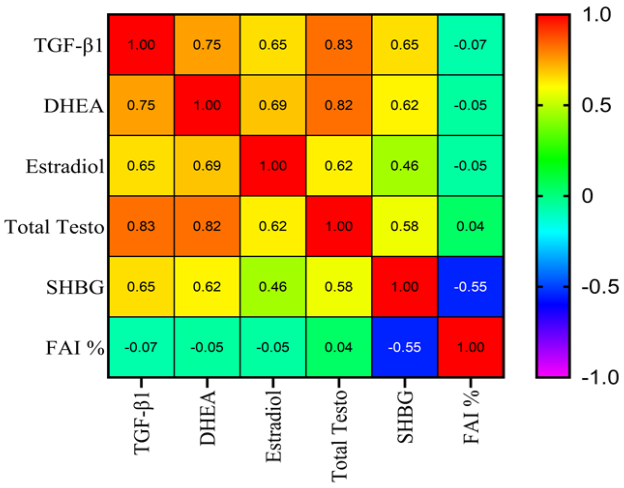

Figure 1. Heatmap of the Pearson correlation matrix for selected hormonal biomarkers among FPHL group. Red means positive correlation, blue negative, and the intensity of the color is proportional to the value of r

Table 6. Binary logistic regression analysis for predicting FPHL risk

Predictor	B	S.E.	Wald	*P*	Odds Ratio (OR)	95% CI for OR
TGF-β1	0.7	0.167	17.5	<0.001	2.016	(1.45 – 2.79)
DHEA	11.8	3.42	11.93	<0.001	-	-
Total Testosterone	**Variable not included in the model**					
SHBG	-0.59	0.19	9.68	<0.001	0.55	(0.378 to 0.801)
Free Androgen Index	**Variable not included in the model**					
Estradiol	-0.15	0.057	7.49	<0.001	0.85	0.76 to 0.95
Constant	-51.06	15.42	10.96	0.0019	-	-

Dependent Variable: Group (FPHL = 1, Combined Controls = 0). Model $\chi^2 = 4.84$, $P < 0.001$, Nagelkerke $R^2 = 0.90$

Discussion

The hormonal and biochemical profiles of women are characterized by complex endocrine dysregulation that extends well beyond mere hyperandrogenism, as reported in this study. Plasma TGF-β1, DHEA, total testosterone, estradiol, SHBG, and FAI levels were measured in both patients and age-matched healthy control subjects, and significant alterations in different hormones and axes were observed. FAI demonstrated outstanding discriminative ability as a biomarker, and the essential role of TGF-β1 in deepening the pathophysiology of FPHL has been reported.

This study demonstrated that FPHL is linked to hyperandrogenism, as indicated by significantly elevated serum levels of total testosterone, DHEA, and FAI, along with reduced SHBG concentrations. These results are consistent with previous reports of hyperandrogenemia in a subset of women with FPHL (10,11). The observed testosterone elevation in this study was greater than that reported in several previous studies (10,11), which may be due to population-specific genetic or environmental factors or the younger age of the participants in this study (13).

The FAI had a sensitivity of 98.3 and a specificity of 90%, uniquely distinguishing patients with FPHL from controls and demonstrating excellent diagnostic value. These values were higher than those reported in other studies on hyperandrogenic disorders (11,14). The high performance of FAI in this study is probably due to its use in the complete hormonal evaluation of androgens, which permits a better estimate of androgen bioavailability. FAI provided a better estimate of androgenic activity than total serum testosterone, particularly when assessing women with hirsutism or acne, in whom SHBG levels determine free hormone concentrations (15). This assertion was supported by the observation that FAI consistently remained elevated across all Ludwig grades despite variations in absolute hormone levels.

In the present study, serum SHBG levels in patients with FPHL were significantly decreased, confirming the inverse correlation with alopecia severity. These results are consistent with a previous report by Bienenfeld et al., and the mean reduction in SHBG levels as the Ludwig classification advances (16). Our study expanded this association by showing that a lower SHBG level is an independent predictor of FPHL in multivariate analysis. The association of SHBG with disease severity is interesting, given the fact that the values were highest in patients with severe (Grade III) alopecia. This was in contrast to the results reported by Chen *et al*., in an Eastern Chinese population, who found a stable negative association (17). This difference may have been caused by ethnic differences in hormone metabolism or differences in the methodological approach used.

The present study provides strong evidence that TGF-β1 is an important mediator in the progression of hair follicle miniaturization, which characterizes FPHL. The gradual increase in hair matrix TGF-β1 levels was consistent with the stage-dependent effect of TGF-β on hair follicles and was related to disease severity, as represented by Ludwig's grading. These findings indicate that TGF-β1 elevation may directly correlate with the severity of the pathological process at the follicular level. The association between TGF-β1 level and severity also agrees with previous experimental evidence showing that TGF-β1 induction causes premature catagen (18). Other recent findings suggest that natural plant extracts may inhibit TGF-β1 expression in cultured human dermal papilla cells and increase their ability to promote hair growth. This result also strengthened the idea that pathogenic FPHL is mediated by high levels of TGF-β1 (19).

Elevated DHEA levels in patients with FPHL support the role of adrenal androgens in the pathogenesis of hair loss, serving as a precursor for conversion to more potent androgens such as testosterone and dihydrotestosterone through 5α-reductase activity (8). Studies on FPHL and androgenetic alopecia have consistently reported elevated adrenal androgens, in contrast to findings in alopecia areata (20). This study indicated that adrenal androgen production may increase as the disease progresses, potentially due to heightened peripheral conversion or changes in HPA axis feedback, in contrast to the existing literature on DHEA and DHEAS.

Lower estradiol levels in patients with FPHL than in controls indicate that estrogen deficiency may contribute to hair loss in women. Estrogens directly regulate the hair cycle, and decreased estrogen levels after menopause are correlated with progressive hair thinning (21,22). The identification of estradiol as a significant protective factor in multivariate analysis indicated that maintaining adequate estrogen levels could serve as a therapeutic strategy to mitigate the progression of FPHL.

In this study, a significant association was found between hormonal measurements and the severity of the Ludwig grade in FPHL. The study indicated that the levels of TGF-β1, DHEA, total testosterone, and estradiol increased with Ludwig grades, reinforcing the hypothesis that FPHL is a continuous disorder driven by hormonal changes. SHBG levels increased in Grade III compared with lower grades, a finding that may indicate compensatory upregulation due to chronic hyperandrogenemia or age-related changes in hepatic SHBG production. In recent decades, the Ludwig type has been reported to be the most common FPHL subtype in large series (23,24). Other morphotypes, such as the Olsen and Hamilton-Norwood phenotypes, were associated with clinical features and comorbidity burden. In this study, only the Ludwig classification was considered because FPHL patients with different patterns of hair loss could have been excluded, and the resulting hormonal findings might not be generalizable to all FPHL phenotypes. The FAI was comparable among Ludwig grades (P=0.7976), suggesting that despite changes in the absolute levels of androgens and SHBG with disease severity, the ratio of androgen to SHBG remained stable.

The current study clearly demonstrates the significant impact of genetics on FPHL. A positive family history was prevalent in 51.7% of patients and showed a strong, statistically significant association with advanced disease severity. This was most evident in patients with Ludwig Grade III; 80% reported affected family members, whereas only 34.8% were noted in Grade I. These findings aligned with previous studies, including Łukasik *et al*., who noted a 62.2% prevalence of positive family

history in their FPHL cohort (25). This study linked a genetic predisposition to a specific hormonal signature. Patients with a positive family history exhibited significantly elevated serum levels of TGF-β1, DHEA, and total testosterone, along with reduced SHBG levels. However, the role of PCOS is complex. In the FPHL cohort, 28.3% had a comorbid PCOS diagnosis. One subgroup exhibited significantly higher testosterone levels; however, PCOS status did not correlate with disease severity. This study indicated that the hyperandrogenic state associated with PCOS was a significant risk factor for the onset of FPHL, but it may not be the primary factor influencing its progression. This aligned with extensive studies, including Quinn *et al*., who observed that androgenetic alopecia occurred in 22% of women with PCOS, although the link to biochemical hyperandrogenemia was not consistently direct (26).

Significant positive correlations were found between TGF-β1, DHEA, total testosterone, estradiol, and SHBG levels, highlighting the interconnected hormonal environment in the pathogenesis of FPHL. The positive correlation between androgens and estradiol indicates common biosynthetic pathways in which testosterone is the substrate for conversion into estradiol. The positive associations observed in the present study are consistent with novel systems biology views that consider hormonal networks (rather than just single pathways) to be of greater importance in complex endocrine diseases (8). Owecka *et al*., suggested that FPHL is a reflection of altered hormonal homeostasis among androgens, estrogens, thyroid hormones, and stress hormones, involving crosstalk signaling networks (3). This suggests that endocrine disorders may produce a cascade of interrelated regulatory events, resulting in coordinated adjustments within other hormonal systems.

Significant independent predictors of FPHL in the current study were TGF-β1, DHEA, SHBG, and estradiol, indicating the strong explanatory ability of the model. Total testosterone and FAI levels were excluded from the final model, suggesting that their diagnostic value was largely captured by the combined assessment of SHBG, DHEA, and TGF-β1. Thus, it may be speculated that wider hormonal screening would give a better diagnostic yield compared with the routine androgen-focused investigation. Comparison with other studies that employed logistic regression to predict the risk of FPHL have revealed discrepancies. Yi *et al*., employed the ordinal logistic regression analyses to evaluate the possible risk factors for FPHL severity and demonstrated that behavioral and metabolic parameters were related to FPHL (27). Other studies have emphasized the role of vitamin D deficiency, ferritin levels, and insulin resistance (28). This study demonstrated that endocrine markers contribute to previous results by showing that hormonal parameters possess independent predictive value and that they might be combined with nutrition-related and metabolic factors in multivariate risk evaluation models.

Strengths, limitations, and future directions

This study delineates the endocrine complexity of FPHL, implicating several other axes in addition to androgens. One important limitation of this study is that we did not have an independent external validation cohort. While we have internally validated our models through bootstrapping to demonstrate their robustness, the results of this study need to be validated in an independent prospective cohort to assess their applicability in different patient populations. In addition, the cross-sectional nature of the study limits the potential to determine causality definitively, and the used biomarkers (systemic serum hormones) might not completely represent local endocrinology or enzyme activity at the level of the follicle. Multiethnic, multicenter prospective studies are needed to confirm this panel of biomarkers, assess its value as a diagnostic tool prospectively, and to investigate the genetics of familial susceptibility.

Clinical integration

The identification of a comprehensive biomarker panel involving TGF-β1, DHEA, SHBG, and estradiol offers a promising avenue for enhancing diagnostic accuracy and tailoring patient management in dermatology clinics. The inclusion of these biomarkers in the standard clinical assessment may lead to an earlier and more accurate diagnosis of FPHL and its differentiation from other types of alopecia. In addition, knowledge of the exact hormone and growth factor status of a particular patient enables physicians to tailor treatments to that patient. For example, a patient with markedly elevated levels of TGF-β1 may be a candidate for treatments that antagonize this pathway, while one with severe estrogen deficiency might be considered for topical estrogenic therapies. A multi-marker approach such as this could allow FPHL management to evolve from a largely empiric approach to a precision medicine approach, with improved outcomes for patients.

In this exploratory study, our results suggest that FPHL could be a multifactorial endocrinopathy with disturbances in several hormonal axes and not only a condition of androgen excess. These findings

demonstrate the applicability of a multi marker panel for diagnostic purposes, where the Free Androgen Index is a good candidate to discriminate between FPHL-patients and controls in this material. The significant associations of hormonal and growth factor markers with disease severity and family history suggest that hormonal, genetic, and growth factor mediated pathways may act synergistically in FPHL. While these results lay the groundwork for subsequent mechanistic studies further confirmation in larger, ethnically diverse populations is required prior to the full integration of these precision medicine strategies for the clinical diagnosis and management of this intricate disease.

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Local Ethics Committee at Babylon Medical College (Approval Reference: BMC-LEC-4122; Date: March 1, 2024).

Acknowledgements

The author would like to thank the director and personnel of the Dermatology Unit in the Marjan Teaching Hospital for their cooperation and facilitation in the recruitment of patients and collection of samples in the course of this study.

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