Original Research Article

Evaluation of the Efficacy of Topical Minoxidil in Treatment of Male Androgenetic Alopecia

ABSTRACT

Aims: This study was conducted to evaluate the efficacy of topical minoxidil 5% in treating male androgenetic alopecia.
Study design: a prospective, randomized study
Place and Duration of study: Outpatient clinic of Dermatology and Venereology Department, Tanta University Hospitals between January 2020 and March 2021.
Methodology: 15 male patients with androgenetic alopecia (age range 23 – 31 years) were included and received topical minoxidil 5% (twice daily) for 24 weeks. Efficacy of treatment was assessed clinically, trichoscopically, and by histopathology (H&E stain).
Results: Patients were classified between grades Ⅲ- Ⅵ at baseline, after 24 weeks of treatment: patients were classified between grades Ⅱ- Ⅴ A. There was an improvement in 60% of patients with no statistically significant difference in the clinical grading before and after treatment. The statistical significance was apparent in the percentage of the terminal and vellus hair and hair density and width after treatment. There were 31.7% and 42.56% increases in hair density and width, respectively. There was a decrease in the miniaturized hair follicles and perifollicular inflammatory infiltrate by H&E stain.
Conclusions: Topical minoxidil 5% was found to be an effective method of treatment of male androgenetic alopecia, which affects both hair density and width.

Keywords: Androgenetic alopecia, topical minoxidil, trichoscopy, histopathology

1. INTRODUCTION

Androgenetic alopecia (AGA) is the most common hair loss disorder, in which there is progressive miniaturization of terminal scalp hair (thick, pigmented) to vellus (fine, non-pigmented) hair. In men, it affects temporal and vertex regions[(1)]. It is generally related to androgenetic effect on the hair follicles in a genetically susceptible patient[(2)]. AGA is common in Caucasians more than in other populations[(3)]. Under trichoscopy, AGA is diagnosed by the presence of hair diameter diversity >20% which corresponds to vellus transformation of hair[(4)], yellow dots, scalp honeycomb pigmentation, brown peripilar sign, white peripilar sign and focal atrichia[(5)]. There are different modalities of AGA treatment, but only two are approved by both United States Food and Drug Administration (FDA) and European Medicines Agency. They are topical minoxidil and oral finasteride. In addition, low level laser therapy is the only non-medicine treatment that is approved by FDA[(3)]. Minoxidil alters the hair cycle, as it prevents the anagen phase shortening, by promoting the regrowth of hair follicles, increasing the duration and the thickness of follicles in the anagen phase and it reduces the telogen follicles[(6)]. The exact mechanism of minoxidil is not fully understood, but it is thought to be through increased cutaneous blood flow by opening the potassium

channels[7]. Also, minoxidil increases the expression of vascular endothelial growth factor (VEGF) mRNA in dermal papillae[8], in addition to inducing hypoxia-inducible factor, upon which VEGF induction depends[9]. Eventually leading to increased vascularity and size of dermal papillae. Moreover, it activates prostaglandin synthase-1, resulting in increased levels of prostaglandins within dermal papillae[10]. Minoxidil has been approved by FDA as a treatment of AGA at concentrations of 2% and 5%. It is recommended to be applied twice daily in a dose 1 mL of 5% minoxidil solution, on a clean and dry scalp. Treatment should be continued permanently, as cessation of treatment leads to re-falling of hair within 3 to 4 months[8].

In the current study we evaluated the efficacy of topical minoxidil 5% in treating male androgenetic alopecia assessed clinically, trichoscopically and histopathologically.

2. MATERIAL AND METHODS

2.1. participants. This study enrolled 15 patients who were presented and diagnosed with AGA at the outpatient clinic of Dermatology and Venereology Department, Tanta University Hospitals, between January 2020 till March 2021. Inclusion criteria were as follows: male patients with androgenetic alopecia, Norwood–Hamilton grade II–IV, who did not receive any treatment within at least 24 weeks before the start of the study, patients who accepted to be enrolled in the study and were ready for regular follow-ups and photographs and signed informed consent. Patients were excluded if presented with any concomitant scalp diseases such as alopecia areata, history of cardiac, hepatic, renal, hypertensive disease, or any major medical illness, Patients with history of bleeding disorders or on anticoagulant medications (Aspirin, Warfarin or Heparin), patients with keloidal tendency and hypersensitivity to minoxidil or its components (as propylene glycol).

2.2. Study Design. Before the beginning of the study and in accordance with the local regulation followed, the protocol and all corresponding documents were declared for Ethical and Research approval by Tanta University Institutional Review Broad. Informed consents were obtained from all participants after full explanation of the procedure, risks and purpose of the study. All patients received the treatment for 24 weeks. Digital photographs and trichoscopic pictures were taken for every patient before and after 24 weeks treatment.

2.3. Minoxidil application. Each patient was subjected to 2- 3 mL of topical minoxidil 5% spray, applied twice daily directly on the scalp, for a duration of 24 weeks.

2.4. Clinical assessment. Patients were graded according to Norwood-Hamilton classification before and after 24 weeks treatment. Digital photographs of the frontoparietal area and the back view of the head using an iPhone XR camera (12 megapixels, Designed by Apple in California, Assembled in China).

2.5. Trichoscopic assessment. Trichoscopic pictures obtained by a digital microscope (CompareView Hair version 1.5.06, written by Prof Steven Abbott 2011-13, USA) by which a computer system analyses the data. A 50-fold magnification was used for evaluating the density of hair per cm^2, terminal and vellus hairs. A 200-fold magnification was used to assess hair shaft diameters. Evaluations were done at 3 different points of the scalp as follows **Figure (1)**: Point A: located at the mid-sagittal line, 9 cm away from the nasion, Point B: located 3 cm posterior to point A and Point C: located at the vertex, and the mean value out of the 3 points was calculated for both hair density and hair shaft diameter at the start of the study and after 24 weeks treatment.

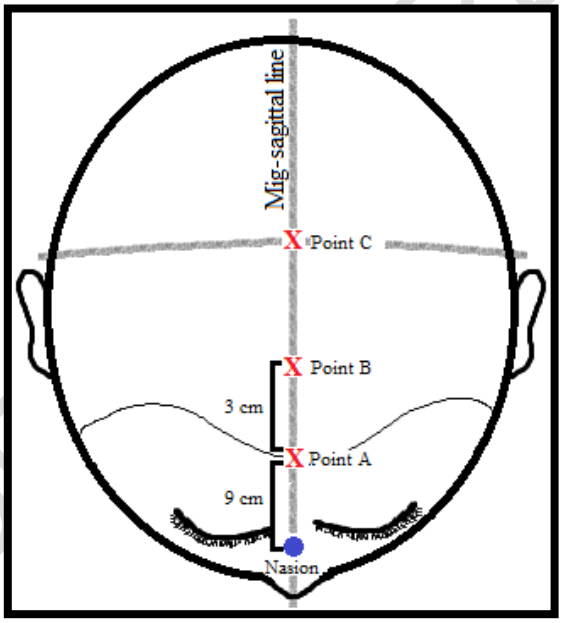

Figure (1): Sites of trichoscopic evaluation

2.6. Histopathologic assessment. Two biopsies were taken using 5 mm biopsy punches from the vertex area one before and one after 24 weeks treatment. Biopsies followed the direction of the hair shafts and the tissue specimens were fixed in 10% neutral

buffered formalin, then embedded in paraffin blocks. Serial sections were subjected to hematoxylin and eosin (H&E) staining and examined to confirm the diagnosis of AGA.

2.7. Statistical analysis. Data were fed to the computer and analyzed using IBM SPSS software package version 20.0**. (**Armonk, NY: IBM Corp**)** Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Significance of the obtained results was judged at the 5% level.

3. Results

A total of 15 male patients with AGA were enrolled to the study. The age of patients in this study ranged from 23 – 31 years with a mean ± SD 26.8 ± 2.81. The disease duration ranged from 1.67 – 8 years with a mean ± SD 3.37 ± 1.77.

3.1. Clinical assessment. Patients were classified between grades Ⅲ- Ⅵ at baseline as follow: 3 patients (20%) were grade Ⅲ, 3 patients (20%) were grade Ⅲ vertex, 3 patients (20%) were grade Ⅳ, one patient (6.67%) was grade Ⅳ A, 3 patients (20%) were grade Ⅴ A, and 2 patients (13.33%) were grade Ⅵ. After 24 weeks treatment: patients were classified between grades Ⅱ- Ⅴ A as follow: 3 patients (20%) were grade Ⅱ, 6 patients (40%) were grade Ⅲ, 2 patients (13.33%) were grade Ⅳ, one patient (6.67%) was grade Ⅳ A, and 3 patients (20%) were grade Ⅴ A. There was improvement in 60% of patients **(Table 1 and Figure 2)**. There was no statistically significant difference in the clinical grading before and after treatment.

Table (1): Comparison between before and after 24 weeks treatment according to clinical grading

Clinical grading	Before ttt		After ttt		MH	P. value
	No.	%	No.	%		
II	-	-	3	20	35.5	0.071
III	3	20	6	40		
III A	-	-	-	-		
III vertex	3	20	-	-		
IV	3	20	2	13.33		
IV A	1	6.67	1	6.67		
V	-	-	-	-		
V A	3	20	3	20		
VI	2	13.33	-	-		

P: P value for comparing between the studied groups before and after treatment
MH: Marginal homogeneity test

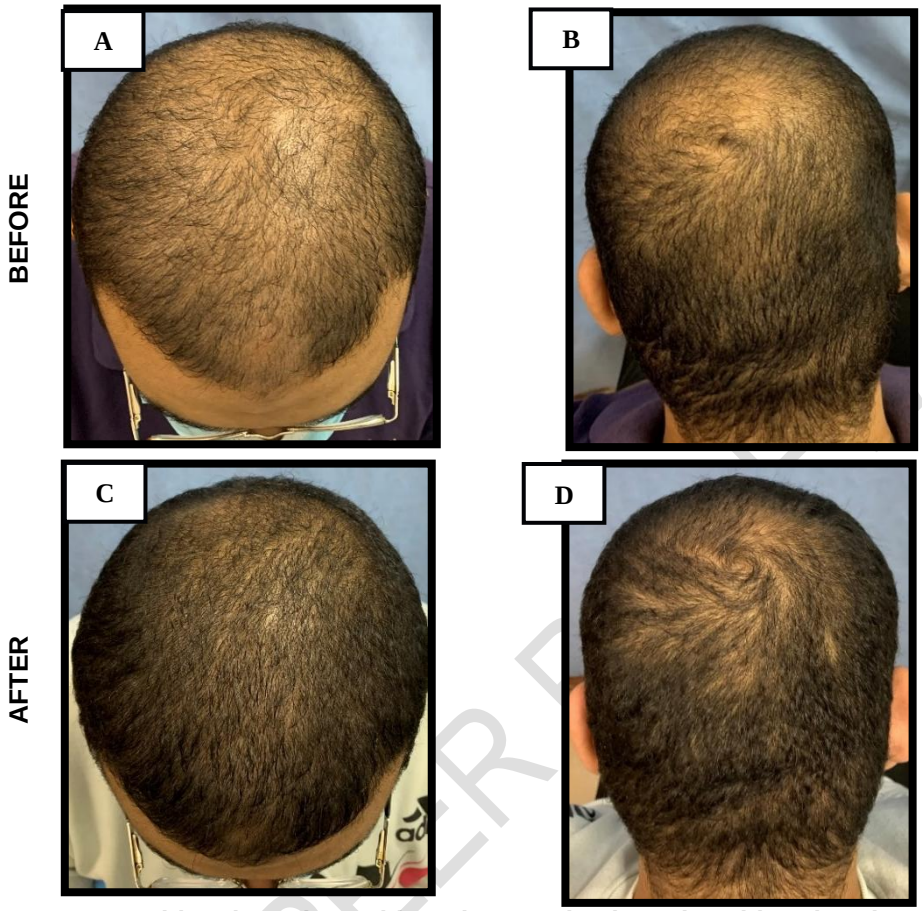

Figure (2): A 30-year-old male patient with androgenetic alopecia, with a duration of 2 years, with positive family history
(A, B) before treatment
(C, D) after 24 weeks treatment with excellent improvement (80%)

3.2. Trichoscopic assessment. Hair density ranged from 80.3 - 178.8, with a mean ± SD 116.24 ± 33.38 at the baseline, and ranged from 118.2 - 187.6, with a mean ± SD 148.07 ± 24.96 after 24 weeks treatment. The mean of difference between before and after treatment ± SD was 31.84 ± 19.13, representing 31.7% ± 20.78 increase in hair density **Figure (3)**. There was statistically significant improvement in density of hair after treatment. The percentage of terminal hair ranged from 38.3 – 76.3, with a mean ± SD 65.39% ± 14.26 at the baseline, and ranged from 76.1 – 89.3, with a mean ± SD 82.15% ± 5.63 after 24 weeks treatment. There was statistically significant improvement in the percentage of terminal hair after treatment. The percentage of vellus hair ranged from 23.7 – 61.7, with a mean ± SD 34.61% ± 14.26 at the baseline, and ranged from 10.7 – 23.9, with a mean ± SD 17.85% ± 5.63 after 24 weeks treatment. There was statistically significant reduction in the percentage of vellus hair after treatment. As for hair width, it ranged from 0.009 – 0.023, with a mean ± SD 0.016 ± 0.004 at the baseline, and ranged from 0.012 – 0.032, with a mean ± SD 0.022 ± 0.005 after 24 weeks treatment. The mean of difference between before and after treatment ± SD was 0.006 ± 0.005, representing a 42.56% ± 34.73 increase in hair width **Figure (4)**. There was statistically significant improvement in width of hair after treatment **Table (2).**

Table (1): Comparison between before and after 24 weeks treatment according trichoscopic parameters

	Before ttt	After ttt		P. value
Hair density			**t**	
Min – Max	80.3 – 178.8	118.2 – 187.6	6.446*	<0.001*
Mean ± SD	116.24 ± 33.38	148.07 ± 24.96		
Median (IQR)	110.3 (96.58 – 114.7)	148.7 (123.7 – 162.9)		
Terminal hair			**Z**	
Min – Max (%)	38.3 – 76.3	76.1 – 89.3	3.42*	0.001*
Mean ± SD	65.39 ± 14.26	82.15 ± 5.63		
Median (IQR)	72.2 (66.55 – 73.6)	79 (77.9 – 88.5)		
Vellus hair			**Z**	
Min – Max (%)	23.7 – 61.7	10.7 – 23.9	3.422*	0.001*
Mean ± SD	34.61 ± 14.26	17.85 ± 5.63		
Median (IQR)	27.8 (26.4 – 33.45)	21 (11.5 – 22.1)		
Hair width			**t**	
Min – Max	0.009 – 0.023	0.012 – 0.032	4.666*	<0.001*
Mean ± SD	0.016 ± 0.004	0.022 ± 0.005		
Median (IQR)	0.016 (0.014 – 0.02)	0.022 (0.019 – 0.024)		

IQR: Inter quartile rangeSD: Standard deviation t: Paired t-test
P: P value for comparing between the studied before and after
*: Statistically significant at P. value ≤ 0.05
Z: Wilcoxon signed ranks test

BEFORE **AFTER**

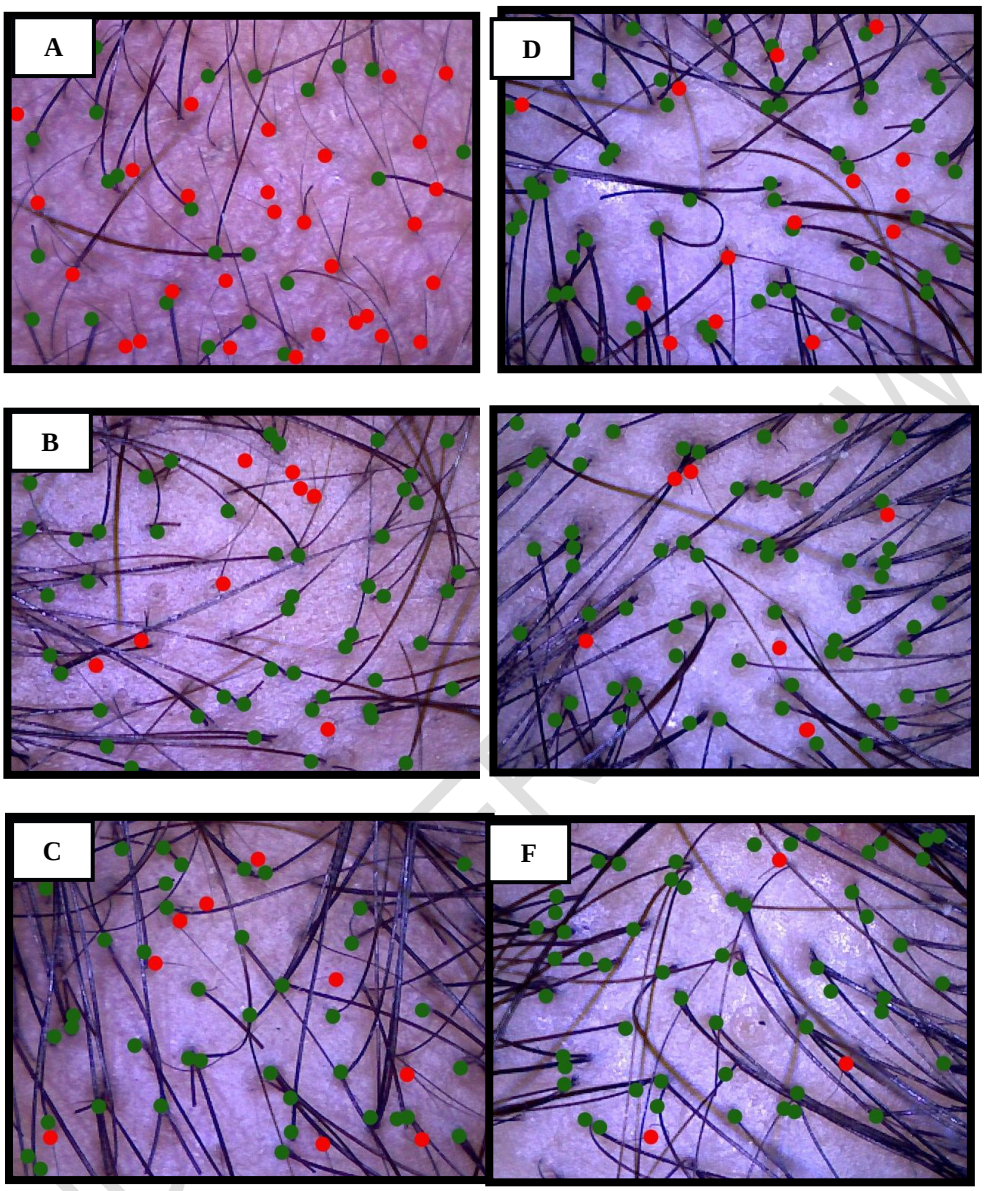

Figure (3): Hair density by the trichoscope at three different points of the scalp
(A, B, C) hair density before treatment. The red dots (vellus hair), green dots (terminal hair) denoting more hair thinning.
(D, E, F) showing improvement after 24 weeks treatment indicated by the increase in green

BEFORE **AFTER**

dots and decrease in red dots.

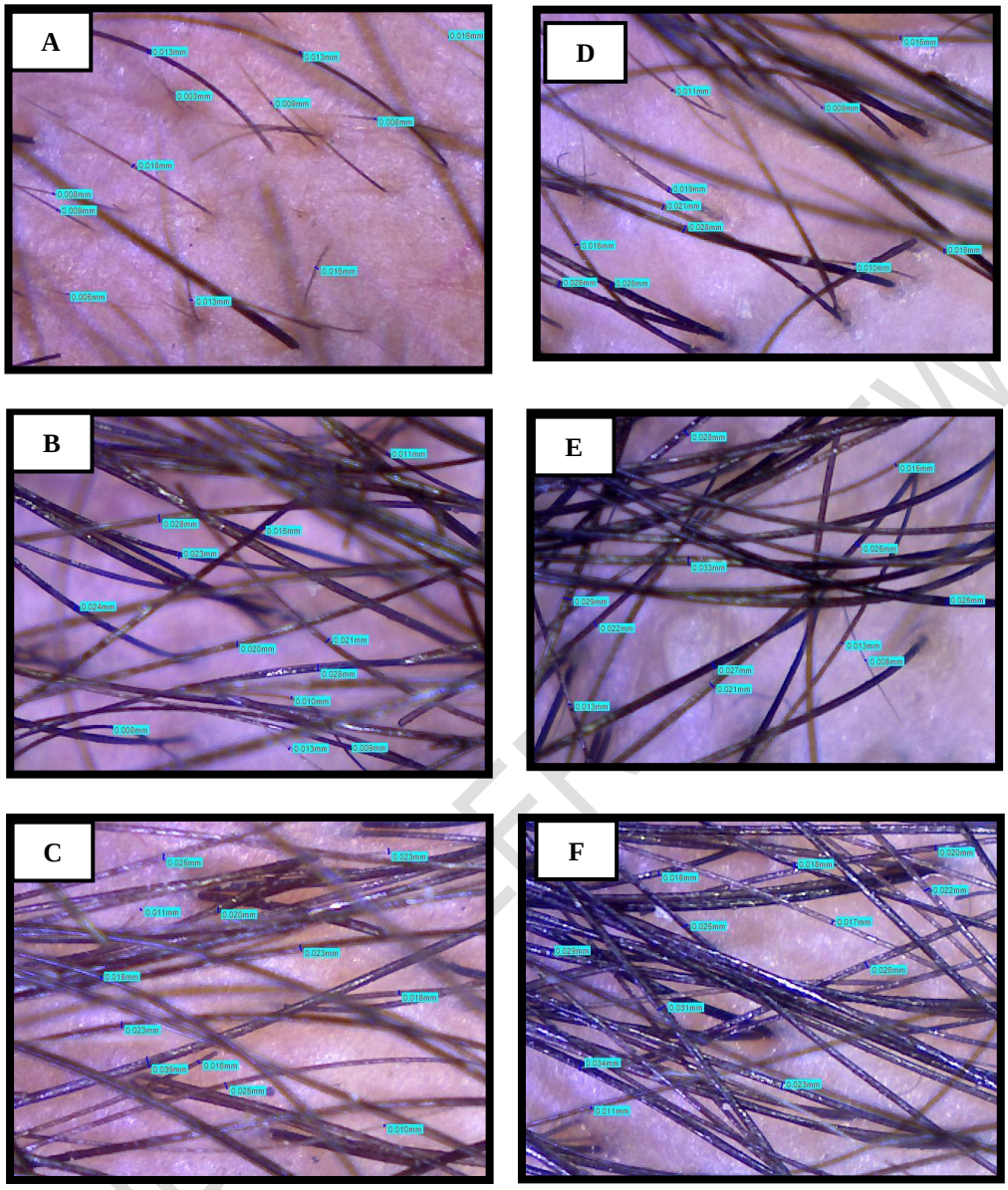

Figure (4): Hair width by the trichoscope at three different points of the scalp
(A, B, C) hair width before treatment
(D, E, F) showing improvement after 24 weeks treatment.

3.3. Histopathologic assessment. By H&E staining, the biopsy obtained at baseline showed a variation in the sizes and miniaturization of the hair follicles with an increased number of vellus hair follicles. There were a sebaceous glands prominence, fibrous tracts in the subcutis, seborrheic folliculitis and reduction in the dermal papilla size **Figure (5,6)**. After 24 weeks treatment, there were more hair follicles with less variation in their sizes and less perifollicular inflammatory infiltrate **Figure (7,8)**.

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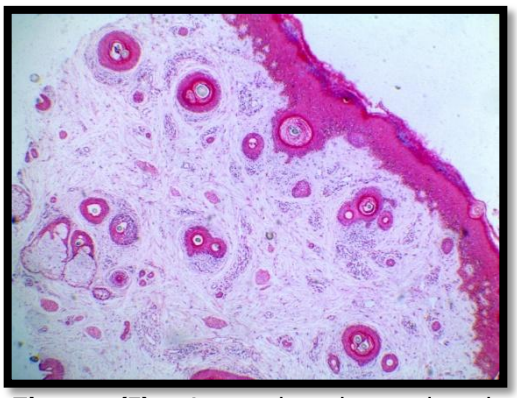

Figure (5): A **s**ection in scalp showing miniaturization with varying size of hair follicles and decrease in size of dermal papilla and Increased number of vellus and telogen hairs **[H&E X40].**

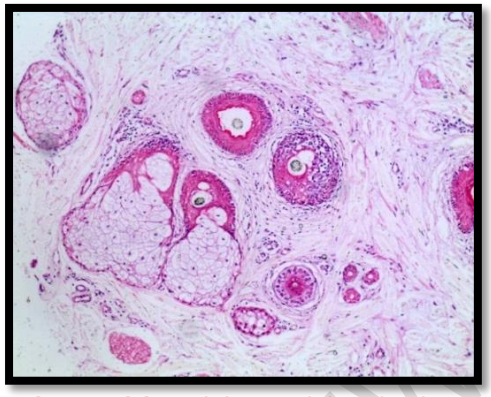

Figure (6): Higher miniaturization with varying size of hair follicles, prominent sebaceous glands, folliculitis and fibrous tracts in the subcutis **[H&E X100]**

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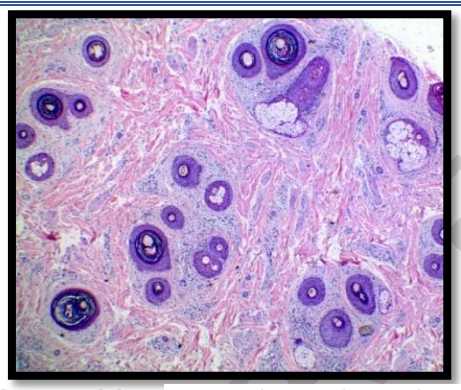

Figure (7): A section of scalp biopsy demonstrates more hair follicles with less variation in size of miniaturized hair follicles **[H&E X40].**

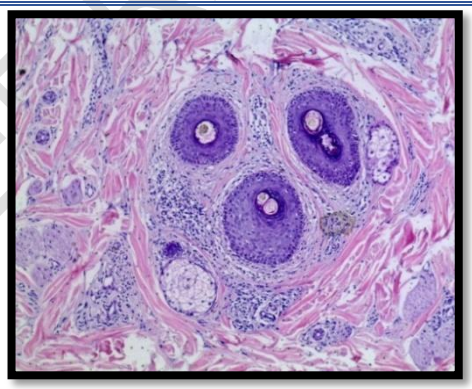

Figure (8): A higher power view of the above section showing the less variation in hair shaft diameter and perifollicular inflammatory infiltrate **[H&E X100].**

4. Discussion

Androgenetic alopecia is the most common hair loss disorder in males, in which there is progressive thinning of hair that mostly starts at puberty with an increasing possibility to develop with age[12]. There are different factors implicated in the pathogenesis of AGA, mainly a genetic background that is influenced by multiple endogenous and exogenous factors. The conversion of testosterone to dihydrotestosterone (DHT) by the 5-α reductase (5-αR) enzyme at the site of the hair follicles and the decrease of the scalp blood flow are the major factors responsible for the development of AGA[13]. Finasteride is FDA- approved medical treatment of AGA, as it blocks 5-αR type Ⅱ enzyme thus inhibiting the production of DHT[14]. As for topical minoxidil, another FDA- approved medical treatment, it depends on opening the K^+ channels located on the blood vessels, resulting in vasodilatation of the scalp blood vessels[8]. Minoxidil promotes the growth of hair follicles, increases the duration and thickness of the anagen follicles, and decreases the telogen follicles thus prevents the anagen phase from shortening[6].

The aim of this study was to evaluate the clinical, trichoscopic and histopathologic changes in male AGA after treatment with topical minoxidil 5%.

There were 15 male patients with AGA of grade (Ⅱ- Ⅵ) according to Norwood-Hamilton classification included in the study; their ages ranged from 23- 40 years with a mean ± SD 28.96 ± 4.35. The disease duration ranged from 1- 13.5 years, with a mean ± SD 3.75 ± 2.48.

In this present study, it was observed that the treatment with topical minoxidil 5% gave moderate improvement on the clinical grading of AGA according to Norwood- Hamilton classification in 60% of patients after 6 months. This result was supported by the studies held by **Ghonemy et al., 2019**[7] and **Pakhomova et al., 2020**[15] which showed 88.8% and 59% improvement, respectively. **Abdel-Raouf et al., 2021**[16] reported improvement in 90% of patient after the application of topical minoxidil 5%.

Trichoscopic monitoring of patients in the present study revealed statistically significant improvement in hair density by 31.7%, as the mean increase at the end of 24 weeks ± SD was 31.84 ± 19.13 per cm^2, in addition to a statistically significant increase in the percentage of terminal hair and reduction in the percentage of vellus hair. **Kumar et al., 2018**[17] reported a mean increase by 1.89 (±8.94) per $inch^2$ in hair density after 12 weeks of treatment. The superiority in the increase noticed in the present study may be attributed to the longer duration of treatment, thus better improvement. The study held by **Ghonemy et al., 2019**[7] showed similar results using topical minoxidil 5% for 36 weeks in the form of an increase in the hair density both in the vertex and frontal areas, as well as an increase in the terminal hair count. Moreover, topical minoxidil 5% has positively affected hair width in the present study, the percentage of hair width increase was 42.56%. This effect was also reported in the study held by **Ghonemy et al., 2019**[7] using topical minoxidil 5%. Another study held by **Ray and Sharma, 2021**[18] reported a positive effect on hair width by 11.24% improvement from the baseline.

The diagnosis of AGA was confirmed by histopathological examination of tissue specimens stained with H&E which were obtained at the start of the study. Miniaturization and variation of the sizes of the hair follicles with an increase in vellus hair count, decrease in dermal papillae size and the presence of fibrous tracts and perifollicular lymphocytic cell infiltrate and collagen deposition were the main features observed. After 24 weeks treatment, there was less miniaturization and variation in sizes of the hair follicles, and a reduction in the fibrous tracts and the perifollicular lymphocytic cell infiltrate. The study held by **Abdel-Raouf et al., 2021**[16] reported similar findings in the form of an increase in the anagen follicles, decrease in telogen and vellus hair and increase in the terminal/vellus ratio.

5. Conclusion

Topical minoxidil 5% is an effective treatment of AGA that positively affects hair density and width, as well as the type of hairs as it increases terminal hair over vellus hair. Histopathologically, it decreases the inflammatory cell infiltrate and fibrous tracts.

CONSENT

Informed consent was obtained from all patients before their participation. Every patient was given a code number and his anonymity was preserved (his personal data was omitted).

ETHICAL APPROVAL

All procedures performed were following the ethical standards of our institutional ethical committee and with the 1964 Helsinki declaration and its later amendments or comparable

ethical standards. The study was approved by the ethical research committee of the faculty of medicine - Tanta University under the code: 33568/12/19

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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ABBREVIATIONS

Androgenetic alopecia (AGA)
United States Food and Drug Administration (FDA)
Vascular endothelial growth factor (VEGF)
Hematoxylin and eosin (H&E)
Dihydrotestosterone (DHT)
5-α reductase (5-αR) enzyme