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Quantitative Morphometric And Histochemical Studies Of Hair Follicles In Alopecia Areata

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Abstract

Alopecia areata (localized hair loss) is a common hair follicle disease with unclear pathogenesis. The aim of this work is to clarify the changes in the hair follicles that may occur during alopecia areata using image analysis. The study was conducted on 20 patients with alopecia areata and 5 healthy volunteers. Biopsies were taken after consent, for quantitative histological morphometric measurements and for histochemical analysis of hair follicles. The study revealed that there was a statistically significant decrease in the mean percent of anagen hair follicles and statistically significant increase in the mean percent of catagen hair follicles in 4mm punch biopsies taken from alopecia patients in comparison to that of normal volunteer. The study showed that there were statistically significant decreases in the following; anagenic and telogenic hair follicles areas, telogenic hair follicles perimeter, anagenic outer sheath thickness, hair follicles irregularity (catagen and telogen), and telogenic hair (diameter, area and perimeter). Also there were statistically significant increases in the following; hair follicles irregularities (anagen and telogen), and anagenic hair perimeter. Regards histochemical studies, there was a statistically significant decrease in the DNA staining affinity of hair follicles of anagen and catagen.

The study concluded that the morphometric quantitative study of tangential histological sections of scalp biopsies with the aid of image analyzer is a feasible and easily technique for differentiation between of alopecia areata and normal hairs and their subtypes.

Introduction

Alopecia areata is a common disorder characterized by limited patchy hair loss which is non-scarring and reversible. (Tobin et al., 1997). The characteristic initial lesion is circumscribed totally bald smooth patch. Genetics constitution, atopic state, non-specific immune and organ specific autoimmune reactions and emotional stress may be considered as factor induces alopecia areata (Gupta et al., 1990). The normal hair follicle undergoes cycle of growth, involution and rest. There are three phases of the hair growth cycle; ***Anagen*** (where the kertinocytes of bulb proliferate rapidly and penetrates deeper into the level of subcutaneous fat), ***Catagen*** (the hair shows gradual thinning and lightening of the pigment at the base of hair shaft, the melanocytes undergo

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apoptosis and the follicular papillae rest at the bottom of the permanent portion of the hair follicles) of and ***Telogen*** (the hair has a club shaped proximal end and is typically shed from the hair follicles) (Lavker et al., 1999). In alopecia areata the majority of hair follicles are in telogen or late Catagen, some anagen hair bulbs are situated at higher levels in the dermis than normal. A peribulbar lymphocytic infiltrate is seen around follicles (Peerebom-Wynia et al., 1989).

Patients and Methods

Twenty patients complaining of alopecia areata (16 males and 4 females) were selected from dermatology outpatient clinic at Kaser El-Aini Hospital. Five healthy volunteers (3 males and 2 females) were selected as control group. A written consent from all individuals was taken. The clinical assessment was performed, fulfilling; clinical type of alopecia, extent and numbers of patches and the presence of other skin lesions. A punch biopsy of $4mm^2$ area was extracted from scalp. The biopsy was subdivided into 2 parts; the first, fixed in neutral buffered formol for paraffin sections preparations, and the second for freezing histochemical preparation. Preliminary longitudinal sections were cut from each sample for determination of stages of the hair follicles. Twenty micrometers thickness paraffin sections were cut serially and tangentially by a microtome at the level of the sebaceous gland. The paraffin sections were stained with Hx and E. stain and Mallory trichrome stain (for quantitative morphometric analysis and collagen content). Methyl green pyronin stain was used for demonstration of DNA staining affinity. The 20μm frozen sections were incubated for acid and alkaline phosphatase enzymes detection. The quantitative morphometric study included (the number of hair follicles and their subtypes per mm^2 surface area, hair follicles (diameter, perimeter, area and irregularity), hair shaft (diameter, perimeter, area and roundness) and root sheath diameters (inner and outer). The measurements were done by the aid of Leica Image Analyzer. The evaluation of DNA staining affinity, acid and alkaline phosphatase enzymes activities were done by image analyzer as mean optical density. The obtained data were tabulated, statistically analyzed using paired student T-test and graphically represented.

Results:

Quantitative morphometric results are summarized in the Tables 1-2 and figures 1-11.

- The mean total hair follicles count in 4mm punch biopsies taken from patients with alopecia was 15.44 ± 4.30, while in the volunteers was 24.56 + 3.97, these decrease was a statistically significant $p<0.01$.
- There was a statistically significant decrease ($p<0.001$) in the percentage of anagen hair follicles, and a statistically significant increase ($p<0.001$) in the percentage of catagen hair follicles, in 4mm punch biopsies of patients with alopecia in comparison to that of volunteers.
- Statistically significant decrease in the mean hair follicle area (anagen and telogen) ($p<0.05$ and $p<0.005$ respectively) of the patients with alopecia in comparison to that of volunteers.

- Statistically significant decrease ($p<0.005$) in the mean telogenic hair follicle perimeter in the patients of alopecia in comparison to that of volunteers.
- Statistically significant decrease ($p<0.05$) in the mean anagenic outer sheath thickness of the patients with alopecia in comparison to that of volunteers.
- The mean hair follicles irregularity was statistically significant increase (anagen and telogen) ($p<0.05$), while in catagen there was a statistically significant decrease ($p<0.05$), in the patients with alopecia in comparison to that of volunteers.
- Statistically significant decrease in the mean telogenic hair diameter and area ($p<0.05$ and $p<0.001$ respectively) of the patients with alopecia in comparison to that of volunteers.
- The mean hair perimeter irregularity was statistically significant increase (anagen) ($p<0.05$), while in catagen there was a statistically significant decrease ($p<0.05$), in the patients with alopecia in comparison to that of volunteers.
- There were non-statistically significant change ($p>0.05$) in the mean optical density of collagen content, acid and alkaline phosphatase enzymes activities in the hair follicles of the patients with alopecia in comparison to that of volunteers.
- There was a statistically significant decrease in mean optical density of DNA staining affinity of anagen and telogen hair follicles ($p<0.001$ and $p<0.005$ respectively) of the patients with alopecia in comparison to that of volunteers.

Table 1- Statiscal Quantitative Morphological Comparison of Hair Follicles in Control and Alopecia

Mean / Type	Control			Alopecia		
	Anagen	**Catagen**	**Telogen**	**Anagen**	**Catagen**	**Telogen**
Hair Count (%)	35.89	12.37	52.91	16.32	32.89	50.80
T-Test	Alopecia VS Control			1.42E-05	2.13E-05	0.316
p-Value				p<0.001	p<0.001	p>0.05
Significance				**Sign. Dec.**	**Sign. Inc.**	**NS**
Hair Follicle Diameter (um)	906.22	217.72	418.02	806.30	225.64	456.62
T-Test	Alopecia VS Control			0.327	0.471	0.362
p-Value				p>0.05	p>0.05	p>0.05
Significance				**NS**	**NS**	**NS**
Hair Follicle Area (um2)	637677.10	84806.07	348902.13	282335.795	121540.68	153129.56
T-Test	Alopecia VS Control			0.049	0.075	0.001
p-Value				p<0.05	p>0.05	p<0.005
Significance				**Sign. Dec.**	**NS**	**Sign. Dec.**
Hair Follicle Perimeter (um)	2669.34	1199.37	2334.34	2039.70	1316.29	1505.78
T-Test	Alopecia VS Control			0.099	0.218	0.002
p-Value				p>0.05	p>0.05	p<0.005
Significance				**NS**	**NS**	**Sign. Dec.**
Outer Sheath Thickness (um)	102.10	98.82	107.72	58.64	256.80	94.31
T-Test	Alopecia VS Control			0.042	0.109	0.161
p-Value				p<0.05	p>0.05	p>0.05
Significance				**Sign. Dec.**	**NS**	**NS**
Inner Sheath Thickness(um)	65.10	27.38	55.39	60.00	63.28	25.11
T-Test	Alopecia VS Control			0.443	0.160	0.058
p-Value				p>0.05	p>0.05	p>0.05
Significance				**NS**	**NS**	**NS**
Hair Follicle Irregularity	0.055	0.254	0.104	0.106	0.160	0.154
T-Test	Alopecia VS Control			0.010	0.027	0.025
p-Value				p<0.05	p<0.05	p<0.05
Significance				**Sign. Inc.**	**Sign. Dec.**	**Sign. Inc.**
Hair Diameter (um)	173.15	69.03	294.09	79.67	58.34	131.69
T-Test	Alopecia VS Control			0.110	0.387	0.032
p-Value				p>0.05	p>0.05	p<0.05
Significance				**NS**	**NS**	**Sign. Dec.**
Hair Area (um2)	33509.33	4754.00	93921.50	7836.32	8360.73	15957.37
T-Test	Alopecia VS Control			0.139	0.146	0.0003
p-Value				p>0.05	p>0.05	p<0.001
Significance				**NS**	**NS**	**Sign. Dec.**
Hair Perimeter (um)	686.31	319.90	1144.58	3583.06	393.64	500.00
T-Test	Alopecia VS Control			0.047	0.125	0.023
p-Value				p<0.05	p>0.05	p<0.05
Significance				**Sign. Inc.**	**NS**	**Sign. Dec.**
Roundness of Hair	0.82	0.57	0.91	0.35	0.66	0.54
T-Test	Alopecia VS Control			0.090	0.270	0.123
p-Value				p>0.05	p>0.05	p>0.05
Significance				**NS**	**NS**	**NS**

Table 2- Statiscal Quantitative Morphological Comparison of Hair Follicles in Control and Alopecia

Mean / Type	Control			Alopecia		
	Anagen	Catagen	Telogen	Anagen	Catagen	Telogen
Mean	Collagen Content					
	1.18	0.92	1.03	1.15	1.08	0.96
SD	0.08	0.06	0.07	0.07	0.07	0.07
SEM	0.02	0.01	0.01	0.02	0.01	0.01
T-Test	Alopecia VS Control			0.077	0.441	0.186
p-Value				p>0.05	p>0.05	p>0.05
Significance				**NS**	**NS**	**NS**
Mean	Acid Phosphatase Enzyme Activity					
	1.19	1.29	1.13	1.28	1.33	1.19
SD	0.07	0.07	0.07	0.07	0.06	0.06
SEM	0.03	0.03	0.02	0.03	0.02	0.02
T-Test	Alopecia VS Control			0.239	0.900	0.165
p-Value				p>0.05	p>0.05	p>0.05
Significance				**NS**	**NS**	**NS**
Mean	Alkaline Phosphatase Enzyme Activity					
	1.78	1.36	1.52	1.64	1.33	1.64
SD	0.05	0.06	0.05	0.07	0.07	0.06
SEM	0.02	0.02	0.01	0.02	0.02	0.02
T-Test	Alopecia VS Control			0.950	0.866	0.789
p-Value				p>0.05	p>0.05	p>0.05
Significance				**NS**	**NS**	**NS**
Mean	DNA Staining Affinity					
	1.21	0.92	1.03	1.03	0.84	1.03
SD	0.06	0.04	0.04	0.03	0.06	0.04
SEM	0.02	0.01	0.01	0.01	0.02	0.01
T-Test	Alopecia VS Control			3.78E-07	2.36E-03	0.405
p-Value				p<0.001	p<0.005	p>0.05
Significance				**Sign. Dec.**	**Sign. Dec**	**NS**

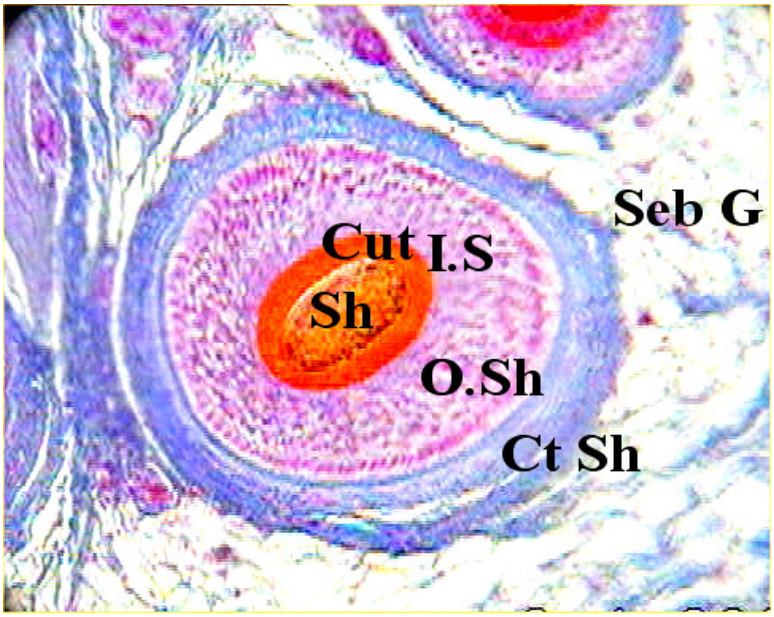


Figure – 1 Computerized photomicrograph of transverse section in normal human scalp skin (Sh=hair shaft, Cut=Cuticle, I.S=Inner sheath, O.Sh=Outer sheath, Ct Sh= Connective tissue sheath, Seb G= Sebaceous gland) (Mallory trichrome stain X 1000)

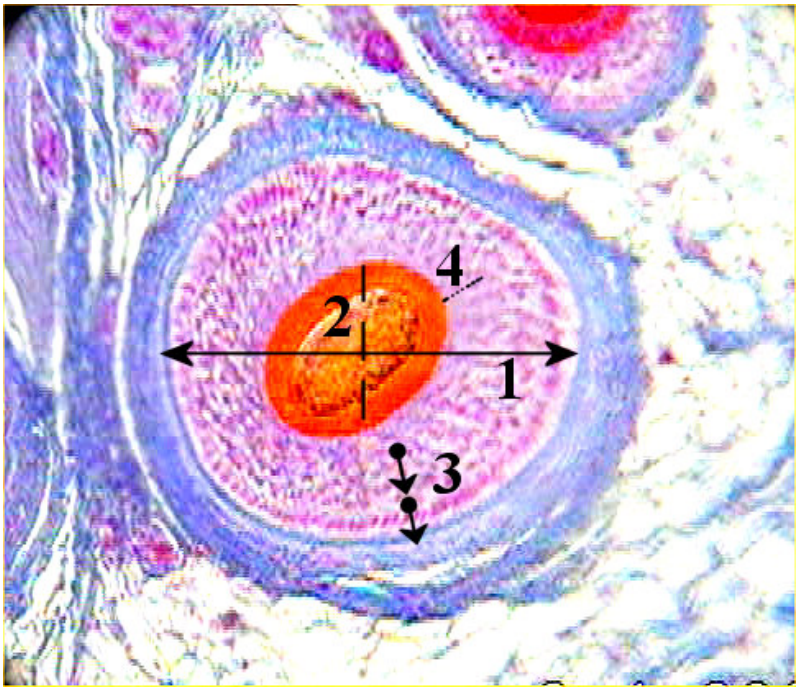


Figure – 2 Computerized photomicrograph of transverse section in human scalp skin (1=Hair follicle diameter, 2=Hair shaft diameter, 3=Outer root sheath diameter, 4=Inner root sheath diameter) (Mallory trichrome stain X 1000)

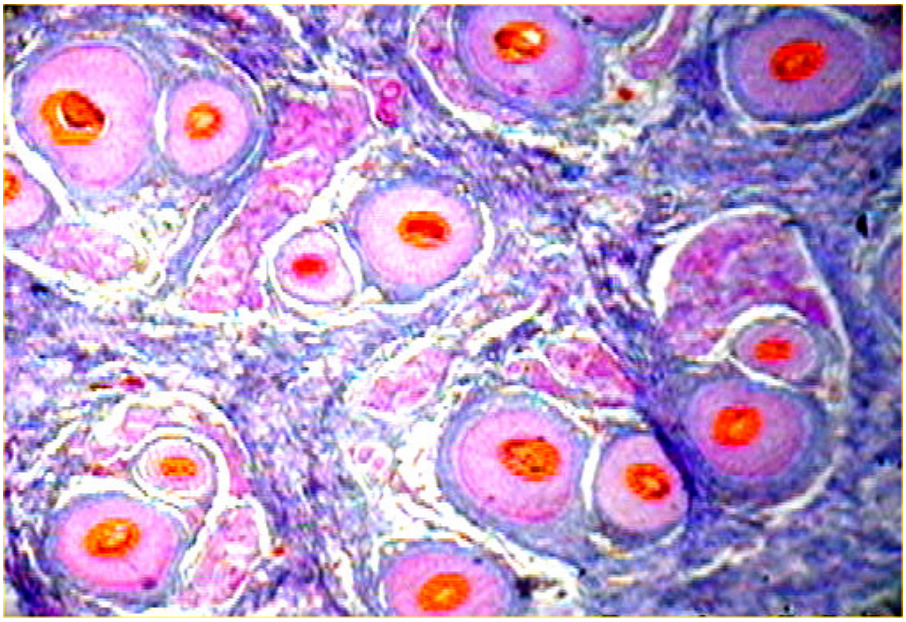


Figure – 3 Computerized photomicrograph of transverse section in normal human scalp skin, showing regular rounded hair follicle and hair shaft at the level of pilosebaceous appendage. (Mallory trichrome stain X 300)

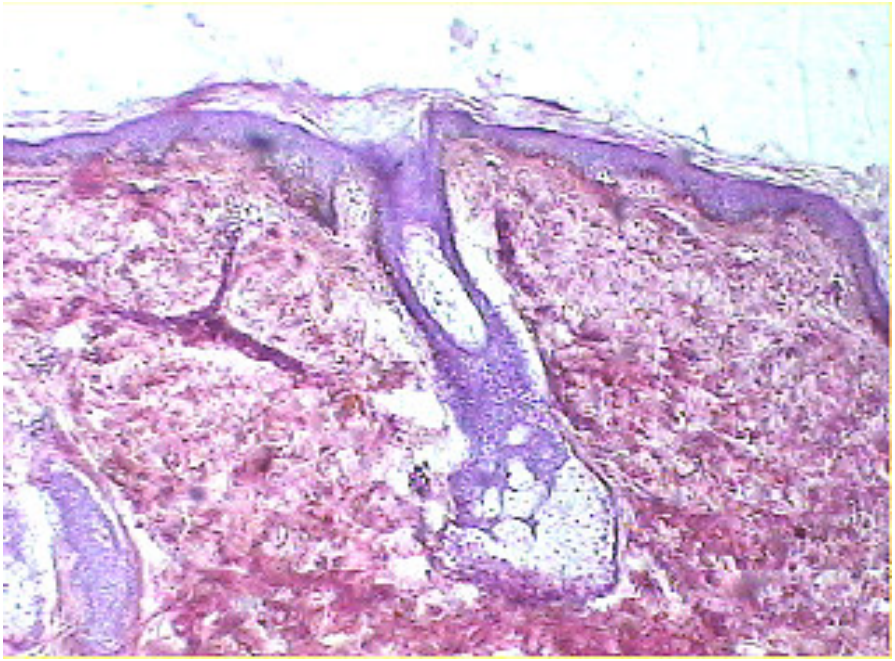
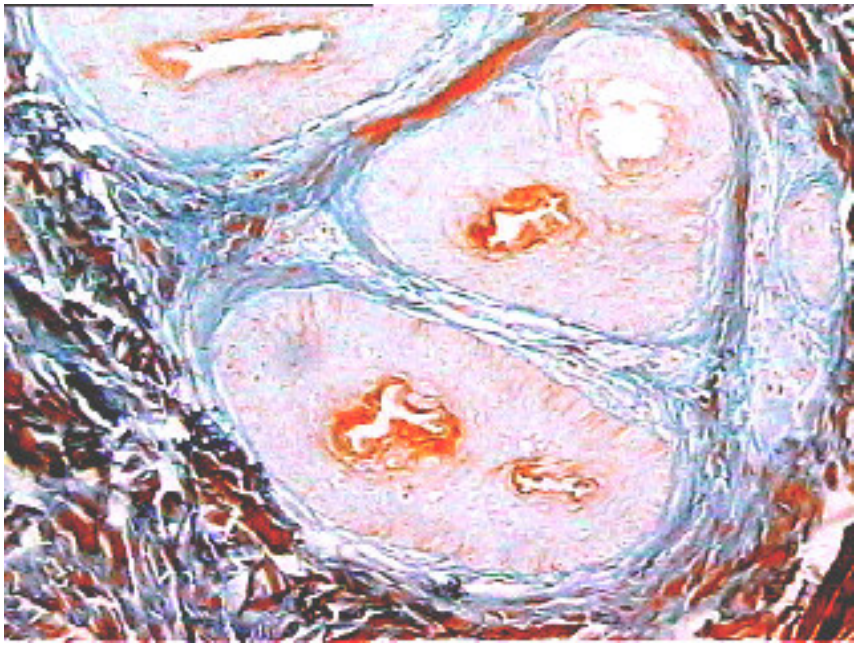
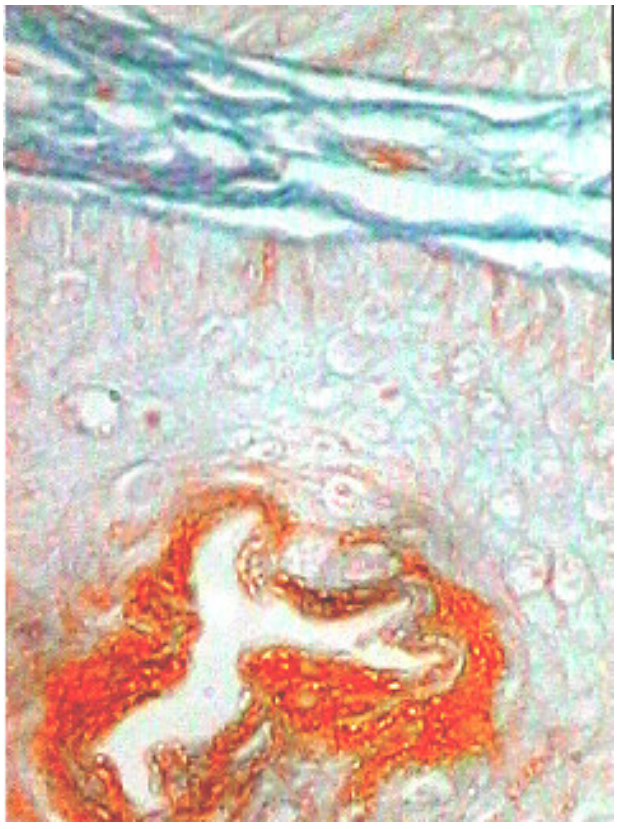


Figure – 4 Computerized photomicrograph of longitudinal section in human scalp skin of patient with alopecia areata, showing catagenic hair follicle above the sebaceous gland. (Hx and E., stain X 450)

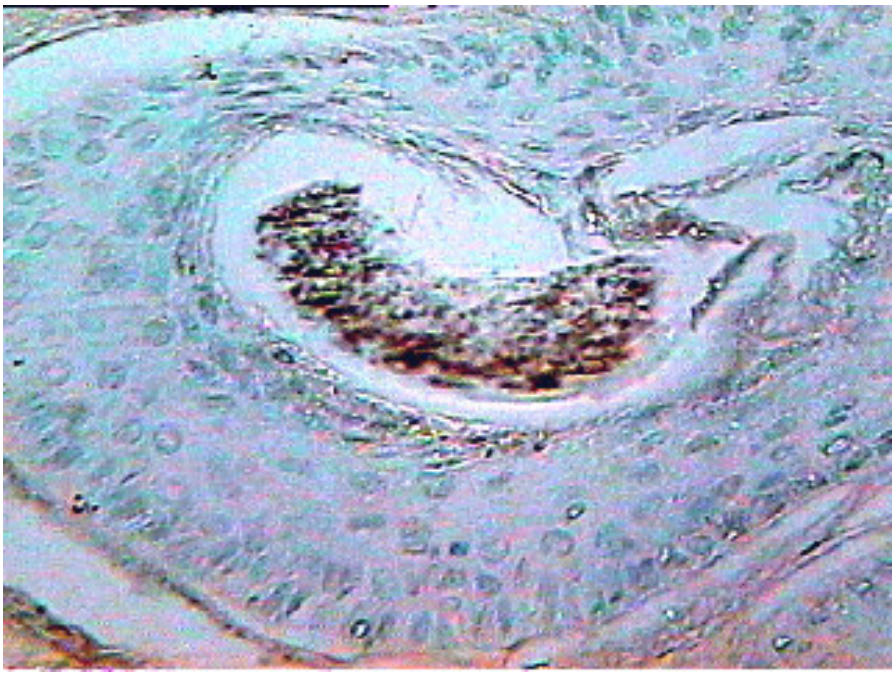


A

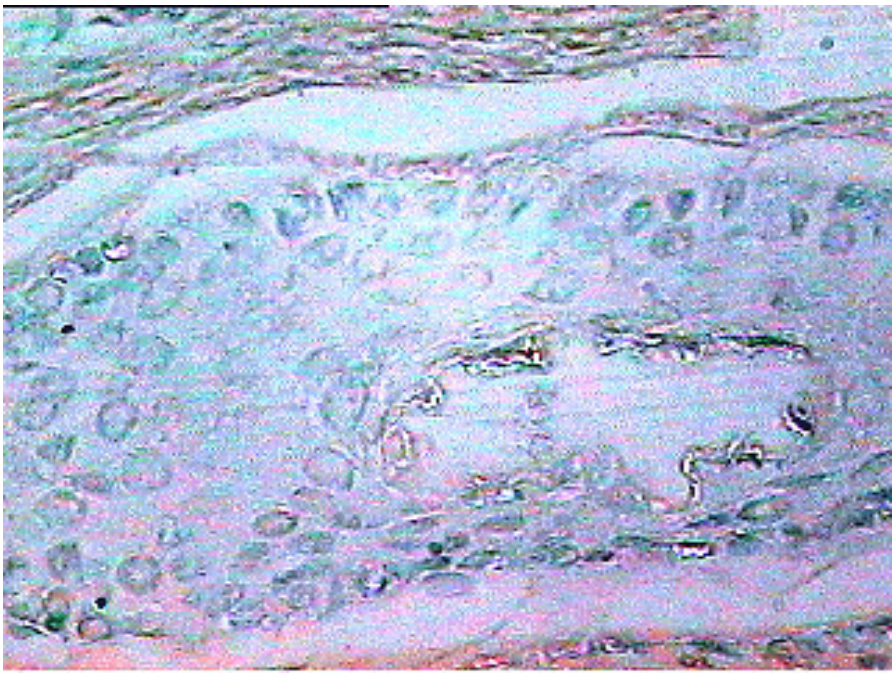


B

Figure –5(A and B) Computerized photomicrograph of transverse section in human scalp skin of patient with alopecia areata, showing 2 catagenic hair follicles showing the most of hair follicles had been replaced by empty fibrous tracts, the residua of previous cycling elements extending from the subcutis. (Mallory trichrome stain X 450 and 1000)



A



B

Figure – 6(A and B) Computerized photomicrograph of transverse section in human scalp skin of control (above) and patient with alopecia areata (below), showing a decrease DNA staining affinity of catagenic hair follicle in alopecia in comparison to control. (Methyl green pyronin stain X 1000)

Fig .7- Comparison of Hair Follicles Types, Diameter and Areas in Control and Alopecia

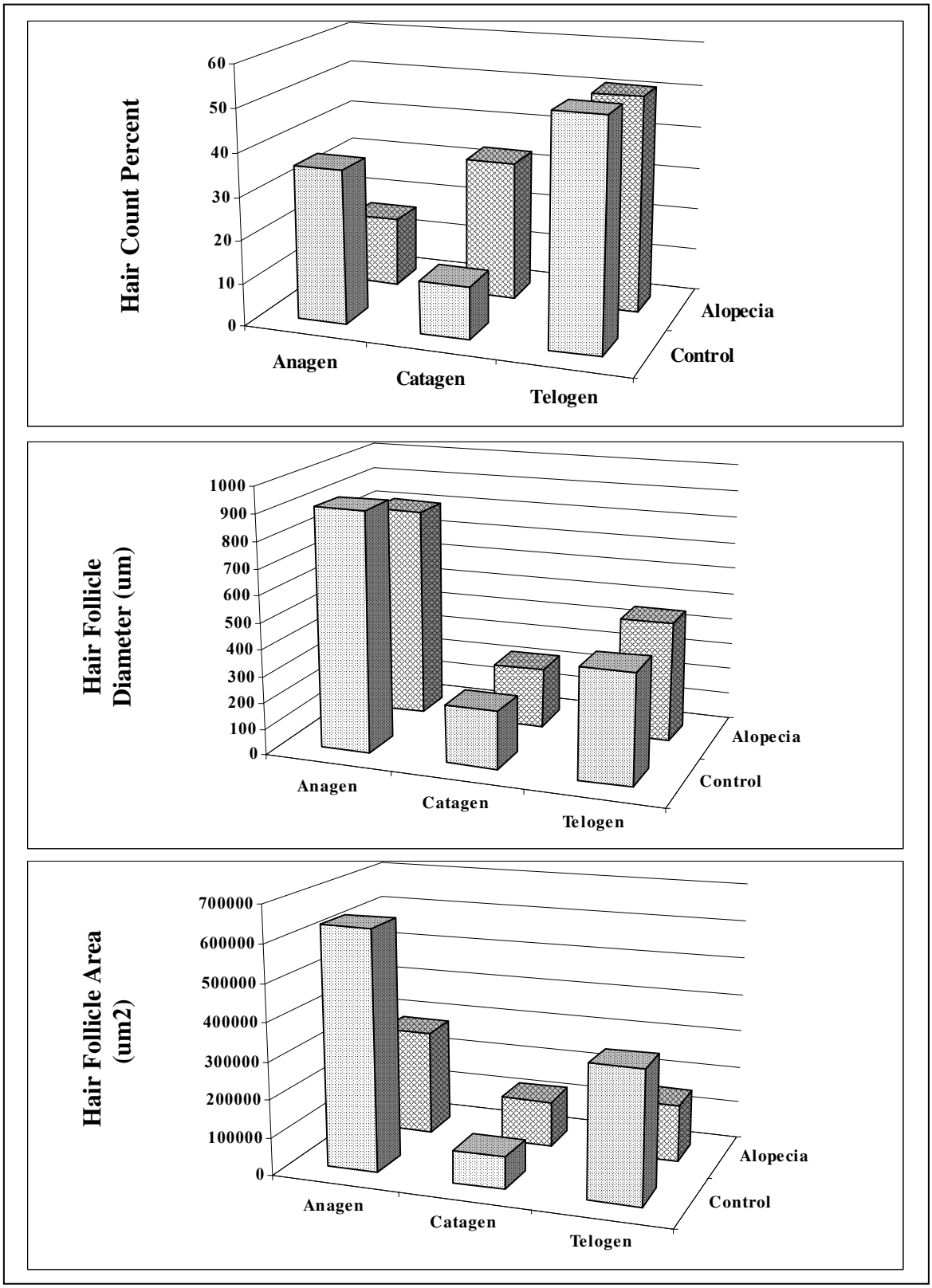


Fig .8- Comparison of Hair Follicles Perimeter, Outer and Inner Hair Root Sheath Diameters in Control and Alopecia

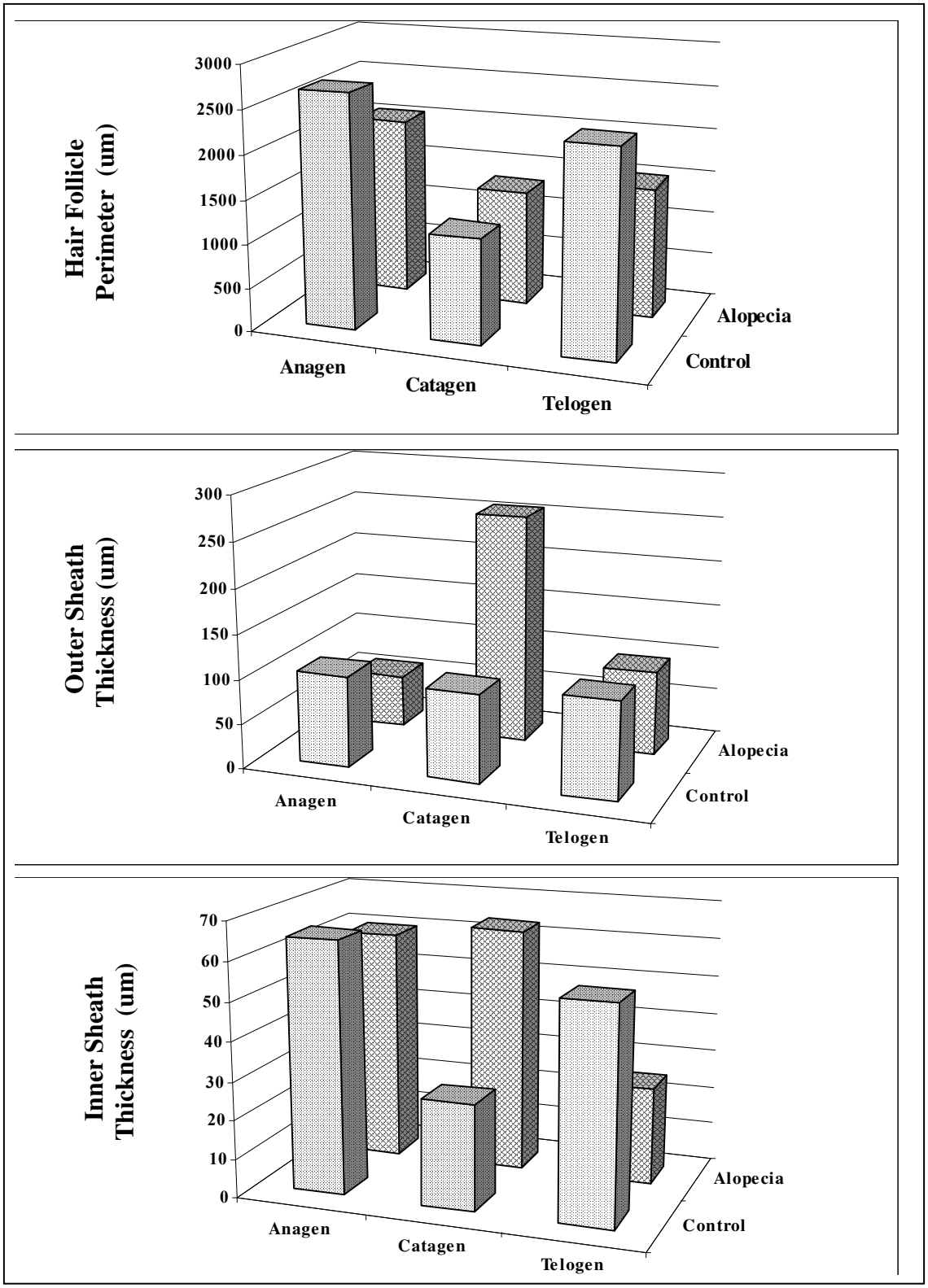


Fig .9- Comparison of Hair, Diameter, Areas and Perimeter in Control and Alopecia

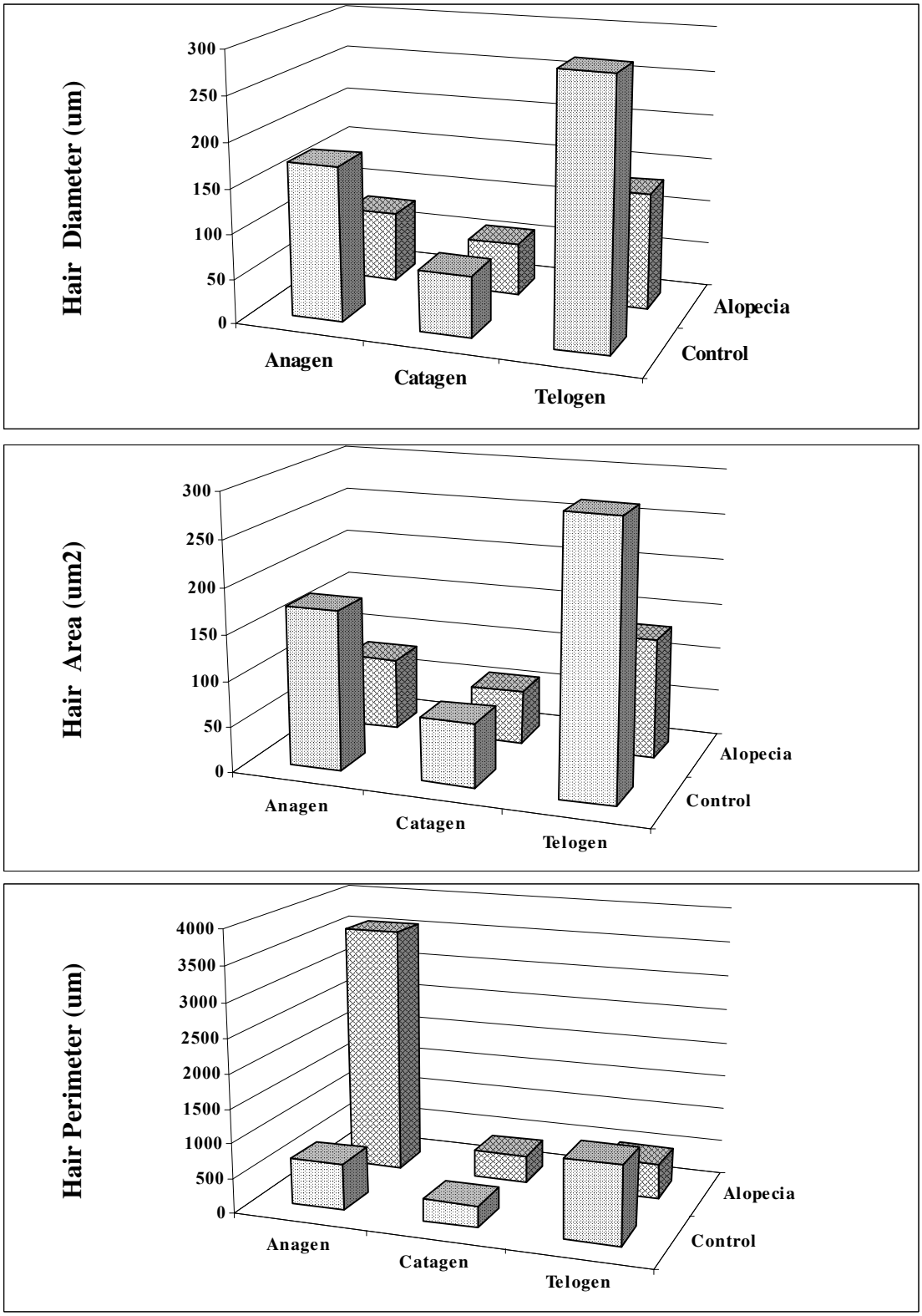


Fig .10- Comparison of Hair Follicles Irregularity, Hair Roundness and Collagen Content in Hair Follicles of Control and Alopecia

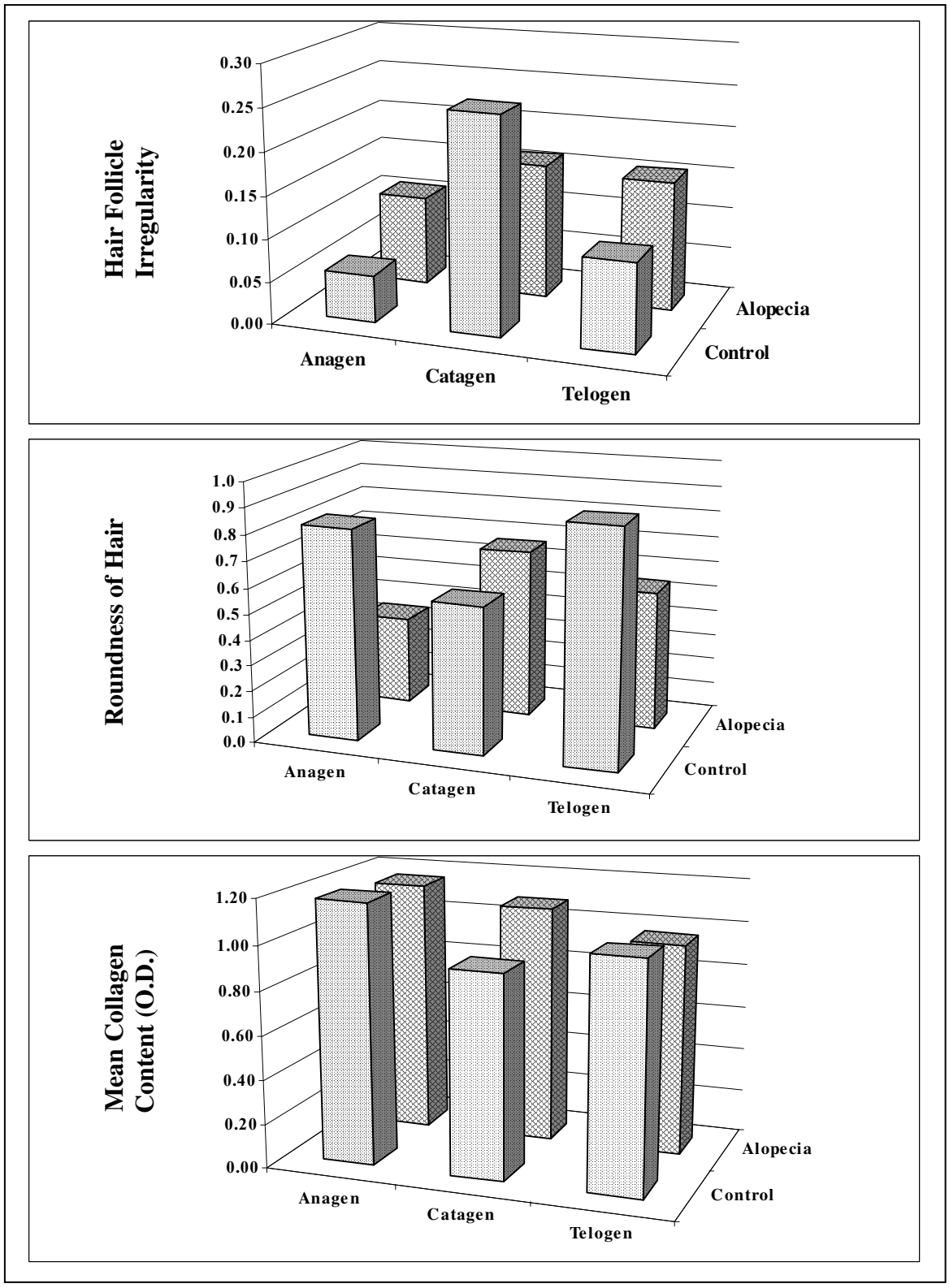
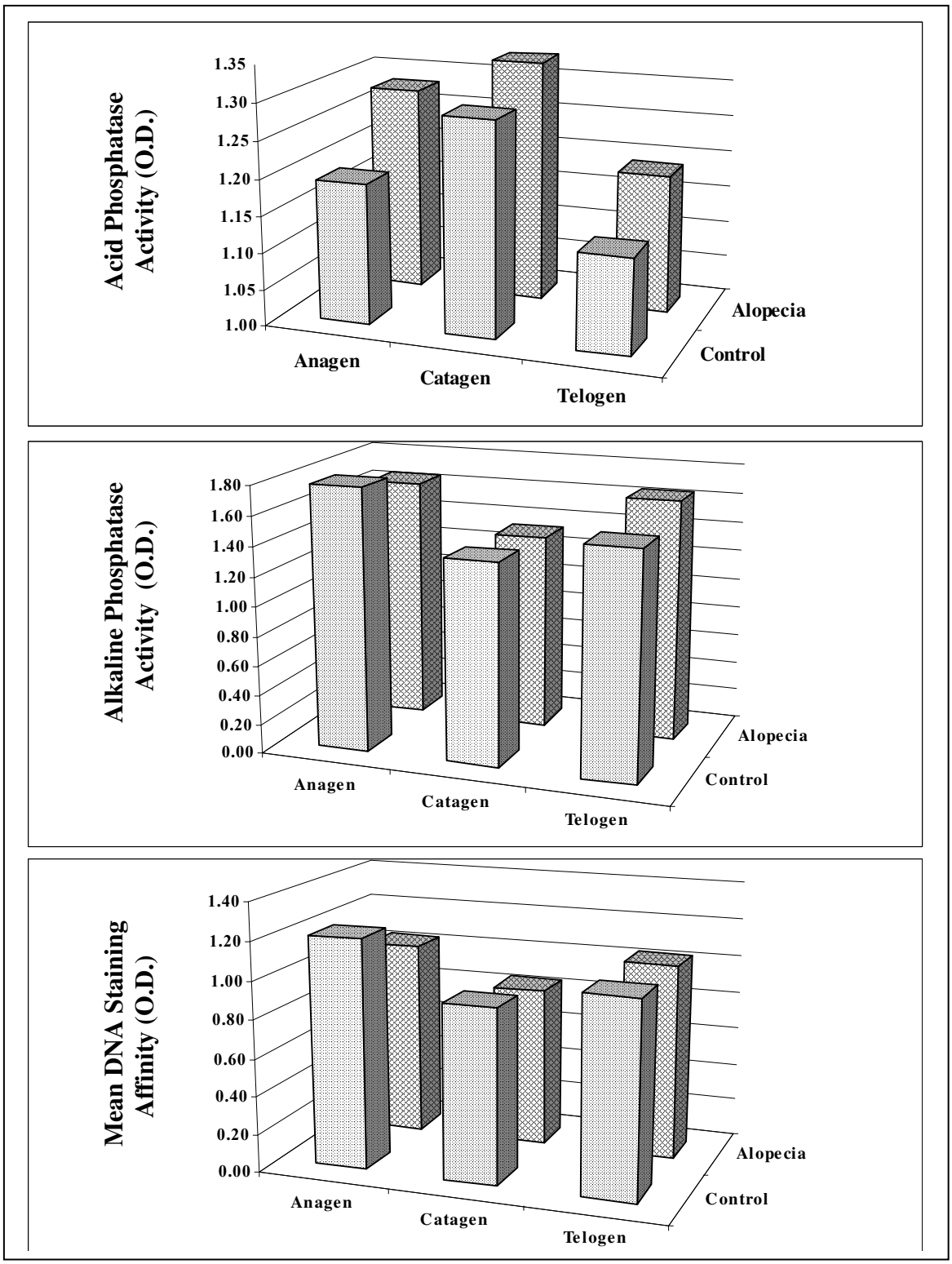


Fig .11- Comparison of Acid, Alkaline Phosphatase Enzymes Activities and DNA Staining Affinity Of Hair Follicles of Control and Alopecia



Discussion

Transverse sectioning of scalp biopsies taken from patients with alopecia areata gives a simultaneous overview of many hair follicles. This technique requires sectioning at several levels of skin since hair follicles present in different depths, depends on the type of hair and part of the cycle they are in. (Olsen et al., 1999).

Whiting, (1995) reported that transverse sections of scalp biopsies in alopecia areata provide more diagnostic and suitable information for the quantitative and morphometric analysis of hair structures than longitudinal sections.

Kim et al., (1999) stated that quantitative assessment of hair follicle morphology using image analyzer is particularly valuable for the diagnosis and disease progress of hair disorders and represents a methods of quantifying the effective uses of hair growth promoters in clinical trials.

The present study showed a significant decrease in the mean total count of terminal hairs, with variations in hair follicles types' percentage, in 4mm punch biopsy of patients with alopecia areata in comparison to that of control and these findings were consistent with the findings of Whiting (1995). However, Kim et al., (1999) showed that there was a little difference between the numbers of terminal hairs in both patients with alopecia areata and normal persons, and the proportions of anagen and telogen hairs were significantly higher in the areata areas than in the controls.

Lavker et al., 1999, showed that the biopsies taken from the edges of expanding bald patches were in late catagen and telogen and the proportion of follicles in catagen to telogen was at least 50% and in one biopsy was 100%. Also he stated that, in the early course of the disease the majority of follicles were in telogen or late catagen, while in established lesions there was no reduction in overall follicle numbers.

The study showed a significant decrease in mean value of anagen hair follicles area, and outer sheath thickness, and a significant increase in mean hair follicle irregularity and mean hair shaft diameter in the patients of alopecia in comparison to that of anagen hair follicles of volunteers.

Regards catagen hair follicles, there was a significant decrease in the hair follicles irregularity in comparison of that of catagen hair of volunteers.

The telogen hair follicles showed a statistically significant decrease in the; hair follicles (area and perimeter), and an increase in the hair follicle irregularity. The hair shaft showed a statistically significant decrease in diameter, perimeter and area in comparison of that of telogen hair of volunteers.

Kim et al.,(1999), used image analysis and found a significant decrease in diameter of hair shaft, which is consistent with the present study results. However, the thickness of inner root sheath and diameter of outer root sheath of patient with alopecia were significantly smaller than in control group in the work of Kim et al.,(1999), while in the present study there was non significant difference between 2 parameters in both groups.

Messenger et al (1986) found that hair follicles in the patients of alopecia were generally smaller than normal follicles, and this more pronounced in longstanding disease.

In the present study, histochemical analysis of acid and alkaline phosphatase enzymes activities in patients with

alopecia areata showed non statistically significant change with that of normal hair follicles.

Lutz et al., (1991) had shown reduced or absent alkaline phosphatase activity of hair follicles in the early stages of alopecia areata. The biological function of alkaline phosphatase in the hair follicle metabolism, growth, differentiation and remodeling has remained obscure.

The fact that alkaline phosphatase activity tends to be high in metabolically active tissues suggests a role for alkaline phosphatase in the tissue remodeling associated with cyclical hair follicle growth.

Handjiski et al., (1994), reported that the pilosebaceous unit displays a prominent alkaline phosphatase activity in patients with alopecia areata, for unknown reasons. This finding could not be verified in the present study.

Vermorken et al., (1978) found in their study in their study done on 121 individual normal hairs roots a considerable variation in acid phosph - atase activity.

The DNA staining affinity was statistically significant decreased in the anagen and catagen hair follicles of patients with alopecia in comparisons of control. These decrease may be accomp - anied the hydropic degeneration of basal cells of hair follicles of patient with alopecia.

Conclusions:

Transverse sections of scalp biopsy provide more information than longitudinal sections.

Image analyzer is a feasible technique for the assessment of hair disorders.

The value of histochemical analysis of acid and alkaline phosphatase enzymes give a little or no information on hair follicles in alopecia areata, while DNA staining affinity may be of value in diagnosis of progress of disease

Recommendations:

Morphometric image analysis is a useful technique to be applied to hair disorders biopsy and to determine the stage of the disease.

References

1. **Gupta AK, Cooper KD, and Ellis CN.,(1990):** Lymphocytic infiltrates of the skin in association with cyclosporine therapy. J Am Acad. Dermatol.; 23: 1137-41
2. **Handjiski BK, Eichmuller S, Hofmann V, Czarnetzki BM and Paus R., (1994):** Alkaline phosphatase activity and localization during the murine hair cycle. Br. J. Dermatol.; 131: 303-10.
3. **Kim IH, Jo HY, Cho CG, Choi HC and Oh CH (1999):** Quantitative image analysis of hair follicles in alopecia areata. Acta Derm. Venereol (Stockh.); 79: 214-16.
4. **Lavker RM, Bertolino AP, Freedberg IM, and Sun TT (1999):** Biology of hair follicles. In Fitzpatrick's Dermatology In General Medicine. New York, McGraw-Hill , p. 230-238.
5. **Lutz G, Dauer H, Zimmerman-Brunzel A and Kaiser HW, (1991):** (Translated from German): Reduzierte Aktivitat der alkalischen phosphatase bei alopecia areata. Z Hautkr; 66: 702-707.
6. **Messenger AG, Slater DN, and Bleehen SS (1986):** Alopecia areata: alterations in the hair growth cycle and correlation with the follicular pathology. Br J Dermatol.; 114 : 337-347.

7. **Olsen EA, Hordinsky M, McDonal-Hull S, Price V, Roberts J, Shapiro J and Stenn K,(1999):** Alopecia areata investigational assessment guidelines. J Am. Acad. Dermatol. 40: 242-6
8. **Peerebom-Wynia JDR, Koerten HK and Van Joost TH, (1989):** Scanning electron microscopy comparing exclamation mark hairs in alopecia areata with normal hair fibers, mechanically broken by traction. Clin. Exp. Dermatol.; 14: 47-50
9. **Tobin JD, Sundberg JP, King LE, Boggess D and Bystryn JC (1997):** Autoantibodies to hair follicles in C3H/HeJ mice with alopecia areata like hair loss. J. Invest. Dermatol.; 109: 329-333
10. **Vermorken AJM, Weterings PJJM, Spierenburg GT, Bennekom CA, Wirtz P, Bruyn CHMM and Oei TL (1978):** Fabry's disease: Biochemical and histoc - hemical studies on hair roots for caries detection. Br. J Dermatol; 98: 191-196
11. **Whiting DA (1995):** Histopathology of alopecia areata in horizontal sections of scalp biopsies. J. Invest. Dermatol.; 104: 268-278.

دراسة كمية مورفومترية وهستوكيميائية لبصيلات الشعر فى مرض الثعلبة

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مرض الثعلبة مرض شائع يصيب بصيلات الشعر ويظهر على شكل مساحة خالية من الشعر دون ان تترك ندبة على فروة الرأس .

يظهر مرض الثعلبة أما على شكل مساحة محددة خالية من الشعر أو فى صورة تساقط كامل للشعرفوق فروة الرأس أو تساقط الشعر فوق جميع انحاء الجسم ويعود الشعر للنمو مرة اخرى فى اغلب الحالات .

لازال السبب الرئيسى لسقوط الشعرفى مرض الثعلبة غير محدد،وان كان ضمن العوامل المفترضة :عوامل وراثية ، واجسام مناعية ضد بصيلات الشعر وقد يلعب الضغط العصبى أيضا دورا فى ظهور المرض .

وقد أجريت هذة الدراسة الاحصائية على عشرين مريض بداء الثعلبة لدراسة التغيرات التى تحدث فى الدورة الطبيعية للشعر خلال المرض ونشاط الأنزيمات المختلفة فى بصيلات الشعر المصابة بداء الثعلبة .

وقد اخذت عينات من فروة الرأس من المرضى من خمسة أشخاص أصحاء كمجموعة ضابطة ، وتم تقطيعها عرضيا ثم تحليلها عن طريق جهاز تحليل الصورة بالكمبيوتر لمقارنة التغيرات الكمية والمورفومترية و الهستوكيميائية فى بصيلات الشعر . وقد اظهرت الدراسة الكمية انخفاض عدد بصيلات الشعر فى المرضى مقارنة بالأصحاء ، واظهرت الدراسة المورفوترية تناقص مساحة ومحيط بصيلة الشعر وكذلك مساحة الشعرة فى المرضى عنها فى الأصحاء ، بينما تزايد عدم انتظام البصيلة فى المرضى المصابين بداء الثعلبة . أما الدراسة الهستوكيميائية ، فقد أظهرت عدم وجود فارق ذو دلالة أحصائية فى نشاط كل من انزيمى الفوسفاتاز الحمضى والقلوى وكمية الكولاجين فى بصيلات الشعر فى كل من الاشخاص الأصحاء والمرضى . وبالنسبة الى قابلية الحمض النووى (DNA) فى بصيلات الشعر الى الصبغ فقد أظهرت الدراسة عن وجود أنخفاض ذو دلالة أحصائية فى المرضى مقارنة بالأصحاء.