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ORIGINAL PAPER

Hair follicle changes following intense pulsed light axillary hair reduction: histometrical, histological and immunohistochemical evaluation

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Abstract Intense pulsed light (IPL) has been used for years in hair reduction; however, no previous studies discussed quantitative histological and immunohistochemical changes of hair follicles after IPL. Accordingly, this study aims to objectively quantify histological and immunohistochemical changes of hair follicles after IPL hair reduction. Right axillae of 21 volunteers were subjected to 6 IPL sessions using Quanta system IPL and evaluated at 1 week and 1 month after last session (i.e., 3 and 4 months from the start of treatment, respectively) in comparison to baseline and left control axillae. Using hair count, histological and immunohistochemical assessment of vertical and serial transverse sections coupled with computerized morphometric analysis, determination of hair reduction percentage, measurement of hair shaft (HS) diameter, calculation of percentage of hair follicle types and quantitative evaluation of PCNA, Ki67 and P53 markers were performed. After IPL, there was significant decrease of hair count, HS diameter, percentage of terminal anagen follicles, terminal/vellus (T/V) ratio, anagen/telogen (A/T) ratio and expression of PCNA and Ki67; however, significant increase of percentage of terminal telogen and total vellus follicles with vellus-like type and P53 expression was identified. So, reduction of hair number and thickness occurred after IPL by induction of telogenesis and miniaturization through decreased hair follicle proliferation and increase in DNA damage that could favor apoptosis.

Keywords Hair reduction · Intense pulsed light · Ki67 · P53 · PCNA · Proliferation

Introduction

Normal but unwanted hair growth is cosmetically uncomfortable to many people. Accordingly, various devices have been used for hair reduction [1]. Intense pulsed light (IPL) provides lower cost and more flexible alternative to laser, while delivering similar clinical results [2, 3]. IPL technology uses high-output flash lamps to emit polychromatic, non-coherent, high-intensity pulsed broadband light (400–1200 nm), which is modified by application of band pass filters to pass the determined wavelength spectrum with blocking emission of shorter wavelength light [2, 4].

The working basis of IPL hair removal rests on the principle of selective photothermolysis in which light, absorbed by the target chromophore (melanin) in hair shaft (HS) and hair bulb of anagen hair follicles, produces thermal energy destroying hair-producing papilla with sparing the epidermal melanin [5].

The hair growth cycle is composed of three stages including anagen, catagen and telogen. The effects of IPL on hair follicles are still confusing, with many theories ranging from induction and prolongation of telogen stage [2], induction of miniaturization [6] up to follicular necrosis [5].

On reviewing the literature, there were no evidence-based publications that discussed IPL effects on objective histological backgrounds using serial transverse sectioning

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technique for quantitative evaluation of follicular types at the most suitable level. However, some IPL studies performed transverse sections, but without serial cutting and assessment at multiple levels [5, 6]. Moreover, little is known about molecular mechanisms driving hair follicle proliferation and regression after IPL. Accordingly, the present study aims to objectively quantify histological and immunohistochemical changes of hair follicles after IPL for hair reduction.

Patients and methods

Patients

The present study included 21 female healthy volunteers with dark black hairs, seeking for hair reduction of normal but unwanted axillary hairs. They were selected from Dermatology Out-patient Clinic of Minia University Hospital. Their age ranged from 17 to 40 years. Females with signs of hyperandrogenism (severe acne vulgaris, hirsutism and androgenetic alopecia) were excluded from the study. An informed consent was taken from each individual and the study was approved by Scientific Committee for Postgraduate Studies and Research of faculty of Medicine, Minia University.

Treatment protocol

All right axillae were treated with 6 IPL sessions at 2-week intervals, using Quanta IPL system, which has high-intensity pulsed flash lamp and three filters (400, 590 and 625 nm) with spectral output of 400–1200 nm (QLJ0034-0204, Eterna Giovinezza, Italy). Meanwhile, this study was carried out by 625-nm cut-off filter, which was specified for hair removal, eliminating wavelengths lower than 625 nm. This means that the emitted wavelengths range from 625 up to 1200 nm (the peak emission of the energy lies between 625 and 825 nm). According to the device manufacturer, the pulse train profile used for photoepilation consisted of three successive sub-pulses (10-ms pulse duration for each) with interpulse delay (pulse interval) between each one of 30 ms or more. The fluence ranged from 23 to 37 J/cm^2. Left axilla was shaved every session and served as a control.

Clinical, histometrical and histological assessment were performed for both axillae at baseline, 1 week (i.e., 3 months from the start of treatment) and 1 month after last treatment session (i.e., 4 months from the start of treatment) by two blinded independent dermatologists. Meanwhile, immunohistochemical evaluation was done for specimens of right axillae of ten volunteers at same points of examination.

Clinical assessment

The mean hair count was calculated in five areas of each axilla, using 1-cm^2 grid. The percentage reduction in hair count was calculated:

$$\frac{\text{Hair count at baseline} - \text{Hair count after treatment} \times 100}{\text{Hair count at baseline}}$$

Histometrical assessment

Hairs were pulled out and mounted on glass slide with immersion oil. A computer-assisted program (analysis® Five Olympus Soft Imaging Solutions GmbH, Johann-Krane-Weg 39, D-48149, Munster, Germany) was employed for measurement of mean HS thickness by obtaining five measurements for each hair.

Histological assessment

Skin biopsy specimens were obtained, using 4-mm punch probes from all volunteers. Each biopsy was immediately fixed in 10% formalin and embedded in paraffin block. Biopsies were sectioned with ordinary microtome into 5-µm sections both vertically (parallel to long axis of hair follicle) and transversely (perpendicular to long axis of follicle and parallel to epidermis down towards subcutis). Sections, from different levels, were stained with H&E and immunohistochemical markers. Light microscope [AccuScope #3025 five headed (A3025-5), Olympus, Tokyo, Japan] with a built-in camera (digital camera E-330 SLR, Olympus) was used.

Longitudinal sections were used for observation of hair follicle types and inflammatory infiltrate. Meanwhile, transverse sections were evaluated at four levels. At infundibular level, total follicular number and appearance of infundibulum were demonstrated. At sebaceous level, follicular units' appearance and count of total terminal and vellus follicles (true vellus and vellus-like) as well as calculation of terminal/vellus ratio (T/V) were identified. At eccrine level, count of terminal anagen, catagen, telogen and vellus-like follicles and calculation of anagen/telogen ratio (A/T) were performed. Subcutis level showed bulbs of terminal anagen follicles, if present. The percentage of any follicle type was determined:

$$\frac{\text{The count of any type of hair follicle} \times 100}{\text{The count of total hair follicles}}$$

Immunohistochemical assessment

Sections were stained according to the manufacturer's instructions with: monoclonal mouse anti-human PCNA [7] (MS-106-P, Lab Vision, Fremont, CA, USA, diluted

at 1:100), Ki67 [5] (275R-16, Cell Marque, Rocklin, CA, USA, diluted at 1:100) and P53 protein [7] (N1581, ready-to-use 1ry antibody, DAKO, USA). A ready-to-use detection system was used (K0673, DAKO LSAB2 system, peroxidase, DAB, DAKO).

The expression of the three markers was nuclear and evaluated in accordance with the scoring system of Liang et al. [8]. This score ranged from 0 to 3 for both degree of positivity (percentage of positively stained cells: 0, <1%; 1, 1–10%; 2, 10–50%; 3, >50%) and degree of staining intensity (faint-brown [score 1] to deep brown [score 3]). The sum of the two scores was taken as level of expression, which was calculated for all follicles in the section with determination of its mean value.

Statistical analysis

Data were statistically analyzed using SPSS for windows (version 16.0.1, Chicago, IL, USA). Mean ± standard deviation (SD) was calculated for quantitative data; however, qualitative data were expressed as percentage. Chi-square test was used to examine relation between qualitative variables (e.g., percentage of biopsies showing follicular plugging). For not normally distributed quantitative data, non-parametric tests were applied as Mann–Whitney test in comparison between two axillae, Wilcoxon test in comparison between two related samples (e.g., before and after treatment in right axilla) and Friedman test in comparing more than two samples (before, after 1 week and after 1 month). Correlation coefficient using Pearson's formula (r) was used to reveal association between different variables (positive or negative). Statistical significance for all tests was defined as $P \leq 0.05$.

Results

All the included female healthy volunteers (21) completed the study.

Clinical results

At 1 week and 1 month after IPL, right axillae showed significant decrease in hair count compared to baseline and control ($P \leq 0.05$), but with significant increase at 1 month compared to 1 week ($P \leq 0.05$). Accordingly, the mean reduction percentage was 89.26 ± 13.51% and 75.90 ± 17.40% after 1 week and 1 month, respectively, with significant decrease after 1 month ($P = 0.01$) (Fig. 1; Table 1). Most of re-grown hairs after IPL appeared finer and lighter than untreated ones.

Histometrical results

At right axilla, HS diameter was significantly decreased at 1 week and 1 month after IPL compared to baseline and control ($P \leq 0.05$), with further significant decrease after 1 month ($P \leq 0.05$) (Fig. 1; Table 1).

Histological results

In longitudinal sections, pre-treated biopsies showed more terminal anagen follicles; however, terminal catagen, telogen and vellus follicles were observed after IPL (Fig. 2). In transverse sections, different hair follicles types were identified at four levels (Figs. 3, 4; Table 2; Supplemental Table 1).

Infundibular level

After IPL, right axillae showed insignificant decrease of total follicular number ($P > 0.05$). Meanwhile, most follicular infundibula, in 19 post-treated biopsies (90.5%), showed complete keratotic plugging and small, homogenized or retracted intraluminal HSs compared to baseline [5 biopsies (23.8%); $P < 0.001$] and control [after 1 week, 4 biopsies (19.04%); after 1 month, 5 biopsies (23.8%); $P < 0.001$] with smaller hair cavities in right axilla at 1 month after IPL.

Sebaceous level

Follicular units were roughly hexagonal, surrounded by collagen network and contained terminal and vellus follicles with sebaceous ducts and glands. At 1 week and 1 month after treatment, right axillae showed significant decrease in terminal follicle percentages and T/V ratio in addition to significant increase in vellus follicle percentages compared to baseline and control ($P \leq 0.05$) with subsequent significant change at 1 month after IPL ($P \leq 0.05$).

Eccrine level

At 1 week and 1 month after treatment, right axillae showed significant decrease in terminal anagen percentages and A/T ratio and significant increase in terminal telogen percentages ($P \leq 0.05$) without significant change in terminal catagen percentages ($P > 0.05$) compared to baseline

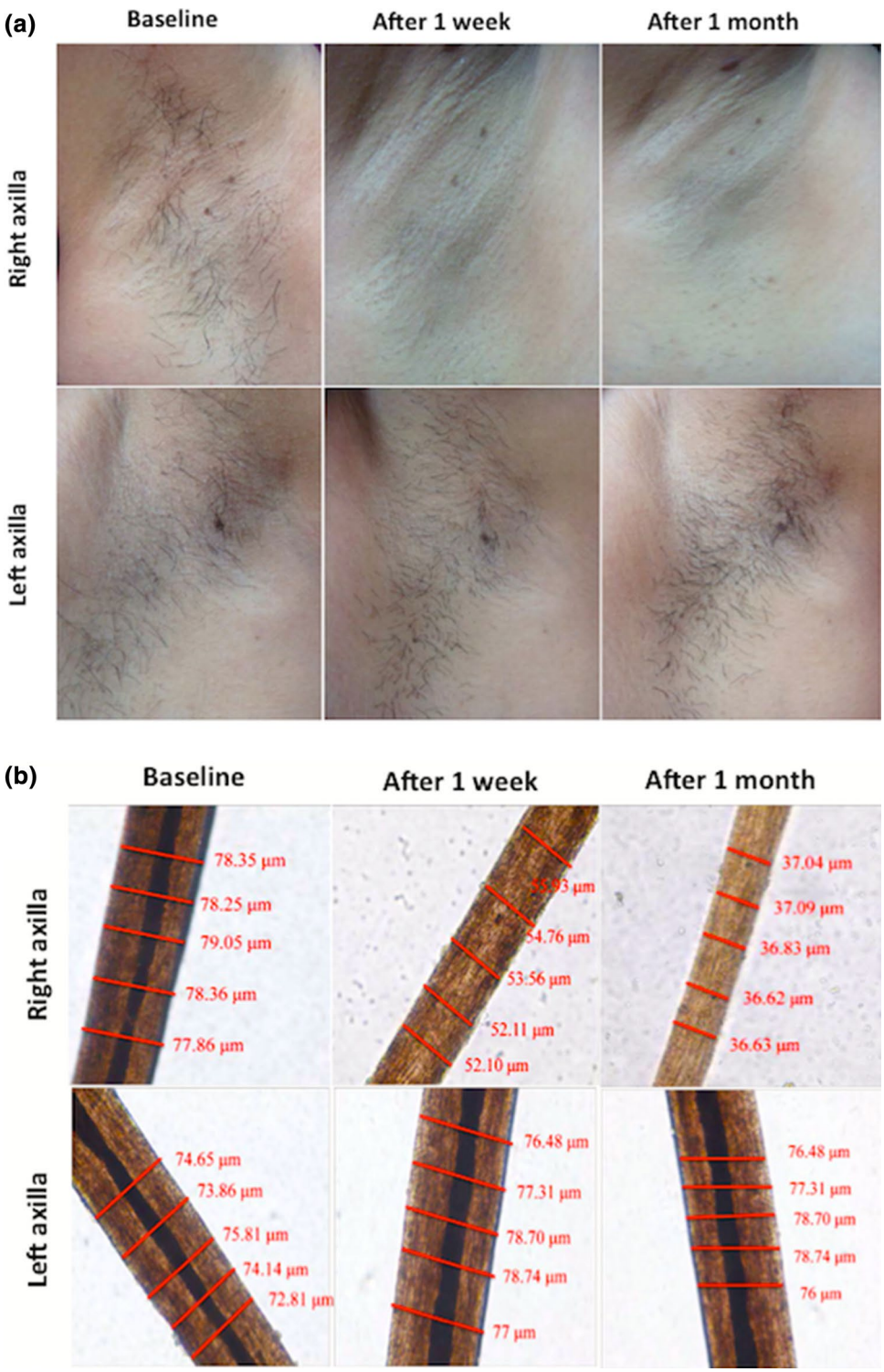

Fig. 1 **a** Clinical evaluation of volunteers. Representative photographs on both axillae showing significant improvement at 1 week and 1 month after IPL in right axilla compared to baseline and left control axilla. **b** Histometry of HS from both axillae showing significant decrease in HS diameter at 1 week with more significant decrease at 1 month after IPL in right axilla compared to insignificant change in left control axilla (Original magnification, ×400)

and control. At baseline, there were no vellus-like follicles; however, they started to appear in IPL-treated axilla after 1 week with further significant increase after 1 month ($P \leq 0.05$).

Subcutis level

Bulbs of terminal anagen follicles were hardly noticed in IPL-treated axillae.

Table 1 Comparison of hair count and hair diameter of right and left axillae among the studied volunteers at baseline, 1 week and 1 month after treatment

	Baseline	After 1 week	After 1 month	P Friedman	P1 Wilcoxon	P2 Wilcoxon	P3 Wilcoxon
Hair count							
Right axilla							
Range	9–15	0–6	0–7.5	<0.001	<0.001	<0.001	<0.001
Mean ± SD	11.5 ± 1.5	1.3 ± 1.7	2.9 ± 2.2				
Left axilla							
Range	8–15	7.8–17.30	8–17.5				
Mean ± SD	11.4 ± 1.7	10.8 ± 2.4	12.03 ± 2.1				
P4 (Mann–Whitney test)	0.8	<0.001	<0.001				
Hair diameter (μ)							
Right axilla							
Range	63.93–92.22	35.97–69.82	11.56–67.09	<0.001	<0.001	<0.001	<0.001
Mean ± SD	78.1 ± 8.7	51.02 ± 6.7	35.3 ± 13.3				
Left axilla							
Range	62.99–91.11	62.11–91.09	62.85–90.92				
Mean ± SD	77.8 ± 8.8	77.6 ± 8.8	77.4 ± 8.6				
P4 (Mann–Whitney test)	0.9	<0.001	<0.001				

P Baseline vs 1 week vs 1 month after IPL in right axilla, *P1* Baseline vs 1 week after IPL in right axilla, *P2* Baseline vs 1 month after IPL in right axilla, *P3* 1 week vs 1 month after IPL in right axilla, *P4* Right axilla vs left axilla either at baseline, 1 week or 1 month after IPL

Morphological changes of hair follicles

After IPL, there were no morphological changes in inner root sheath (IRS), outer root sheath (ORS) and bulbs of the present anagen follicles. However, perifollicular fibrosis was demonstrated, mostly around vellus follicles, in eight specimens (38%) at 1 month after IPL (Fig. 2).

Perifollicular inflammatory infiltrate

At baseline, perifollicular lymphocytic infiltrate was sparse. At 1 week and 1 month after IPL, it was moderate in 23.81% and 4.76% of biopsies, respectively, mostly in superficial compartments (infundibular, sebaceous and eccrine levels), around vellus follicles (Fig. 2).

Immunohistochemical results

Proliferation markers (PCNA, Ki67)

At baseline, both nuclear PCNA- and Ki67-expressions were seen in hair matrix and ORS during anagen stage to be decreased and even negative in late catagen and telogen stages. However, Ki67 expression was limited to outer layers of ORS. After 1 week and 1 month, follicular expression of PCNA and Ki67 was significantly decreased compared to baseline ($P \leq 0.05$), without significant change after 1 month ($P > 0.05$) (Figs. 5, 6; Table 3). There was significant positive correlation between hair count and PCNA expression at 1 week ($r = 0.7$, $p = 0.01$) and 1 month after IPL ($r = 0.8$, $p = 0.004$).

P53 expression

At baseline, nuclear P53 expression appeared in ORS and hair matrix in lower follicles around dermal papilla (DP) during anagen transition to catagen to be increased in epithelial strand of catagen stage. In addition, prominent P53 expression was detected in upper follicles after IPL. Accordingly, there was significant increase in P53 expression at 1 week and 1 month after IPL compared to baseline ($P \leq 0.05$), but with significant decrease at 1 month compared to 1 week after IPL ($P \leq 0.05$) (Fig. 7; Table 3).

There was significant negative correlation between P53 and either PCNA or Ki67 at 1 week after IPL [($r = -0.7$, $P < 0.001$); ($r = -0.5$, $P = 0.02$) respectively]; however, at 1 month after IPL, the same correlation was detected only between P53 and PCNA ($r = -0.7$, $P < 0.001$).

Discussion

The hair growth cycle, composed of three stages including anagen, catagen and telogen, is different according to body location, and even independent for each individual hair follicle. Accordingly, hair reduction requires multiple IPL sessions [9] as performed in the present study.

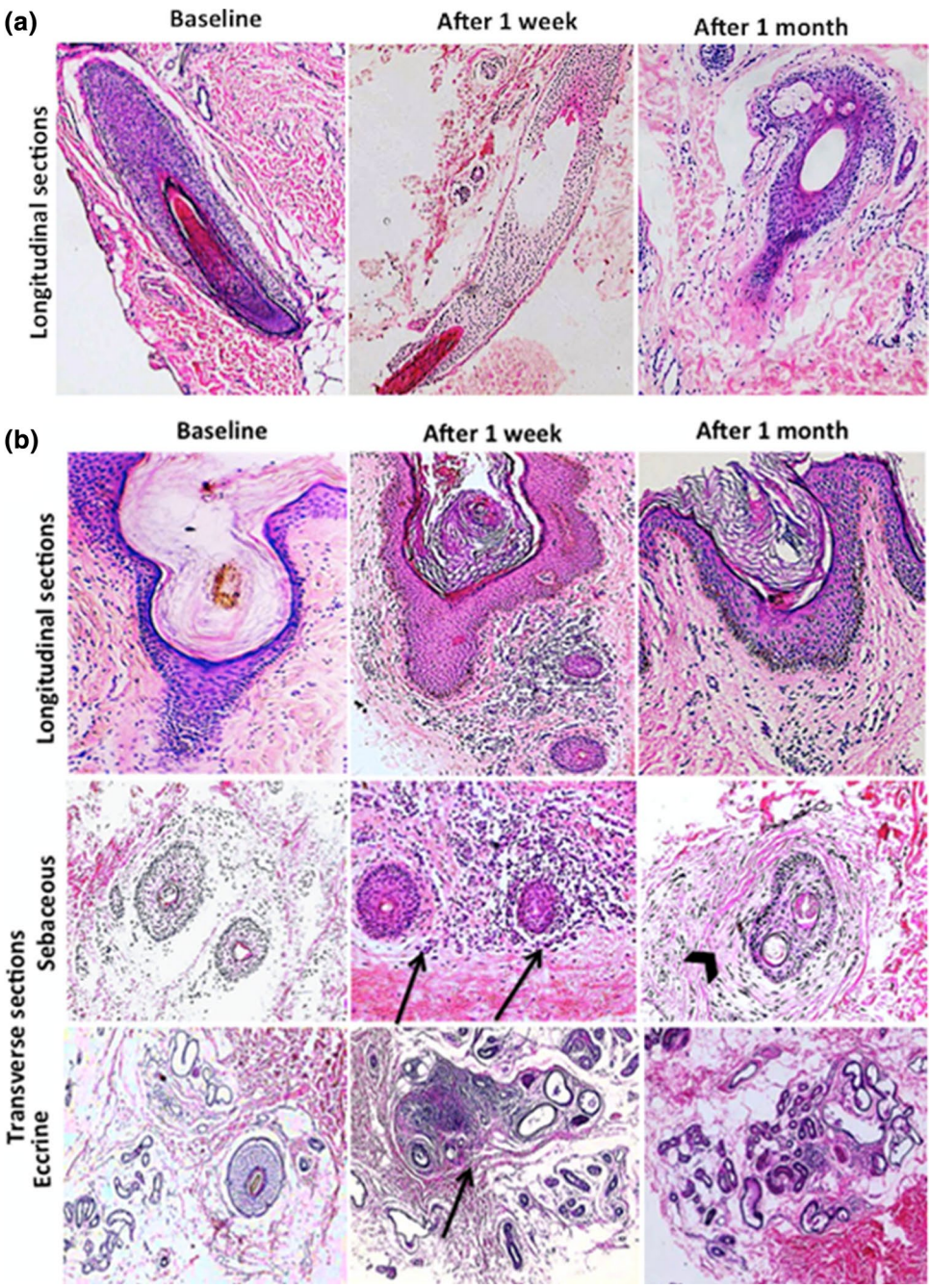

Fig. 2 **a** Longitudinal sections of skin biopsy taken from right axilla. At baseline, terminal anagen hair follicle is identified in deep dermis with basophilic hair matrix surrounding DP. At 1 week after IPL, terminal catagen hair follicle is shown in lower dermis with moderately thickened vitreous layer and epithelial column. At 1 month after IPL, terminal telogen hair follicle is identified in upper dermis with telogen germinal unit in lower follicle. **b** Moderate periappendegeal inflammatory infiltrate, in both longitudinal and transverse sections (sebaceous, eccrine levels), is apparent at 1 week after IPL, mostly around vellus follicles (*arrows*); however, sparse infiltrate is shown at baseline and 1 month after IPL. Note the perifollicular fibrosis around two vellus hair follicles at 1 month (*head arrow*) (H&E, ×100)

At 1 week and 1 month after IPL (i.e., 3 and 4 months from the start of treatment, respectively), hair reduction percentage was 89.26 and 75.90%, respectively, with significant increase in hair count after 1 month, but still less than baseline. On reviewing IPL studies on axilla, variable hair reduction percentages ranged from 72% [6] up to 92% [6, 10], irrespective of regimens and IPL parameters used.

Most of re-grown hairs in right axilla were fine and light colored with significant decrease in their mean HS diameter at 1 week (51.02 µm) and 1 month (35.3 µm) after IPL compared to baseline and control axilla. Our results agree with Kim et al. [11]. Accordingly, we suggested miniaturization of terminal hairs since their diameter was similar to that of vellus-like hairs [0.03–0.06 mm (i.e., 30–60 µm)] [12]. Tse [13] explained previous characters of re-grown hairs after IPL by hair bulb damage inducing catagen or telogen stage. On the contrary, Sadick et al. [5] noticed no significant change in HS diameter after single IPL session.

Histologically, vertical-sectioned biopsy is adequate for assessing inflammatory infiltrate with detection of 10% of the already present follicles [14], especially in axilla. This is because the hair follicles, which grow at an angle, are

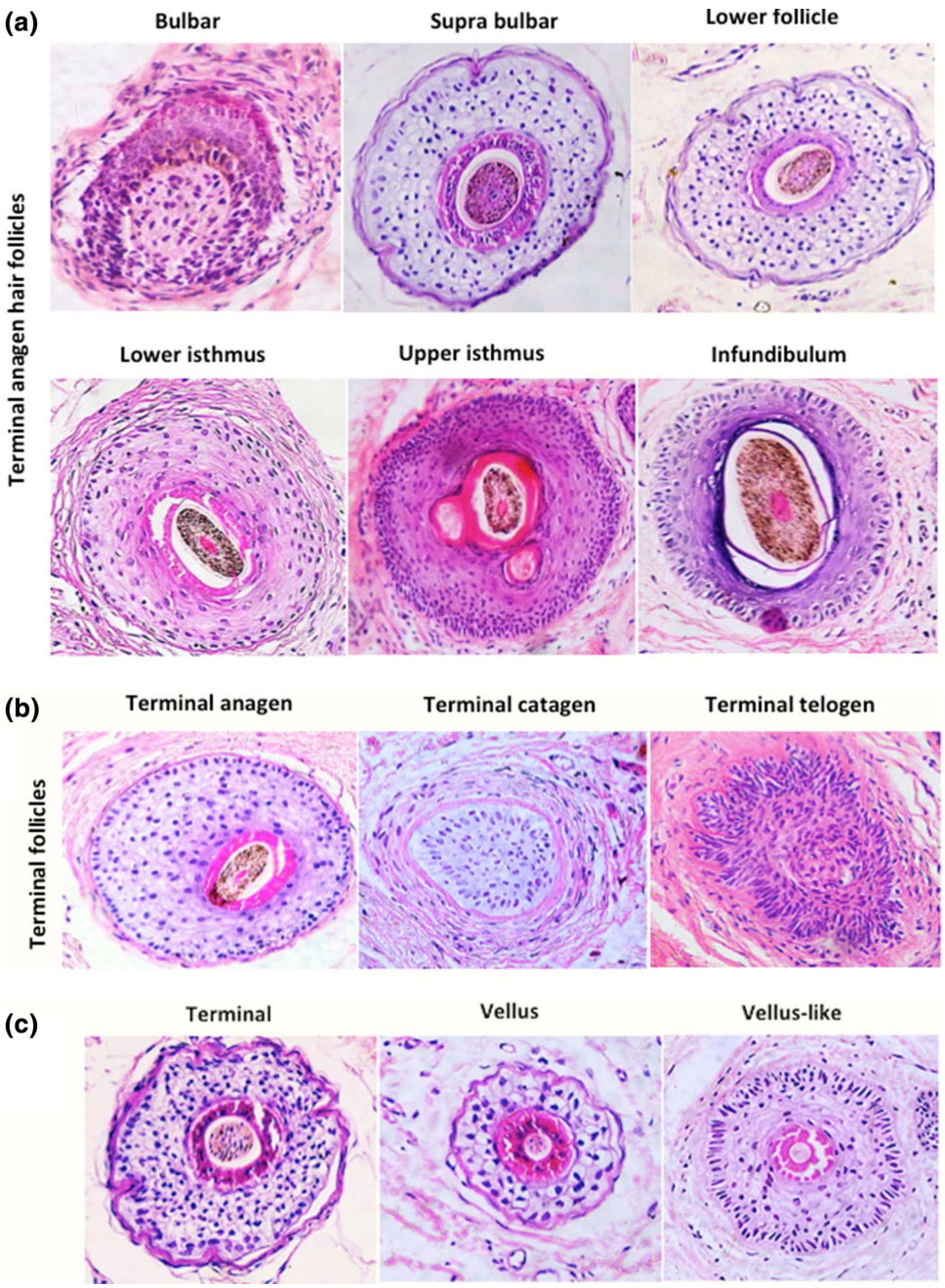

Fig. 3 **a** Transverse sections of terminal anagen hair follicles at different levels at baseline. Bulbar level showing central DP surrounded by hair matrix cells, ORS and fibrous sheath. Start of IRS keratinization appears at supra-bulbar level to be complete at lower follicle level with start of IRS desquamation at lower isthmus level. Trichilemmal keratinization of ORS and compact keratin without granular cell layer start at upper isthmus level; however, lamellar keratin with granular cell layer appears at infundibular level. **b** Transverse sections of terminal hair follicles at different stages. Terminal anagen hair follicle at lower isthmus level; terminal catagen with the characteristic thickened hyaline vitreous membrane around shrinking ORS; terminal telogen with characteristic telogen germinal unit containing central baseloid cells with peripheral palisading of the nuclei. **c** Transverse sections of terminal anagen, vellus and vellus-like (miniaturized) hair follicles. The central terminal HS has a diameter larger than thickness of IRS. The central vellus HS has a diameter slightly smaller than thickness of IRS and its ORS is fairly thin about two to three cell layers. The central vellus-like HS is slightly equal to the thickness of IRS and the ORS is fairly thick about three to five cell layers (H&E, ×400)

cut tangentially and so they cannot be visualized in their entirety [15]. Meanwhile, transverse sectioning technique allowed visualization of all follicles [14, 16] throughout their entire length with quantitative evaluation of follicular cycling [17]. So, all biopsies in our study were sectioned vertically and transversely at multiple levels (infundibular, sebaceous, eccrine and subcutis).

After IPL, right axillae showed insignificant decrease of total follicular number indicating no hair follicle destruction; however, most follicular infundibula, in 90.5% of biopsies, showed complete keratotic plugging and small, homogenized or retracted intraluminal HSs compared to baseline and control with smaller hair cavities after 1 month indicating miniaturization. These HS changes were attributed to the photothermal effect as observed by Trelles et al. [6] in their immediate and late biopsies after IPL

Normally, vellus hairs in axilla are few in females in childbearing period due to their conversion into terminal hairs after puberty [12]. At 1 week and 1 month after IPL, sebaceous levels of right axillae showed significant increase in vellus follicles percentage and significant decrease in terminal follicles percentage and T/V ratio compared to baseline and control with further significant change after 1 month. On the contrary, Sadick et al. [5] reported no

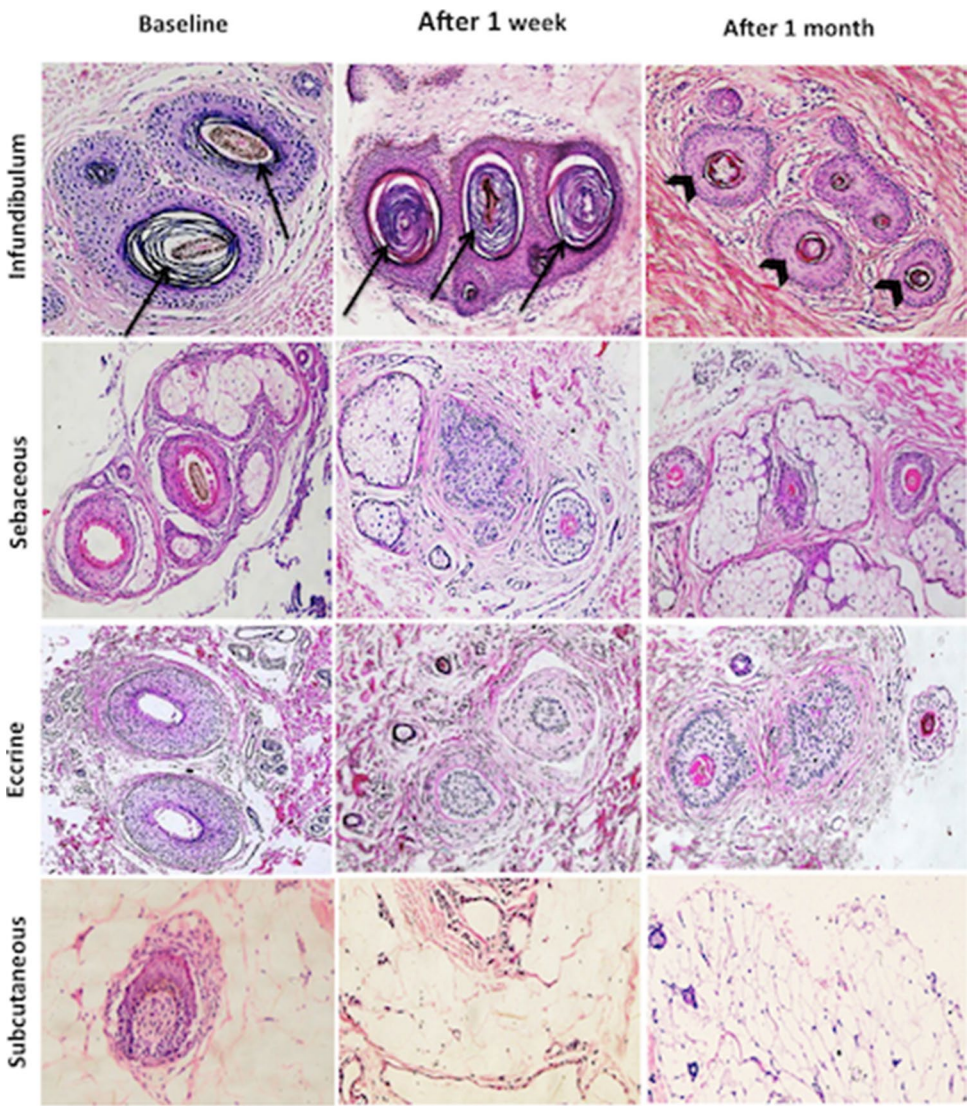

Fig. 4 Transverse sections of right axillae at infundibular, sebaceous, eccrine and subcutaneous levels. At baseline, the follicular infundibulum shows incomplete filling with keratin and normal HS (*arrows*); however, after 1 week and 1 month, the infundibulum shows complete keratotic plugging and homogenized, lightly pigmented HSs (*arrows*) with smaller follicular cavity at 1 month (*head arrows*). At sebaceous level, baseline state shows more terminal anagen follicles beside sebaceous glands; however, after 1 week and 1 month, terminal telogen and vellus follicles are apparent. At eccrine level, baseline state shows more terminal anagen hair follicles; however, after 1 week and 1 month, terminal catagen, telogen and vellus-like hair follicles are apparent. At subcutis level, at baseline, hair bulb of terminal anagen hair follicle is apparent with absence at 1 week and 1 month after IPL (H&E, ×200)

significant change in T/V ratio in immediate and late biopsies after single IPL session; however, the level of counting was not determined.

For further determination of terminal follicles types, examination at eccrine level was performed, since recognition is only possible at lower follicle below "bulge" level, because of the presence of IRS or trichilemmal club and their absence at upper follicle with appearance of keratinized HS [18].

At eccrine level, right axillae revealed significant decrease in terminal anagen percentage and A/T ratio and significant increase in terminal telogen percentage without significant change in terminal catagen percentage at 1 week and 1 month after IPL compared to baseline and control. This agrees with Trelles et al. [6], who subjectively observed an increase in telogen follicles in IPL-treated axillae. On the other hand, Sadick et al. [5] detected no significant difference in A/T ratio. The non-significant change of terminal catagen percentage after IPL may be due to brief transitional catagen phase (2–3 weeks) [12].

Vellus-like follicles were absent at baseline; however, they started to appear after 1 week with more significant increase after 1 month, which coincided with subsequent significant decrease in HS diameter compared to 1 week after IPL. This goes with Trelles et al. [6], who subjectively observed some miniaturized hairs.

The present study showed no morphological changes in IRS, ORS and DP of the present anagen hair follicles after Quanta IPL, excluding follicular necrosis and resorption [5].

The hair follicles undergo successive cycles of growth (anagen) followed by regression (catagen) and rest (telogen) by modulating the balance of follicular proliferation and apoptosis [19]. Among the proliferation markers used in this study were PCNA and Ki67 [20], which were significantly decreased after IPL compared to baseline. This

Table 2 Transverse sections of hair follicles of right axilla at infundibular, sebaceous and eccrine levels at baseline, 1 week and 1 month after IPL

	Baseline	At 1 week after IPL	At 1 month after IPL	P Friedman test	P1 Wilcoxon test	P2 Wilcoxon test	P3 Wilcoxon test
Infundibular level							
Total number of hair follicles							
Range	5–7	4–7	5–7	0.7	0.8	0.7	0.3
Mean ± SD	5.90 ± 0.59	5.95 ± 0.62	6.0 ± 0.45				
Sebaceous level							
Terminal hair follicles (%)							
Range	75–100	42.86–71.42	40–53	<0.001	<0.001	<0.001	<0.001
Mean ± SD	94.56 ± 9.13	59.69 ± 6.91	46.59 ± 4.83				
Vellus hair follicles (%)							
Range	0–16.67	12.5–57.14	33.33–60	<0.001	<0.001	<0.001	<0.001
Mean ± SD	4.11 ± 6.73	37.72 ± 10.20	49.75 ± 8.16				
Terminal/vellus hair ratio							
Range	3–7	0.75–2.5	0.50–1.33	0.003	0.03	0.03	0.001
Mean ± SD	4.48 ± 1.2	1.54 ± 0.44	1.002 ± 0.33				
Eccrine level							
Terminal anagen (%)							
Range	33.33–66.67	0–50	0–37.5	<0.001	<0.001	<0.001	0.4
Mean ± SD	53.33 ± 11.26	11.55 ± 13.65	13.44 ± 12.15				
Terminal catagen (%)							
Range	0–20	0–16.67	0–16.67	0.9	0.6	0.8	0.3
Mean ± SD	3.33 ± 7.07	4.87 ± 7.07	4.02 ± 6.56				
Terminal telogen (%)							
Range	20–50	37.5–57.14	28.57–57.14	0.003	0.004	0.004	0.2
Mean ± SD	35.24 ± 10.46	46.19 ± 5.33	44.25 ± 6.87				
Anagen/telogen ratio							
Range	0.5–3	0–1.3	0–2	<0.001	<0.001	0.001	0.2
Mean ± SD	1.33 ± 0.61	0.27 ± 0.34	0.34 ± 0.46				
Vellus-like hair (%)							
Range	0–0	12.5–50	14.48–60	<0.001	<0.001	<0.001	0.02
Mean ± SD	0.0 ± 0.0	34.56 ± 10.65	41.06 ± 9.98				

P Baseline vs 1 week vs 1 month after IPL in right axilla, *P1* Baseline vs 1 week after IPL in right axilla, *P2* Baseline vs 1 month after IPL in right axilla, *P3* 1 week vs 1 month after IPL in right axilla

means that IPL could work, after the initiation of photothermolysis, by decreasing follicle proliferation, which was confirmed by the presence of significant positive correlation between PCNA and corresponding hair count at 1 week and at 1 month after IPL. However, Sadick et al. [5] reported non-significant decrease in follicular Ki67 expression following single IPL session. To the best of our knowledge, there were no previous IPL studies evaluating follicular PCNA expression.

P53, a tumor suppressor gene, activates apoptosis and controls DNA replication and repair [21]. In controlling cellular proliferation, its wild type can cause cell cycle arrest in response to DNA damage allowing time for DNA repair, or cell destruction by an irreversible apoptotic pathway before replication renders the damage permanent [8].

Normally, during transition to catagen stage, P53-positive cells appeared in lower follicles leading to shortening of cyclic portions from subcutis to bulge level denoting an apoptotic role of P53 [22]. In the present study, prominent P53 expression was demonstrated also in upper follicles after IPL with significant increase in its expression compared to baseline. Accordingly, P53 overexpression might result from normal transition to catagen by DP growth factor withdrawal and in response to DNA damage resulting from IPL [23]. On the contrary, Sadick et al. [5] detected non-significant difference in P53 expression after IPL.

At 1 month after IPL, there was significant decrease in follicular P53 expression without significant change in PCNA and Ki67 compared to 1 week after IPL indicating continuous stable decrease in proliferation; however, DNA

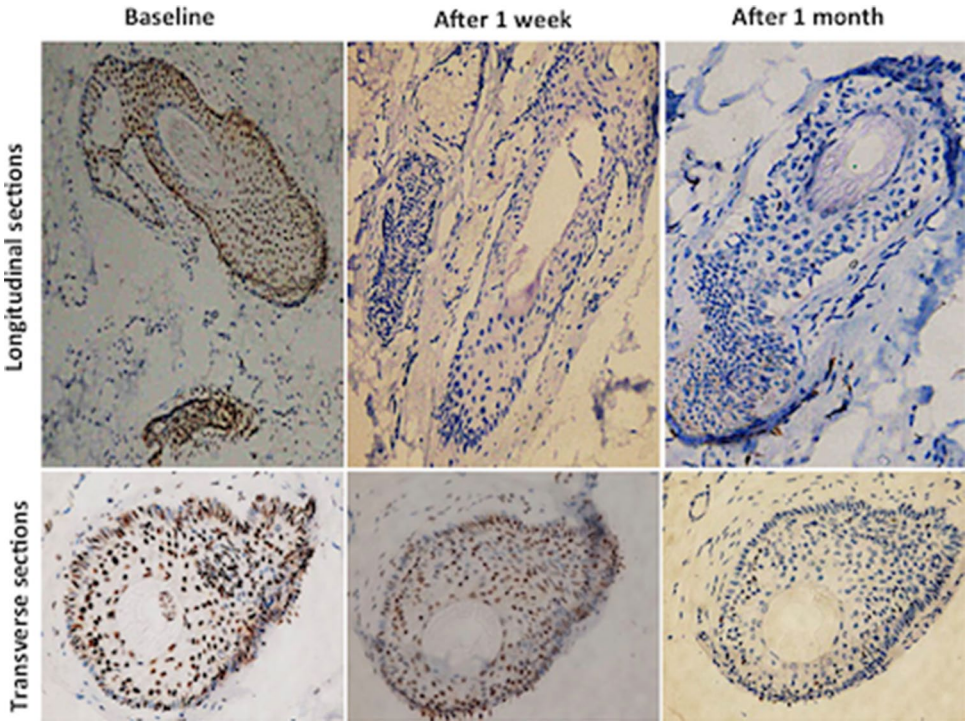

Fig. 5 Expression of PCNA in longitudinal and transverse sections in right axilla showing higher expression at baseline; however, lower or no expression is apparent at 1 week and 1 month after IPL (IHC, ×200)

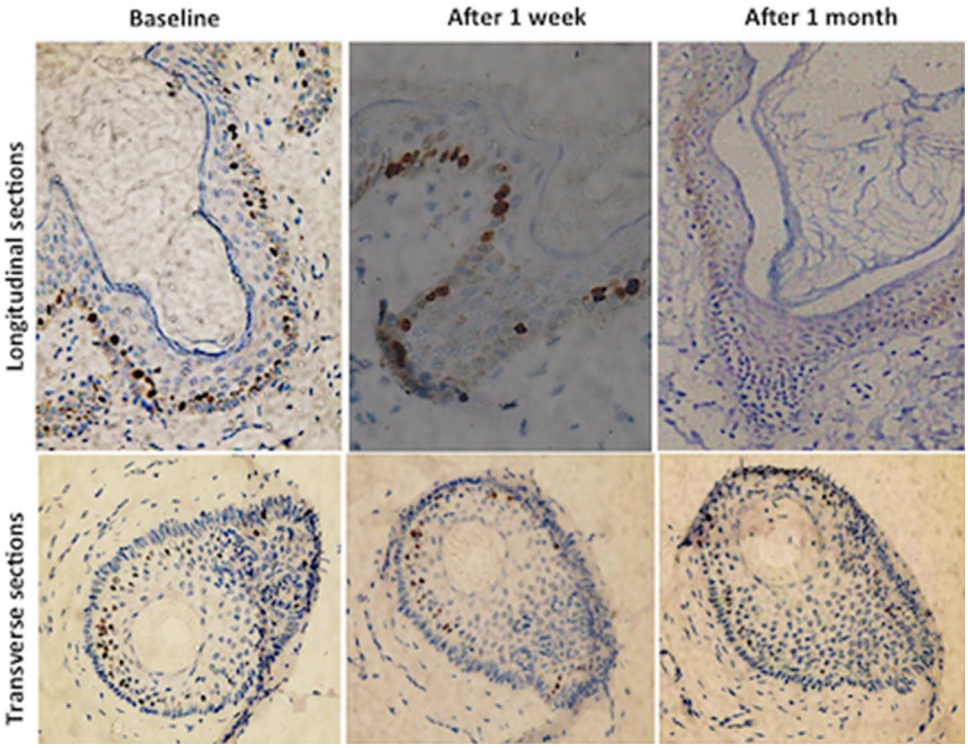

Fig. 6 Expression of Ki67 in longitudinal and transverse sections in right axilla showing higher expression at baseline; however, lower or no expression is apparent at 1 week and at 1 month after IPL. Note that the positivity of Ki67 is restricted to outer layers of ORS (IHC, ×200)

damage or apoptosis was about to be recovered, but still more than baseline.

There was significant negative correlation between P53 and either PCNA or Ki67 at 1 week after IPL; however, the same correlation was detected only between P53 and PCNA after 1 month. This denotes that the higher is the follicle proliferation, the lower is the P53 expression and vice versa. So, this agrees with the physiological balance between cell proliferation and death [19].

Table 3 PCNA, Ki67 and P53 expression of hair follicles of right axilla at baseline, 1 week and 1 month after IPL

	Baseline	After 1 week	After 1 month	P Friedman test	P1 Wilcoxon test	P2 Wilcoxon test	P3 Wilcoxon test
PCNA							
Range	2.51–4.27	0.4–1.75	0.4–1.97				
Mean ± SD	3.30 ± 0.47	1.10 ± 0.47	1.30 ± 0.54	<0.001	0.005	0.005	0.2
Ki67							
Range	0.68–2.33	0.18–0.92	0.23–0.84				
Mean ± SD	1.2 ± 0.49	0.43 ± 0.27	0.49 ± 0.20	<0.001	0.005	0.005	0.1
P53							
Range	0–0.43	0.28–0.69	0.25–0.64				
Mean ± SD	0.17 ± 0.14	0.49 ± 0.13	0.41 ± 0.14	<0.001	0.005	0.007	0.005

P Baseline vs 1 week vs 1 month after IPL in right axilla. *P1* Baseline vs 1 week after IPL in right axilla, *P2* Baseline vs 1 month after IPL in right axilla, *P3* 1 week vs 1 month after IPL in right axilla

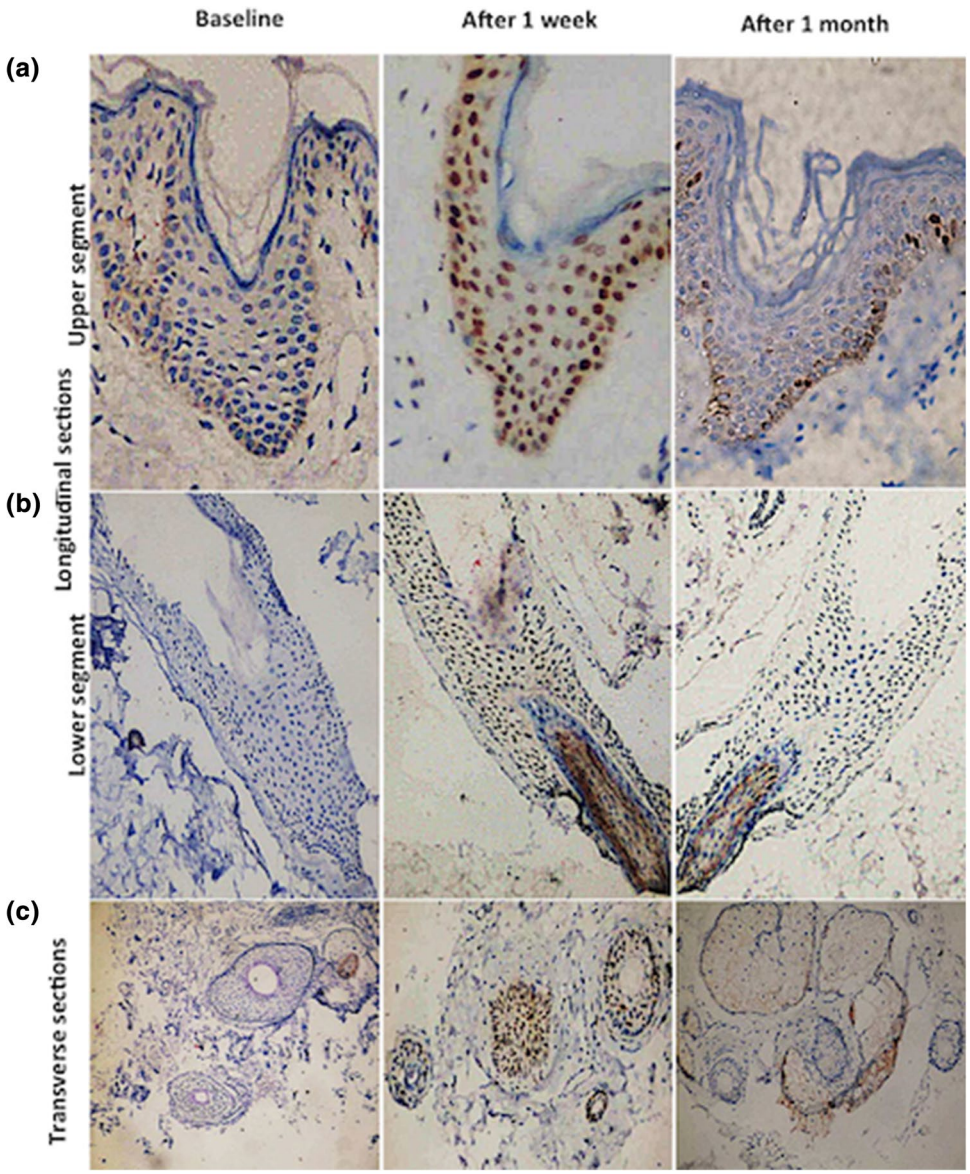

Fig. 7 Expression of P53 in longitudinal (**a, b**) and transverse sections (**c**) in right axilla. Negative expression is apparent at baseline in both upper and lower segments of longitudinal (**a, b**) and transverse sections (**c**); however, higher expression is evident in both upper and lower segments of longitudinal hair follicles (**a, b**) and transverse sections (**c**) at 1 week to be decreased again at longitudinal sections (**a, b**) or even absent at transverse sections at 1 month after IPL (**c**) [IHC, (**a**, ×400); (**b**, **c**, ×200)]

It is important to note that miniaturized follicles observed after laser or lPL treatment correspond to that of androgenetic alopecia histometrically [12], histopathologically [24] and immunohistochemically with lower proliferation rate (decreased PCNA) and also apoptosis detected by P53 overexpression [7].

Observed HS changes in the present study may suggest that IPL starts its action after photoepilation by selective photothermolysis effect on melanin of hair follicles [5], which leads to decreased hair follicle proliferation (confirmed by decreased PCNA and Ki67) and induced overexpression of P53 that implies an increase in DNA damage that could favor apoptosis [25]. These in turn could cause induction and prolongation of telogen stage and induction of vellus-like hair follicles resulting in decrease in both hair count and diameter. This agrees with some previous studies, which suggested subjectively induction of telogen [2] and miniaturization [6] after IPL, but without objective measurements and explanation on molecular basis. On the contrary, Sadick et al. [5] observed follicular necrosis and resorption after IPL, which were excluded in the present study since no morphological changes in IRS, ORS and DP were detected.

In conclusion, IPL is an effective hair reduction therapy causing decrease of hair number and thickness by induction of miniaturization and telogenesis. IPL could act on the principle of selective photothermolysis through inhibition of hair follicles proliferation confirmed by decrease in PCNA and Ki67 markers. Meanwhile, p53 overexpression after IPL implies an increase in DNA damage that could favor apoptosis.

Compliance with ethical standards

Conflict of interest None declared.

Funding sources None.

Ethical approval The study was ethically approved by Scientific Committee for Postgraduate Studies and Research of faculty of Medicine, Minia University.

Informed consent An informed consent was taken from each individual.

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