Does Estradiol Level in Platelet-Rich Plasma Improve Efficacy of Androgenic Alopecia Treatment?

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Level of Evidence: 2 (Therapeutic)

Abstract

Background: Although there are some studies in the literature reporting that autologous and homologous platelet-rich plasma (PRP) can be used in treatment of androgenic alopecia (AGA), there is no study examines estrogen concentration of prepared PRP.

Objective: We aimed to determine the presence of estrogen in PRP and to investigate the effect of estrogen concentration of PRP on AGA treatment, in this study.

Methods: Between 2017-2018, 30 male patients with hair loss complaints were included in this prospective study. Autologous PRP was injected to patients in Group 1. Homologous PRP with high estrogen levels was injected to the patients in Group 2. PRP injected to both groups 4 times in 0, 1st, 3rd, and 6th months. The obtained photographs were evaluated and hair densities of each patient at controls were calculated.

Results: The mean estrogen level measured in PRP was statistically significantly higher in Group 2. In both groups, the increase in hair density was observed from the first month, but this increase was statistically significantly higher in all controls in Group 2. In Group 2, there was a statistically significant increase in the 1st and 3rd months compared with the previous control, but there was no difference between the 6th and 12th months and the 3rd month.

Conclusions: It has been determined that increase in hair density is higher and earlier in the group use estrogen-rich PRP than the group use autologous. We think that estrogen-rich PRP may be used in the treatment of AGA in the presence of an appropriate donor.

Androgenic alopecia (AGA) is characterized by a reduction in the number of androgen-sensitive hair follicles secondary to high levels of dihydrotestosterone (DHT), increased 5-α reductase activity and increased androgen receptor expression.[1] Testosterone typically enters papillae, outer root sheath (ORS) and sebaceous gland cells where it is converted into DHT by 5-α reductase activity. Then, DHT binds to the androgen receptors and activates the synthesis of proteins harmful to the follicle and disrupts the normal hair growth cycle. As a result, the hair cycle changes to shorten the anagen phase, resulting in premature regression of the hair during the catagen and telogen phases.[2]

Platelet-rich plasma (PRP), which is one of the treatment options of AGA, is the concentrated form of human platelets in small amounts of plasma. Platelets contain many growth factors such as fibroblast growth factor (FGF), platelet growth factor (PDGF), transforming growth factor beta (TGF-β), vascular endothelial growth factor (VEGF), etc.[3] It is thought that the effect of PRP on alopecia treatment is based on the principle of increasing cell rejuvenation through these growth factors. It increases angiogenesis, epithelization, cell division and macrophage chemotaxis.[4] Considering the hair cycle physiology, PRP as a treatment method focuses on increasing the molecular cell growth mechanisms and preventing the onset of apoptotic processes.[5]

Estrogen is one of the female reproductive hormones. In addition to its activities in human physiology, it is well-known that estrogen plays some important roles in human hair cycle. Although the mechanism of female-type hair loss has not been fully resolved, it is thought that the almost discontinuation of ovarian estrogen production during postmenopausal period causes changes in hair growth characteristics.[6] It has been suggested that the number of follicles at telogen phase is higher than the number at anagen phase in the postmenopausal period.[7] It has been shown that ligands acting on estrogen receptor suppresses proliferation of endothelial cells and prostate cancer cells which proliferate DHT-dependent and also act as DHT antagonists.[8]

Although there are some studies in the literature, report autologous and homologous PRP can be used in treatment of AGA,[9] little is known about estrogen concentration of the prepared PRP.

In this study we aimed to both determine the presence of estrogen in PRP and to investigate the effect of estrogen concentration of PRP on AGA treatment.

METHODS

Thirty married male patients between the ages of 25-35, who made an application to our outpatient clinic with hair loss between August 2017 and September 2018 and who were evaluated as Noorwood type 3 and 4, were included in this prospective study. We eliminated patients who get current or previous treatment for hair loss, who have known endocrine, metabolic or dermatological diseases, who have vitamin deficiency, who is smoker and who did not accept homologous PRP from the study. Patients who did not attend follow-ups and PRP sessions regularly were excluded from the study. The ethical approval was obtained from local ethics committee (Necmettin Erbakan University). All procedures were performed and all data were collected by the same surgeon. All patients gave written and oral consent to participate.

The patients were divided into two randomized groups according to the order of their application. Patients with an odd number of application formed Group 1, while patients with an even number of application formed Group 2.

Autologous PRP prepared from their own blood was injected into the patients in Group 1. Spouses of the patients in Group 2 were included in the study as PRP donors. Homologous PRP with high estrogen levels obtained during the ovulation period from volunteer female donors, who are hepatitis B, C and HIV (-), between 25-40 years of age and has the same ABO and rH blood groups with recipients, was injected to the patients in Group 2. The luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol levels were examined before blood drawing from donors in order to show that donors are at ovulation time period. In addition, estrogen levels at PRP's were also studied from PRP samples taken from both of the groups after PRP preperation. PRP injected to the patients in both groups 4 times in 0, 1st, 3rd and 6th months. Before all PRP injections and at the 12th month after the first injection, the patients were asked to cut their hair in appropriate shortness and standard photographs, which were taken with the same camera and taken with the same dermatoscope during dermatoscopic examination was obtained. The obtained photographs were evaluated with NEO Image Analysis program and hair densities (n / cm^2) of each patient at 0, 1st, 3rd, 6th and 12th months were calculated. Hair density calculations were made from three fixed regions previously determined for each patient. In each session, 4-5ml PRP was obtained by using a commercially available PRP kit (TrueCell CGF, Inovatek, Malatya, Turkey). PRP injections were made intradermally with 26 G injector approximately 0.1 ml to each cm^2. All the patients were called for a satisfaction survey, 6

months after the last follow-up. Patient satisfaction was graded on a linear analogue scale of 1-10 (1 = worst, 10 = best result) and recorded for both of the groups.

Statistical Analysis

The statistical significance of the differences between the mean values was analyzed using SPSS 24.0 (USA) statistical software. Bonferroni-adjusted sample test in paired samples was used to compare the improvement in hair density and patient satisfaction scores between the groups and paired *t* test was used to compare the increase in hair density for each group between sessions. P values of < 0.05 were considered statistically significant.

RESULTS

Two patients from Group 1 and 3 patients from Group 2 were excluded from the study due to lack of regular attendance to follow-ups. The mean age of Group 1 was 29.8 years (range, 25-34 years), while that of Group 2 was 27.9 years (range, 25-33 years). There were no complications and no systemic side effects in any of the patients. All of the patients were followed up for 12 months in both groups.

Mean thrombocyte concentration of prepared PRPs for Group 1 was 803.000 (712.000-877.000) / ml, and for Group 2 was 794.000 (754.000-898.000) / ml. Mean estradiol concentration of the PRP prepared for Group 1 was 38.58 (16.4-43.58) µg / L, while the estradiol concentration in PRP prepared from donors in Group 2 was 160.1 (117.2-214.3) µg / L (Figure 1).

In Group 1, increase in the hair density was calculated as 9.21% in the first month, 13.23% in the 3rd month, 16.84% in the 6th month and 24.63% in the 12th month. In Group 2, this increase was calculated as 36.02% in the first month, 51.38% in the 3rd month, 54.45% in the 6th month and 55.17% in the 12th month (Figures 2-4).

Mean estrogen level measured in PRP was statistically significantly higher in Group 2 ($p < 0.05$). In both of the groups, the increase in hair density was seen after first month, but this increase was statistically significantly higher for all controls in Group 2 ($p < 0.05$). In addition, hair density increase of Group 2 in the first month was statistically significantly higher than hair density increase of Group 1 in the 12th month ($p < 0.05$). When increase in hair densities were compared within the groups themselves, it was seen that hair densities increased statistically significantly in all controls in Group 1 when compared with the previous control ($p < 0.05$). But in Group 2, there was a statistically significant increase in the

1st and 3rd months compared with the previous control, but no statistically significant difference was found between the 6th, 12th months and the 3rd month ($p > 0.05$).

Mean patient satisfaction score was 7.1 ± 0.9 in Group 1 and 7.9 ± 1.1 in Group 2, at patient satisfaction survey.

DISCUSSION

AGA occurs secondary to the effects of DHT on hair follicles. Locally high concentrations of DHT cause changes in the distribution of hair follicles in the anagen, catagen and telogen phases, and leads to turn of more follicles into the telogen phase. It has been claimed that the amount of hair loss varies with the region, which means that hair follicles of each region have different androgen sensitivities. In men, AGA is more common in the vertex, occipitoparietal and frontal regions, whereas women are affected without a specific region.[10,11] AGA is seen more frequently in men between the ages of 25-40, that is why the patients who included in this study were between this age gap.

Estrogen is produced via conversion of androstenedione or testosterone by the aromatase enzyme.[12] Combined oral contraceptive drugs (COC) which contain estrogen or progestogen increase the levels of sex-hormone-binding globulin. In addition, COC send negative feedback signals that suppress both hypothalamic secretion of gonadotropin-releasing hormone and pituitary secretion of FSH and LH. As a result, androgen production decreases and androgen effects on hair follicles reduce.[13] It has been claimed that ethinyl estradiol and spironolactone treatment is effective in pre-menopausal women with female pattern hair loss.[12] In our study, it was suggested that high-estrogen concentrated PRP injection is effective in AGA treatment.

The therapeutic effect of PRP depends on its ability to prolong the anagen phase of the hair follicles and prevent follicles entry into the early catagen phase.[5] In addition, estrogen has been shown to shorten the duration of telogen phase and increase the length of the anagen phase in human scalp.[6] For this reason, we aimed to compare the efficacy of estrogen-rich homologous PRP (e-rhPRP) with autologous PRP (aPRP) which has well-known positive effect on AGA treatment.

We tried to determine whether there is estradiol in PRP and what effect it has on hair loss treatment, if any. We did not add extra estradiol to prepared PRPs. The addition of estradiol may increase the duration of the effect or it may reduce the number of PRP sessions. Effectivity of estradiol alone or estradiol added PRP on hair loss treatment could be studied in future researches.

It was suggested that homologous PRP is more effective than autologous PRP in the treatment of AGA. The fact that, platelet count in homologous PRP is higher than autologous one revealed as a reason for this circumstance.[9] However, in that study, the sex of the donors for homologous PRP or the concentration of estrogen in the PRP was not studied. e-rhPRP used in our study is also a homologous PRP. Since they are prepared with the same PRP kit, there was no statistically significant difference between the platelet intensities of the PRPs injected to both groups, so this advantage of homologous PRP has been eliminated. In our study, while platelet concentrations were similar in both groups, there was about 4-fold difference between estrogen concentrations of the PRP's.

Before the blood was drawn from female donors to prepare PRP, blood FSH, LH and estrogen levels of the donors had been measured to make sure that donors were at ovulation period at that moment. So, the highest estrogen level was tried to be obtained. Although PRPs in both groups were similar in terms of mean platelet count and patient age, hair density increase was statistically significantly higher in Group 2. We think that this difference is due to the high levels of estrogen in the PRP's has both DHT antagonistic effect[7] and effect of increasing the number of hair follicles in anagen phase.[6]

When the rates of increase in number of hair follicles in both groups were compared, the increase in hair densities of Group 2 was statistically significantly higher than Group 1. When PRPs prepared for two groups were compared with each other, it was found the amount of estrogen in Group 2 was approximately 4 times higher than other group, while other parameters were similar. In addition, in one of the studies about this topic, PRPs prepared from men and women bloods, were compared with each other and it has been shown that there is no significant difference in terms of platelet counts and growth factor amounts of PRPs.[14] Considering all these data, the DHT antagonistic effect of estrogen in Group 2 could be the reason for the differences in hair density between these two groups. Injections can be made after adding synthetic estradiol into PRP. But in order to do this, advanced clinical studies are required.

Hair density increased in terms of number of follicles by square centimeter and we determined this by computer examination of the photographs taken. Improvement of the hair density in Group 2 was continued until 3rd month, but there was no statistically significant increase in the 6th and 12th month measurements. In Group 1, despite the fact that there was a gradual increase in the measurements at 1st, 3rd, 6th, and 12th months, the maximum increase was statistically less than Group 2. e-rhPRP was more effective in AGA treatment than aPRP and its maximum effect had seen earlier. In Group 2, there was no statistically significant

increase in hair density after 3rd month, which might be due to the absence of more viable hair follicles in PRP injected areas. From this point of view, it can be considered that PRP injections will be beneficial in the prophylaxis of hair loss as well as treatment.

Although it was reported that an average of 3 injections were needed to increase hair density in AGA treatment,[15] the mean hair density measured in the first month in Group 2 was higher than the measurement in 12th month in Group 1. It can be thought that, hair densities can be increased with even only one e-rhPRP injection.

Marx et al[16] have recommended to use of PRP which contains 1 million platelets per milliliter, but it has been reported that PRP may be effective in the treatment of AGA even if it contains lesser concentration of platelets.[2] Many studies have reported quite different platelet concentrations in PRP, so there is no consensus on the ideal platelet concentration that PRP should contain.[12]

Although the infection parameters are examined, there may be a risk of infection with micro-organisms during incubation period. However, since the spouses of the patients were taken as donors in our study, the risk of infection can be ignored.

This study will pave the way for a significant change in the AGA treatment. The treatment models used to date have PRP and esradiol coexistence. In our study, this association was found to be more effective and a value related to the concentration of estradiol that the com-pound should contain was obtained. In the future, this compound may be commercially available.

Microscopic examination is the gold standard for determining hair thickness and quality. However, any kind of biopsy material was not used to be taken in our study. Lack of taking biopsy to evaluate the results can be considered as one of the limitations of our study. Furthermore, half-life of local effects of estrogen on hair follicles could not be evaluated in our study. This can be added to the list of disadvantages of the study, also.

To summarize, use of e-rhPRP not only reduces the androgenic effects in hair follicles and stops the hair loss, but also by the growth factors it contains it stimulates the development of new hair follicles.

CONCLUSION

In conclusion, autologous and estrogen-rich homologous PRPs have been shown to be effective in the treatment of AGA. It has also been determined that increase in hair density is higher and earlier in the group use e-rhPRP than the group use aPRP. We think that e-rhPRP may be used in the treatment of AGA in the presence of an appropriate donor.

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Figure Legends

Figure 1. Characteristics of prepared PRP's according to the two groups.

Figure 2. Increase in hair density for months according to the two groups.

Figure 3. A 26-year-old male patient from Group 1. (A) Before treatment. (B) Three months after treatment. (C) Twelve months after treatment. (D) Before treatment, closer view. (E) Twelve months after treatment, closer view.

Figure 4. A 28-year-old male patient from Group 2. (A) Before treatment. (B) Three months after treatment. (C) Twelve months after treatment. (D) Before treatment, closer view. (E) Twelve months after treatment, closer view.

Figure 1

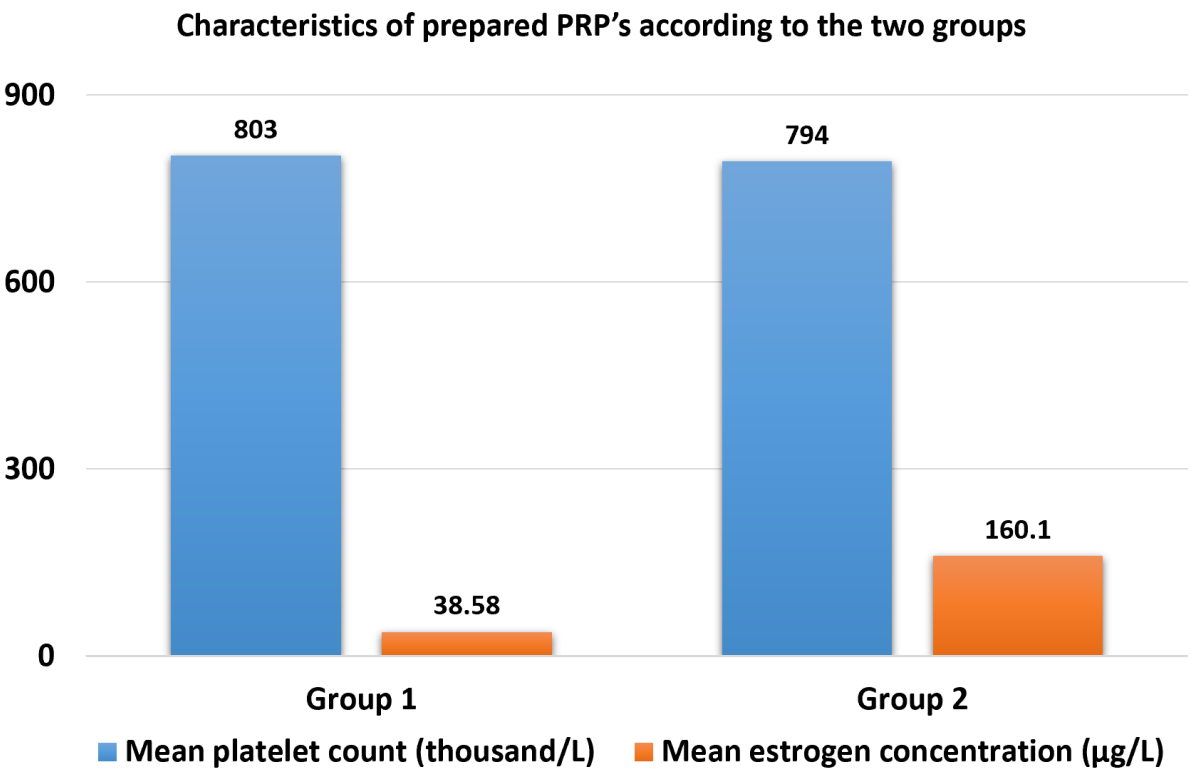


Figure 2

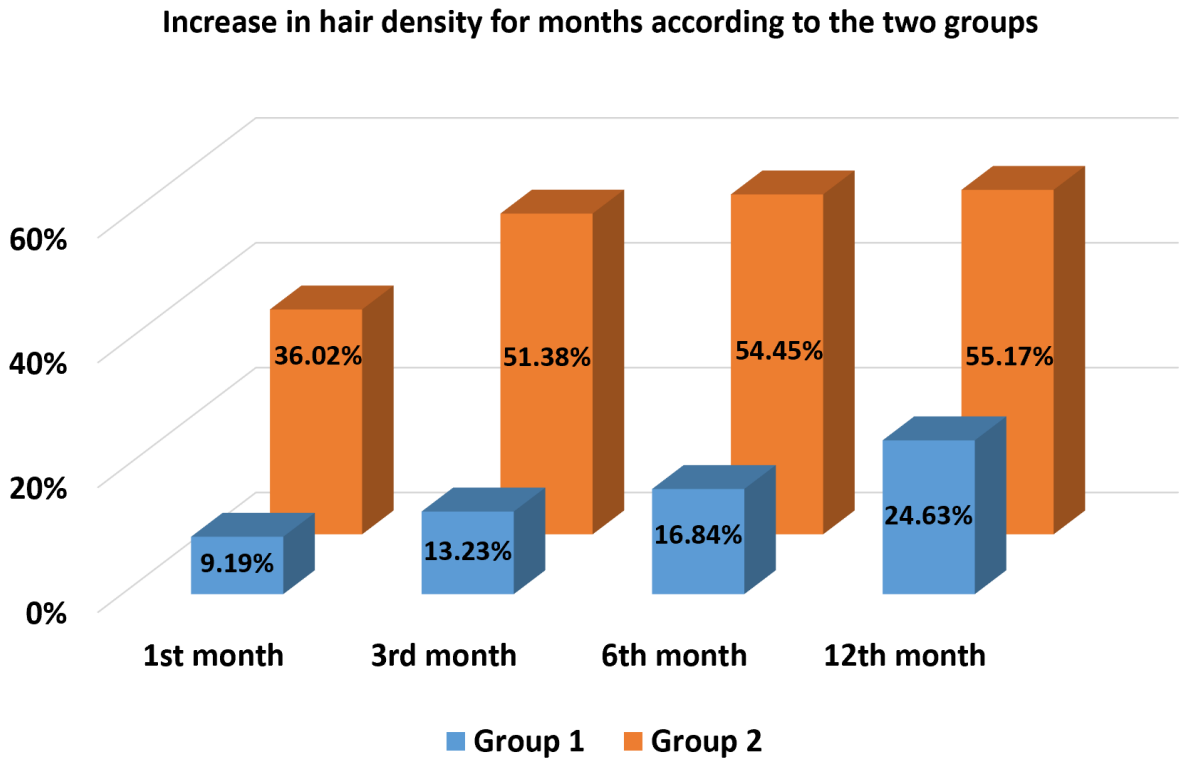
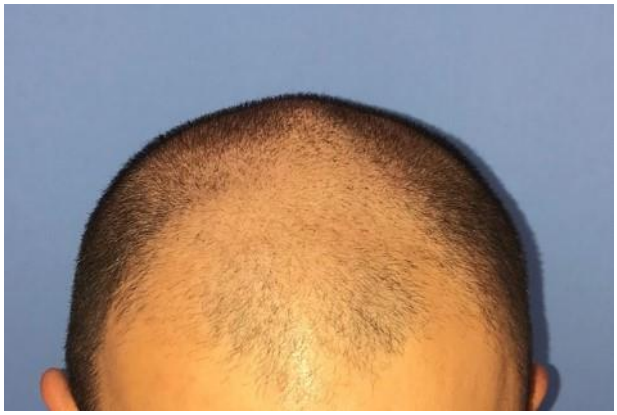


Figure 3a



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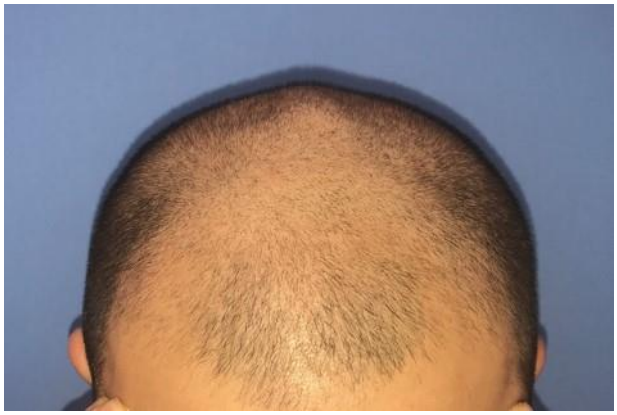

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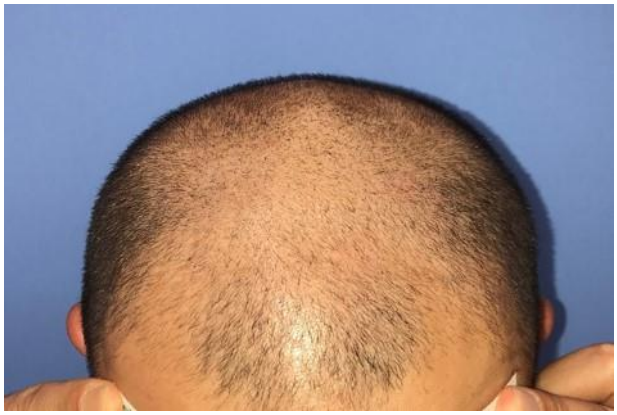

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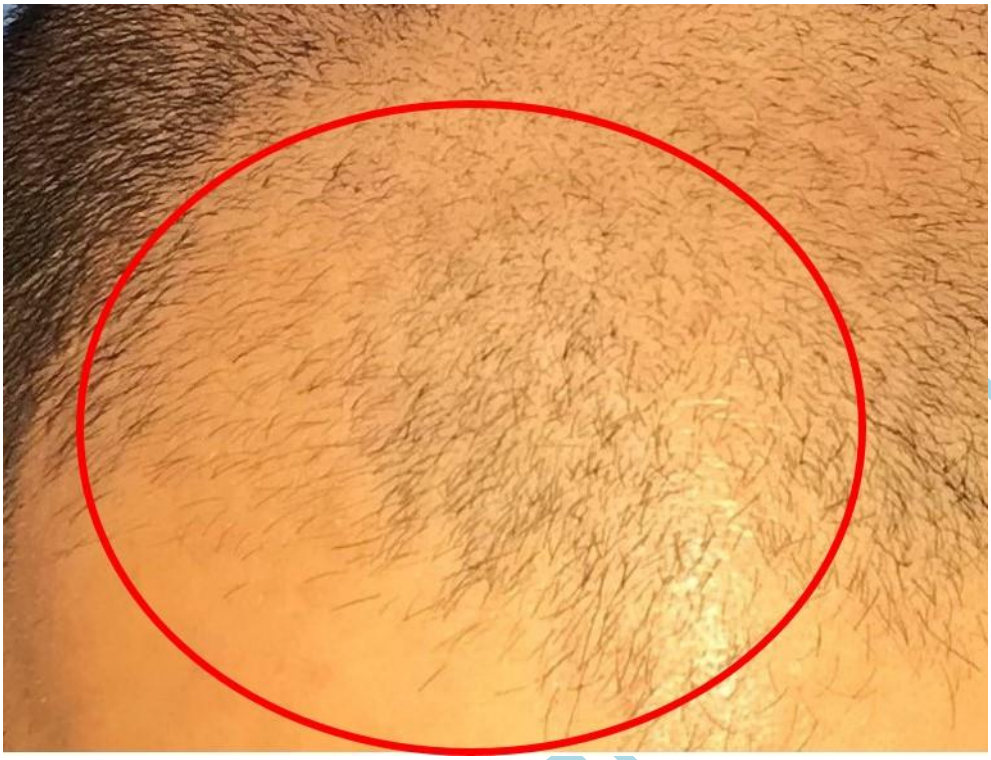

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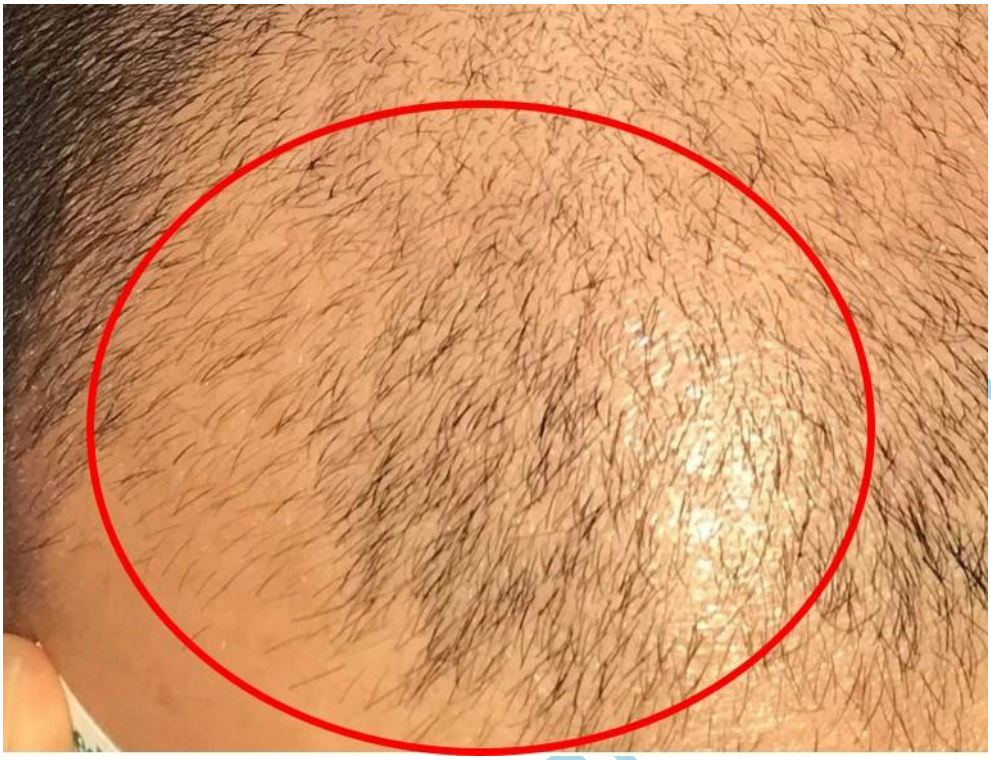

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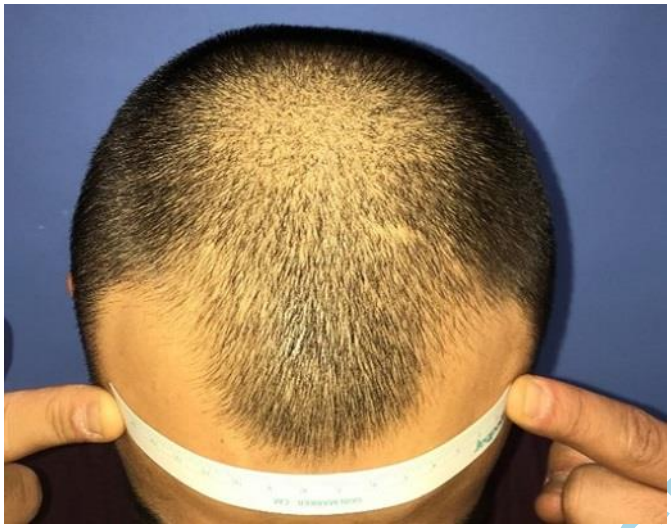

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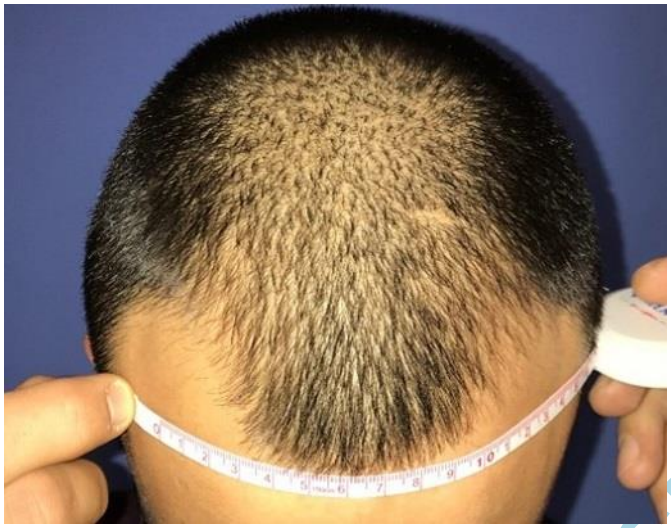

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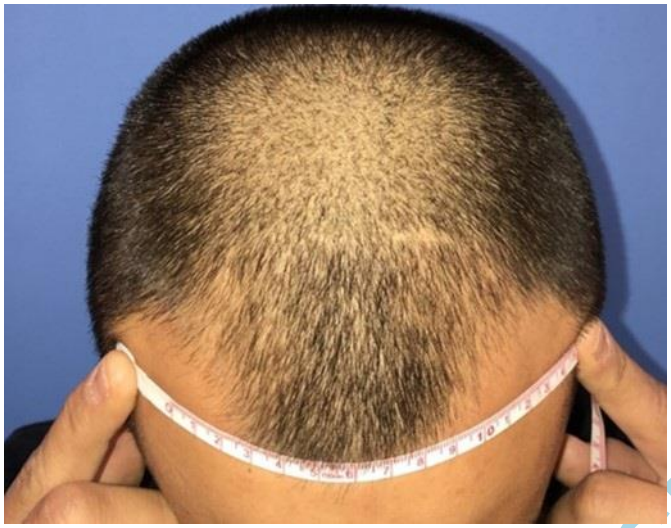

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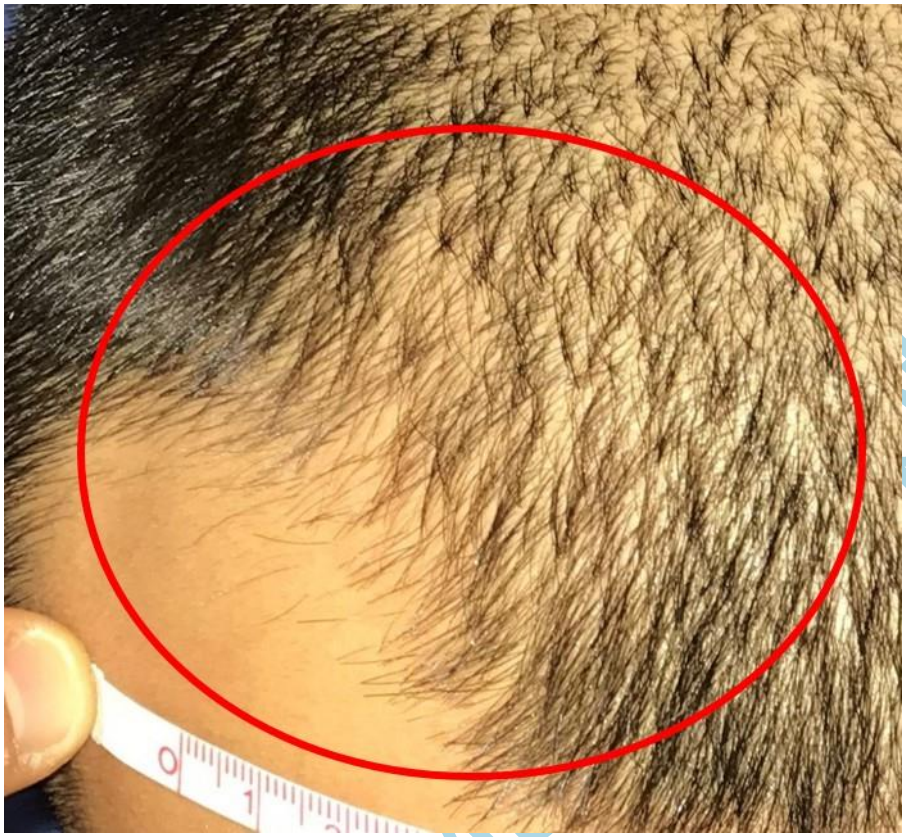

Figure 4e