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ESTROGEN RECEPTORS OF HAIRS BLACKS AND WHITES

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Background: Aging is termed as same as degenerative process, in which all part of tissue organs retarted the microstructure either macrostructure, forming and function even the colour, including black hair change to white hair. Several researchers have been recommended that estrogen hormone be able ease black to white hair, but hormone without any presenting of receptor won't be work properly. The main aim of this study were to determine amount of estrogen receptor contents in famales and males black and white hairs included the microscopically structure. **Method:** Twelve females and males there were 50 -56 years old each pairs black and white head hairs were plucked along with follicles. This estrogen receptors analyzed using radioreceptor binding assay there were 5mm eah hair follices including the root cutted and each pair put its in 2 ml glass tube already filled in with 500 µl ^{125}I-estradiol and incubated in 37^{o}C for 3 hrs. Following times were over the tube flushed twice carefully the hair won't be flushed. Then count by putting in the gamma counter chamber for 1 minute each. The values that shown in the monitor as CPM (count per minute), recorded as receptor of estradiol. **Results:** Mean (±SD) sum estrogen receptor in females black and white hairs were 479.3 ± 37.5 and 387.7 ± 33.0, but significantly decreased in male black hair was 316.9±17.8 and 274.0 ± 19.8. All those pairs significantly different either female black and white hairs or male black and white hair and also significantly different among groups. **Conclusion:** The lowest estrogen receptors recorded in male white hairs and microstructure decreasing of melanin contents.

Keywords: estrogen receptor; black; white hairs; microstructure

INTRODUCTION

Histologically human hair consisted with hair shaft and hair follicles, in which at growing period the hair follicle and shaft filled up with melanin as seen as black or brown. The changes from black/brown to white its an indicator a person got older there are a physiologically should not be avoided and all cells organs turning on in retarding structure, functioning, forming or even the colour. A study reported that women at young age belonging white hairs, can be predicted to be as a osteoporosis 4 times risk than averages.[1] The hair follicle is a miniorgan composed of an epithelial appendage of ectodermal origin. Morphologically begins with catagen (regression stage or apoptosis-driven involution). The transformations from telogen through six stages of anagen (growth and production stage) and eight stages of catagen, followed again by telogen (relative resting stage). Hair follicle is a target and source of prolactin, estrogen, cortisol, thyroid hormone and erythropoietin. Ovarian hormones can significantly influence the hair cycle. Estrogen can increase melanin synthesis and tyrosinase activity in human skin melanocytes culture.[2] Estrogen alter hair follicle growth and cycling by binding estrogen receptors (ERs), also modifies androgen metabolism within hair follicle. During growth hair period estrogen as mitotic and proliferation cells act needed more than the other hormones, therefore androstenedione and testosterone can be converted into E2 (estradiol) or E1 (estrone) by aromatase enzyme. The role of estrogen in regulation of the hair follicle cycle is still debated mainly regulation and mechanism of action or hormonal function in certain estrogen receptor. There are two distinct isoform of the ER as well as ERα and ERβ in which each kind of receptor localized in different part of hair follicles. In human hair follicles from male and female nonbalding scalp skin, ERβ expression was found to be localized in nuclei of outer root sheath and epithelial matrix keratinocytes, but ERα and androgen receptor were

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expressed in papilla cells. During the telogen period, ERα is maximally expressed.[3] Estrogen receptor β (ERβ) is widely expressed in the hair follicle, but the role of ERβ has yet to be explored. Active hair follicle pigmentation occurs only during active hair growth (anagen stage) so the duration of this stage could have direct implications for follicular melancyte homeostasis. No melanin pigment is actively produced during telogen (the relative-resting stage of the hair cycle). Melatonin interacts with melatonin receptor, androgen and estrogen receptor. Melatonin-IR (immunoreactivity) has been found in the hair-follicle bulb. Melatonin has been described to increase number of melanocytes in culture. Hair shaft pigmentation is generated by melanocytes of the hair follicle pigmentation unit and melanogenic activity is coupled to hair follicle cycling (anagen III-VI).[4] There is still minimal data of mechanisms melanocyte loss in order to melanin production related to estrogen receptor in certain with age. The objective of this study were to determine of estrogen receptors in females and males black and white hairs involved on microscopically structure to a complete better understanding of estrogen in the regulation of the hair follicle life cycle because is still under debate.

MATERIALS AND METHOD

Twelve females and males 50 to 56 years old already having two colour hairs, their head hairs were taken 2 peaces each white and 2 peaces black by plucking along with the follicles from frontal area. Most of those females already in perimenopause and postmenopause period, and were not differenteated to the other. The estrogen receptor analized using radioreceptor binding assay,[5,6] performed at Endocrinology Laboratory, Veterinary Obstetric of Reproduction Department, Faculty of Veterinary Medicine, Airlangga University. Each two peaces of those white and black hairs were cutted totally 5mm consisted of the hair roots and shaft from frontal area of the head, then put it into small glass tubes that containing with 500µL ^{125}I-estradiolequal with 10425CPM (count per minutes) (Diagnostic Product Corporation/DPC, CA-USA). All those speciment tubes were incubated in 37^{o}C in incubator for 3 hrs to allow the estrogen tracer to bind on receptors that containing in the hair cells. Following 3 hrs over the estrogen tracer in the speciment tubes were paw in the radioactive waste container, then rinse twice with 1ml aquadest each. Each speciment tube then put it in the Gamma counter chamber for 1 minute each , the cpm show up on the monitor recorded as a main data receptor. The data were presented in descriptive statistic and to differentiate among mean was analyzed using ANOVA 5% confidency then followed with LSD (SPSS-20 programme) and the curve builded using exell programme .

RESULTS AND DISCUSSIONS

From twelve female and males 50 to 56 years old those each pair black and white hair having the sum of estrogen receptors Mean (±SD) each two peaces in females black and white hair were 479.3±37.5 with range value between 399–516 and 387.7±33.0 with range values between 328–455 respectively. In males black and white hair were 316.9±17.8 with range values between 287–341 and 274.0±19.8 with range values of 254–309, respectively (Table 1).

Table 1
Sum of estradiol receptors (ERs) in females and males black and white hair

Parameters	ER Hairs of Female		ER Hairs of Male	
	Blacks	Whites	Blacks	Whites
N	12	12	12	12
Mean	479.33^{a}	387.70^{b}	361.90^{c}	274.00^{d}
SD	37.46	33.00	17.80	19.80
Range	399-516	328-455	287-341	254-309

Superscipts a,b,c and d in one raw were significantly different (F= 120.69 ; p<0.05)
SD = standard deviation

It was found decreasing ability of white hair to fix on the estrogen receptors compared to the black hair even though in famales or males. Appearly a significantly also found that black or white hair in females containing higher estrogen receptors (ER) than males black and white hair (Figure 1).

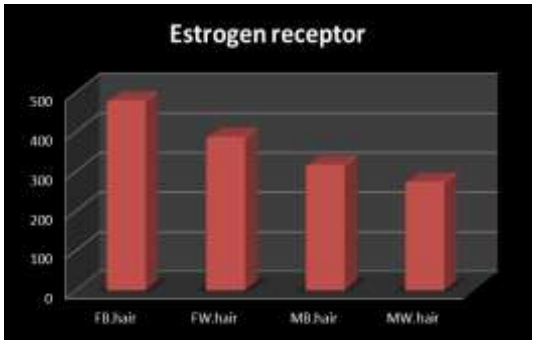

Figure 1
Estrogen Receptors (ERs) in females and male black and white hairs
FB: Female black; FW: Female white; MB: Male black; MW: Male white

Aromatase level in hair follicles from women were approximately six times higher than from male.[2] After stimulation with E2, ER expression was greatly enhanced in female scalp follicles, but no up-regulation ERβ expression was seen in male scalp hair follicles. E2 effects on human frontotemporal scalp hair follicle show significant differences between the sexes. There is sex-dependence differences in gene regulation of scalp

hair follicles.[3] Commonly existing of ER in the cytoplasma cells there are physiologically just a certained amount, then these concentrations to be increased when there are a stimulation by a similar antigen.[7] The influence of estrogen on pigmentation is still poorly understood and the functional role of ERα and ERβ in regulation of hair cycle is unknown. The presence of ER in melanocytes has been demonstrated using binding studies, immunocytochemistry and RT-PCR.[8] The biological process of the physiological aging appears to be associated with progressive loss of the melanocytes. During estrus or fertile period, mice and also women under controlled of higher estrogen concentration reported had also higher ER than the other estrus cycle, moreover in menopause mice this estrogen flat down in basal level and the ER found lowest compared to the other estrus cycles.[6] Existing of phytoestrogen in certain level in oil hair is useful to maintain persistently longer of black hair, that why estrogen known as anti white hair.[9] Phytoestrogen contents in several leafs as well as Pegaga and green clover extacts had been confirmed by proof using Radioimmunoassay (RIA) that the estradiol concentration higher 10 times in green clover compared to pegaga leaf.[10] It has been reported that in animal study after applied topical estrogen, regrowth of normally pigmented hair shafts in chemotherapy-induced alopecia group was accelerated compared to control group.[11] In human scalp hair follicle, topical estrogen decrease the telogen rate and prolongs the anagen phase.[12] As shown in Figure 2 below, the hair shaft quite clear be able differentiated between histological structure, black hair look so solidy black and white hair has empty melanin inside.

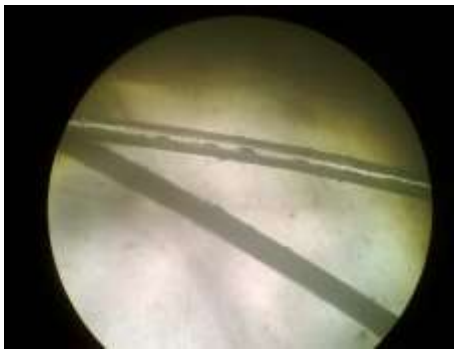

Figure 2
Representative histopathology of human head blacks and whites hairs (magnification 4x10)
Below position is a black hair shaft with solidy black colour.Upper position is a white hair shaft with empty or some melanin droplet inside.

In Figure 3, hair follicle and shaft of the white hair much tinner and narrow than black hair, with empty melanin inside. All 12 times observations microscopically result that the black hair solidy hair follicle and shaft containing with melanin as a black structure without any space shown in, but white hair besides of the hair follicle and hair shaft look much thinner than black hair, also clearly the hair shaft almost empty or just a part filled with melanin.

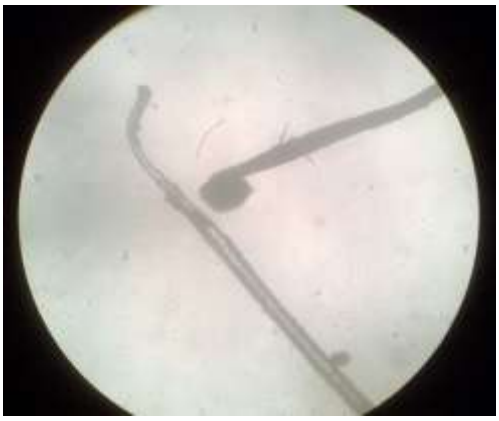

Figure 3
Representative histopathology of human blacks and whites hairs (magnification 2x10)
Upper position is black hair, hair follicle bigger and solidy black, follicle and shaft with melanine inside. Below left position is white hair, hair follicle and shaft with empty melanin inside.

This decreasing of melanin level to be able change black to white hair, but this condition be able eased or slowed down the changes by using estrogen is due to estrogen act actively stimulate tyrosin enzyme that can be catalyzed melanin formed.[1,13,14] Follicular melanin synthesis and pigment transfer to bulb keratinocytes are modified by hormonal signals. The chief part of melanocytes is in close contact with the dermal papilla, the prominent site of ERα.[3,15] The influence of estrogen on pigmentation is still poorly understood and the functional role of ERα and ERβ in regulation of hair cycle is unknown. The presence of ER in melanocytes has been demonstrated using binding studies, immuno cytochemistry and RT-PCR.[8] There is still minimal data of mechanisms melanocyte loss with age. Deficient of anti-apoptotic protein Bcl2, could loss of melanocyte stem cells. The other study in mice reported that accumulation of irreparable DNA damage with age may block melanocyte stem cells renewal.[16,17] BERKO mice display an accelerated catagen development with an increase in the number of apoptotic hair follicle keratinocytes. Estrogen receptor β (ERβ) may protect hair follicle from oxidative stress by inducing a antioxidative enzymes.[12] Other authors have suggested that estradiol effects on keratinocytes are mediated via a membrane ERα that activates the MAPK pathway.[3] By consuming extract of semanggi leaf (*Marsilea crenata* Presl) in menopause mice, the estrogen level detected by radioimmunoassay increased, and ERα and ERK1/2 expression in osteoblast from

proximal femur also increased.[18] Non genomic effect of estrogen is important in nonreproductive tissues. In one study reported that prevention of osteoblast apoptosis are mediated by non-genomic action of estrogen, involving the MAPK-signaling pathway mediated by ERα, ERβ and androgen receptor.[3] The role of non-genomic effects in hair follicle is as yet unknown. Hair follicle is controlled by complex signaling networks within the skin, the NF-kβ pathway and Wnt/β-catenin signaling networks. Transforming growth factor-β-activated kinase 1 (TAK1) is a member of MAP3 kinase family and a member of NK-kB pathway, involved in IL1 and TNF-α induced activation of NF-kB and MAP kinase. Sayama et al., (2010) reported in their study that the inflammatory mediator TAK1 regulates hair follicle, is required for anagen induction and maintenance.[19] In bone remodeling, the Wnt signal has a role to osteoblast differentiation and function, the mechanism may through cannocial β-catenin and BMP2 interaction.[19] Before this study between estrogen and its action in target hair no yet any clarification what the changes occurred, so now this finding make among estrogen mechanism of action and the receptors estrogen changes in white hair appearly to be much more clear. Although melanin doesn't analyzed quantitatively as well as ER in this study, however from microscopically analysis show much more the hair change to white the ER decrease and also followed with reducing of melanin.

CONCLUSIONS

The lowest estrogen receptor had been found in males white hair and then followed to be higher in males black hair, females white hair and the highest receptor in female black hair. Microscopically can not be differentiated between female and males white hair there almost the same stucture showing a little droplets or almost empty follicle hair from melanin.

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