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Serum Estrogen and Estrogen Receptor Beta Levels in Female and Male Patients with Vitiligo

Nagwan A. Sabek[1,*], Moustafa M Eyada[2], Shiymaa M Abdel Aziz[2], Shimaa M Demerdash[2], Amal H. Goma[2], Shereen Fikry[2]

[1]Medical Biochemistry Department, Suez Canal University, Ismailia, Egypt
[2]Dermatology & Venereology Department, Suez Canal University, Ismailia, Egypt
*Corresponding author: Nagwan_yasser@yahoo.com

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Abstract Background: Vitiligo is a common skin disease characterized by cutaneous white macules. The exact pathogenesis of vitiligo is not yet known. Estrogen receptor β (ERβ) was expressed in the melanocytes and has a possible role in pathogenesis of autoimmune diseases. Objective: This study aimed to assess serum estrogen and ERβ levels in female and male vitiligo patients and studied their association with the disease activity and severity; to our knowledge this study is the first one that assessed the serum level of estrogen and estrogen receptor β in patients with vitiligo. Subjects and Method: the study was conducted on 30 female and 30 male patients with vitiligo and 30 female and 30 male healthy controls. All patients were subjected to full history taking and clinical assessment; serum estrogen and ERβ were measured using enzyme linked immune-assay (ELISA) kits. Results: Our results showed that serum estrogen level was statistically significant higher in female vitiligo patients compared to controls (46.3± 42.0 vs 30.0±28.9, P=0.028 respectively). Serum ERβ level was statistically lower in vitiligo patients compared to controls (96.5±52.2 vs 118.2 ± 76.0, P=0.035, respectively), also there was significantly difference in serum ERβ levels among different pattern of vitiligo (P=0.049), also we found serum estrogen levels were significantly higher in male vitiligo patients compared to controls (30.3 ±17.9 pg/ml, 21.3±11pg/ml, p=0.022). There was statistically significant difference in serum estrogen level in patients with different disease pattern (P=0.003). Conclusions: In our study population, there was a statistically significant difference in serum estrogen and ERβ in vitiligo female and male patients compared to their controls, these results might be highlight their possible role in the pathogenesis of vitiligo.

Keywords: *Vitiligo, Estrogen, Estrogen receptor β (ERβ), melanocytes, vitiligo area severity index (VASI), vitiligo disease activity score (VIDA), enzyme linked immune-assay (ELISA)*

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1. Introduction

Vitiligo is an acquired disorder of the skin and mucous membranes that is characterized by well circumscribed, de-pigmented macules and patches that occurs secondary to selective destruction of melanocytes [1].

It may appear at any age; cases have been reported as early as 6 weeks after birth with approximately 0.5% to 1% of the population is affected and almost half present before 20 years of age. Its prevalence appears to be equal between men and women, and there is no difference in rates of occurrence according to skin type or race [2].

Generalized vitiligo is the most common type and is characterized by few to many widespread macules, universal vitiligo involves more than 80% of the body [3]. The actual pathogenesis of vitiligo is under debate and had been attributed to autoimmune causes, oxidative stress, and/or sympathetic neurogenic disturbance [4].

There are many theories about the etiology of vitiligo, including the self-destruct, biochemical, neural, autoimmune, and genetic hypothesis. Autoimmunity has been suggested to play a role in the development of the vitiligo [5].

Estrogen-induced immunomodulation is mediated via estrogen receptors alpha and beta (ER α/β) that are expressed on most immune cells. ERs have prominent effects on immune function in both the innate and adaptive immune responses [6].

ER β appears to be more widely expressed and has shown to be present in both male and female reproductive tissues in addition to non-reproductive tissues including the lung, bladder, heart, adrenal, thymus, kidney, pituitary, hypothalamus and skin [7].

The estrogen receptor (ER) belongs to a superfamily of nuclear receptors including those for steroid , thyroid hormones, vitamin-D3 and retinoic acid. The two estrogen receptors are not generated from alternate transcription sites of the same gene, as progesterone receptor [8]. ERβ

is localized on human chromosome 14, while ERα is found on chromosome 6 [9].

The number of ER has been reported to vary in different parts of the body, with receptor levels higher in facial skin than in skin from the thigh or breast [10]. The primary mechanism of 17β estradiol (E2) action is mediated by transcriptional activity of the intracellular estrogen receptors (ER); ER α and ERβ, the mRNA expression of ERα and ERβ had been demonstrated in human peripheral blood lymphocytes (PBLs) [11].

Based on female predominance of autoimmune diseases, the role of gender and sex hormones in the immune system is of long-term interest. ERs are nuclear hormone receptors which bind directly to estrogen response elements in gene promoters or serve as cofactors with other transcription factors, cytoplasmic estrogen receptors impact specific kinase signaling pathways [6].

Melanocytes are the cell producing melanin also they are present in retinal pigment and melanocyte secretes melanosome to keratinocyte [12]. Tyrosinase is key enzyme required for melanin synthesis, which catalyze the hydroxylation of tyrosine to dihyroxyphenylalanine (DOPA) which undergo oxidation to dopaquinon [13].

For a long time, it has been suggested that estrogens may be involved in the depigmentation process of vitiligo because the initiation/progression of the disease is observed at pregnancy, or after the use of oral contraceptives/hormonal substitution [14].

Human T lymphocytes express both ERα and ERβ. Furthermore, estrogen mediates effects on lymphocytes to modulate cytokine production through transcriptional regulation [15]. Estrogens are degraded by oxidative metabolism via the enzyme cytochrome P450 to generate H_2O_2 which induce DNA as shown in human peripheral blood lymphocytes [16].

The role of H_2O_2 in pathogenesis of vitiligo depends on its ability to cause various changes in the immune system and protein structures which contribute in the pathogenesis of vitiligo. The higher concentrations of H_2O_2 are potent competitive inhibitor of human tyrosinase enzyme which regulates the melanin synthesis [17].

As vitiligo is a common disorder of pigmentation affects about 1% of the world population and causes a tremendous impact on the quality of life in affected patients so this study aimed to assess serum estrogen and estrogen receptor β levels in female and male vitiligo patients and to determine its association with the pattern and severity of the disease.

2. Subjects and Methods

The present study was carried out as case control study among 30 female and 30 male vitiligo patients attending the outpatient clinic of dermatology and 30 female and 30 male healthy controls. Patients with other autoimmune or liver diseases and or receiving hormonal therapy, PUVA, radiation or psoralen were excluded. The study protocol was approved by Ethics Committee of faculty of medicine of Suez Canal University hospital and written informed consents were obtained from all participants included in the study.

2.1. Methods

History was obtained taking into account age, education, duration, course and onset of disease, precipitating factors, and any previous forms of therapy whether systemic or topical and general medical status. All patients were examined to determine the site, distribution, number, and approximate surface area of the lesions. The vitiligo area severity index (VASI) [18] and vitiligo disease activity score (VIDA) [19] were used for assessment of severity and activity of vitiligo respectively. Vitiligo has been classified based on the extension and distribution of the lesions into; localized, generalized, and universal. Localized vitiligo is further subdivided into focal and segmental. Focal vitiligo involves one or more patches in one area but not in a segmental pattern, while the segmental vitiligo involves one or more macules in a dermatomal distribution [3]. Human estrogen receptor ELISA Core Kit (Biopark, Optics Valley, Wuhan, CHINA) and Human estradiol ELISA Core Kit (Biopark, Optics Valley, Wuhan, CHINA) were used for measuring both serum estrogen and ERβ [20]. Expected values for estrogen receptor as follow: In females: postmenopausal phase <18 pg/ml. Early follicular (30-100 pg/ml), late follicular (100-400 pg/ml), luteal phase (60-150 pg/ml) pregnant (normal up to 35,000 pg/ml) and prepubertal children (normal <10 pg/ml) [21]. Expected values for serum estrogen: In females: postmenopausal phase < 18 pg/mlEarly follicular 30-100 pg/ml Late follicular 100-400 pg/mlluteal phase 60-150 pg/mlpregnant , normal up to 35,000 pg/ml prepubertal children, normal < 10 pg/ml [21].

2.2. Assessment of Serum Estrogen Receptor and Estrogen by ELISA

Concentration in serum was determined by ELISA as recommended by the manufacturer. The micro-titer plate provided in this kit has been pre-coated with an antibody specific to ER-beta or estrogen. Standards and samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for ER-beta or estrogen and Avidin conjugated to Horseradish Peroxidase (HRP) (RPN1231, Amersham-Pharmacia, Buckinghamshire, UK) is added to each microplate well and incubated. Then a Tetramethylbenzidine (TMB) chromogen substrate solution is added (Sigma Fast OPD, Sigma, St Louis, MO) to each well. Only those wells that contain ER-beta or estrogen, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of ER-beta or estrogen in the samples is then determined by comparing the optical density of the samples to the standard curve [20].

2.3. Statistical Analysis

Gathered data were processed using SPSS version 15 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as means ± SD while qualitative data were expressed as numbers and percentages (%). Student *t* test was used to test significance of difference for quantitative variables and Chi Square was used to test significance of difference for qualitative variables. A probability value (p-value) ≤ 0.05 was considered statistically significant.

3. Results

Our results showed that the mean age of the studied patients was 30.5 ± 17.7 years old while the control was 29.1 ± 12.5 years, 76.7% of the cases within the child bearing period while 80% among control females, as in Table 1. The majority of studied patients demonstrate a progressive course of vitiligo (90%), most of our studied patients had a vulgaris type of vitiligo (70%), There was statistically significant difference in serum estrogen receptor β levels among the different pattern of vitiligo disease (p=0.049) as in Table 2.

Table 1. Distribution of age in female group

Age	patients group (n = 30)		Control group (n = 30)		p-value
	No	(%)	No	(%)	
Pre-pubertal	2	6.6%	2	6.6%	0.936
Child bearing	23	76.7%	24	80%	
Post-menopausal	5	16.7%	4	13.4%	
Mean ± SD	30.5 ± 17.7		29.1 ± 12.5		

P< 0.05 is statistically significant; student t- test was used.

Table 2. Serum Estrogen Receptor β Levels According to Vitiligo Types in Female Patients

Type of vitiligo	Female patients (n = 30)	Estrogen receptor β level Mean ± SD	p-value
Vulgaris	70%	81.4 ± 33.8 pg/ml	0. 049*
Acrofacial	13.3%	158.3±125.7pg/ml	
Segmental	10%	100 ± 56.5 pg/ml	
Focal	6.7%	127.5 ± 14.4 pg/ml	

P< 0.05 is statistically significant; student t- test was used.

Serum estrogen receptor and estrogen levels: Our results showed that the mean serum estrogen level was significantly higher in all age groups compared to control group (22.5 ± 10.6 pg/ml, 51.7 ± 46.4 pg/ml and 31.0 ± 12.5 pg/ml versus 10.0 pg/ml, 35.0 ± 30.6 pg/ml and 30 ± 28.9 pg/ml respectively)(P=0.028) as in Table 3. Also our results showed that the mean of serum estrogen receptor β level in female patients was significantly lower compared to control group (96.5 ± 52.0 pg/ml versus 118.0 ± 76.0 pg/ml respectively) (P=0.035) Table 4.

Table 3. Serum Estrogen Levels in Female Patients.

Estrogen levels pg/ml Mean ± SD	patients (n = 30)	Control *(n = 30)*	P-value
Pre-pubertal (<18 pg/ml)	22.5 ± 10.6	10.0 ± 0	0.028*
Child-bearing (30-100 pg/mL)*	51.7 ± 46.4	35.0 ± 30.6	
Post-menopausal (<10 pg/ml)*	31.0 ± 12.5	11.3 ± 2.5	
	46.3±42	30.0 ± 28.9	

P< 0.05 is statistically significant; student t- test was used.

Table 4. Serum estrogen receptor β levels in Female Patients.

Estrogen receptor β level (pg/ml) Mean ± SD	patients (n = 30)	Control (n = 30)	P-value
Pre-pubertal	220 ± 113.1	87.5± 38.9	0.035*
Child bearing	88.91 ± 36.3	121.5± 83.1	
Post-menopausal	82 ± 30.1	112.5 ± 40.9	
	96.5 ± 52.0	118 ± 76.0	

P< 0.05 is statistically significant; student t- test was used.

According to the duration of vitiligo; there were no statistically significant differences in serum estrogen and estrogen receptor β levels (p>0.05) According to VASI; the highest mean serum estrogen receptor β levels (131.7±70.4 pg/ml) was associated with VASI (10-25) and the lowest mean (72.5± 31.8pg/ml) was associated with VASI (>25-50). The differences between serum estrogen receptor β levels according to VASI were not statistically significant (p>0.05). According to VIDA score; the differences between serum estrogen and estrogen receptor β levels were not statistically significant differences (p>0.05).

Results of male studied group; our results showed that the mean age for male vitiligo patients and controls were 26.3 ± 14.5 and 23.3 ± 11.4 years respectively. Serum estrogen level was significantly higher in vitiligo male patients as compared to controls with mean 30.3 ±17.9 pg/ml and 21.3 ± 11.0 pg/ml respectively (p= 0.022). Regarding serum ERβ level, our results showed that there was lower non- significant difference in serum ERβ in patient compared to controls (91.2 ± 33.2 pg/ml vs. 99 ± 42.8 pg/ml respectively, P=0.431) as shown in Table (5). Regarding to the disease severity, the VASI score ranged from 0.10 to 7.50 with mean score 2.73±2.76. We found no significant correlation between serum level of ERβ and severity (using VASI). Our results also showed that the highest mean level of estrogen according to VIDA scoring system was found in +2 (new lesions appeared in last 3-6 m) with mean level 41.7 ± 20.2 pg/ml, while the lowest mean level of estrogen was found in 0 activity (the disease is stable for 1 year or more) with mean 23.9 ± 10.2 pg/ml. There was no statistically significant difference in serum level of estrogen among patients with different disease activity (*p* =0.341). Our results also showed statistically

significant correlation between serum concentration of estrogen and severity of vitiligo using VASI (r=0.51, P= 0.004).

Table 5. Serum estrogen and ERβ levels among male vitiligo patients and controls

Mean ± SD	patients (n = 30)	Control (n = 30)	*P-value*
estrogen levels pg/ml	30.3 ± 17.9	21.3 ± 11.0	0.022*
ERβ level (pg/ml)	91.2 ± 33.2	99 ± 42.8	0.431

*Statistically significant at $p \leq 0.05$, student t- test was used.

4. Discussion

Vitiligo is a multifactorial disorder related with genetic, environmental, local, and endocrine factors. The causes of vitiligo were focused on three different mechanisms: autoimmune, autocytotoxicand neural factors [22]**.** The destruction is thought to be due to an autoimmune problem, multiple immunological, neurological, and genetic components have been considered in the pathogenesis of the disease [23].

Cultured human epidermal melanocytes hava shown to contain estrogen receptors by ligand-binding studies [24]. A biological effect of 17β-estradiol on these cells, demonstrating that estrogens can increase epidermal melanocyte cell numbers, while decreasing melanin content and tyrosinase activity [25].

Estrogens have prominent effects on immune functions and both ERα and ERβ are expressed in immune cells [26]. It seems that estrogen may indeed be associated with a predisposition for autoimmune disorders in men [27] and play important roles in pathophysiology of autoimmune rheumatic diseases [28]. Estrogens are confirmed as one of the risk factors in autoimmunity but details about the role of ERβ have yet to be fully characterized [29].

Up till our knowledge our study is the first study which assesses the serum estrogen and ERβ levels in female and male vitiligo patients. In our study the results revealed that serum ERβ levels were statistically lower in the female and male vitiligo patients compared to controls, and the serum estrogen levels were statistically higher in patients compared to controls. Our results were consistent with the study done by Akihiro et al., [30] who found that expression of ERβ by peripheral blood mononuclear cells (PBMC) from SLE patients was lower compared with controls.

Our results were in agreement with Doukas et al., [27] who reported increasing of serum estrogen levels in men with autoimmune disorders than controls.

Our results revealed that there were no association between serum ERβ and estrogen level and the duration of vitiligo in female patients, also we found the mean duration of the disease was 10.7 years which not in accordance with Jin et al., [31], who reported the mean duration of the disease was 27.7 years.

Naresh et al., 2013 showed that oxidative stress is one of the main principal causes of vitiligo [32]. The generation of H_2O_2 by estrogens and other aromatic steroids (e.g. progesterone) can contribute to DNA damage as shown in human PBLs and in spermatozoa .Therefore, it was tempting to ask the question whether peripheral blood lymphocytes in patients with vitiligo would be more susceptible to estrogen-induced DNA damage compared to healthy controls [16].

Schallreuter et al., 2006 [33] showed that estrogens had contribute to the oxidative stress via H_2O_2 in lymphocytes, leading to DNA damage in these cells. However, in addition, a direct effect of the estrogens, which was not prevented by catalase, demonstrating that semiquinone and orthoquinone metabolites from the hormone can contribute to DNA damage.

Our results are in accordance with Cutolo et al.,[28] who found that synovial fluid concentration of free estrogens was elevated in Rheumatic Arthritis patients. While our results are not in accordance with McMurray and May [34]; they didn't find significant differences in estrogen levels of male patients with SLE compared to controls**.**

The explanation for our study results regarding the association of serum estrogen and serum ERβ in female and male patients with vitiligo suggested that the pathogenesis of the disease occur due to deficiency in ERβ with increase in serum estrogen levels which were significantly higher in our cases than controls.

In conclusion, our study supports the hypothesis that estrogen and estrogen receptor β play an important role in pathogenesis of vitiligo.

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Conflict of Interests

All author have no conflict of interest

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of Ethics Committee of faculty of medicine of Suez Canal University hospital

Informed Consent

Informed consents were obtained from all participants included in the study

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