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RESEARCH ARTICLE

Estimation of Serum Levels of Testosterone and Estrogen in Vitiligo Patients

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Abstract

Vitiligo is an idiopathic systemic autoimmune disorder affecting skin, hair and oral mucosa. This genetic moreover acquired disease distinguished by melanin absence causes morbidity over all races. Although thyroid disorder has been known as a key trigger of this pathology, arange of other factors plays serious role in its presentation. Several hormones such as, testosterone and estrogen have been suspected as drivers of this disorder. The present study aimed to determine the levels oftestosterone, estrogen and the ratio of testosterone/ estrogen in serum of patients with vitiligo compared to matches control group .The study included50 patients from Egyptian population with lesions of generalized vitiligo (29 males and 21 females; median age 35.0 years; range 28–40 years and 50 healthy volunteers as control group (22 males and 28 females; median age 36.1 years; range 25–40years). All patients of vitiligo were recruited from Medical research center of excellence of National Research Centre, Egypt, during a period of 4 months (February 2019 to May 2019). The mean duration of the disease in the vitiligo group was 5.00years.Vitiligo diagnosis is based on if areas of patient's skin, hair or eyes lose coloring and confirmation is done using Wood's lamp. Blood samples were obtained from both the patient and control groups, testosterone, estrogen and the ratio of testosterone/ estrogen. Results: The present results declared significant elevation of testosterone (+144.5), estrogen (+116.048) as well as testosterone/ estrogen ratio compared to control subjects. Conclusion: Some endocrine markers play a principle role in pathogenesis and/or consequences of vitiligo. The abnormally disturbed levels of these markers lead to melanocyte damage and/or depigmentation.

Keywords: *Autoimmune disease, Melanin loss, Oxidative stress, Vitiligo, Estrogen, Testosterone, Testosterone /Estrogen ratio*

Introduction

One of the acquired diseases of the skin and mucous membranes is the vitiligo which is well characterized by circumscribed, macules depigmentation and patches which occurs as a results of distinct damage in melanocytes. It may happen at any age; patients have been demonstrated as early as six weeks post birth. Population about 0.5 - 1% is affected, and nearly half found before 20 years of age. Its prevalence seems to be similar between men and women, and there is no variance in rates of appearance according to type of skin or race [1].The pathogenesis of actually occurrence of vitiligo is not well understood. There are several hypothesis about the vitiligoetiology, involving the most predominant involving autoimmune, neurohumoral, and autocytotoxic [2]. None are completely exclusive, and it is similarly that they each partially contribute. The convergence theory states that stress, toxic accumulation of compounds, infection and autoimmune diseases, mutations changed the environment of the cells, and the impairment

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of the melanocyte migration can all implicate to the pathogenesis of vitiligo [1,3].

Vitiligo is a genetic acquired disease causing morbidity over all races. Although thyroid disorder has been known as a key trigger of this pathology, arange of other factors plays serious role in its presentation. Several hormones (adrenocorticotropic hormone, α-melanocyte-stimulating hormone, melatonin, testosterone, estrogen), genes (Human leukocyte antigen (HLA), Forkhead box D3 (FOXD3), Estrogen receptor (ESR) 1, Cyclooxygenase-2 (COX2), Vitiligo-associated protein 1 (VIT1)), and lifestyle choices (stress, diet, cosmetic products, and medications) have been assumed as drivers of this disorder [4].

It was found that the factors regulating the production of pigment in skin are complicated and not well - understood. It has been known for a long a period that the colour of the skin often differs during pregnancy, which supposes that sex steroid hormones may be implicated in this event, so that estrogen elevates the production of pigment in melanocytes of human, while progesterone reducesit [5].

Estrogens may be implicated in the process of depigmentation of vitiligo because the disease's initiation/progression is observed at pregnancy, or post contraceptives/hormonal substitution handling [6]. Immunomodulation is observed to be mediated by estrogen via receptors alpha and beta (ER α/ß) of estrogen that are strongly expressed on most cells of immune system. ERs have effects mainly on the function of immune system in both the innate immune response and adaptive one [7].

The levels of serum ERß were previously found to be statistically lower in the vitiligo female and male patients compared to controls and also, the serum estrogen levels were higher in patients compared to controls [8].The effect of a biological 17ß- estradiol on these cells, elucidating that, estrogens can enhance the numbers of melanocyte epidermal cell, while reducing the content of melanin and activity of tyrosinase [9].

The pathogenesis of the steroid hormone; testosterone-mediated pigmentation is till now unknown. Histologic investigation demonstrates that, epidermal cells of melanocytes might have been induced by testosterone and exposure to sun, which might have elevated the production of melanin. Previously Shuttle worth et al. [10]indicating the relationship between methyl testosterone and acanthosisnigricans [10] Hence, the present study aimed to assess the role of both estrogen and testosterone , the serum levels of both hormones as well as declaring the testosterone/ estradiol ratio in 20-40 years old of Vitiligo patients (Non-segmental and segmental).

Subjects and Methods

Subjects

Depending on Hintze [11] who found that Mean± SE of the Testosterone (nmol/L) in control and stable vitiligo 10.42 ± 1.85 and 5.20 ± 0.81 respectively, and assuming the power= 0.80 and α=0.05, and by using PASS 11th release the minimal sample size for an equal size a controlled clinical trial is 50 in each group.

The study was carried out among 100 subjects attending the outpatient Dermatology Clinic of Medical research centre of excellence, Egypt, during a period of 4 months (February 2019 to May 2019). Written informed consents were obtained from all participants included in the study. The study included 50 patients with lesions of generalized vitiligo (29 males and 13 females; median age 35.1 years; range 20–40 years and 50 healthy volunteers as a control group (22 males and 14 females; median age 36.2 years; range 25-40 years). None of the patients had been treated for vitiligo.

A general dermatological examination was performed for all patients. The location of vitiligo lesions was recorded. Clinical subtypes of vitiligo were defined as segmental or non-segmental. All patients were examined to determine the site, distribution, number, and approximate surface area of the lesions. Vitiligo was diagnosed clinically (presence of white milky macules and patches and confirmed with wood's light examination).Inclusion criteria including, male and female patients with vitiligo, age from 20 to 40 years, stable disease for 1 year, patients not suffering from other autoimmune diseases, receiving hormonal therapy in the last 6 months, not receiving ultraviolet therapy and psoralen in the last 6 months, not receiving any topical

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or systemic treatments in the last 6 months. However, exclusion criteria including, patients age < 20 years or > than 40 years, unstable disease ,patients suffering from other autoimmune diseases, patients who received hormonal therapy in the last 6 months, patients who received ultraviolet therapy and psoralen in the last 6 months, patients who received any topical or systemic treatments in the last 6 months.

History has been 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. Blood samples were obtained from both the patient and control groups. Testosterone, estrogen levels and testosterone /estrogen ratio were examined, in serum samples. The study protocol followed the Declaration of Medical Division, National Research Centre (NRC); all subjects were informed about the study protocol, and written consent was obtained from all participants. The study was approved by Ethics Committee of NRC, Cairo, Egypt with no" 19- 031.

The active phase of vitiligo was defined as the progression or appearance of new lesions within the previous 6 months.

Laboratory assessment

Peripheral blood samples from patients with vitiligo were obtained from Dermatology Dep., Excellence Centre Clinic, NRC, Egypt. In addition to peripheral blood samples from clinically healthy individuals were obtained in our Excellence Centre Hospital (NRC). Blood samples were collected in EDTA-containing tubes and anticoagulant-free tubes after an overnight fast. After immediate centrifugation (3,000 g) for 10 min at 4oC, plasma and serum were separated in Eppendorf tubes and frozen immediately at −80oC until analysis.

Human estradiol ELISA Core Kit and testosterone ELISA kit (Biopark, Optics Valley, and Wuhan, CHINA and ALPCO immunoassays, USA respectively) were used for measuring serum estrogen and testosterone.

Results

Table 1: Testosterone, Estrogen and Testosterone/ Estrogen ratio in vitiligo patients compared to matches control

Markers	**Control**		**Stable vitiligo patients (SVP)**	
	Male	**Female**	**Male**	**Female**
Testosterone(nmol/L)	30.90±2.77[a]	1.60± 2.77[b]	11.66±1.10[c]	2.00±0.33[b]
% Change				
Estrogen (Pmol/L)	100.30±4.40[d]	1200±40.00[e]	125.40±9.00[d]	1350.00±59.0[f]
Testosterone/ Estrogen ratio	7.0 /14.0[g]		18.0/29.0[h]	

Data are expressed as Mean ±SD . Statistical analysis is carried out using SPSS computer program version 8 combined with co-state computer program , where different letters are significant at $p \leq 0.05$.

Table 1 : revealed significant decrease in testosterone level in serum of SVP compared to control .While marked significant increase in estrogen levels in female of SVP patients compared to female control subjects . Also, significant increase in testosterone/ estrogen ratio was recorded in SVP compared to control subjects.

Discussion

The vitiligoetiology is still complicated and unknown. Different hypothesis were suggested to illustrate the loss of function of melanocyte [12].As a results of the disturbances in neural and endocrinal status , other incorporated reasons involving ; an autoimmune disorders , an alteration in the homeostasis of tetrahydrobiopterin (BH4) [13], stressors of psychological condition [14]and defective in the defense mechanism for free radical on melanin production [15]. Several neural, emotional and/or stressful factors appear to have and play an essential role in initiation of vitiligo or exacerbation. Schallreuter [16] showed that keratinocytes of human are completely able to produce and/or destroy catecholamines. In this mechanism, tyrosine was spilt into melanin and catecholamine type neurotransmitters by tyrosine hydroxylase enzyme (signaling of

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neural molecules; DA, NE, etc, that regulate both central and peripheral nervous systems)[17]..

The present study declared significant decrease in the levels of testosterone in male of SVP compared to control, while significant increase in estrogen levels in female of SVP related to their corresponding control. Besides, the ratio of testosterone/ estrogen in SVP is significant higher compared to control. In a good connection with the present results El-Sayed et al.[15], indicated that the relationship between elevated levels of catecholamine and the process of depigmentation was illustrated by a biochemical theory demonstrated perturbations in the biopterins metabolic system due to the increased levels of (6R)-l-erythro 5,6,7, 8- tetrahydrobiopterin (6BH4) and its isomer 7BH4 in epidermis of vitiligo which occurred as a consequence of enhancement in the catecholamine biosynthesis on the expenditure of melanin and development of H2O2, which is melanocytes toxic agents and by autoimmune theories (where a destructed melanocyte elicits an autoimmune response).

Moreover, biopterins act as inhibitors of the phenylalanine hydroxylase and tyrosinase enzymes implicated in the process of melanogenesis [18]. In this context, the products of stress; reactive oxygen species (ROS) can be induced by several provocations such as catecholamine. Additionally, increased catecholamine abnormality caninitiate vasoconstriction causinghypoxia for epidermal-dermal tissue, and may be probablyoxidized through several oxidative system with the production of radicals and oxyradicals of quinones and semiquinone, .However, increased production of systemic H2O2 levels via catecholamine have the ability to change homeostasis of calcium, so disturbing the l-phenylalanine(precursor of the amino acid; tyrosine in melanocytes)uptake,.

It is practical to speculate that high oxidative radicals levels resulting from monoamine oxidation as well as their metabolites might contribute to early phase of melanocyte damage of vitiligo pateints [18,19]. Moreover, El Sayed et al. [15],illustrated that ,elevated catecholamines, DA, estrogen, and prolactin levels as a consequence of hyper-H2O2 production leads either to stimulation of immune reaction versus melanocytes (depigmentation) or inhibition to BH2 reductase and BH4 cofactor (reduced pigmentation), enhanced catecholamines stimulate biosynthesis of melatonin or activate its receptors. Further, the high level of estrogenin SVP might be occurred as a consequence of oxidative stress particularly H2O2 high levels are detected in the skin and even in VP blood cells as demonstrated by Rokos et al. [20], which is probably because of increased levels of estrogens (as observed in the current study) and this strongly implicated in the procedure of depigmentation of vitiligo [21].

Also, important enzymes, BH2 reductase (EC 1.6.99.7), which was entangledinre cycling of (6R)-l-erythro-5, 6, 7, 8-BH4 cofactor and was disabled by H2O2 [22, 23]. However, melasma (facial pigmentation of grayish brown colour) may often appear in women using oral pills of contraceptive containing analogs of steroid hormone supposing that, human pigmentation might be regulated with a factor other than α-MSH like estrogen. Therefore, estrogen acts as ligands, effects on production of melanin through its receptors on melanocytes [15].

Accordingly, women at child-bearing age due to high estrogen level could be less likely to develop vitiligo as described by Kang and Ortonne [24]. Also, El-Sayed et al.[15]declared the association between the lack of fertility and vitiligo, so in hypogonadism of male; the inadequate production of testosterone hormone leads to infertility. The same authors added that, the reduction in testosterone levels in serum of males with active or stable VP may be probably due to emotional affliction and low self-esteem which disturbs sexual life of patients.

In conclusion the current study probably presents novel competent approach for determination and diminution of the progress of vitiligo through a trial to understood the mechanism(s) of occurrence and the analysis of specific biomarkers contribute to its progress. Also, it is recommended that every dermatologist must take neurological, psychological, and endocrine diseases into account because the probable reasons concomitant with vitiligo disease are able to attenuate the advancement of the disease, and improve vitiligo management. Jointly, we can further address these neuro-

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endocrinal diseases connected by vitiligo disease.

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