DETECTION OF PLASMA AND URINARY CATECHOLAMINES AND THEIR METABOLITE LEVELS IN VITILIGO

Thesis

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ABSTRACT

Vitiligo is one of the oldest diseases known to mankind. Although not being life threatening or complicated by any serious internal manifestations, it still represents one of the most troubling diseases to both patient and physician. For the patient, it is a very serious disease with serious psychological implications. For the physician, it is a very difficult disease to treat with unpredictable prognosis. Catecholamines are chemical compounds derived from the amino acid tyrosine. Their name is derived from the fact that they contain catechol and amine moieties. They are water-soluble and are 50% bound to plasma proteins, so they circulate in the blood stream. They have been implicated in many dermatoses, but their role in the etiopathogenesis of vitiligo still remains largely obscure. Our aim is to evaluate the role of the neural factor in the pathogenesis of vitiligo by measuring catecholamines and their metabolites in the plasma and urine of patients suffering from vitiligo and to correlate these factors with the onset and the activity of the disease.

Key words: vitiligo, catecholamines, neural aspects

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$6BH_4$	(6R)-L-erythro-5,6,7,8-tetrahydrobiopterin
AAADC	aromatic L-amino acid decarboxylase
CLA	Cutaneous lymphocyte antigen
COMT	Catechol-O-methyltransferase
DA	Dopamine
DHPG	(S)-3,5-Dihydroxyphenylglycine
DOPA	L-3,4-dihydroxyphenylalanine
EDTA	Ethylenediaminetetraacetic acid
EP	Epinephrine
5-HIAA	5-Hydroxyindoleacetic acid
HLAs	Human leukocyte antigens
HPLC-ED	High-performance liquid chromography and electrochemical detection
5-HT	5-hydroxytryptamine
5-HTP	5-Hydroxy-L-tryptophan
HVA	Homovanillic acid
KCS	Keratinocytes
L-DOPA	L-3,4-dihydroxyphenylalanine
MAO	Monoamine oxidase
MCs	Melanocytes
MHPG	3-methoxy-4-hydroxyphenylglycol
MHPG-SO4	3-methoxy-4-hydroxyphenylglycol sulfate
MN	Metanephrine
MN-SO4	Metanephrine sulfate
mRNAs	Messenger ribonucleic acids
NAS	N-acetylserotonin
NB-UVB	Narrow Band Ultraviolet B
NE	Norepinephrine
NMN	Normetanephrine
NMN-SO4	Normetanephrine sulfate
NPY	Neuropeptide Y
PAH	Phenylalanine hydroxylase
PUVA	Psoralen-ultravioelt A
SULT1A3	Sulfotransferase type 1A3
TH	Tyrosine hydroxylase
TPH	L-Tryptophan-5-monooxygenase
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
VMA	Vanillylmandelic acid

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Background

Vitiligo is an acquired pigmentary disorder of the skin and mucous membranes, and it is characterized by circumscribed depigmented macules and patches. It is a progressive disorder in which some or all of the melanocytes in the affected skin are selectively destroyed. Vitiligo affects 0.5-2% of the world population, and the average age of onset is 20 years **(Bleehen and Anstey, 2004).**

Catecholamines are chemical compounds derived from the amino acid tyrosine. Their name is derived from the fact that they contain catechol and amine moieties. Some of them are biogenic amines (**Purves et al., 2008**). They are water-soluble and are 50% bound to plasma proteins, so they circulate in the bloodstream. The most abundant catecholamines are epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine, all of which are produced from phenylalanine and tyrosine. Tyrosine is created from phenylalanine by hydroxylation by the enzyme phenylalanine hydroxylase (PAH). Tyrosine is also ingested directly from dietary protein. It is then sent to catecholamine-secreting neurons. Here, many kinds of reactions convert it to dopamine (DA), to norepinephrine (NE), and eventually to epinephrine (EP) (**Joh and Hwang, 1987)**

Hypothesis

The aetiology of vitiligo is still controversial and several hypotheses have been proposed to explain the loss of melanocytes. These include: the intrinsic genetic susceptibility (**Boissy, 2000**), an autoimmune mechanism (**Bystryn, 2000**), an autotoxic destruction of melanocytes (**Hann and Chun, 2000),** an altered tetrahydrobiopterin homeostasis (**Schallreuter et al., 2000**), and neural hypothesis (**Orecchia, 2000**). The latter is supported by clinical, physiological, microscopic, ultrastructural,

immunohistochemical and biochemical findings **(Castanet and Ortonne, 1997).** Moreover, the catecholamines, which are consistently released as a consequence of emotional and or stressful events, are considered as being strictly related to the onset or worsening of the disease (**Kovacs, 1988**).

Different strategies have been adopted to study these neural markers and their metabolites. **Morrone et al. (1992)** first reported that significant increases in the urinary concentrations of catecholamine metabolites homovanillic acid (HVA) and vanillylmandelic acid (VMA) characterized the onset and progression of vitiligo, irrespective of the clinical type**. Schallreuter et al. (1994)** noticed an increase in NE production in both plasma and urine. Also higher urinary excretion values of indol metabolites in vitiliginous patients had been evidenced by **Chakraborty et al. (1996).** Other studies on these markers have focused on cellular levels with increased production of catecholamines being observed in lesional keratinocytes (**Schallreuter et al., 1991).**

Previous studies noted that patients during a recent disease onset showed significantly higher concentrations of plasma and urinary monoamines and their metabolites than long-term suffers and subjects in a stable phase (**Cucchi et al., 2000).** Urinary excretion of indole metabolites has been previously showed in vitiliginous patients **(Chakraborty et al., 1996)**.

Aim of the work

The aim of the present study is to evaluate the role of the neural factor in the pathogenesis of vitiligo by measuring catecholamines, their metabolite and 5-Hydroxyindoleacetic acid (5-HIAA) (serotonin metabolite) in the plasma and urine of patients with vitiligo and to correlate these factors with the onset and the activity of the disease.

VITILIGO

VITILIGO

Definition

Vitiligo is a common, acquired skin disorder characterized by well-demarcated, depigmented lesions with variable size and shape that have a tendency to expand over time **(Gupta and Kumar, 2003).**

Epidemiology

Vitiligo affects 0.5-2% of the world population, and the average age of onset is 20 years **(Bleehen and Anstey, 2004)**.

Pathophysiology

Vitiligo is a multifactorial polygenic disorder with a complex pathogenesis. It is related to both genetic and nongenetic factors (**Youn et al., 2000)**.

Although several theories have been proposed about the pathogenesis of vitiligo, the precise cause remains unknown (**Le Poole et al., 2000)**.

Generally agreed upon principles are an absence of functional melanocytes in vitiligo skin and a loss of histochemically recognized melanocytes, owing to their destruction (**Kovacs, 1998**). However, the destruction is most likely a slow process resulting in a progressive decrease of melanocytes (**Spritz, 2008**).

Theories regarding destruction of melanocytes include autoimmune mechanisms, cytotoxic mechanisms, an intrinsic defect of melanocytes, oxidant-antioxidant mechanisms, neural mechanisms and genetic mechanism (**Hsin-Su, 2002**).

a) Autoimmune theory

This theory is supported by the fact that vitiligo is commonly associated with autoimmune diseases. Initial studies failed to demonstrate specific antibodies against melanocytes **(Howanitz et al., 1981)**.

However, subsequent research clearly detected the existence of antibodies against melanocytes surface antigen in vitiligo patients and proved that the extent of depigmentation is correlated with the incidence and level of antibodies against melanocytes **(Naughton et al., 1983).**

Antibodies against pigmented cells were detected in the sera of patients with vitiligo. These antibodies were found in 78% of patients compared to only 14% of controls. Several antibodies were detected but the most common were against 40-45-KD, 75-KD and 90-KD antigens. These antigens were thought to be pigment cell surface antigen **(Kemp et al., 2007)**. Antityrosinase antibodies were found in patients with focal as well as those with generalized vitiligo. Antibodies activity was more apparent in active rather than stable vitiligo **(Baharav et al., 1996).**

It is likely that both humoral and cellular immunity co-operate in the destruction of melanocytes (**Ongenae et al., 2003)**.

Concerning cellular immunity, an important role has been given to the infiltrate underlying the pigmented lesional skin, where CD4 and CD8 positive T cells were detected, also expressing activation molecules **(Al Badri, 1993)**. A substantial number of infiltrating T cells express the cutaneous lymphocyte antigen (CLA) typical of skin homing T cells **(Al Badri, 1993)**, and a recent study localized CLA positive cytotoxic T cells in apposition to disappearing melanocytes in the perilesional skin **(Van den Wijngaard et al., 2000).** In vitiligo patients, high frequencies of Melan-A/Mart1 (a melanosomal antigen) specific CD8 positive T cells were detected in peripheral blood **(Ogg et al., 1998)**. Interestingly, Melan-A/Mart1 specific CD8 positive T lymphocytes were identified in inflammatory lesions of melanocyte destruction following infusion of Melan-A/Mart1 specific CD8 positive T-cell clones in melanoma

patients: this finding gives direct evidence of T-cell-mediated vitiligo **(Yee et al., 2001)**.

b) Intrinsic defect of melanocytes theory

Vitiligo melanocytes may have an intrinsic defect leading to melanocyte death. These melanocytes demonstrate various abnormalities, including abnormal, rough endoplasmic reticulum and incompetent synthesis and processing of melanocytes. In addition, homing-receptor dysregulation has also been detected. Early apoptosis of melanocytes has also been suggested as a cause of reduced melanocyte survival; however, subsequent investigation found that the relative apoptosis susceptibility of vitiligo melanocytes was comparable with that of normal control pigment cells (**van den Wijngaard et al., 2000**).

c) Disturbance in oxidant-antioxidant theory

Oxidant stress may also play an essential role in the pathogenesis of vitiligo. Studies suggest that accumulation of free radicals toxic to melanocytes leads to their destruction (**Halder and Chappell, 2009).**

Because patients with vitiligo exhibit a characteristic yellow/green or bluish fluorescence in clinically affected skin, this led to the discovery that the fluorescence is due to accumulation of two different oxidized pteridines (**Mosher et al., 1987)**. The overproduction of pteridines led to the discovery of a metabolic defect in tetrahydrobiopterin homeostasis in patients with vitiligo, which results in the accumulation of melanocytotoxic hydrogen peroxide **(Schallreuter et al., 2008**).

d) Genetic theory

The inheritance of vitiligo may involve genes associated with the biosynthesis of melanin, a response to oxidative stress, and regulation of autoimmunity (**Halder and Taliaferro, 2008**).

Human leukocyte antigens (HLAs) may be associated, but not in a consistent manner (**Zhang et al., 2004**).

e) Neural theory

This theory presupposes that there is a chemical mediator liberated at the nerve endings that destroys the melanocytes or inhibits production of melanin pigment (**Grimes, 2004).**

Lerner (1959) first proposed the neural theory which was based on:

1- Case reports of patients having nerve injury and vitiligo with decreased or absent skin findings in the denervated areas.

2- Segmental and dermatomal vitiligo.

3- Increased sweating and vasoconstriction in vitiligenous areas implying increased adrenergic activity.

Degeneration and regeneration of autonomic nerves with thickened basement membrane of dermal Schwann cells was detected in the center and periphery of depigmented patches (**Al'Abadie et al., 1995).**

Segmental vitiligo frequently occurs in a dermatomal pattern, which suggests that certain chemical mediators are released from nerve endings that affect melanin production. Further, sweating and vasoconstriction are increased in depigmented patches of vitiligo, implying an increase in adrenergic activity (**Slominski et al., 2004**).

Estimation of the urinary levels of HVA (a dopamine metabolite) and VMA (a norepinephrine and epinephrine metabolite) was found to be significantly higher in patients with early active vitiligo. This may be either the cause of vitiligo or due to stress **(Cucchi et al., 2003)**.

Depigmentation induced by acetylcholine has been reported, but acetylcholine has not been shown to be melanocytotoxic in vivo or in vitro (**Shelly and Ohman, 1969**).

Injection of EP into rats causes depigmentation but this may be related to vasoconstriction (**Selye, 1967**).