

## Interrelation of Immune, Antioxidant and Hormonal System Indices and their Influence on Melanogenesis in Patients with Skin Hyper Pigmentation

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### ABSTRACT

According to the results obtained after immunologic, hormonal and biochemical status investigations has been discussed peculiarities of melanogenesis in 135 patients with skin hyper pigmentation (78 patients with pigmented nevus and 57 – with melasma). The average age consisted – 36,5 year. Of 135 patients 32 were men and 103 women. Patients with pigmented nevus revealed increased rate of CD4<sup>+</sup>, CD25<sup>+</sup>, CD69<sup>+</sup>, HLA-DR<sup>+</sup>, IL-1, IL-6, IL-8, ACTH, MSH, DC, MDA, SOD, GP, CL and decrease in CD3<sup>+</sup>, CD8<sup>+</sup>, CD16<sup>+</sup>, CD95<sup>+</sup>, IL-2, cortisol levels. In the group of patients with melasma increased level of CD20, CD25, HLA-DR+, MSH, ACTH, DC, MDA and reduced concentration of CD3+, CD4+, CD8+, CD16+, CD69+, CD95+, IL-1, cortisol, SOD and CL were registered. Comparison of component levels of LP-AP systems and suppressive (CD8<sup>+</sup>) activity of lymphocytes in patients with hyper pigmentation revealed significant negative correlation and presence of close positive relation between CD25<sup>+</sup> and CD95<sup>+</sup> cells and indices of LP-AP systems and pituitary-adrenal gland systems as well. Has been detected positive correlation between IL-1 and cortisol in patients with melasma and negative correlation in patients with pigmented nevus. Patients with pigmented nevus revealed decreased production of IL-2 and on this background reduced concentration of cortisol was registered. In case of hyper pigmentation increased level of CD95<sup>+</sup> cells resulted in suppression of apoptosis and supported increase in quantity of melanocytes. Consequently, in case of hyper pigmentation insufficiency of apoptosis was obvious. Thus, in case of hyper pigmentation, interrelation of indices and intercellular regulations of immunologic, hormonal and antioxidant systems exists.

**KEYWORDS:** skin hyper pigmentation, melanogenesis, antioxidant system

In skin proceed both, common and specific only for skin processes, for example process of melanogenesis. Melanogenesis - high-regulated and complicated process has been detected in specialized cells - melanocytes [7,8]. Production of melanin is the specific defensive reaction of skin in humans that protects organism from ultra violet radiation (UVR). UV-rays, having damaging effect on skin, can directly affect on melanocytes or indirectly, owing to the products of keratins in the form of cytokines or melanotropic hormones [4,5,9,11]. Mechanisms, involved in the process of melanin synthesis inhibition via the cytokines and paracrine factors is still obscure.

Proceeding from the afore-said, the aim of our study was detection of peculiarities of melanogenesis via the investigation of immunologic, hormonal and biochemical statuses at skin hyper pigmentation.

### MATERIAL AND METHOD

A total of 78 patients with pigmented nevus (intradermal nevus, fibro-epithelial and Setton's nevus) and 57 – with melasma were under observation. The average age consisted – 36,5 year. Of 135 patients 32 were men and 103 women. Control group involved 40 practically healthy individuals with normal skin pigmentation.

Parameters of cellular immunity were detected with the use of monoclonal antibodies (firm "Sorbent", Russia) labeled FITC, by the method of flowing cyto-fluorometry on apparatus "Coulter Epix XL" firm "Becton Dickinson" (USA); Indices of humoral immunity (IgA, M, G) – by the method radial immune-diffusion by Mancini. Interleukins IL-1 $\beta$ , IL-2, IL-6 and IL-8 were investigated by the firm-phase immune-ferment method using the test systems OOO "Protein contour" (Russia) spectrophotometer "Multiscan".

For investigation of systems - lipid peroxidation-antioxidant protection (LP-AP), the fresh taken venous

blood, stabilized with heparin was used. In order to separate plasma and erythrocytes investigated blood underwent centrifugation - 3000 rpm during 20 minute. After erythrocytes' separation they were washed up with cold saline solution three times.

Have been studied parameters common for free radical formation - malonic dialdehyde (MD) and dienic conjugates (DC). Ferment status was evaluated by glutathione peroxidase (GP), superoxide dismutase (SOD) and catalase (CL) activity with the use of spectrophotometry method. Level of lipid peroxidation was discussed according to DC and MD accumulation in plasma and erythrocytes. Indices of antioxidant system - SOD, CL, and GP were defined in 10% erythrocytes' suspension.

Hormones were studied by the method of radio-immunologic analysis (RIA). Concentration of melanocysts' stimulating hormone ( $\alpha$ -MSH) in blood was detected by means of standard sets of firm Immuno Nuclear Corporation (USA), adrenocorticotrophic hormone (ACTH) – CIS (France, Italy), and cortisol – Steron (Belarus) with the use of  $\gamma$ -counter LKB-Walls 1181 (Switzerland).

Obtained data were analyzed statistically – program Statistica (StatSoft, USA).

### RESULTS AND DISCUSSION

Patients with pigmented nevus revealed increased concentration of CD4<sup>+</sup>, CD25<sup>+</sup>, CD69<sup>+</sup>, HLA-DR<sup>+</sup>, IL-1, IL-6, IL-8, ACTH, MSH, DC, MD, SOD, GP, CL and decreased concentration of CD3<sup>+</sup>, CD8<sup>+</sup>, CD16<sup>+</sup>, CD95<sup>+</sup>, IL-2, cortisol. In the group of patients with melasma increased concentration of CD20, CD25, HLA-DR+, MSH, ACTH, DC, MD and decreased concentration of CD3+, CD4+, CD8+, CD16+, CD69+, CD95+, IL-1, cortisol, SOD, CL were detected (Tab.1).

Indices	Patients with pigmented nevus (n=78)	Patients with melasma (n=57)	Control (n=40)
CD3 <sup>+</sup>	67,2±4,0*	68,0±6,1*	74,8±9,8
CD4 <sup>+</sup>	48,0±13,2	44,5±11,4	46,0±10,3
CD8 <sup>+</sup>	26,3±7,2	26,6±6,6	27,5±7,1
CD16 <sup>+</sup>	12,7±2,8*	13,9±3,3	14,5±3,2
CD20 <sup>+</sup>	8,7±0,6	9,9±1,0	9,2±1,3
CD25 <sup>+</sup>	25,7±4,3	24,6±3,2	23,1±4,9
CD69 <sup>+</sup>	41,5±4,2	34,8±4,7	37,1±5,1
CD95 <sup>+</sup>	40,5±10,3	40,9±13,2	42,9±14,9
HLA-DR <sup>+</sup>	16,1±2,3*	15,4±2,0	13,6±3,5
IgG, g/l	11,6±1,0	10,3±0,6	9,8±0,5
IgA, g/l	1,81±0,15	1,80±0,12	1,77±0,21
IL-1β, pg/ml	61,4±8,2*	51,6 ±4,5	52,4±4,9
IL-2	19,8±4,2	21,7±5,0	22,5±4,8
IL-6	59,6±3,2	55,1±2,6	54,4±2,0
IL-8, pg/ml	53,1±4,7*	48,4±6,0	48,0±5,8
DC, n.mol/ml	161,1±8,3*	165,8±3,7*	151,2±2,0
MD, n.mol/ml	150,2±9,0*	147,7±5,7*	130,3±2,6
SOD, unit/min	50,0±2,8	38,4±3,2	44,6±5,2
CL, unit/min	23,5±2,9	20,1±2,6	21,7±1,7
GP, mc.mol/mg	13,7±1,7	12,6±0,6	12,2±0,4
α-MSH, ng/l	49,8±3,8	52,5±4,9	48,7±7,5
AKTf, p.mol/l	15,3±2,7	19,0±3,7*	13,4±0,55
Cortisol, n.mol/l	363,6±26,9	355,1±31,1*	365,4±22,8

Note: \* - statistically reliable compared to control (p<0,05)

**Tab.1** Indices of immunity, antioxidant and pituitary-adrenal gland systems in patients with hyper pigmentation.

Correlative analysis of the above-listed indices has been carried out. Results have shown in Tab.2.

After comparison of components of LP-AP system with suppressive (CD8<sup>+</sup>) activity of lymphocytes in patients with hyper pigmentation revealed significant negative correlation. It is known that T-lymphocytes are high sensitive to reactive forms of oxygen [10]. Probably, activation of lipid peroxidation affects on membranes, which in turn alters interaction of immune competent cells eventually leading to immune status disorders. So, we could explain disbalance of immune competent cells not only by their redistribution as a result of stress reaction, but by disorder and death as a result of toxic effect of lipid peroxidation products. It is supposed that high level of DC and MD in membranes of immune competent cells disorders and interferes with immune globulin synthesis that has been revealed in patients with skin hyper pigmentation. High intensity of lipid peroxidation points on reduced antioxidant protection of organism in patients with melasma. However, in the group of patients with pigmented nevus increased intensity of both, lipid

peroxidation and antioxidant protection was stated. Probably, such kind of confrontation points on impossibility to proliferate.

Susceptibility of target-cells to cytotoxic effects of humoral autoimmune attacks is modulated by local cellular factors. The one of such factors could be various expressions of membrane antigens - receptors by melanocytes, mediating cytotoxic effects of auto-reactive immunocytes. Thus, one of the phenomenon includes apoptosis, selection of T-lymphocytes. Removal of disordered cells via the apoptosis provides minimal disorders of tissue in comparison with other mechanisms of death. Disorders of cellular death processes may lead to pathological state and disease accompanied by alterations of degenerative or proliferative nature. Alterations of skin pigmentation could be the result of disordered processes of melanocytes programmed death or destruction as a result of susceptibility to oxidative stress, which is the result or toxic intermediate link of melanin, melanocyte-determined protein or other sources.

Indices	Pigmented nevus	Melasma
CD3+-SOD	-0,177	+0,254
CD3+-MD	-0,317	-0,288
CD3+-MSH	-0,310	-0,247
CD3+-ACTH	-0,380	-0,405
CD4+-SOD	+0,372	+0,431
CD8+-DC	-0,431	-0,371
CD8+-MD	-0,521	-0,414
CD8+-GP	-0,301	-
CD816-MSH	-0,387	-0,501
CD16+-MD	-0,325	-0,306
CD16+-SOD	-0,455	+0,266
CD16+-ACTH	-0,512	-0,457
CD25+ - MD	+0,551	+0,527
CD25+ DC	+0,427	0,462
CD25+ -MSH	+0,522	+0,518
CD25+ - ACTH	+0,511	+0,477
CD25+ CL	+0,353	+0,333
CD95+ - MD	-0,283	-0,318
CD95 - DC	-0,317	-0,245
CD95+ GP	-0,278	-
CD95+ SOD	-0,277	+0,314
IL1 - MD	+0,462	-0,270
IL-1 - DC	+0,487	-0,282
IL-1 - cortisol	-0,320	+0,471
IL-1 - ACTH	+0,326	-0,228
HLA-DR+-MD	+0,410	+0,267

**Tab.1** Coefficient of correlative analysis of immunologic, hormonal and LP-AP systems in patients with hyper pigmentation.

Along with that, on the background of immune competent cells shifting in case of pigmented nevus and melasma, especially in patients with intradermal nevus, symptomatics were absent.

Formation of disbalance in the process of physiologic apoptosis during this latent period may become in future the one of the reasons of clinical manifestations.

It is remarkable that there is a close positive correlation between CD25<sup>+</sup> and CD95<sup>+</sup> cells and indices of LP-AP systems, and pituitary-adrenal gland systems as well. Low affinity receptor for IL-2 molecule CD25 partly is the marker of B-lymphocytes [3]. Increased level of CD25<sup>+</sup>-cells points on that continued activation of T-cells is involved in the progressive process of depigmentation. It must be mentioned that these cells play a major role in regulation of apoptosis as far as they perform the function of signals' conduction towards apoptosis [2].

In our opinion, HLA-DR cells that are the target cells for cytotoxic T-cells, influence on the process of melanocytes destruction in the pigment derangements. High level of HLA-DR cells is detected in case of hyper pigmentation.

These cells help T-cells to recognize foreign antigens. At the same time the positive correlation between these cells and MD is revealed.

Of humoral factors, regulating number of cellular population in organs and tissues, hormones and cytokines are of great importance. Apoptosis can be stimulated by excess of hormones. It is characteristic especially for glucocorticoids and for cortisol in particular. Immune cells are under influence of glucocorticoids. They regulate number of immune globulin producing cells.

Supposed multidirectional nature of processes, connections between immune and endocrine systems, in which lymphokines and monokines are discussed as information messengers to endocrine structures about current state and activity of immune system. Pituitary gland, owing to its location is ideally adjusted for integration of central and peripheral stimuli [1].

As is known, IL-1 serves as main endogens mediator of immune response, and activates immune competent cells [6,10]. At the same time, an important component of multifaced biological activities of IL-1 are disorders in

neuro-endocrine system, partly leading to increase in blood cortisol level. Thus, the positive correlation between IL-1 and cortisol is obvious in patients with melasma and negative correlation - in patients with pigmented nevus. In turn, high concentration of cortisol suppresses immune reactions. Probably, the increased level of cortisol, under influence of IL-1, is more important for limitation of farther synthesis of endogenous IL-1, to avoid toxic effects of its high concentration, and not for immune suppressive actions.

As a result of glucocorticoids action, production of IL-2 decreases. In the process of investigation, decreased production of IL-2 was detected in the group of patients with pigmented nevus and on this background decreased concentration of cortisol was registered. It should be taken into consideration that one and the same cytokin in various situations may have opposite affect on one and the same cellular systems.

Thus, IL-2 has a dual regulatory action controlling both survival and programmed death of natural killers. For T- and B-lymphocytes IL-2 in general is the immune stimulator.

In stress situations, especially in chronic stress situations (note, any disease is the chronic stress), increased production of ACTH glucocorticoids suppresses secretion of IL-1 in macrophages, which removes stimulating affect of IL-1 in hypothalamus.

Thus, feedback mechanism between ACTH and IL-1 is obvious. Analysis of correlations revealed moderate positive correlation between IL-1 and ACTH in patients with pigmented nevus and negative correlation in the group of patients with melasma. Probably, increased secretion of ACTH is the result of cytokines, IL-1 and IL-6 in particular secreted by follicular-star cells in anterior pituitary gland. Correlation between IL-6 and ACTH in patients with pigmented nevus points on the above-mentioned ( $r=+0,363$ ,  $p<0,05$ ).

In case of hyper pigmentation, increased level of CD95<sup>+</sup> cells leads to suppression of apoptosis and supports increase in melanocytes concentration. Consequently, in case of hyper pigmentation insufficiency of apoptosis is observed.

### CONCLUSION

Thus, in case of hyper pigmentation there is correlation of indices and intercellular regulations of immune, hormonal and antioxidant systems. Taking into consideration the afore-said, investigation of these systems would be of great value as far as detection of mechanisms leading to biochemical disorders, would help us to clarify character of the course of processes and elaborate adequate methods of their correction.

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## **Взаимосвязь показателей иммунной, антиоксидантной и гормональной систем и ее влияние на меланогенез у больных гиперпигментацией кожи**

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### **Р Е З Ю М Е**

На основании изучения иммунологического, гормонального и биохимического статуса рассмотрены особенности меланогенеза у 135 больных гиперпигментацией кожи (78 пациентов пигментным невусом и 57 - мелазмой). Средний возраст составил -36,5 лет, мужчин было 32, женщин - 103. У пациентов пигментным невусом отмечен рост числа CD4<sup>+</sup>, CD25<sup>+</sup>, CD69<sup>+</sup>, HLA-DR<sup>+</sup>, ИЛ-1, ИЛ-6, ИЛ-8, АКТГ, МСГ, ДК, МДА, СОД, ГП, КТ и уменьшение CD3<sup>+</sup>, CD8<sup>+</sup>, CD16<sup>+</sup>, CD95<sup>+</sup>, ИЛ-2, кортизола. В группе пациентов мелазмой зарегистрировано увеличение уровня CD20, CD25, HLA-DR<sup>+</sup>, МСГ, АКТГ, ДК, МДА и низкое содержание CD3<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup>, CD16<sup>+</sup>, CD69<sup>+</sup>, CD95<sup>+</sup>, ИЛ-1, кортизола, СОД, КТ. Сопоставлением уровня компонентов системы ПОЛ-АОС с супрессорной (CD8<sup>+</sup>) активностью лимфоцитов у пациентов с гиперпигментацией обнаружена отчетливая отрицательная корреляция и наличие тесной положительной связи между CD25<sup>+</sup> и CD95<sup>+</sup> клетками и показателями системы ПОЛ-АОС, а также системы гипофиз-надпочечники. Выявлена выраженная позитивная корреляция между ИЛ-1 и кортизолом у пациентов мелазмой и отрицательная - у пациентов пигментным невусом. У больных пигментным невусом обнаружилась сниженная продукция ИЛ-2 и на фоне его - снижение содержания кортизола. При гиперпигментации увеличенный уровень CD95<sup>+</sup> клеток сопровождался ослаблением апоптоза, обуславливая увеличение количества меланоцитов. Следовательно, при гиперпигментации установлены недостаточный апоптоз. Таким образом, в условиях гиперпигментации существует сложная взаимосвязь показателей и межклеточных регуляций иммунологической, гормональной и антиоксидантной систем.

**Ключевые слова:** гиперпигментация кожи, меланогенез, антиоксидантная система