

## Immune System Behavior during Herpesvirus Infection in Childhood

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### Abstract

**Background:** Persistently infected cells by herpesvirus (HSV, CMV, EBV) and their dysfunction are associated with different disorders. The immune and endocrine systems are involved in pathogenesis of herpesvirus infection. Changes of IFN- $\gamma$ , IL-4, DHEAS and cortisol level are a part of the regulatory mechanisms of the immune and endocrine system responses in persistent herpesvirus infection.

**Objectives:** The aim of this study was to identify immunoregulatory mechanisms (clinical manifestations, cytokines level-IFN- $\gamma$ , et IL-4, hormones level-DHEAS, cortisol, parameters of oxidative metabolism, enzymes of cell injury) and their consequences concerning clinical disease expression (low level of hemoglobin (Hb), leucocytopenia, high level of monocytes, hyper gammaglobulins, low level of NBT-test).

**Study design:** 40 children (1-16 years old) with herpesvirus infection were analyzed. In patients routine laboratory investigations were performed: white blood cells count, lactate dehydrogenase-LDH, creatinin-kinase-CPK, oxidative metabolism of the blood phagocytes, the ability of phagocytes nitro-blue tetrazolium-NBT reduction and immunoglobulin serum level. Serum levels of IFN- $\gamma$ , IL-4, DHEAS and cortisol were measured by ELISA test. Normal healthy controls had negative ELISA tests for CMV, HSV and EBV

**Results:** The high level of LDH, CPK, low ability of NBT reduction and hypergammaglobulins were detected. The increases of IFN- $\gamma$ , IL-4 level, as well as, the decrease of DHEAS and cortisol level were present. The patients with the high level of IFN- $\gamma$ , had the high level of LDH, CPK, GOT, GPT, the low level of Hb, leucocytopenia and monocytes.

**Conclusions:** Two groups of patients were presented. First with the high level of IFN- $\gamma$  (70%) and second with the high level of IL-4. The patients in the first group, with the high level of IFN- $\gamma$ , had anemia, respiratory infections, BHR, labial HSV, stomatitis aphthosa. Vasculitis, anginae recidivans, was clinical manifestation characteristics for patients in the second group with the high level of IL-4. The high level of LDH, CPK, the low ability of NBT reduction and hypergammaglobulins were detected. The patients with the high levels of IFN- $\gamma$ , had the high level of LDH, CPK, GOT, GPT, the low level of Hb, leukopenia and monocytosis. The decreased level of DHEAS (50%) and the normal level of cortisol (76%) were present. The low levels of DHEAS followed the IgG high values while leukopenia (78%), was followed by high values of DHEAS. The patients with normal cortisol values had the significant monocytosis (80%) while patients with the low cortisol level had the high IgG (85%) level.

**Keywords:** Herpes virus infection; DHEAS; Cortisol; IFN- $\gamma$ ; IL-4; Immune system

### Introduction

Infection with viruses that have the capacity to modulate or evade the immune response can result in persistence, which can lead to a variety of chronic problems including neoplasia, immune suppression, autoimmune-like syndromes, and selective organ failure. An important characteristic of the herpes group viruses is their ability to persist in the tissues of their hosts for many years after the initial infection.

Disorder of regulation and chronic stimulation of immune system are very often part of herpesvirus infection.

Virus infection (CMV, HSV, EBV) has been estimated as our factor of etiopathogenesis. Bradford et al. [1] showed that in children with symptomatic congenital CMV infection involving the CNS, viremia during early infancy is associated with hearing loss and systemic CMV disease. The spectrum of disease caused by HSV includes primary and recurrent infections of mucous membranes (e.g. gingivitis, herpes labialis, and genital HSV infections), keratoconjunctivitis, neonatal HSV infections of the immune compromised host, encephalitis, Kaposi like of varicella eruption, and an reminds with erythema multiforme [2] Disease symptoms (HSV-1) can range from mucocutaneous lesions to life-threatening encephalitis or systemic disease involving multiple organ system [3]. Leaute-Labreze et al. [4] conclude that childhood erythema multiforme is related to herpes infection, as recurrent erythema multiforme.

It is well known that the herpes group viruses are intracellular viruses and can persist in the tissue of their host for many years after

the initial infection [5]. Based on data that herpes viruses have the ability to induce chronic persistent infection, and have a remarkable similarity in terms of viral kinetics [6], we can speculate different clinical manifestation as a part of chronic reactivation herpes virus infection. Because persistently infected cells whose dysfunction is associated with disease are themselves not injured, then conceptually clearing the virus infection might be expected to restore normal cell function [7].

Cytokines regulate cellular survival, may induce antiviral functions in the cell and modulate the development of a local inflammation. Therefore, it is not surprising that targeting cytokines is one strategy of viruses to evade clearance by the immune system [8,9]. Following primary infection of the skin or mucosa, herpes simplex virus type 1 (HSV-1) invades sensory neurons and is transported by retrograde axonal transport to the neuronal cell bodies in the sensory ganglia. Thus, recurrent disease results from an awakening of latent virus rather than from exogenous re-infection [6]. The uniform expression of IFN- $\gamma$ , and TNF $\alpha$  in latently infected suggests persistent stimulation of the cells producing these cytokines. Neurons up-regulation MHC class I expression during HSV-1 infection, but MHC class I expression diminishes to an undetectable level when the virus enters a latent state. This suggests concordant regulation of MHC class I and viral gene expression in neurons [6].

The hypothalamic-pituitary-adrenal (HPA) axis and the immune system interact in a bidirectional manner providing the basis for the regulation of the immune response due to a pathogenic stimulus [10]. Also the hypothalamic-pituitary-adrenal (HPA) axis has an important role in the regulation of the anti-inflammatory response. Dihydroepiandrosterone sulfate (DHEAS) and cortisol are multifunctional adrenal hormones with immune modulating properties. Chronic inflammatory diseases such as rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, and others are accompanied by low levels of dehydroepiandrosterone (DHEA) and its sulfated derivative DHEAS [11]. It has been proposed that proinflammatory cytokines such as tumor necrosis factor (TNF) and intra-adrenal immune cells may change the microenvironment leading to lower adrenal and gonad hormone secretion [11].

The aim of the study was to analyze the parameters of chronic immune system stimulation during herpes virus infection.

## Materials and Methods

The study included 40 children (aged from 1 to 16 years) with signs of herpes virus (Citomegalo virus-CMV, herpes simplex virus- HSV, Epstein Barr virus-EBV) infection medical history of all patients showed frequent inflammatory events such as: labial herpes recidivans, seizures, urticaria, vasculitis, BHR recurrent respiratory tract infections. Including criteria were age and positive ELISA test for one of the following viruses: CMV, HSV and EBV. None of the children were under any treatment before the check-up and blood collection for analysis.

Blood was taken from patients and controls after their verbal consent. In addition, a general consent is also obtained from all patients in this hospital for all investigations as a part of a routine patient processing.

Controls group of patients (20) were normal healthy.

## Quantification of hematological and biochemical parameters

White blood cell, hemoglobin level, LDH, CPK, GOT, GPT were measured using micro analyzer Culter drift II (Instrumental Laboratory) following the manufacture instruction's.

## Quantification of NBT test/Tetrazolium reduction test

The Nitro-blue-tetrazolium (NBT)-test of peripheral blood phagocytes was performed according to the method to Park et al. [12]. The activation of monocyte-macrophage system and the measurement of metabolic oxidative explosion of phagocytes were observed through NBT-test.

## Quantification of IFN- $\gamma$ , and IL-4 level in plasma

IFN-  $\gamma$ , and IL-4 serum levels were measured by the ELISA test using kits of Bio Source Europe S.A. (Zoning Industrial B-6220 Fleurus Belgium), stock number KHM0009 following the manufacture instruction's.

## Quantification of DHEAS and level of cortisol in plasma

DHEAS and serum levels of cortisol were measured by the ELISA test using kits of Humana-Human Gesellshaff fur Biochemical und Diagnostic mbH, stock number 55050 (cortisol) and 55060 (DHEAS).

## Serologic survey herpesviruses using ELISA assay

Concentration of antibody (IgM and IgG) for HSV, CMV, EBV were determined by commercial ELISA test (Humana-Human Gesellshaff fur Biochemical und Diagnostic mbH) following the manufacture instruction's.

## Statistical analysis

Data are reported as mean  $x \pm sd$  and as percentage of certain parameters. The statistical significance of differences was estimated by using Student's t-test. The correlation analyses were calculated using Persons' linear correlation model. Microsoft program SSPS version 7,5 was used for statistical calculation of data.

## Results

The study included 40 children and control group of healthy children (20).

## Clinical manifestations

The children had different clinical manifestations in our study (Table 1).

clinical manifestations	n	(%)
Labial HSV infection	4	10
Urticaria recidivism	4	10
Wheezing bronchitis	4	10
Laryngitis	4	10
Angina	3	7.5
Anaemia	3	7.5

BHR	2	5
Atopic dermatitis	2	5
Stomatitsaftosa	2	5
Vasculities	2	5
Erythemaanulare	2	5
Purpura	2	5
Pneumonia	2	5
Encephalitis	1	2.5
IgA deficient	2	5
Syndrome decrease ability of immunity	1	2.5

**Table 1:** Clinical manifestations

All of clinical manifestations were very heterogeneous which comprised different organs and systems.

### ELISA test of viruses: CMV, HSV and EBV

In order to point out etiological factors we used an ELISA test to detect the presence of IgG and IgM antibodies against CMV, HSV and EBV. We found that 43% of the patients were positive for anti-HSV IgG, 67,86% for anti-CMV IgG and 60% for anti-EBV IgG, whereas only 7% of the patients were positive for IgM against HSV (Table 2 and 3).

HSV		CMV	EBV
IgM +	IgG +	IgG +	IgG +
7%	43%	67.86%	60%

**Table 2:** Positive ELISA test for CMV, HSV and EBV in patients

	IFN- $\gamma$ (IU/l)	DHEAS ( $\mu$ g/ml)	IL-4 (pg/ml)	Cortisol ( $\mu$ g/ml)
study group(n=40)	0.45 $\pm$ 0.28	0.23 $\pm$ 0.47	1.61 $\pm$ 0.44	47 $\pm$ 53.17
control group (n=40)	0.23 $\pm$ 0.42	0.34 $\pm$ 0.51	1.38 $\pm$ 0.23	95 $\pm$ 49.72
Results show as mean values SD; n-number of patients				

**Table 3:** Mean values of IFN-, IL-4, DHEAS and cortisol. Mean value IFN-, IL-4, DHEAS and cortisol in serum

Means values of IFN- $\gamma$  and IL-4 were high levels in relation on control group, while means values DHEA-S and cortisol were low in relation of control group.

Results showed significant low level of mean value of IFN- $\gamma$  ( $p < 0.01$ )-Student t-test. There was no normal level of IFN- $\gamma$  and high level of cortisol too. Values of IL-4 were significant high and low ( $p < 0.01$ ). There was no significant different in means values of DHEAS in any of groups of patients. Values of cortisol were significant low ( $p < 0.05$ ).

Higher value of IFN- $\gamma$  was about 70% of patients and high value of IL4 was about 48% of patients. Lower value of DHEAS was about 50% of patients and lower value of cortisol was about 23% of patients (Table 4).

Values of parameters IFN- $\gamma$ , IL-4, DHEAS and cortisol were comparative analysed. High values of IFN- $\gamma$  followed change in haematologic parameters as low values of Hb (66%), leukopenia (25%), monocytosis (90%).

	low Hb	Leukopenia	Monocytosis
high IFN- $\gamma$	66%		90%
high DHEAS		78%	
low DHEAS			
high IL-4	57%		40%
low cortisol			
normal cortisol			80%

**Table 4:** Comparison between haematological parameters and IFN- $\gamma$ , IL-4, DHEAS and cortisol. Interleukins, hormones and low levels of Hb, leukopenia, monocytosis

There was no change in values of DHEAS in patients with low values of haemoglobin and monocytosis. Low levels of DHEAS followed high values IgG while high values of DHEAS followed leukopenia (78%). We used low level of hemoglobin about 80-90 g/lit, in relation to the age of the children, in our study.

Low values of Hb (57%) and monocytosis (40%) were associated with high values of IL-4. At there was significant monocytosis (80%) in patients with normal values of cortisol (Table 5).

	low LDH	low CPK	high GOT	high GPT
high IFN- $\gamma$	70%	81%	69%	66%
high IL-4			50%	
low IL-4		60%		

**Table 5:** Comparison between enzymes, IFN $\gamma$  and IL-4. Interleukins (IFN-, IL-4) and enzymes (LDH, CPK, GOT, GPT)

Comparison of interleukins values (IFN- $\gamma$  and IL-4) and hormones (DHEAS and cortisol) with values of LDH, CPK, GOT i GPT is on table 6. This results show that high levels of IFN- $\gamma$  followed high levels of LDH (70%), CPK (81%), GOT (69%) i GPT (66%). There was no significant deviate at low values of IFN- $\gamma$ . High values of IL-4 associated with high values of GOT (50%), but low level of IL-4 associated with high values of CPK (60%) (Table 6).

	low spont. NBT-test	low. stim. NBT-test
high IFN- $\gamma$	84%	76%
normal cortisol	86%	78%

**Table 6:** Comparison between NBT-test, IFN- $\gamma$ , and cortisol. Interleukine IFN- $\gamma$ , cortisol and NBT-test

Domination of low values of NBT (spontaneous and stimulation) in patients with high values of IFN- $\gamma$  and normal values of cortisol (Table 7).

	IFN-g and DHEAS	IFN-g and IL-4	IFN-g and cortisol	DHEAS and IL-4	DHEAS and cortisol	IL-4 and cortisol
r	-0.228	-0.04	-0.188	-0.125	0.206	-0.175
n	34	33	34	33	34	33
t	1,32	0,22	1,08	0,70	1,19	0,99
p	0.1946	0.8254	0.2859	0.4883	0.2426	0.331

**Table 7:** Correlation between measured parameters (IFN-, IL-4, DHEAS, cortisol). Correlation IFN-, IL-4, DHEAS and cortisol

Negative correlation between all parameters is evident (beside DHEAS and cortisol) and no statistical significant (coefficients of correlation-r are: for IFN- $\gamma$  and IL-4  $r=-0,040$ , IFN- $\gamma$  and cortisol  $r=-0,188$ , DHEAS and IL-4  $r=-0,125$ , IL-4 and cortisol  $r=-0,175$ ). Positive correlation between DHEAS and cortisol ( $r=0,206$ ) is evident.

## Discussion

Our examination of patients pointed alteration of herpesvirus infection and parameters of chronic stimulation of immune system. In this study, patients with different clinical manifestations (labial HSV-10%, urticaria-10%, bronhitis-10%, laryngitis-10%, anemia-7%, angina-7%, BHR-5%, atopic dermatitis-5%) are indicated in Table 1.

In this study ELISA test showed positive test at patients (IgG antibody on HSV-43%, CMV-67, 86% and EBV-60%). Positive antibody (IgM) on HSV was 7% at patients.

Oxidative metabolism and function of phagocytes are activated by virus. Activation of macrophages is the principal and result of cell-mediated immunity and priming for an enhanced respiratory burst is an essential component of that process [13]. Activated immune competent cells and some their mediators (IL-1, IL-2, INF- $\gamma$ , TNF $\alpha$ ), arachidonic acid metabolites like a PGE2 have direct or indirect effects on oxidative burst of peripheral blood phagocytes [13].

In this study the majority of patients with high level of IFN- $\gamma$ , had low NBT-test (spontaneous, stimulation) and normal level of cortisol. Incite us to speculate on possible pathogenic mechanisms occurring during herpes virus infection. Also, we observed that low NBT-test is better for organism, because on this way, oxidative damage of cells is reduced.

Patients with high level of IL-4 had low level of Hb (57%) and high level of monocytes (40%). Higher values of monocytescan indicate a possible infection of virus, while low level of Hb is a part of defense of cell injury during disorders of immune regulation.

Levels of IFN- $\gamma$ , IL-4, DHEAS cortisol were compared with levels of LDH, CPK, GOT, GPT (Table 5). Patients with high level of IFN $\gamma$  had high level of LDH (70%), CPK (81%), GOT (69%) and GPT (66%). The presence of elevated levels of this immune modulation cytokine in persistent infection suggests that when the immune system is unable to mediate viral clearance it may contribute to injury of hepatocyte [14]. Patients with high level of IL-4 had high level of GOT (50%) while patients with low level of IL-4 had high level of CPK (60%). The

presence of elevated levels of CPK may contribute cell injury during herpes virus reactivation.

Cytokines and other inflammatory mediators can signal the brain thus, influencing behaviour and other complex body reactions. For example, mast-cell histamine increases the expression of corticotropin-releasing hormone (CRH) mRNA in the hypothalamus, which activates the hypothalamic pituitary-adrenal (HPA) axis. Moreover, CRH secretion could be triggered by IL-6 and IL-1, both of which are also released from mast cells; conversely, CRH stimulates IL-6 release. Proinflammatory cytokines (e.g. via activation of innate immunity in response to infections) can induce bad behaviour [15].

Chronic stress promotes the development of glicocorticoid resistance, which has been associated with increased cytokine production and which might also release the sympathetic nervous system from inhibitory control, further promoting inflammatory activation [16].

From an immunological point of view, it is important to note that the ratio of dehydroepiandrosterone sulphate (DHEAS) to cortisol fell, and that there was loss of delayed hypersensitivity (DTH) responses with relative sparing of humoral immunity. This also suggested that stress drives a shift in the Th1/Th2 balance towards Th2. A central paradigm in stress research is that the endocrine, immune and neurons "super-system" engage in multiple interactions during the response of the body to acute and chronic stress [15]. Paus et. al. speculated that in the skin, pruritus, excessive sweating (hyperhidrosis) and "flushing" (facial erythema), and many dermatoses such as atopic dermatitis, psoriasis, seborrhoisches eczema, prurigo nodular, lichenplane, chronic urticaria and alopecia areata, can be triggered or aggravated by stress. Interactions between mast cells, nerves and neuropeptides have also been implicated in atopic dermatitis [15].

Rook et al showed that anti-steroid effect is indirectly Th1 enhancing, and DHEA also directly change Th1 T-cell activity [17]. Patients with high level of DHEAS hadleucopenia (78%). A possible explanation for these results is that DHEAS is a part of regulatory mechanism during herpes virus infection.

Our study compared IFN- $\gamma$ , IL-4, DHEAS and cortisol serum level with hematological parameters. Patients with high level of IFN $\gamma$ , had changes in hematological parameters (low-level of Hb-66%), leucopenia (25%), monocytosis (90%). Middle level of IFN- $\gamma$ , were significant low ( $p<0.01$ ). Patients did not have normal level of IFN- $\gamma$ , and high level of cortisol.



Decrease of serum levels of adrenal androgens may lead to a more proinflammatory disease state because DHEAS has been shown to inhibit proinflammatory cytokines such as secretion of tumor necrosis factor (TNF). On the other hand, DHEA seems to type Th1 immune response whereas DHEA inhibits Th2 immune responses.

Middle level of DHEAS and cortisol were low in comparison with control groups. Middle level of DHEAS was no significant different none of groups. Levels of cortisol was significant low ( $p < 0.05$ ) in comparison with control groups

DHEA may exert an anti-inflammatory effect in Th2-driven diseases and a proinflammatory effect in Th1-driven diseases [18] Yu Yang et al showed that administration of DHEA during allergic sensitization could attenuate the subsequent allergic responses elicited by challenge, and that the suppressive effect of DHEA was associated with a down-regulation of Th2 response.

This study points towards an important role of TNF for changes of steroidogenesis in chronic inflammatory diseases [18]. Since TNF was positively associated with the ratio of the serum levels of DHEAS/DHEA only in the chronic inflammatory disease, this particular cytokine can be responsible for an increase of serum levels of DHEAS in relation to serum levels of DHEA. Huge decrease of serum levels of DHEA, DHEAS, ASD in patients with chronic inflammation, which is paralleled by nearly stable serum levels of cortisol. It seems as if cortisol secretion is maintained at the expense of ASD, DHEA and DHEAS secretion. In contrast, in patients with an acute inflammatory stressful disease state, there is an increase of all measured adrenal hormones. There are markedly different hormonal response patterns and hormone shifts in patients with an acute inflammatory stressful disease state as compared with patients with chronic inflammation [18].

An important overview of the role of DHEA in reducing the damage produced by chronic inflammation was recently published by a team of researchers at the University of Regensburg, Germany [11]. The authors point out those patients with chronic inflammatory diseases such as rheumatoid arthritis show adrenal dysfunction that manifests itself both in insufficient levels of cortisol in response adrenocorticotrophic hormone (ACTH) and low levels of DHEA. With cortisol and DHEA being too low, the inflammation progresses and leads to harmful consequences [11].

The current practice is to use synthetic corticosteroids such as prednisolone in an effort to fight chronic inflammation. DHEA remains neglected, in spite of repeated findings of low DHEA levels in patients suffering from chronic inflammatory diseases. But DHEA also plays an important role in preventing inflammation. It is a potent inhibitor of pro-inflammatory cytokines (hormone-like immune chemicals), which in turn signal the immune system and provoke further cellular destruction.

Of special interest is DHEA's ability to inhibit interleukin 6 (IL-6) and tumor necrosis factor (TNF). These pro-inflammatory cytokines rise with age, and are especially high in patients with inflammatory diseases. In addition, IL-6 promotes the production of certain immune cells which attack the body's own tissue in autoimmune conditions such as rheumatoid arthritis. High serum IL-6, as seen in rheumatoid arthritis, for instance, is regarded as a reliable biomarker of inflammation. The finding that DHEA supplementation can lower IL-6 makes it a very promising anti-inflammatory agent, especially for chronic disorders which are characterized by significantly elevated IL-6.

The deficiency of DHEA in inflammatory diseases also implies a deficiency in peripheral tissue of various sex steroids for which DHEA serves as a precursor. These steroids, both estrogenic and androgenic, are known to have beneficial effects on muscle, bone, blood vessels and so forth. The mainstream therapy with corticosteroids is itself known to lower androgen levels. Consequently, the authors argue that hormone replacement for patients with chronic inflammatory diseases should include not only corticosteroids, but also DHEA.

DHEA has long been known to boost the immune function. It is vital for the development of certain mature immune cells and for enhanced antibody production [18]. A new study has found that the number of cells secreting interferon-gamma correlated with serum DHEA levels in men, and that the activity of these cells was associated with DHEA levels in premenopausal women [18]. Thus, DHEA seems to be involved in modulating cytokine production. Because infection produces a rise in cortisol, which in turn suppresses the immune system, it would be logical to try to regulate this immune suppression with anti-glucocorticoids such as DHEA [18].

## Conclusion

Complicated immune regulatory events in different disorders depend on the pathologic process itself and the phase of disease. Immune parameters aberrations in advanced phase of disease, compared to the initial ones, may be significant, making difficult the understanding of basic immune regulatory process of the disease.

Multi organs and multi systems disorders were manifestation during herpesvirus infection. Alteration in hematology and biochemistry parameters during herpes virus infection reflect immune reactivation and manifest cell injury (CPK), but does not give any indication on the mechanism originating the dysfunction. Likewise high level of LDH show on replication or reactivation virus infection. Monocytosis and hypergammaglobulins present chronic activation of immune system. Decreased stimulation of oxidative ability of phagocytosis probably result of stress that cells elapse during virus infection Changes in value of DHEAS and cortisol part of regulatory mechanisms immune response cross endocrine system. In accordance with the fact that during chronic stress serum levels of DHEA decrease, it can be concluded that in our patients, decreased values of DHEAS are the result of a "chronic stress" caused by prolonged activation of the immune system.

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