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Influence of flavonoids on the cellular immunity indices in children and adolescents with influenza and acute respiratory viral infections before and after treatment

■ INTRODUCTION

In recent years, there is an excess incidence of viral and viral-bacterial infections. Among them, first of all, acute respiratory viral infections (ARVI), including the influenza – both in children and adolescents.

Abundant evidences point out that both quantitative and functional immunological disorders and decreased antioxidant activity are observed in case of ARVI and influenza [1, 2, 5]. There are a large number of “trigger mechanisms” that cause immunological responses and draw different types of blood cells and biologically active factors, and therefore it is considered that in patients with ARVI and influenza these mechanisms are also violated, which cause these diseases progression in pathogenetic aspect [3, 4]. ARVI and influenza affect all bodily organs and systems: respiratory, gastrointestinal, cardiovascular, nervous system, etc. However, children, compared with adolescents, have a more severe disease course with the frequent serious complications and chronic pathology development.

Pharmaceutical agents that have combined properties and simultaneous antiviral and immunocorrective activity, proven efficiency and safety, include drugs that contain flavonoids of wild grasses, such as tufted hair grass (Deschampsia caespitosa) and bushgrass (Calamagrostis epigeios), namely Immunoflazid® and Proteflazid®, which subsequently referred to in this article as immunomodulating drugs IMDIF and IMDPF. The drugs have antiviral properties, act as an inhibitor of virus-specific enzymes and neuraminidase of the influenza virus, as well as immunomodulating, antioxidative and apoptosis-modulating actions due to the broad spectrum of action of flavonoids.

■ OBJECTIVE

To improve the treatment efficacy of ARVI and influenza in children and adolescents, to study indicators of the cellular component of immune system and the immune system correctability using modern antiviral and immunomodulating drugs [2, 5].

■ MATERIALS AND METHODS

To evaluate the treatment efficacy, an observational follow-up study was conducted for 2 years (from 2016 to 2017) that enrolled 206 patients, including 106 children aged from 1 to 9 years and 100 adolescents aged from 10 to 18 years.

To estimate the immune status, a comprehensive immunological examination was carried out, which included detection of the mature T lymphocytes (CD3+) and their subpopulations (CD4+, CD8+), immunoregulatory index (CD4/CD8), as well as B lymphocytes (CD22+) and phagocytic activity of monocytes (PHAM).

The method was based on the interaction of monoclonal antibodies labelled with fluorescent tags with lymphocyte surface antigens and the following sample analysis by using the FACSCalibur flow cytometer (BectonDickinson, USA). The phagocytic activity of monocytes (PHAM) was studied using the original pour plate method with calculation of the following indices of phagocyte activity: phagocytic number (PN), phagocytic index (PI) and digestion index (NDI) of neutrophils. The results of the PN survey were expressed in per cent, PI and NDI – in conventional units (conv.units).

In total 106 children were enrolled with ARVI and influenza (group 1 – 100%) with the moderately severe diseases, including 51 children with ARVI (group 1.1 – 48 %) and 55 children with influenza (group 1.2 – 51.9 %) aged from 1 to 9 years, among them there were 56 boys and 50 girls. Besides, 100 adolescents with ARVI and influenza (group 2 – 100 %) aged 10–18 years with moderate diseases were also under observation: 46 of them had ARVI (group 2.1 – 46 %) and 54 patients with influenza (group 2.2 – 54 %), among them there were 55 boys and 45 girls. The control group (K1) of immunological studies consisted of 20 apparently healthy children aged from 1 to 9 years, and the control group (K2) comprised 20 apparently healthy adolescents aged from 10 to 18 years.

The children of groups 1.1 and 1.2 were administered IMDIF in the comprehensive treatment, and the children of group 3 (group of comparison, 46 persons) were administered conventional pharmacological therapy only. The examined adolescents of groups 2.1 and 2.2 were administered IMDPF in the comprehensive treatment, and the adolescents of group 4 (group of comparison, 50 persons) were administered conventional pharmacological therapy only. Patients who were under observation were randomised by age, gender, duration of ARVI and influenza. The diagnosis of ARVI and influenza was established by expertise, taking into account anamnesis, clinical examination and laboratory results.

Aetiotropic antiviral drugs – neuraminidase inhibitors of the influenza virus, as well as paracetamol (as symptomatic therapy with hyperthermic syndrome), decongestants and antitussives (if medically required) were administered as baseline therapy from the first hours of the disease onset in the age-specific dosage variances in compliance with the instructions.

The children aged 1-9 years of the main group were prescribed, along with baseline therapy, antiviral and immunomodulating therapy using IMDIF syrup, 100 ml of which contains 0.32 mg of flavonoids in terms of the equivalent amount of rutin and 0.3 mg of carboxylic acids equivalent to malic acid, 20-30 minutes before meals using a dosing scoop for 14 days, twice daily in the age-specific dosage variances in compliance with the instruction.

The children aged 10-18 years of the main group were prescribed IMDPF in drops for 14 days 10-15 minutes before meals in the age-specific dosage variances in accordance with the instruction.

IMDIF and IMDPF are new original antiviral and immunomodulating drugs that normalize cellular immunity, increase migration and cooperation of T cells and B lymphocytes and the phagocytic activity of macrophages, as well as stimulate cell division and differentiation, have a broad spectrum of antiviral and antioxidative activity, and apoptosis modulating properties.

In the experimental studies, it was found that the cells of phagocytic system are targets for the pharmacological action of flavonoids: monocytes/macrophages and microphages, the functional activity of which plays a leading role in the interferon production and actively influences over the cellular and humoral immunity indices [1, 2, 4].

Using the flow cytometry, it has been shown that IMDIF and IMDPF at 37°C interact with almost all cells of the immune system: lymphocytes, neutrophils and monocytes, but may enter only into neutrophils and monocytes and practically does not enter lymphocytes [1, 5].

When interacting with neutrophils and macrophages/monocytes, a notable activation of functional activity of these cells is observed, which is characterised by increased cytokine synthesis and stimulation of phagocytosis processes [1].

When interacting with peripheral blood mononuclear cells of healthy donors, flavonoids of IMDIF and IMDPF induce the cytokine synthesis by monocytes, while it is important to note that the induction of cytokine production occurs in this case only at their initial low or medium levels; however, in case of the initial elevated levels of cytokines, IMDIF and IMDPF do not affect or even slightly reduce their production.

Another feature of the immunocorrective action of IMDIF and IMDPF is the enhancement of the α-interferon synthesis by peripheral blood mononuclear cells of donors [4, 5].

When interacting with human peripheral blood mononuclear cells, IMDIF and IMDPF enhance the cytotoxicity of NK cells, but only in cases where this cytotoxicity was initially reduced [4].

It is important to emphasize that, generally, the effects of IMDIF and IMDDPF on the immu¬ne system are immunomodulating, that is, dependent on the initial state of its functional activity.

In addition to immunomodulating, they have significant detoxicating, antioxidative, apoptosis modulating and membrane stabilizing effects [1, 2, 5]. The combination of these properties makes IMDIF and IMDPF indispensable in the comprehensive treatment and prevention of ARVI and influenza.

■ RESULTS AND DISCUSSION

When studying the efficacy of IMDIF and IMDPF on the cellular immunity status in patients of both groups, it was found that at the baseline they had a typical clinical picture of the disease, which was characterised by a sudden onset, severe intoxication syndrome (severe headache, body pain, sleep loss, delusion, nausea, vomiting, severe tachycardia, respiratory rate more than 28 per minute, painful cough, retrosternal pain, general weakness and dizziness. In the followed-up patients, there were also typical psychoemotional disorders, which were characterised by symptoms of asthenia or depression. Therefore, among the subjective symptoms, the steady feeling tired was predominant, which was observed in all patients and did not disappear after rest, general weakness and severe distress, which was observed in the overwhelming majority of patients. Characteristic feature was the increased irritability, severe emotional lability, loss of appetite, and dull diffuse pain in muscles and joints, which increased after physical exercise. It was found at the baseline that in the first days of treatment in both groups of patients there was a considerable circulating T-lymphocytes (CD3+) reduction, i.e. T-lymphopenia, decreased number of T-helpers/inductors (CD4+ cells) and an immunoregulatory index CD4/CD8 (Th/Ts); in contrast, the levels of T-suppressors/killer cells (CD8+) and B-cells (CD22+) in most patients were within the normal range (Table. 1, 2).

Consequently, at the baseline of the study in patients with ARVI and influenza (since influenza also belongs to acute respiratory viral diseases), there were well-defined immunological status disorders, which can be characterised as an immune deficiency with a relative suppressor variant, i.e. deficient in T-helpers/inductors (CD4+) and decreased CD4/CD8-coefficient that reflects Th/Ts ratio and is called the immunoregulatory index.

Table 1.
Cellular immunity indices in patients with ARVI and influenza before treatment (X±Sx) in children aged 1-9 years

Immunological indices	Norm	Children (group 1)		Comparison group 3 (n=46)
		1.1 (n=51)	1.2 (n=55)	
CD3+, %	68.2±2.3	50.4±2.1*	51.2±2.2*	49.4±2.1*
g/L	1.3±0.04	0.81±0.03*	0.82±0.03*	0.71±0.03*
CD4+, %	4.8±1.6	39.1±1.8*	38.3±1.7*	34.1±1.8*
g/L	0.86±0.03	0.56±0.03*	0.56±0.03*	0.56±0.03*
CD8+, %	1.9±1.1	18.8±1.2*	17.3±1.3*	18.8±1.2*
g/L	0.43±0.02	0.35±0.02*	0.36±0.02*	0.35±0.02*
CD4/CD8	2.0±0.002	1.61±0.02*	1.58±0.02*	1.71±0.02*
CD22+, %	21.1±1.2	23.6±1.3*	23.8±1.4*	22.6±1.3*
g/L	0.2±0.02	0.38±0.02*	0.38±0.02*	0.38±0.02*
RLBT on PHA, %	68.5±2.5	40.6±2.1*	41.1±2.2*	39.6±2.1*

Note: * - the probability of differences in relation to the norm P<0.05.

Table 2.
Cellular immunity indices in patients with ARVI and influenza before treatment (X±Sx) in adolescents aged 10-18 years

Immunological indices	Norm	Adolescents (group 2)		Comparison group 4 (n=50)
		2.1 (n=46)	2.2 (n=54)	
CD3+, %	69.2±2.3	52.2±2.2*	53.2±2.2*	50.2±2.2*
g/L	1.3±0.04	0.91±0.03*	0.92±0.03*	0.84±0.03*
CD4+, %	45.8±1.6	35.3±1.7*	36.3±1.7*	37.3±1.7*
g/L	0.86±0.03	0.58±0.03*	0.56±0.03*	0.59±0.03*
CD8+, %	22.9±1.1	17.3±1.3*	18.3±1.3*	17.3±1.3*
g/L	0.43±0.02	0.36±0.02*	0.38±0.02*	0.39±0.02*
CD4/CD8	2.0±0.002	1.59±0.02*	1.58±0.02*	1.60±0.02*
CD22+, %	22.1±1.2	23.9±1.4*	26.8±1.4*	24.8±1.4*
g/L	0.42±0.02	0.39±0.02*	0.40±0.02*	0.37±0.02*
RLBT on PHA, %	69.5±2.5	42.1±2.2*	43.1±2.2*	43.1±2.2*

Note: * - the probability of differences in relation to the norm P<0.05.

With repeated immunological tests after the study completion, it was found that in the 1st and 2nd main groups, the members of which were administered IMDIF and IMDPF, there was a significant improvement of immunological parameters (Table 3).

In these patients, there was normalisation of T-lymphopenia, lymphocyte increasing to the lower normal limit value (T-helpers/inducers) and In the comparison group, positive trend of the immunological profile was also noted, however, it was significantly less pronounced.

Therefore, after the treatment completion, T-lymphocyte (CD3+) level in the comparison group of patients remained significantly lower than the normal limit value, as well as the same parameter in the main group. CD3+ cells in the relative measurement in the comparison group was on average 1.3 times less than the norm (P<0.05) and in absolute terms 1.4 times less than the norm (P<0.05). The number of lymphocytes was, on average, in the comparison group during this period of examination was 1.2 times less than the norm in relative measurement (P<0.05) and 1.32 times in absolute terms (P<0.05).

In addition to the quantitative characteristics of the cellular immunity indices in patients, functional indicators characterising the T-cell ativity, in particular, RLBT (blast-transformation reaction) on PHA (phytohemaglutenin), were also studied.

Table 3.
Cellular immunity indices in patients with ARVI and influenza after treatment completion (X±Sx)

Immunological indices	Norm	Groups of examined patients					
		Children (group 1)			Adolescents (group 2)		
		1.1 (n=51)	1.2 (n=55)	Comparison group 3 (n=46)	2.1 (n=46)	2.2 (n=54)	Comparison group 4 (n=50)
CD3+, %	69.2±2.3	69.4±2.2**	64.2±2.1**	49.2±2.1	69.4±2.2**	64.2±2.1**	54.2±2.1
g/L	1.3±0.04	1.28±0.04**	0.92±0.03**	0.92±0.03	1.28±0.04**	0.92±0.06**	0.92±0.036
CD4+, %	45.8±1.6	45.2±1.5**	41.1±1.6***	38.1±1.6	45.2±1.5**	42.1±1.6**	38.1±1.6
g/L	0.86±0.03	0.84±0.03**	0.65±0.03**	0.65±0.03	0.84±0.03**	0.65±0.03**	0.65±0.03
CD8+, %	22.9±1.1	22.8±1.2**	21.2±1.3***	19.2±1.3	22.8±1.2**	21.8±1.3**	20.2±1.3
g/L	0.43±0.02	0.42±0.02**	0.36±0.02**	0.36±0.02	0.42±0.02	0.36±0.02**	0.36±0.02
CD4/CD8	2.0±0.002	1.98±0.03**	1.8±0.02**	1.7±0.02	1.98±0.03**	1.8±0.02**	1.6±0.02
CD22+, %	22.1±1.2	21.5±1.3**	22.8±1.4**	19.8±1.4	22.0±1.3**	21.8±1.4**	20.8±1.4
g/L	0.42±0.02	0.42±0.02**	0.39±0.02**	0.37±0.02	0.42±0.02**	0.39±0.02**	0.39±0.02
RLBT on PHA, %	69.5±2.5	66.8±2.3**	59.1±2.5**	51.1±2.5	65.8±2.3**	58.1±2.5**	51.1±2.5

Note: ** - the probability of differences after the treatment P<0.05.

Table 4.1
PHAM indices in examined patients (children) with ARVI and influenza before and after treatment, X±Sx

PHAM indices	Apparently healthy persons, n=20	Groups of examined patients					
		Children (group 1)					
		1.1 (n=51)		1.2 (n=55)		Comparison group 3 (n=46)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
PI, %	60.3±2.1	50.8± 0.05*	59.6± 0.04**	49.4± 0.03*	58.5± 0.01**	51.8± 0.05*	58.5± 0.01
PN, conv.units	9.2±0.5	5.4± 1.6*	8.6± 1.8**	6.2± 1.4*	8.9± 1.3**	5.9± 1.6*	6.9± 1.3
NBT test, %	30.7±0.4	18.2± 0.6*	27.5± 0.8**	19.5± 0.4*	28.8± 0.9**	18.2± 0.6*	18.8± 0.9

Таблица 4.2
Показатели ФАМ у обследованных пациентов (подростки) с ОРВИ и гриппом до и после лечения, X±Sx

PHAM indices	Apparently healthy persons, n=20	Groups of examined patients					
		Adolescents (group 2)					
		2.1 (n=46)		2.2 (n=54)		Comparison group 4 (n=50)	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
PI, %	60.3±2.1	52.8± 0.05*	58.4± 0.01**	53.8± 0.05*	59.5± 0.01**	49.8± 0.05*	50.5± 0.01
PN, conv.units	9.2±0.5	5.6± 1.6*	6.1± 1.3**	6.9± 1.6*	8.9± 1.3**	6.0± 1.6*	7.9± 1.3
NBT test, %	30.7±0.4	19.2± 0.6*	20.8± 0.9**	18.7± 0.6*	28.8± 0.9**	17.2± 0.6*	18.8± 0.9

Notes:
* - the confidence level of PHAM indices before treatment as compared to the controls, P<0.05;
** - the confidence level of PHAM indices after treatment as compared to the controls, P<0.05;

It was found that at the baseline, RLBT in the main group was 1.7 times less than the norm (P<0.05), and in the comparison group it was 1.68 times less (P<0.05). At the same time, no significant difference between RLBT in these groups was detected. In the patients of groups 1 and 2, who additionally were administered IMDIF and, accordingly, IMDPF, a 1.6-fold increase in RLBT on average compared with the basal value (P<0.05) and the normal lower limit value achievement (P<0.05) were detected. In the children and adolescents of the comparison group during this period of examination, RLBT, though increased by 1.25 times compared with the basal value (P<0.05), however, remained significantly lower than the norm – by 1.35 times.

When studying the indices of PHAM (Table 4.1, 4.2), it was proved that at the baseline in the main group in children and adolescents there was an immune deficiency, especially concerning indices of phagocytosis, which were as follows: a decrease in PN by 2.2 times, PI – by 1.9 times, NDI – by 2.6 times.

The obtained data suggested that at the baseline of the study, a significant inhibition of the phagocytic reaction was identified in the patients. On treatment completing, in the 1st and 2nd main groups, there was an increase in PHAM indices as compared to the lower physiological normal thresholds. Therefore, in the control groups at the time of the baseline therapy completion, there was a significant difference in the test parameters, particularly with regard to the phase of digestion of PHAM. When using IMDIF and IMDPF in association with the conventional therapy in patients with ARVI and influenza, the phagocytosis indices normalised as compared to the control groups who were admininstered the baseline therapy.

■ CONCLUSION

The data obtained in the clinical and immunological studies suggest that, in the context of the evidence-based medicine, treatment with IMDIF and IMDPF improves both subjective and objective clinical symptoms among the examined patients with ARVI and influenza, both in children (from 1 to 9 years), and in adolescents compared with the control groups, which was generally characterised by the normalisation or reduction of the severity of intoxication, asthenovegetative symptoms, as well as exacerbation signs of the chronic inflammatory process in various organs and systems.

Improvement of the clinical status of patients who were administered IMDIF and IMDPF occurs secondary to the cellular immunity index normalisation (or a pronounced tendency to it).

Based on the immunological data obtained, it can be considered the inclusion of modern antiviral IMDIF and IMDPF in the comprehension treatment and prevention of patients with ARVI and influenza as aetiopathogenetically evidence-based and clinically promising.

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