IMMUNOLOGICAL ASPECTS OF THE IMPACT OF ORGANOCHLORINE PESTICIDES DURING PESTICIDE PENETRATION INTO THE BODY THROUGH THE GASTROINTESTINAL TRACT

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The paper presents data on the analysis of breast milk samples collected from 201 women living in urban and cotton-growing areas of the Osh Province of the Kyrgyz Republic. Breast milk (BM) samples were screened for organochlorine pesticides (OCPs) in the course of their penetration into the body through the gastrointestinal tract and excretion from the body with BM considering lymph nodes and immune-forming eubiotics damaged by these pesticides in gastrointestinal tract. Reduced detoxification function of the liver and low levels of secretory immunoglobulins (SIgA and SIgM) in GM were also taken into account.

Keywords: immunological aspects, pesticides, gastrointestinal tract, breast milk, cotton-growing areas, Osh Province, Kyrgyz Republic

The higher the concentration of OCPs in the BM, the lower the level of secretory immunoglobulins SIgA and SIgM; the higher the concentration of OCPs in the gastrointestinal tract, the lower the level of eubiotics and liver detoxification function and the higher the risk of developing dysbiosis and the frequency of disturbances in biochemical parameters.

Background. The words spoken by L.I. Medvedev in 1970 “Even if we stop using DDT, people will remain it’s carriers for many years” are still relevant today and will remain true in the future [3]. In this respect, in Kyrgyzstan, sites polluted by organochlorine pesticides (OCPs) that were used in the 1970s for cotton and tobacco pest control (cotton and tobacco were strategically important raw materials in the Soviet Union) as well as for the protection of forest plantations, etc. [6, 7] are a constant source of concern. The impact of OCPs on human body, individual organs and systems were studied by A.I. Shtenberg, M.N. Rybakov [1970], P.P. Lyarskiy [1970], E.P. Krasnyuk et al. [1970], A.P. Schitskov et al. [1970], B.A Revich [2000, 2004]. M.B. Shpirt et al. studied the effect of different doses of DDT on immunological reactivity of experimental animals [8] in 1969. Despite the numerous works devoted to toxic chemicals and pesticides, many questions still remain unanswered.

It was found that in the environment of the Osh Province, more than 95% of pesticides penetrate into the body through the gastrointestinal tract [7]. We have not met with the papers devoted to the comprehensive study of the impact of OCPs on the damage of immunity “defenders” during the penetration of OCPs and their excretion from the body depending on the functions of the body. Studying this problem may contribute to the development of methods of protection and prevention of “primary” damaged organs, systems and beneficial microbes-eubiotics of the gastrointestinal tract from the toxic effects of pesticides.

The present study has been conducted and is being conducted under the projects of the Institute of Medical Problems of the Southern Branch of the National Academy of Sciences of the Kyrgyz Republic: “Studying the influence of negative factors of the environment and working environment on the health of the population” (2001–2005) and “Medical and biological aspects of health preservation and improvement of the gene pool of the population living in ecologically unfavourable areas of the Kyrgyz Republic with the development of complex measures using local raw materials” GR № 0000465, (2006–2016).

Materials and methods of research

Pesticides, when they get into the body through the gastrointestinal tract, first come into contact with the intestinal microflora that is involved in all processes in the gastrointestinal lumen, including the generation of antibodies and alter their enzyme and antagonist properties leading to variability of the intestinal microflora including eubiotics. Subsequently, OCPs get into the blood stream and lymph nodes (where immunoglobulins are formed) through the mucous intestine wall and damage lymph system (i.e., the immune system) and they then get into the liver by the portal vein disturbing its functions, including antitoxic function. In other cases, pesticides extracting with breast milk affect the level of antibodies in breast milk. On this basis, the state of the intestinal microflora (stool samples were collected for dysbacteriosis), liver function (liver tests were performed) and the levels of secretory immunoglobulins in breast milk have been studied. Concentrations of OCPs in breast milk were also measured.

Measurements of OCPs (hexachlorocyclohexane (HCH) and its isomers (α-HCH, γ-HCH, β-HCH and δ-HCH), 1,1,1-trichloro-2,2-di (n-chlorophenyl) ethane (DDT), 4.4-dichlordiphenyldichloroethylene (DDE), dichlorodiphenildichloroethan (DDD), aldrin and dieldrin, heptachlor) in breast milk were performed by thin-layer gas-liquid chromatography using methodical instructions "Guidelines on the detection of trace amounts of pesticides in food stuffs, biological media, feed and the environment" [Part 17. Moscow, 1988, P. 389] and revised edition by M.A. Klisenko, A.A. Kalinina, D.B. Girenko et al. Pesticides test were carried out in the Laboratory of Toxicology, Radiology, Morphology and Ecology of the Institute of Medical Problems, South Branch of the Kyrgyz National Academy of Sciences (Osh) and Osh Province SES (Osh).

A total of 201 women living in urban and cotton-growing areas of the Osh Province of the Kyrgyz Republic were examined. Of them, 172 women with OCPs detected in breast milk were allocated into Group I. Group II consisted of 29 women with no traces of OCPs in breast milk. Examined women in Group I were allocated into 4 subgroups depending on the concentration levels of OCPs in breast milk. Subgroup I comprised 15 women with OCPs concentration level > 0,1 mg/l; Subgroup II – 66 women, OCPs concentration levels ranged 0,01–0,099 mg/l. Subgroup III consisted of 75 women with OCPs concentration levels 0,001–0,0099 mg/l and 4 women were allocated in Subgroup IV (OCPs concentration levels < 0,001 mg/l). All patients (201) were screened for secretory immunoglobulins – SIgA and SIgM (g/l) in breast milk. Stool samples were collected and tested for bacteriosis.

Levels of secretory immunoglobulins (SIgA and SIgM) in breast milk were measured by ELISA.

Microbiological studies aiming at identifying and detecting the level of dysbiotic disorders of the gastrointestinal tract of the examined women were performed according to the guidelines by R.V. Litvak and F.P. Vilshanskaya (1977) [1] supplemented by V.M. Bondarenko V.G. Likhoded (2007).

Liver tests were performed on 68 women. Of them, Group I consisted of 39 women with the detected OCPs in breast milk, Group II – 29 women (no traces of OCPs were detected in breast milk). Blood samples were taken from the ulnar vein (5 ml) in the morning on an empty stomach. Biochemical parameters in blood serum were measured by ELISA "Multisan", biochemical analyzer «BIO-Chemsa» 3/№ E117209, p. (Italy). ELISA was performed using the diagnostic test analyzer (ELISA) Stat Fax 4200 (USA). In order to avoid false positive results, ELISA positive serum samples were re-tested using confirmatory test systems.

Liver biochemical parameters were determined by the standard technique: total bilirubin – by Iendrashek – Grof method, ALT (alanine aminotransferase), AST (aspartat aminotransferase) – by using Reitman – Frankel procedure, cholesterol – by Ilka method, total protein – by biuret test, the serum protein and thymol fractions – by turbidimetric method. Further details of the research and values detected are presented in Table 1.

Results of research and their discussion

Of the 201 women living in cotton-growing and urban areas and screened for OCPs, OCPs were detected in 172 (Group I), accounting for 85,6%. Group II consisted of 29 (14,4%), who showed no traces of OCPs in breast milk samples.

Data on OCPs concentrations in breast milk of women in Subgroups of Group I are presented in Table 2.

Table 1

Standards and techniques for the measurement of biochemical parameters

Test type	Method/Technique	Normal values
Total protein	Biuret test	65,0–85,0 g/l
Total bilirubin	Iendrashek – Grof	8.5–20,5 μm/l
Thymol	Turbidimetric method	0–5 UI
Cholesterol	Fermentative method	2,9–5,2 μm/l
ALT (alanine aminotransferase)	Reitman – Frankel	0,12–0,68 μm/l hr
AST (aspartat aminotransferase)	Reitman – Frankel	0,12–0,68 μm/l hr
Protein fraction	Turbidimetric method	
Albumin	–//–	55,0–60,0 %
α	–//–	15,0–16,0 %
β	–//–	9,0–12,0 %
γ	–//–	16,0–18,0 %

Table 2

Allocation of the surveyed women with detected OCPs in breast milk into Subgroups, depending on OCPs concentrations

Subgroups of Group I	OCPs concentrations mg/l	Number of women	%
Subgroup I	> 0,1	15	8,7
Subgroup II	0,01–0,099	66	38,4
Subgroup III	0,001–0,0099	75	43,6
Subgroup IV	0,0001–0,00099	16	9,3
Total		172	100

Table 3

Comparative data on the altered secretory immunoglobulins SIgA SIgM levels in breast milk depending on the concentration of OCPs

Group	SIgA, g/l		SIgM, g/l	
	M	P	M	P
I	7,85 ± 0,917	< 0,01	7,11 ± 1,35	< 0,01
II	11,5 ± 0,14	< 0,01	11,6 ± 0,15	< 0,01
Subgroups				
I	5,3 ± 1,2	< 0,05	4,55 ± 1,76	< 0,05
II	7,1 ± 0,93	< 0,01	6,3 ± 1,23	< 0,01
III	8,6 ± 0,81	< 0,01	7,8 ± 0,76	< 0,01
IV	**9,85 ± 1,1**	**< 0,05**	**9,65 ± 1,79**	**< 0,05**

Of the OCPs detected, HCH was found in 54,86 %, DDE – in 53,5 %, DDT and DDD – in 4,5 and 4,86 %, respectively. Aldrin was detected in 1,7 % and Hexochloride – in 0,34 %. Dieldrin was not found.

The levels of secretory immunoglobulins in breast milk of nursing mothers depended on the concentration and type of OCPs in breast milk. The more detailed information is given in Table 3.

As shown in Table 3, in Group I, secretory immunoglobulins SIgA and SIgM levels in breast milk ranged 7,85 ± 0,917 and 7,11 ± 1,35 g/l vs. 11,5 ± 0,14 and 11,6 ± 0,15 g/l ($P < 0{,}01$) in Group II, i.e. secretory immunoglobulins SIgA SIgM levels in breast milk decrease 1,4 to 1,6 times in Group I with the detected OCPs in breast milk.

The lowest concentration of immunoglobulins SIgA SIgM was found in breast milk samples where OCPs concentration level in breast milk was > 0,1 mg/l: SIgA 5,3 ± 1,2 g/l, SIgM 4,55 ± 1,76 g/l ($P < 0{,}05$), i.e. the higher the concentration of OCPs in breast milk, the lower the concentration of secretory immunoglobulins SIgA SIgM. This may be due to the fact that OCPs inhibit the synthesis of immunoglobulins when they get into the lymph nodes.

Another equally important factor involved in protecting the body is the state of intestinal biocenosis; eubiotics in the intestinal tract not only possess antagonist properties against pathogenic and opportunistic pathogenic microorganisms, but also directly involved in the formation of secretory immunoglobulins in the digestive tract. OCPs interact with the intestinal flora, inhibit the colonization resistance of the intestinal tract and increase the number of opportunistic microbes suppressing eubiotics, thereby reducing the formation of secretory immunoglobulins.

The effect of OCPs concentrations in breast milk on the state of intestinal biocenosis is shown in Table 4.

As shown in Table 4, the highest percentage of alterations was detected in Subgroup I, where alterations were revealed in almost all groups of intestinal microflora with the increased number of week-fermenting *E.coli* and decreased number of bifidoflora, i.e. bacteria involved in the development of secretory immunoglobulins. This may be due to the fact that OCPs affect the microflora, primarily eubiotics, inhibiting their growth and disrupting the function of the enzyme. Detection of pathogens in control group may be due to the greater sensitivity of pathogenic *E.coli* to OCPs, but this issue requires further detailed studies.

Some antibiotic-resistant, modified microbes – *E. coli* and other opportunistic microbes detected during bacteriological tests are of great interest.

The effect of OCPs concentrations in breast milk on liver biochemical parameters: in Group I, alterations of biochemical parameters of the liver were detected in 18 (46,15 %) of the 39 examined women with the detected OCPs in breast milk; in Group II – in 6,9 %. The more detailed data on the alterations of liver biochemical parameters in Groups I and II are shown in Table 5.

In Group I, liver function abnormalities were detected in 18 of the 39 women examined, accounting for 46,15 %, in Group II, in 2 (6,9 %) of the 29.

Table 4

Comparative data on the alatered intestinal biocenosis depending on the concentration of OCPs in breast milk of nursing women (%)

Number	Alterations of intestinal biocenosis	Group									
		I		II		III		IV		V	
		n = 15	%	*n* = 66	%	*n* = 75	%	*n* = 16	%	*n* = 29	%
1	Detection of coliform bacteria. B N – 0 %	–	–	–	–	–	–	–	–	1	**3,4**
2	Reduction of the total number of coliform bacteria. B N – 300–400 млн/г	13	**86,6**	31	**46,9**	7	**9,33**	2	**15,3**	2	**6,9**
3	Increase of the number of *E. coli* with weakly expressed enzymatic properties. B N – < 10 %	15	**100**	48	**72,7**	8	**10,6**	3	**23,0**	4	**13,8**
4	Increase of the number of lactose-negative enterobacteria. < B N – до 5 %	10	**66,6**	32	**48,4**	10	**13,3**	2	**15,3**	3	**10,3**
5	Detection of hemolytic *E. coli*. B N – 0 %	7	**46,6**	6	**9,09**	4	**5,33**	1	**7,69**	1	**3,4**
6	Increase of the number of coccal forms in a total amount of microbes. B N – < 25 %	9	**60,0**	30	**45,4**	12	**16**	1	**7,69**	1	**3,4**
7	Detection of hemolyzing *S.aureus* in relation to all coccal forms. B N – 0 %	8	**53,0**	11	**16,6**	6	**8**	3	**23,0**	1	**3,4**
8	Reduction of bifidobacteria. < 10^7	14	**93,3**	30	**45,4**	15	**20**	2	**15,3**	3	**10,3**
9	Detection of *Candida*. B N – 0 %	12	**80,0**	15	**22,7**	10	**13,3**	2	**15,3**	1	**3,4**
10	Detection of *Proteus* species B N – 0 %	5	**33,3**	6	**9,09**	4	**5,33**	1	**7,69**	1	**3,4**

Table 5

Comparative data on the alterations of liver biochemical parameters in Groups I and II (%)

Group	Number of examined	Number of liver function alterations	%	Decreased serum cholesterol level		Increased thymol		Other values (ALT, AST and bilirubin)	
					%		%		%
I	39	18	46,15	12	**30,76**	4	**10,5**	17	43,6
II	29	2	6,9	0	**0**	0	**0**	2	6,9

In Group I, increased thymol was found in 4 (10,5 %), sublimate – in 4 (10,5 %), reduced cholesterol – in 12 (30,76 %), increased ALT and AST – in 17 (43, 6 %), increased total bilirubin – in 4 (10,5 %): direct bilirubin – in 2 (5,5 %) and indirect bilirubin – in 2 (5,5 %). Reduced albumin was detected in 5 (12,82 %), increased levels of gamma globulin – in 5 (12,8 %). The ratio of albumin to globulin (A/G ratio) was within the range of 0,8–1,0. Changes in liver tests depended on the concentration and type of OCPs found in breast milk, but this issue requires a more detailed study on more samples.

In Group II, 2 of the 29 women had alterations of liver function, accounting for ,9 %. One patient showed a moderate increase in ALT and AST, the other one – a moderate increase in total bilirubin.

Thus, OCPs, when they get into the body through the gastrointestinal tract, primarily affect the microflora which participate in the formation of immunoglobulins disrupting its colonization resistance; when OCPS get into the liver, they disrupt protein-formation and neutralizing function of the liver; and eventually, excreting with breast milk, OCPs reduce secretory immunoglobulins SIgA and SIgM levels. The higher the concentration of OCPs in breast milk, the higher the occurrence of alterations of the colonization resistance of intestinal biocenosis, liver and the lower the levels of immunoglobulins in breast milk. It is therefore strongly recommended to examine breast milk of all lactating women living in cotton-growing and urban areas in the environment of the Osh Province for OCPs. It is also necessary to take appropriate measures to protect women from further penetration of OCPs and develop the ways of their elimination from the body.

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