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RELATIONSHIPS BETWEEN IMMUNE PARAMETERS OF SALIVA AND BLOOD

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Summary

Background. The study of the relationships between the immune parameters of saliva and blood is of both theoretical and practical importance. The first is to study the relationships between systemic and local immunity, while the second is interesting as a non-invasive method of assessing the immune status and the influence of immunotropic drugs, including balneological, ecological etc. **Material and Methods**. The object of observation were 34 men and 10 women 24-70 y, who came to the Truskavets' spa for the treatment of chronic pyelonephritis combined with cholecystitis in remission. The testing was conducted twice, before and after balneotherapy for 7-10 days. Immune parameters of blood evaluated on a set of I and II levels recommended by the WHO. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD56. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Circulating Immune Complexes and Immunoglobulins classes M, G, A. Parameters of phagocytic function of neutrophils estimated in tests with cultures of Staphylococcus aureus and Escherichia coli. The set of immune parameters of saliva was IgG, IgA, secretory IgA and

Lysozyme. **Results.** The correlations of the registered immune parameters of saliva as effective traits with the immune parameters of blood as factor traits were screened. The level of IgA in saliva, which is 88% (R=0,943) determined by immune parameters of blood, was found to be most closely related to them. Dependence on blood parameters of the level of secretory IgA was weaker, but still strong enough (R=0,801). Salivary IgG is associated with blood parameters to a similar extent (R=0,795), but the factor structure of the connection is significantly different from that of secretory IgA. The weakest were the connections of immune parameters of blood with the level of lysozyme in saliva (R=0,717). **Conclusion**. Saliva levels of IgG, both forms of IgA, and lysozyme are closely related to the immune parameters of the blood, so they can be markers of systemic immunity and its reactions to immunotropic effects.

Key words: Immunity, Saliva, Blood, Relationships.

INTRODUCTION

The study of the relationships between the immune parameters of saliva and blood is of both theoretical and practical importance. The first is to study the relationships between systemic and local immunity [1,4], while the second is interesting as a non-invasive method of assessing the immune status and the influence of immunotropic factors, including ecological [5,6,19], balneological [6,7,9,12-16,18], chronic stress [3,6].

In an excellent review by P Brandtzaeg [1] it is said that the two principal antibody classes present in saliva are secretory IgA (SIgA) and IgG; the former is produced as dimeric IgA by local plasma cells (PCs) in the stroma of salivary glands and is transported through secretory epithelia by the polymeric Ig receptor (pIgR), also named membrane secretory component (SC). Most IgG in saliva is derived from the blood circulation by passive leakage mainly via gingival crevicular epithelium, although some may be locally produced in the gingiva or salivary glands. Gut-associated lymphoid tissue (GALT) and nasopharynx-associated lymphoid tissue (NALT) do not contribute equally to the pool of memory/effector B cells differentiating to mucosal PCs throughout the body. Thus, enteric immunostimulation may not be the best way to activate the production of salivary IgA antibodies although the level of specific SIgA in saliva may still reflect an intestinal immune response after enteric immunization. It remains unknown whether the IgA response in submandibular/sublingual glands is better related to B-cell induction in GALT than the parotid response. Such disparity is suggested by the levels of IgA in submandibular secretions of AIDS patients, paralleling their highly upregulated intestinal IgA system, while the parotid IgA level is decreased. Parotid SIgA could more consistently be linked to immune induction in palatine tonsils/adenoids (human NALT) and cervical lymph nodes, as supported by the homing molecule profile observed after immune induction at these sites. Several other variables influence the levels of antibodies in salivary secretions. These include difficulties with reproducibility and standardization of immunoassays, the impact of flow rate, acute or chronic stress, protein loss during sample handling, and uncontrolled admixture of serum-derived IgG and monomeric IgA.

Despite these problems, saliva is an easily accessible biological fluid with interesting scientific and clinical potentials.

Earlier, we found that salivary levels of lysozyme, IgG and sIgA, but not IgA negatively correlated with the level of caries intensity [11]. In another study, we found that combined gastroenterological and dental pathology is accompanied by lower activity of lysozyme in saliva than incompatible [15]. In last study, we found that lysozyme saliva level correlated with some neural and hemato-immune factors [19].

This study was conducted in line with previous ones.

MATERIAL AND METHODS

The object of observation were 34 men and 10 women aged 24-70 years old, who came to the Truskavets' spa for the treatment of chronic pyelonephritis combined with cholecystitis in remission. The survey was conducted twice, before and after balneotherapy (drinking bioactive water Naftussya three times a day, ozokerite applications, mineral baths every other day for 7-10 days [12,13]).

Immune parameters of blood evaluated on a set of I and II levels recommended by the WHO as described in the manuals [5,8]. For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which adsorbed monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD56 from company "Granum" (Kharkiv) with visualization under light microscope with immersion system. Subpopulation of T cells with receptors high affinity determined by test of "active" rosette formation. The state of humoral immunity judged by the concentration in serum of Circulating Immune Complexes (by polyethylene glycol precipitation method) and Immunoglobulins classes M, G, A (ELISA, analyser "Immunochem", USA).

Parameters of phagocytic function of neutrophils estimated as described by SD Douglas and PG Quie [2] with moderately modification by MM Kovbasnyuk [7,14]. The objects of phagocytosis served daily cultures of Staphylococcus aureus (ATCC N 25423 F49) as typical specimen for Gram-positive Bacteria and Escherichia coli (O55 K59) as typical representative of Gram-negative Bacteria. Both cultures obtained from Laboratory of Hydro-Geological Regime-Operational Station JSC "Truskavets'kurort". Take into account the following parameters of Phagocytosis: activity (percentage of neutrophils, in which found microbes - Hamburger's Phagocytic Index PhI), intensity (number of microbes absorbed one phagocytes - Microbial Count MC or Right's Index) and completeness (percentage of dead microbes - Killing Index KI). On the basis of the recorded partial parameters of Phagocytosis, taking into account the Neutrophils (N) content of 1 L blood, we calculated the integral parameter - Bactericidal Capacity of Neutrophils (BCCN) by the formula [6]:

$$BCCN\ (10^9\ Bacteras/L) = N\ (10^9/L) \bullet PhI\ (\%) \bullet MC\ (Bact/Phag) \bullet KI\ (\%) \bullet 10^{-4}$$

The set of immune parameters of saliva was IgG, IgA, secretory IgA (ELISA, analyser "Immunochem", USA) and Lysozyme. The activity of the latter was evaluated by the bacteriolysis test Micrococcus lysodeicticus (nephelometric method) [5,15].

Results processed using the software package "Statistica 5.5".

RESULTS AND DISCUSSION

Abstracts are published in the conference proceedings [17].

First of all, it was stated that the relationships between the immune parameters of saliva are positive, and their strength is in the range r 0,43 ÷ 0,87 (Table 1).

Table 1. Matrix of correlations between salivary immune parameters

Parameters (n=88)	SI-gA	IgA S	IgG S	Lys S
SIgA Saliva	1	,53	,44	,87
IgA Saliva	,53	1	,77	,64
IgG Saliva	,44	,77	1	,43
Lysozyme Saliva	,87	,64	,43	1

The levels of sIgA and Lysozyme were most closely related (Fig. 1).

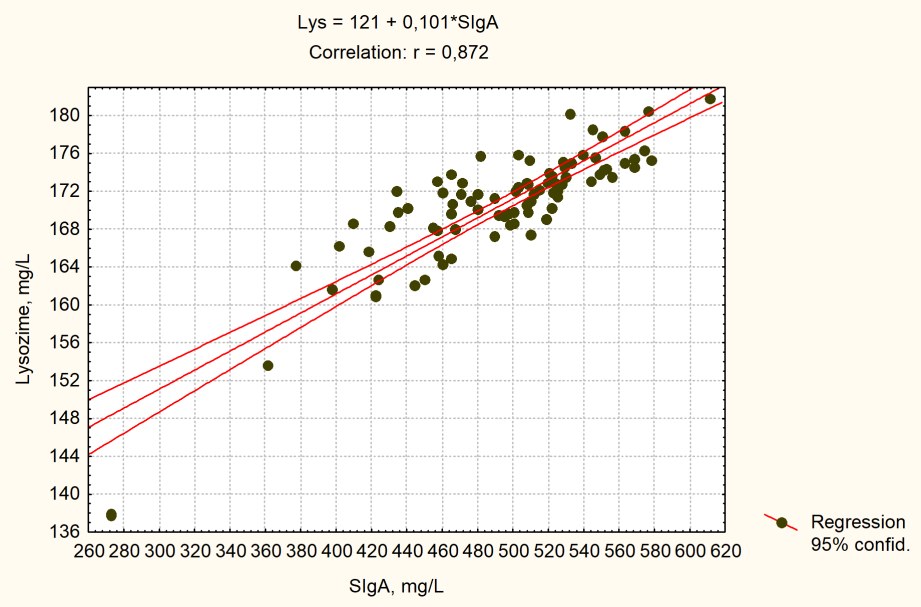


Fig. 1. Scatterplot of correlation between Saliva sIgA (X-line) and Saliva Lysozyme (Y-line)

In the table. 2 shows the result of screening correlations between immune parameters of saliva and blood.

Table 2. Matrix of correlations between immune parameters of saliva and blood

	Parameters of Saliva			
Parameters of Blood	SI-gA	IgA S	IgG S	Lys S
Leukocytes Blood	-,04	-,08	-,06	-,07
Polymorphonucleary Neutrophils	,31	,49	,22	,28
Stubnucleary Neutrophils	,14	,40	,26	,11
Eosinophiles	,07	,14	,08	,07
Monocytes	-,18	-,29	-,10	-,07
Phagocytosis Index vs Staph. aureus	,25	,45	,41	,25
Microbial Count for Staph. aureus	,28	,15	,28	,26
Killing Index vs Staph. aureus	-,28	-,38	-,27	-,27
Bactericidity vs Staph. aureus	,15	,12	,12	,12
Phagocytosis Index vs E. coli	,50	,56	,29	,53
Microbial Count for E. coli	,50	,42	,49	,50
Killing Index vs E. coli	-,41	-,48	-,33	-,43
Bactericidity vs E. coli	,02	,02	,06	-,02
Pan Lymphocytes	-,31	-,52	-,26	-,30
$CD3^+$ T-active Lymphocytes	,16	,36	,22	,12
$CD4^+$ T-helper Lymphocytes	,12	,87	,68	,17
$CD8^+$ T-cytolytic Lymphocytes	,04	,04	,10	-,00
$CD22^+$ B-Lymphocytes	-,10	-,10	-,06	-,16
$CD56^+$ NK-Lymphocytes	-,11	-,67	-,58	-,12
0-Lymphocytes	,01	-,39	-,34	,04
IgG Serum	-,12	,15	-,04	-,01
IgA Serum	,24	,44	-,08	,15
IgM Serum	-,24	,25	,01	,23
Circulating Immune Complexes	,01	,10	,10	-,09

We now analyze the correlations of each immune parameter of saliva as a productive features with the immune parameters of the blood as a factorial features. The level of secretory IgA is most closely related to the intensity of phagocytosis of E. coli directly (Fig. 2), while with its completion inverse (Fig. 3).

The combined effect of both parameters of phagocytosis determines the level of sIgA by 33% (Fig. 4).

The regression model, built by stepwise exclusion of variables to reach the maximum Adjusted R2, identified seven of them, the cumulative effect of which determines the level of sIgA by 61% (Table 3 and Fig. 5).

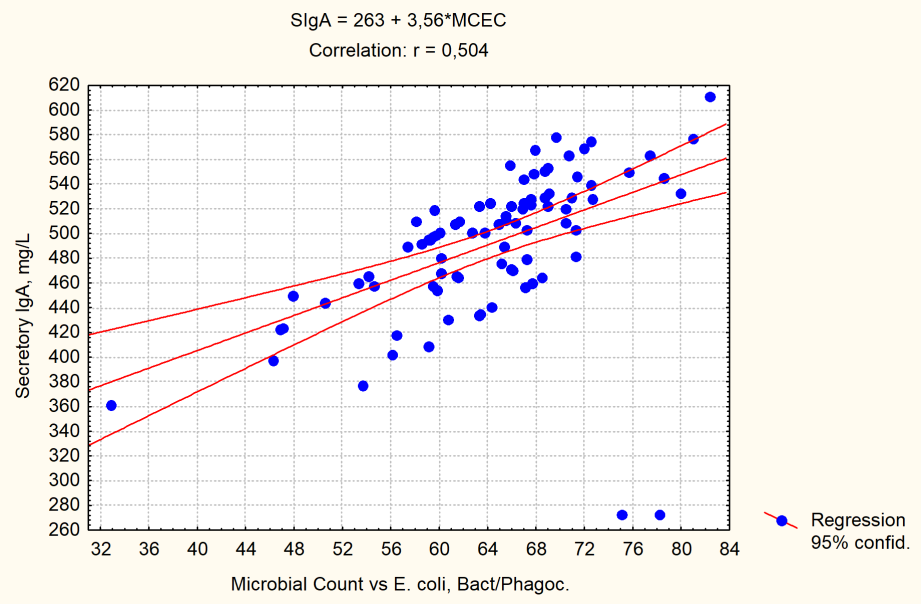


Fig. 2. Scatterplot of correlation between Intensity of Phagocytosis of E. coli (X-line) and sIgA Saliva (Y-line)

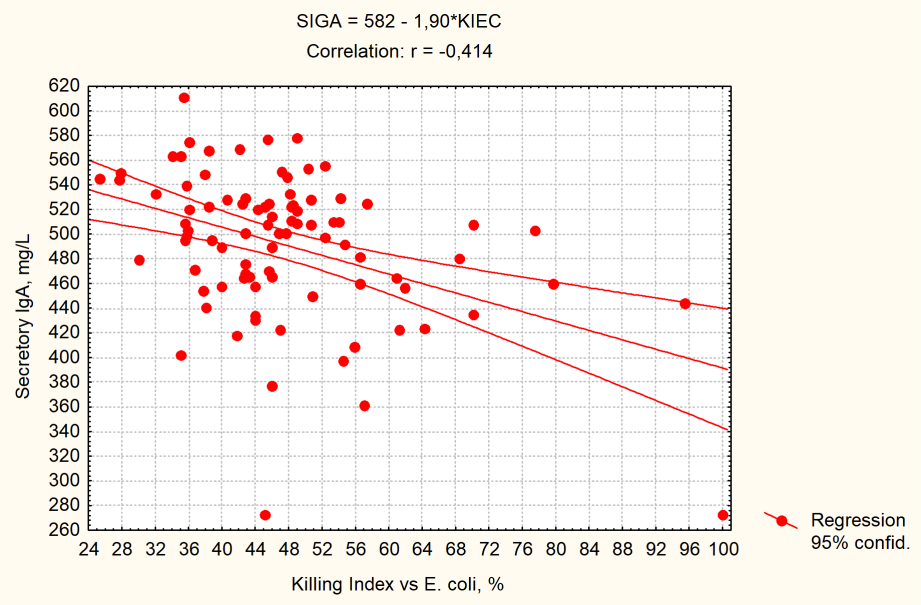
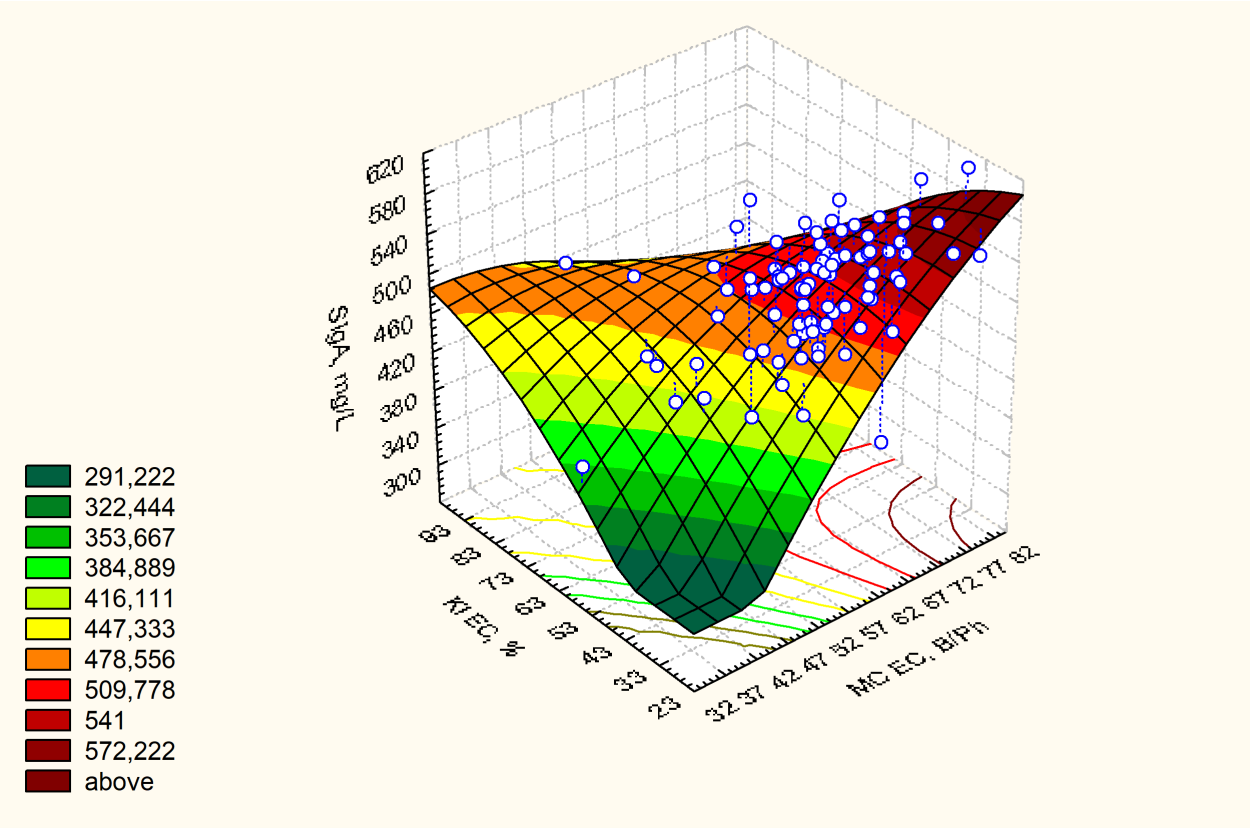


Fig. 3. Scatterplot of correlation between Completeness of Phagocytosis of E. coli (X-line) and sIgA Saliva (Y-line)

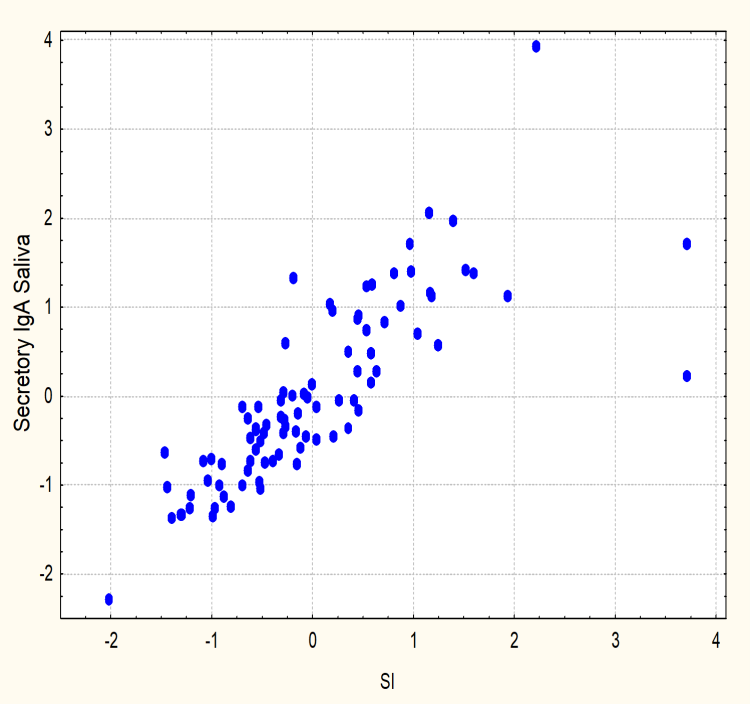


SIgA=365+3,03*MC–1,42*KI; R=0,587; R^2=0,345; Adjusted R^2=0,329; $F_{(2,9)}$=22,4; $p<10^{-5}$; SE: 48,6 mg/L

Fig. 4. Scatterplot of correlation between Intensity (X-line) and Completeness (Y-line) of Phagocytosis of E. coli and sIgA Saliva (Z-line)

Table 3. Regression Summary for Dependent Variable: SIgA Saliva
R=0,801; R^2=0,641; Adjusted R^2=0,610; $F_{(7,8)}$=20,4; $p<10^{-5}$; SE: 37 mg/L

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(80)}$	p-level
Independent Variables	r		In-tercpt	-1596	415	-3,85	10^{-3}
Phagocytose Index vs E. coli, %	0,50	,404	,081	17,61	3,52	5,00	10^{-5}
Microbial Count for E. coli, Bac/Phag	0,50	,391	,079	2,76	,56	4,93	10^{-5}
Polymorphonucleary Neutrophils, %	0,31	,537	,276	4,01	2,06	1,94	,055
Killing Index vs E. coli, %	-0,41	-,360	,081	-1,65	,37	-4,45	10^{-4}
Pan Lymphocytes, %	-0,31	,459	,268	3,57	2,08	1,71	,090
IgM Serum, g/L	-0,24	-,432	,071	-85,65	14,12	-6,07	10^{-6}
Monocytes, %	-0,18	,190	,095	5,39	2,69	2,00	,048



R=0,801; R^2=0,641; $\chi^2_{(7)}$=84,5; $p<10^{-6}$; Λ Prime=0,359

Fig. 5. Scatterplot of canonical correlation between sum of Immune parameters Blood (X-line) and sIgA Saliva (Y-line)

The level of IgG saliva is most closely related to the level of T-helpers in the blood (Fig. 6).

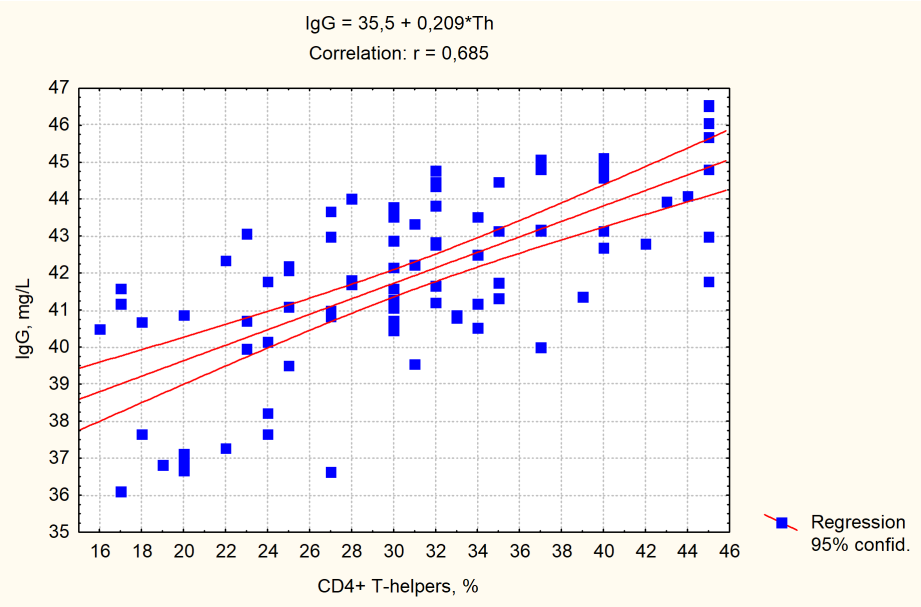
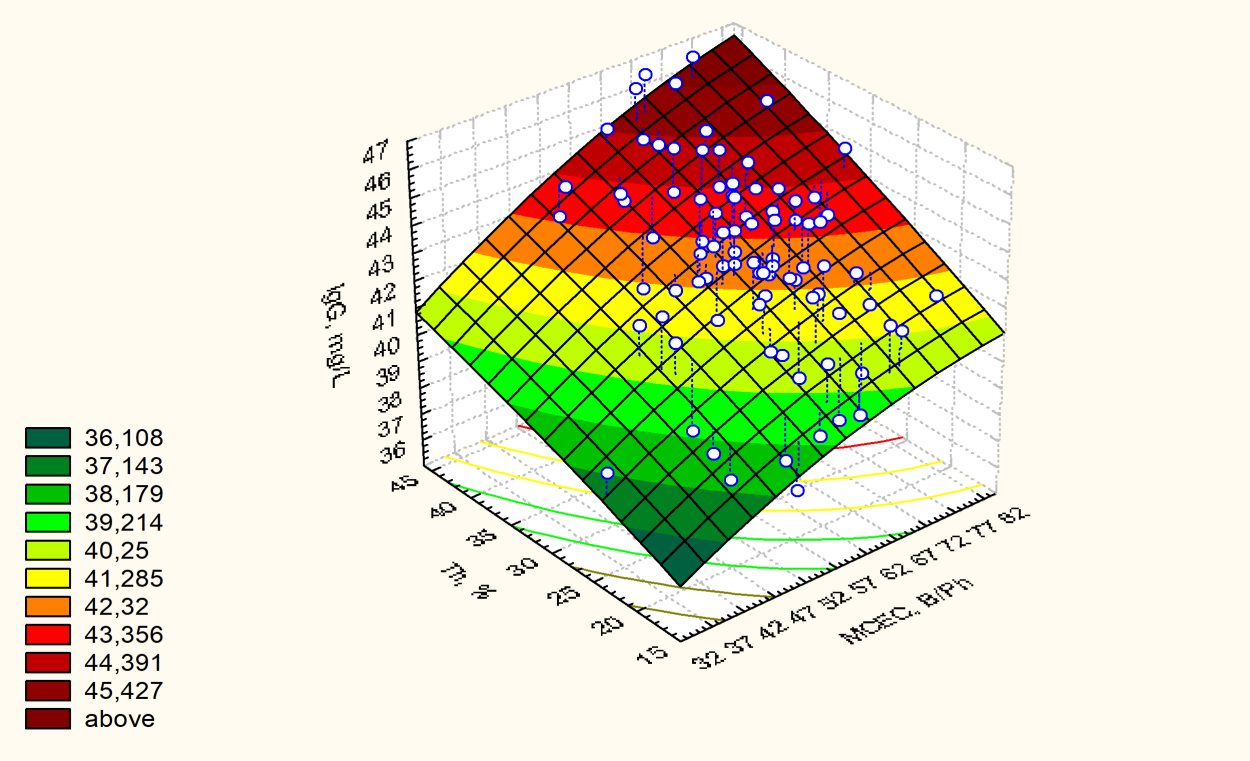


Fig. 6. Scatterplot of correlation between T-helpers Blood level (X-line) and IgG Saliva (Y-line)

Together with the intensity of phagocytosis of E. coli, T-helpers determine the level of IgG saliva by 60% (Fig. 7).



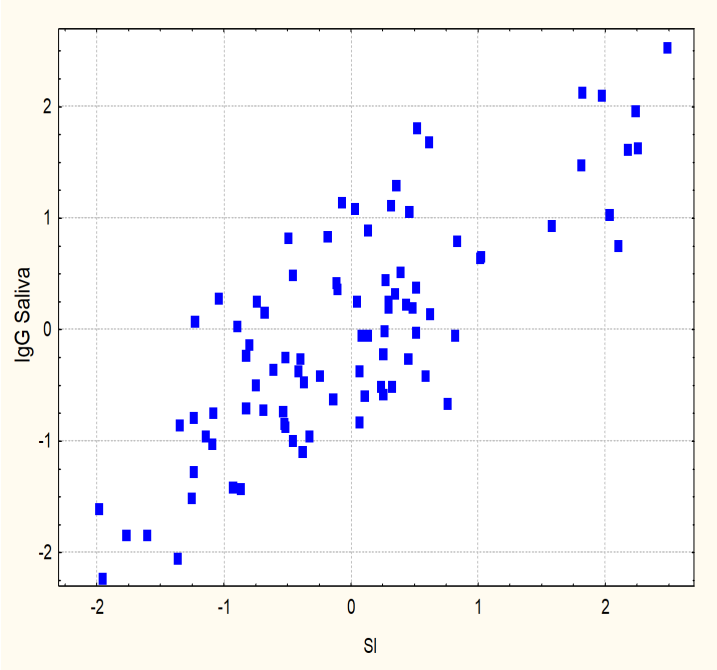
IgG=29,6+0,102*MC+0,187*Th; R=0,772; R^2=0,597; Adjusted R^2=0,587; $F_{(2,9)}$=62,9; $p<10^{-5}$; SE: 1,5 mg/L

Fig. 7. Scatterplot of correlation between Intensity of Phagocytosis of E. coli (X-line) and T-helpers Blood (Y-line) and IgG Saliva (Z-line)

Additional inclusion in the regression model of four more immune blood parameters that characterize the phagocytosis of E. coli and Staph. aureus, increases the coefficient of determination to only 61% (Table 4 and Fig. 8). By the way, we could not confirm the data of JF Johnsen et al [4] about a strong positive correlation (r=0,7; p<0,001) between saliva IgG (mean±SD: 0,2±0,11 g/L) and serum IgG (32,1±11,94 g/L) founded in neonatal calves.

Table 4. Regression Summary for Dependent Variable: IgG Saliva
R=0,795; R^2=0,633; Adjusted R^2=0,605; $F_{(6,8)}$=23,2; p<10^{-5}; SE: 1,5 mg/L

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(80)}$	p-level
Independent Variables	r		Intercpt	52,41	12,88	4,07	,0001
$CD4^+$ T-helper Lymphocytes, %	,68	,677	,079	,207	,024	8,60	10^{-6}
Microbial Count for E. coli, Bac/Phag	,49	,337	,094	,094	,026	3,60	,0005
Phagocytose Index vs E. coli, %	,29	-,156	,081	-,270	,140	-1,93	,057
Microbial Count for St. aur, Bac/Phag	,28	,159	,088	,046	,025	1,81	,074
Killing Index vs E. coli, %	-,33	-,147	,097	-,027	,018	-1,51	,134
Killing Index vs Staph. aureus, %	-,27	,160	,096	,044	,026	1,66	,100



R=0,795; R^2=0,633; $\chi^2_{(6)}$=83; p<10^{-6}; Λ Prime=0,367

Fig. 8. Scatterplot of canonical correlation between sum of Immune parameters Blood (X-line) and IgG Saliva (Y-line)

The level of IgA in saliva was also closely related to the level of T-helpers in the blood (Fig. 9).

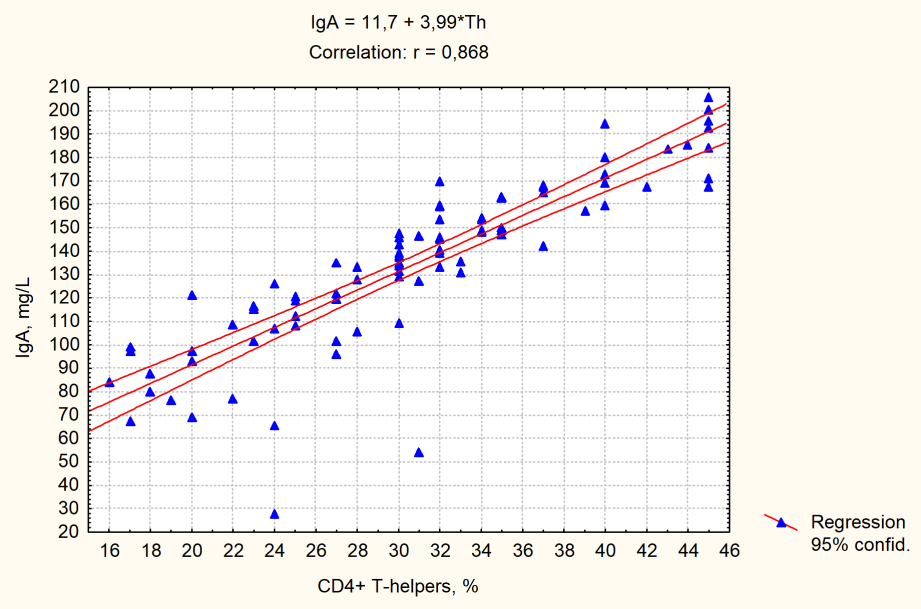
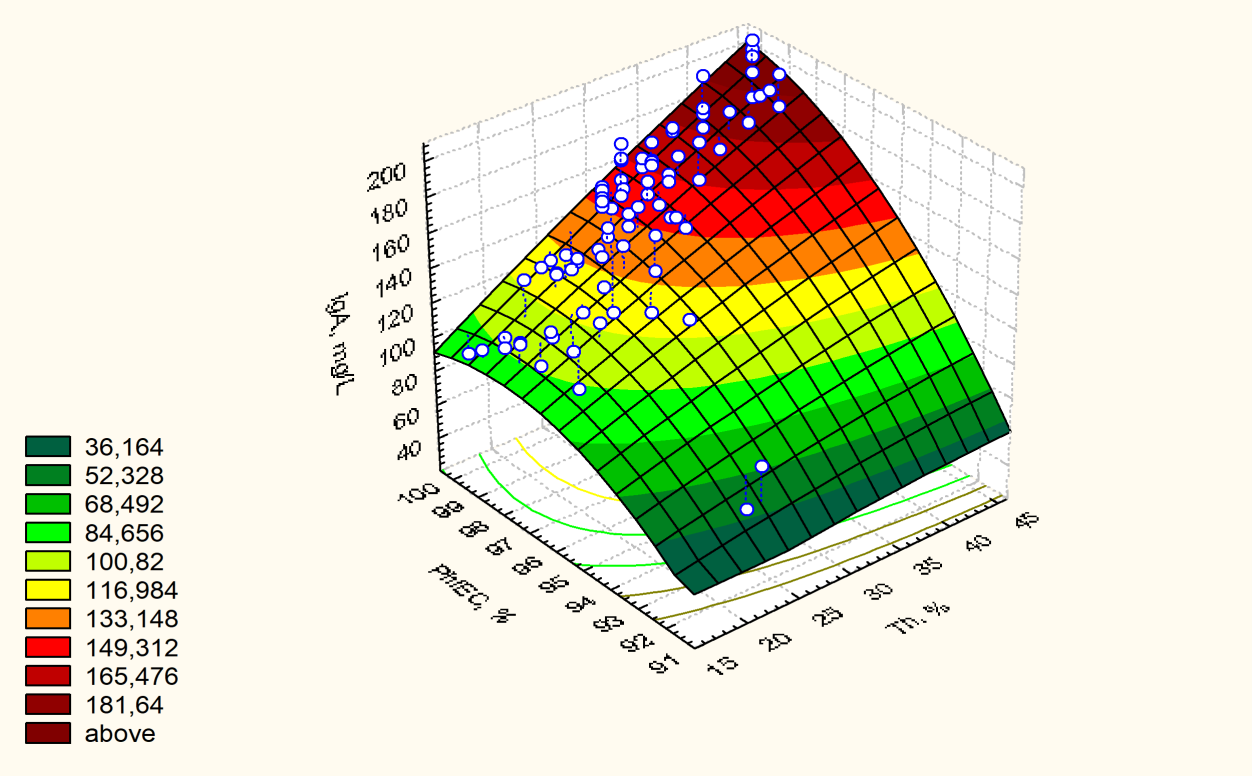


Fig. 9. Scatterplot of correlation between T-helpers Blood level (X-line) and IgA Saliva (Y-line)

The latter together with the activity of phagocytosis of E. coli determine the level of IgA by 81% (Fig. 10), and together with four other parameters by 88% (Table 5 and Fig. 11).

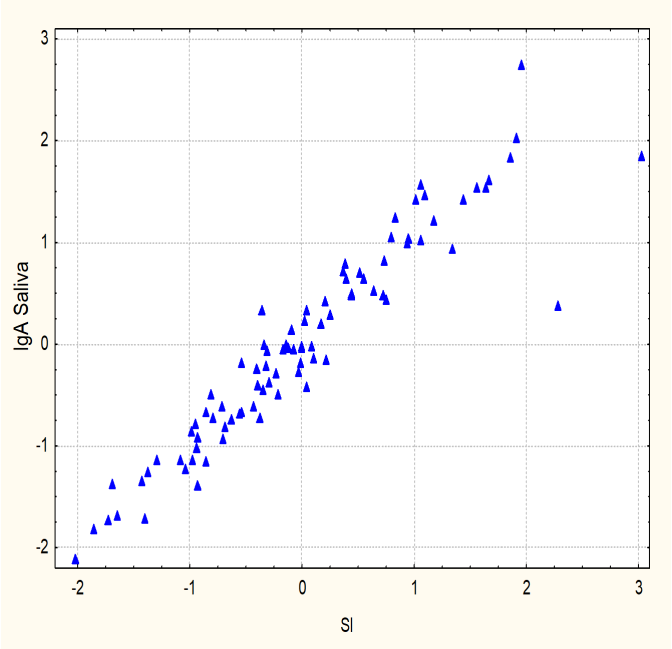


IgG=-659+6,92*PhI+3,52*Th; R=0,902; R^2=0,813; Adjusted R^2=0,809; $F_{(2,9)}$=185; $p<10^{-4}$; SE: 15,5 mg/L

Fig. 10. Scatterplot of correlation between T-helpers Blood (X-line) and Activity of Phagocytosis of E. coli (Y-line) and IgA Saliva (Z-line)

Table 5. Regression Summary for Dependent Variable: IgA Saliva
R=0,943; R^2=0,889; Adjusted R^2=0,881; $F_{(6,8)}$=108; p<10^{-4}; SE: 12 mg/L

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(80)}$	p-level
Independent Variables	r	Intercpt		-670,6	113,9	-5,89	10^{-6}
$CD4^+$ T-helper Lymphocytes, %	,87	,714	,046	3,282	,213	15,43	10^{-6}
Phagocytosis Index vs E. coli, %	,56	,270	,048	7,012	1,239	5,66	10^{-6}
Polymorphonucleary Neutrophils, %	,49	,085	,047	,379	,208	1,82	,073
IgA Serum, g/L	,44	-,159	,049	-11,71	3,62	-3,23	,002
Microbial Count for E. coli, Bac/Phag	,42	,131	,043	,553	,180	3,07	,003
Killing Index vs E. coli, %	-,48	-,194	,043	-,532	,118	-4,52	10^{-4}



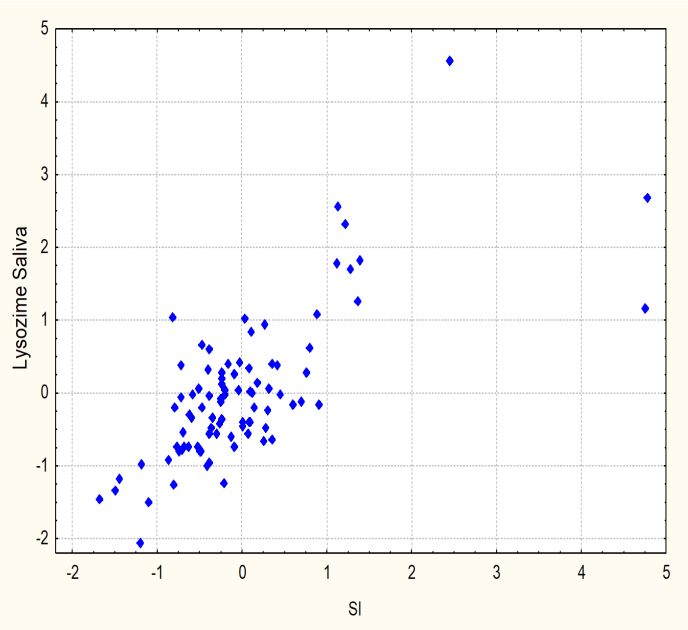
R=0,943; R^2=0,889; $\chi^2_{(6)}$=183; p<10^{-4}; Λ Prime=0,111

Fig. 11. Scatterplot of canonical correlation between sum of Immune parameters Blood (X-line) and IgA Saliva (Y-line)

Salivary lysozyme activity, in contrast to immunoglobulin levels, was associated with phagocytosis parameters of both E. coli and Staph. aureus, as well as IgM weaker, which together determine it by 48% (Table 6 and Fig. 12).

Table 6. Regression Summary for Dependent Variable: Lysozime Saliva
R=0,717; R^2=0,514; Adjusted R^2=0,478; $F_{(6,8)}$=14,3; $p<10^{-5}$; SE: 5 mg/L

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(81)}$	p-level
Independent Variables	r	In-tercpt		44,06	55,51	,79	,430
Phagocytose Index vs E. coli, %	,53	,490	,096	2,458	,484	5,08	10^{-5}
Microbial Count for E. coli, B/Phag	,50	,219	,112	,178	,091	1,97	,053
Microbial Count for St. aur., Bac/Ph	,26	,131	,105	,110	,088	1,25	,216
Phagocytose Index vs Staph. aur., %	,25	-,225	,109	-1,315	,636	-2,07	,042
IgM, g/L	,23	,096	,081	2,194	1,853	1,18	,240
Killing Index vs E. coli, %	-,43	-,340	,083	-,180	,044	-4,11	10^{-4}



R=0,717; R^2=0,514; $\chi^2_{(6)}$=60; $p<10^{-6}$; Λ Prime=0,486

Fig. 12. Scatterplot of canonical correlation between sum of Immune parameters Blood (X-line) and Lysozime Saliva (Y-line)

CONCLUSION

Saliva levels of IgG, both forms of IgA, and lysozyme are closely related to the immune parameters of the blood, so they can be markers of systemic immunity and its reactions to immunotropic effects.

ACKNOWLEDGMENT

We express sincere gratitude to administration of JSC “Truskavets’kurort” and “Truskavets’ SPA” as well as clinical sanatorium “Moldova” for help in conducting this investigation.

ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants. For all authors any conflict of interests is absent.

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