The Assessment of Biological Parameters and Remineralisation Potential of Saliva in Pregnancy

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Pregnancy determines changes of saliva parameters. The assessment of these parameters is useful in the analysis of the affectation degree of salivary function both in pregnancy without general pathology and with general pathology and allows the evaluation of the applied preventive and therapeutical methods. Resting salivary rate (RSF), stimulated salivary rate (SSF), saliva buffering capacity (BC), microcrystallization index (IMK) were evaluated before and after using Recaldent tharapy. This nonopertive method offers a real improvement of saliva remineralisation potential.

Keywords: saliva, remineralisation, biological parameters, microcrystallization index, pregnancy

Saliva has a major role in preserving the oral cavity homeostasis. As other body biological fluids (blood, lympha), saliva is a biomarker used in the assessment of various pathological conditions of human organism. The crystallization phenomenon of biological fluids is a process influenced by genetic factors, and related to quantity, to quality, to organic/mineral ratio and to composition of biological fluids [1]. Biological fluids as saliva are composed by lipotropic crystals and any external influence determines a specific change that defines the crystallographic structure [2].

Actually, the researches of biological fluids using crystallographic method represent an useful instrument. Salivary parameters can be used as accurate indicators in various systemic diseases and in evaluation of pathological conditions, including those from oral cavity [3]. Evaluation using microcrystallization index (IMK) can be applied both in the assessment of general homeostasis to quantify the efficicency of preventive measures [4] and to establish the prognosis or evolution of systemic diseases including the dental disease [5, 6].

The pregnancy represents a physiological periode in women life associated with numerous changes in body systems, including oral cavity. These changes are more visible in the pregnancy with associated pathology, when general condition is modified by hormonal imbalances that influence oral cavity homeostasis.

In pregnancy can be influenced by measures related to oral hygiene, diet and genetic factors. The existence of a quantitative and qualitative salivary support can positively influence the cariogenic risk of pregnant women. The local application of remineralisation products with calcium, phosphate and fluor ions can be beneficial due to synergic action of the three minerals [7, 8].

The assessment of remineralisation potential of saliva using microcrystallization index (IMK) and other salivary parameters in pregnancy periode represent a real aid in the assessment of the carioactivity specific to pregnant women as well as in the quantification of the applied preventive measures.

The objectives of the researches are as follows: the assessment of salivary parameters related to pregnancy with or without systemic pathology (RFS, SSF, BC, IMK) and the efficiency of the remineralisation therapy on salivary parameters.

Experimental part

The study was performed on 45 women, selected from patients treated in Clinical Base M.Kogalniceanu UMF Iasi and in private dental practice, divided in three study groups: two research groups (Group I - pregnancy without associated pathology (n = 15), Group II – pregnancy with associated systemic pathology (n = 15)) and control group (Group III – women without pregnancy (n = 15), with same age category (20 – 40 years) and medium cariogenic risk. The study was performed accordingly to the legislation established by Ethics Committee of Ministry of Health and UMF Gr. T.Popa, Iasi. The informed written consent was obtained for each subject included in this research study.

For all subjects a preventive - therapeutical treatment protocol was applied using GC MI Paste Plus (GC Corporation), based on Recaldent technology, with high content of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). The local applications were performed for 14 days (two applications daily: morning, evening).

The assessment of salivary biological parameters was performed before and after 14 days of treatment: resting salivary rate (RSF), stimulated salivary rate (SSF), saliva buffering capacity (BC), microcrystallization index (IMK). The saliva buffering capacity was measured using Dentobuff Strip.

The salivary microcrystallization index (IMK) was calculated by P.A. Leus method [10, 11], modified by L.V.

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RSF assessment						
Groups/ Number	Group I(SFP)		Group II(SCP)		Group III(M)	
	baseline	post-treatment	baseline	post-treatment	baseline	post-treatment
1	0.7	0.8	0.6	0.7	0.7	0.8
2	0.6	0.7	0.5	0.6	0.5	0.6
3	0.8	0.8	0.7	0.8	0.7	0.9
4	0.9	0.9	0.6	0.8	0.6	0.7
5	0.5	0.7	0.5	0.6	0.6	0.6
6	0.6	0.7	0.5	0.7	0.6	0.7
7	0.7	0.7	0.6	0.6	0.6	0.7
8	0.7	0.8	0.5	0.7	0.5	0.6
9	0.9	0.9	0.6	0.7	0.6	0.7
10	1.0	1.0	0.9	0.9	0.8	0.9
11	0.8	0.9	0.4	0.6	0.7	0.8
12	0.8	0.8	0.5	0.7	0.8	0.8
13	0.6	0.7	0.5	0.6	0.6	0.7
14	0.6	0.6	0.4	0.6	0.4	0.6
15	0.9	0.8	0.6	0.8	0.8	0.9

Table 1
RSF VALUES

RSF	Group I baseline	Group I post	Group II baseline	Group II post	Group III post	Group III post
N Valid	15	15	15	15	15	15
Missing	30	30	30	30	30	30
Mean	**.7400**	**.7867**	**.5600**	**.6933**	**.6333**	**.7333**
Std. Error of Mean	.03754	.02737	.03207	.02482	.03034	.02873
Std. Deviation	.14541	.10601	.12421	.09612	.11751	.11127

Table 2
RSF MEAN VALUES AND STANDARD DEVIATIONS AT BASELINE AND AFTER TREATMENT

RSF	Group I baseline	Group II baseline	Group III baseline	Group I final	Group II final	Group III final
Group I baseline	0.32	0.49	0.52	0.35	0.33	0.28
Group II baseline	0.49	0.42	0.58	0.15	0.01	0.08
Group III baseline	0.52	0.58	0.43	0.32	0.17	0.01
Group I final	0.35	0.15	0.32	0.34	0.09	0.19
Group II final	0.33	0.01	0.17	0.09	0.14	0.31
Group III final	0.28	0.08	0.01	0.19	0.31	0.15

Table 3
WILCOXON TEST. COMPARISON OF RSF RESULTS BETWEEN GROUPS I, II AND III AT BASELINE AND AT THE END OF TREATMENT

Belischaia [2]. The results were divided in three microcrystallization categories: I (high), II (medium), III (low), reported to the calculated score [12]:

IMK = 0.6÷1, high microcrystallization level;
IMK = 0.4÷0.6, medium microcrystallization level;
IMK = 0÷0.4, low microcrystallization level.

The statistical analysis was performed using SPSS v.17.

Results and discussions

RSF values obtained for study groups and control groups are presented in table 1.

The medium values and standard deviations of RSF for study groups and control group are presented in table 2.

RSF values for all groups recorded an increase after treatment, with values range from 0.74 to 0.78 in group I, from 0.56 to 0.69 in group II, from 0.63 to 0.73 in group III (table 2). Data were analysed using Wilcoxon test. The significance levels after the comparison groups are presented in table 3. Significant statistical differences were considered for $p < 0.05$. Also significant statistical differences were recorded when values were compared before and post-treatment.

SSF values before and after the treatment are presented in table 4.

SSF mean values and standard deviations for all groups are presented in table 5.

Mean SSF values for all groups increased post-treatment, with range values between 0.79 to 0.85 for Group I, 0.60 to 0.75 for Group II, 0.80 to 0.86 for Group III (table 5). Data were statistically analysed using Wilcoxon test. The significance levels after comparisons between groups are presented in table 6. Significant statistical differences were considered for $p < 0.05$. Also significant statistical differences were recorded when values were compared before and post-treatment.

BC values for the investigated groups before and post-treatment are presented in table 7.

BC mean values for all groups increased post-treatment, with range values between 4.71 to 5.25 for Group I, 4.14 to 4.77 for Group II, 4.58 to 5.47 for Group III (table 8). Data were statistically analysed using Wilcoxon test. The significance levels after comparisons between groups are presented in table 9. Significant statistical differences were considered for $p < 0.05$. Also significant statistical

SSF asssessment						
Groups/ Number	Group I(SFP)		Group II(SCP)		Group III(M)	
	baseline	post-treatment	baseline	post-treatment	baseline	post-treatment
1	0.80	0.80	0.60	0.80	0.80	0.90
2	0.60	0.70	0.80	0.90	0.70	0.60
3	0.70	0.70	0.70	0.80	0.70	0.70
4	1.20	1.10	0.80	0.80	0.90	0.70
5	0.60	0.80	0.60	0.70	0.70	0.80
6	0.40	0.60	0.60	0.80	0.60	0.60
7	0.70	0.70	0.70	0.90	0.70	0.90
8	1.10	1.10	0.40	0.60	1.00	1.10
9	0.90	0.90	0.50	0.70	0.80	0.90
10	1.00	1.10	0.70	0.80	1.20	1.20
11	0.90	0.90	0.30	0.60	0.80	0.90
12	0.70	0.80	0.50	0.70	0.90	1.00
13	0.80	0.90	0.50	0.70	0.70	0.80
14	0.70	0.80	0.60	0.70	0.70	0.90
15	0.80	0.90	0.70	0.80	0.80	0.90

Table 4
SSF VALUES

SSF		Group I baseline	Group I post	Group II post	Group II post	Group III post	Group III post
N	Valid	15	15	15	15	15	15
	Missing	30	30	30	30	30	30
Mean		**.7933**	**.8533**	**.6000**	**.7533**	**.8000**	**.8600**
Std. Error of Mean		.05297	.04008	.03651	.02364	.03904	.04342
Std. Deviation		.20517	.15523	.14142	.09155	.15119	.16818

Table 5
SSF MEAN VALUES AND STANDARD DEVIATIONS AT BASELINE AND AFTER THE TREATMENT

SSF	Group I baseline	Group II baseline	Group III baseline	Group I final	Group II final	Group III final
Group I baseline	0.24	0.31	0.91	0.21	0.18	0.12
Group II baseline	0.31	0.43	0.01	0.08	0.01	0.21
Group III baseline	0.91	0.01	0.06	0.33	0.15	0.58
Group I final	0.21	0.08	0.33	0.12	0.07	0.79
Group II final	0.18	0.01	0.15	0.07	0.09	0.04
Group III final	0.12	0.21	0.58	0.79	0.04	0.09

Table 6
WILCOXON TEST. COMPARISON OF SSF RESULTS BETWEEN GROUPS I, II AND III AT BASELINE AND AT THE END OF THE TREATMENT

BC assessment						
Groups/ Number	Group I(SFP)		Group II(SCP)		Group III(M)	
	initial	post-treatment	baseline	post-treatment	baseline	post-treatment
1	4.7	5.4	3.8	4.5	4.5	5.1
2	4.6	5.5	4.6	5.2	4.6	5.2
3	4.2	4.4	3.8	4.6	4.0	5.3
4	4.3	5.1	3.9	4.4	3.9	4.8
5	4.0	4.8	4.0	4.6	4.5	5.2
6	3.8	4.3	3.9	4.2	4.7	5.5
7	4.6	5.4	4.2	5.3	4.5	5.8
8	4.8	5.3	3.8	4.4	4.6	5.4
9	4.8	5.6	4.1	4.6	3.8	4.7
10	5.1	5.6	4.2	4.9	3.9	5.3
11	5.1	5.3	4.5	5.4	5.4	6.1
12	4.9	5.4	4.6	4.9	5.0	5.9
13	5.4	5.6	4.8	5.6	4.9	5.7
14	5.5	5.6	3.9	4.3	4.8	5.9
15	4.9	5.4	4.0	4.7	5.6	6.2

Table 7
BC FOR ALL STUDY GROUPS

differences were recorded when values were compared before and post-treatment.

The aspects of saliva microcrystallization changes were as follows: micro-networking, tree limb, ovoid or cube forms, dandelion or flake forms, multiple points, banded clusters, combinations of the enumerated forms (figs. 1-3a and b). The IMK values for all study groups (table 10).

BC		Group I baseline	Group I post	Group II baseline	Group II post	Group III baseline	Group III post
N	Valid	15	15	15	15	15	15
	Missing	30	30	30	30	30	30
Mean		**4.7133**	**5.2467**	**4.1400**	**4.7733**	**4.5800**	**5.4733**
Std. Error of Mean		.12493	.10905	.08608	.11061	.13704	.11648
Std. Deviation		.48383	.42235	.33338	.42840	.53077	.45114

Table 8
BC MEAN VALUES AND STANDARD DEVIATIONS AT BASELINE AND AFTER THE TREATMENT

BC	Group I baseline	Group II baseline	Group III baseline	Group I final	Group II final	Group III final
Group I baseline	0.12	0.13	0.34	0.01	0.08	0.14
Group II baseline	0.13	0.04	0.02	0.09	0.01	0.15
Group III baseline	0.34	0.02	0.03	0.02	0.08	0.01
Group I final	0.01	0.09	0.02	0.03	0.07	0.35
Group II final	0.08	0.01	0.08	0.07	0.11	0.01
Group III final	0.14	0.15	0.01	0.35	0.01	0.12

Table 9
WILCOXON TEST. COMPARISON OF BC RESULTS BETWEEN GROUPS I, II AND III AT BASELINE AND AT THE END OF TREATMENT

IMK assessment						
Groups/ Number	**Group I(SFP)**		**Group II(SCP)**		**Group III(M)**	
	baseline	**post-treatment**	**baseline**	**post-treatment**	**baseline**	**post-treatment**
1	0.6	0.8	0.4	0.4	0.6	0.8
2	0.4	0.6	0.2	0.5	0.5	0.7
3	0.5	0.7	0.5	0.7	0.7	0.9
4	0.4	0.6	0.3	0.6	0.3	0.5
5	0.6	0.8	0.2	0.4	0.5	0.7
6	0.2	0.4	0.6	0.9	0.3	0.6
7	0.5	0.6	0.4	0.7	0.4	0.6
8	0.7	0.9	0.5	0.8	0.7	0.9
9	0.6	0.8	0.2	0.5	0.6	0.8
10	0.5	0.6	0.5	0.7	0.8	1.0
11	0.3	0.5	0.4	0.7	0.6	0.8
12	0.4	0.6	0.5	0.8	0.5	0.7
13	0.5	0.7	0.3	0.5	0.4	0.6
14	0.7	0.9	0.2	0.5	0.5	0.8
15	0.2	0.5	0.6	0.8	0.3	0.5

Table 10
IMK VALUES FOR ALL STUDY GROUPS

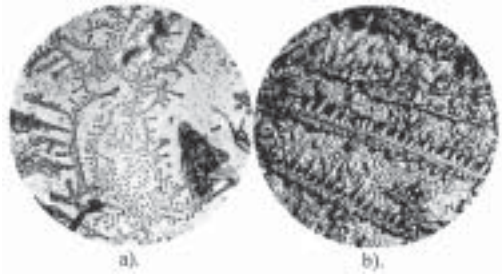

Fig. 1. Aspects of saliva microcrystallization. Group II: **a)**baseline, b) after the treatment

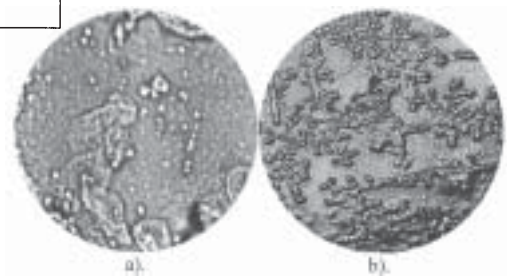

Fig. 2. Aspects of saliva microcrystallization. Group II: **a)** baseline, b) after the treatment

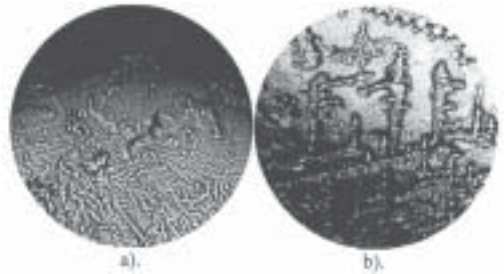

Fig. 3. Aspects of saliva microcrystallization. Group III: a)baseline, b) after the treatment

IMK mean values for all groups increased post-treatment with range values between 0.47 to 0.67 for Group I, 0.38 to 0.63 for Group II, 0.51 to 0.72 for Group III (table 11).

Data were statistically analysed using test *t* for pair samples and test *t* independent samples. The significance levels after comparisons between groups are presented in table 11. Significant statistical differences were considered for $p < 0.05$. Significant statistical differences were recorded between groups when compared values before treatment, excepting Groups I and III. Post-treatment values statistical analysis determined the absence of statistically significant differences. Also significant statistical differences were recorded when IMK values were compared before and afte the treatment (table 12).

IMK		Lot I initial	Lot I dupa	Lot II initial	Lot II dupa	Lot III initial	Lot III dupa
N	Valid	15	15	15	15	15	15
	Missing	30	30	30	30	30	30
Mean		**.4733**	**.6667**	**.3867**	**.6333**	**.5133**	**.7267**
Std. Error of Mean		.04079	.03863	.03763	.04102	.04008	.03838
Std. Deviation		.15796	.14960	.14573	.15887	.15523	.14864

Table 11
IMK MEAN VALUES AND STANDARD DEVIATIONS AT BASELINE AND AFTER THE TREATMENT

IMK	Group I baseline	Group II baseline	Group III baseline	Group I final	Group II final	Group III final
Group I baseline	0.22	0.12	0.49	0.00	0.09	0.13
Group II baseline	0.12	0.00	0.02	0.22	0.00	0.16
Group III baseline	0.49	0.02	0.11	0.08	0.05	0.00
Group I final	0.00	0.22	0.08	0.14	0.09	0.28
Group II final	0.09	0.00	0.05	0.09	0.17	0.10
Group III final	0.13	0.16	0.00	0.28	0.10	0.22

Table 12
IMK RESULTS OF TEST *T* FOR COMPARISON BETWEEN GROUPS I, II [a]I III AT BASELINE AND AT THE END OF TREATMENT

Related to crystals aspects, for high IMK values, in 94.9% cases the forms of microcrystallization present a tree-fern regular aspect, with a tendency of crystals distribution from center to periphery (figs. 1b, 2b and 3b). For low IMK values, in 88.3% cases the forms of microcrystallization present a diffuse structure, multiple points or cubical forms, disposed alone or in clusters, distributed on all visual field or with tendency to gather to the periphery of saliva blob (figs. 1a, 2a and 3a).

Despite the significant increase of literature data committed to the saliva researches [8, 13-15], there are yet unclarified aspects related to this domain. Saliva is a biological active fluid with complex content and multiple assessment possibilities, that allows a non-invasive collecting, unlimited as time, frequency and quantity [16]. The crystallographic methods based on descriptive assessment as well as on interpretation of the biosubstrats formation, represent a simple and accessible method that offers valuable informations in the analysis of physic-chemicall parameters of oral fluids, especially saliva [1, 10-12, 17-19]. These methods allow a global assessment of informational components of biological fluids. The microcrystallization method represents an useful instrument to preserve and transmit information, offering a clear view on the organic components as well as mineral components [20].

The remineralisation capacity of saliva is related to an optimal supply of minerals; an important role is also played by salivary mucines. The salivary mucines have a protective role, participate to transepithelial migration of ions K^+, Na^+, Cl^- and to saliva biocrystallization ability associated to Ca^{2+} ions. The organic mucines matrix offers support to the crystals growth and development and influences the variability of crystals forms [18, 19, 21, 22].

In oral cavity, CPP-ACP molecules link the dental biofilm, enamel hydroxyapatite crystals or deposit on soft tissues, thus representing a permanent reservoir of calcium, phosphate and fluor.

The analysis of various researches [2, 3, 19, 23] determined that crystaloptical properties of saliva can suffer significant changes under the action of external factors (stress, iradiation, intoxications) during crystallization process as well as as response to internal pathological changes of the organism in diverse diseases.

Stress, anxiety, psychic imbalances, associated with cortisol discharges, hormonal imbalances (testosteron), IgA changes and other salivary disfunctions were investigated in diverse researches [6,15, 24-27]. In 2008 A.F. Hamzina determines a direct correlation between specific psychophysiological categories of teenagers and IMK values [3]. The physical exhaustion determines imbalances to salivary level. In 2011 L.V. Belischaia demonstrated, on an athletes group including trainings with different degrees of difficulty, that microcrystallization analysis allows the assessment of general condition and the influence of external factors on body homeostasis [2].

The pregnancy represents a sensitive periode regarding the psychic and emotional condition. Both hormonal and psychic changes can influence the function of salivary glands and homeostasis of oral cavity.

The decrease of the remineralisation potential of saliva is observed in some systemic diseases associated with hormonal disorders (diabetes, thyroid disorders). These disorders are associated to imbalances of salivary calcium, phosphor and pH. The remineralisation therapy applied in these cases can determine the rehabilitation of salivary functions [27].

The results of our study, accordingly to other studies [10, 12, 16, 28] confirm the improvement of salivary parameters following the local applications of remineralisation products. A constant supply of minerals (calcium, phosphate, fluor) offered by MI Paste Plus determines an increase of remineralisation potential of saliva, demonstrated by the assessment of IMK index. High IMK values, associated to the rebalancing of RSF, SSF and BC parameters can contribute significantly to the decrease of cariogenic risk in pregnancy, with or without associated systemic pathology.

The salivary IMK analysis can represent a simple, non-invasive indicator for the assessment of the remineralisation capacity both for healthy organisms and those associated with general pathology.

Conclusions

Pregnancy, especially if associated with systemic pathology, determines changes of saliva parameters.The assessment of these parameters is useful in the analysis of the affectation degree of salivary function both in pregnancy with or without general pathology and allows the evaluation of the applied preventive and therapeutical methods. The use of Recaldent therapy offers a real improvement of saliva remineralisation potential in pregnacy.

References

1.MARTUSEVICH, A.K., KAMAKIN, N.F., Bulletin of Experimental Biology and Medicine, **143**, no.3, 2007, p. 385.
2.BELSKAYA, L.V., GOLOVANOVA, O.A., SHUKAILO, E.S., TURMANIDZE, V.G., Vestnik Otdelenia Nauk o Zemle Ran, **3**, no. 12, 2011, p. 142.
3.HAMZINA, A.F., Izvestia RGPY, **5**, 2008, p. 56.
4.LIKHORAD, E.V., SHAKOVETS, N.V., Med. J., **3**, 2013, p.7.
5.SHAKOVETS, N.V., KREMKO, L.M., BEZROKOVNAIA, V.G., Sov. Stom., **4**, 1998, p.11.
6.DAVIDOVICH, E., AFRAMIAN, D.J., SHAPIRA, J., PERETZ, B., International Journal of Paediatric Dentistry, **20**, no. 4, 2010, p. 235.
7.SMOLISCHAIA, I.V., Beloruss. Med. J., **2**, 2003, p. 90.
8.PIEKARZ, C., RANJITKAR, S., HUNT, D., MCINTYRE, J., Australian Dental Journal, **53**, no.1, 2008, p. 5.
9.LATA, S., VARGHESE, N.O., VARUGHESE, J.M., J Conserv Dent., **13**, no. 1, 2010, p. 42.
10.LEUS, P.A., Autoref. Doc. 14.00.21, (Moscova), 1977, p. 30.
11.LEUS, P.A., Diagnosticescoe znacenie homeostaza sliuni v clinike terapevticescoi stomatologhii, BGMY, (Minsk), 2011, p.67.
12.PANCU, G., LACATUSU, S., CARUNTU, I.D., IOVAN, G., GHIORGHE, A., Rev. Med. Chir. Soc. Med. Na., **110**, no. 1, 2006, p. 206.
13. MANDEL, I.D., Journal of the American Dental Association, **119,** no. 2, 1989, p. 298.
14.HAECKEL, R., HANECKE, P., Annales de Biologie Clinique, **51**, no. 10-11, 1993, p. 903.
15. KIRSCHBAUM, C., WUST, S., HELLHAMMER, D., Rev. Oral. Biol. Med., **4**, no. 3-4, 1993, p. 251.
16. MATTHEWS, D.C., CLOVIS, J.B., BRILLANT, M.G.S., FILIAGGI, M.J., MCNALLY, M.E., KOTZER, R.D., LAWRENCE, H.P., Journal of the Canadian Dental Association, **78**, 2012, p. 3.
17.GONCIAR, F.L., POHODENICO-CIUDACOVA, I.O., Supl. Conf., (Minsk), 2010, p. 70.
18.LEONTIEV, V.K., Stom., **2**, 1999, p. 22.
19.KAMAKIN, N.F., MARTUSEVICI, A.K., **10**, 2002, p. 3.
20.IUSKIN, N.P., GAVRILIUK, M.V., GOLUBEV, E.A., Inform. Bull. RFFI, **4**, 1996, p. 393.
21.DERIABINA, N.I., ZALESSKII, M.G., Vest. Nov. Med. Tehn., **12**, no. 1. 2005, p. 85.
22.BARER, G.M., DENISOV, A.B., MIHALEVA, I.N., Biul. Exp. Biol. Med., **126**, no. 12, 1998, p. 693.
23.BELISCAIA, L.V., Rez. teza Doc. (Omsk), 2009, p. 158.
24.GRANGER, D.A., WEISZ, J.R., KAUNECKIS, D., Journal of Abnormal Psychology, **103**, no. 2, 1994, p. 267.
25.MARTIN, R.B., GUTHRIE, C.A., PITTS, C.G., Behavioral Medicine, **19**, no. 3, 1993, p. 111.
26.DAVIES, R.H., HARRI, S.B.,THOMAS, D.R., COOK, N., READ, G., RIAD-FAHMY, D., British Journal of Psychiatry, **161**, nov., 1992, p. 629.
27.SHIP, J.A., PILLEMER, S.R., BAUM, B.J., Journal of the American Geriatrics Society, **50**, no. 3, 2002, p. 535.
28.GORBULENCO, V.B., SOSTACOVSCHAIA, S.I., IACOVLEVA, V.I., J. Nov. Stom., **2**, 2003, p. 70.

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