Analysis of the severity of oxidative stress and the level of apoptosis marker, annexin a5, in relation to the dental indices in bronchiectasis-associated chronic generalized periodontitis

Análisis de la severidad del estrés oxidativo y el nivel del marcador de apoptosis, anexina a5, en relación con los índices dentales en la periodontitis crónica generalizada asociada a bronquiectasias

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Received: 04/26/2021 Accepted: 07/15/2022 Published: 07/25/2022 DOI: https://doi.org/10.5281/zenodo.7358787

Abstract

Objective. The objective is to study the severity of oxidative stress and the level of apoptosis marker, annexin a5 (AnxA5), in relation to the dental indices in bronchiectasis-associated/non-associated chronic generalized periodontitis (CGP).

Materials and Methods. The study involved 90 CGP patients examined, which were divided into two groups: main group - patients with bronchiectasis-associated CGP, n=50, and experimental group - patients with CGP, n=40. The following dental indices were measured: papillary-marginal-alveolar index (PMA), periodontal index (PI), gingival sulcus bleeding index (Muhlemann-Cowell), and simplified hygiene index (OHI-s). The oral fluid was studied for the level of biomarkers: total superoxide dismutase (SOD), advanced oxidation protein product (AOPP), malondialdehyde (MDA), and annexin A5 (AnxA5).

Results. The analysis of the levels of oxidative stress markers showed high peroxidation of lipid molecules, high modification of protein molecules, low activity of antioxidant protection, and high intensity of apoptosis in all CGP patients, with a greater severity among patients with comorbid pathology. Patients whose total SOD was within the control group values, had their AnxA5 also within the control group values. The presence of correlations between the levels of biomarkers of oxidative stress and apoptosis, AnxA5, with the dental indices were stronger in patients with comorbid pathology. Correlation analysis revealed a positive, statistically significant relationship between the levels of total SOD, MDA, and AnxA5.

Conclusion. The results obtained indicate the relationship between the intensification of oxidative stress and the increase in the intensity of apoptosis with the deterioration of the periodontal tissues. While the presence of bronchiectasis exacerbates periodontal tissue damage in CGP patients. Also, the results of the study assume SOD to be an apoptosis severity protective factor in bronchiectasis-associated and non-associated CGP patients.

Keywords: chronic generalized periodontitis, bronchiectasis, dental indices, oxidative stress, apoptosis

Resumen

Objetivo. El objetivo es estudiar la severidad del estrés oxidativo y el nivel del marcador de apoptosis, la anexina a5 (AnxA5), en relación con los índices dentales en la periodontitis crónica generalizada (PGC) asociada/no asociada a bronquiectasias.

Materiales y métodos. El estudio involucró a 90 pacientes con CGP examinados, que se dividieron en dos grupos: grupo principal - pacientes con CGP asociada a bronquiectasias, n=50, y grupo experimental - pacientes con CGP, n=40. Se midieron los siguientes índices dentales: índice papilar-marginal-alveolar (PMA), índice periodontal (PI), índice de sangrado del surco gingival (Muhlemann-Cowell) e índice de higiene simplificado (OHI-s). Se estudió el nivel de biomarcadores en el fluido oral: superóxido dismutasa total (SOD), producto de proteína de oxidación avanzada (AOPP), malondialdehído (MDA) y anexina A5 (AnxA5).

Resultados: El análisis de los niveles de marcadores de estrés oxidativo mostró alta peroxidación de moléculas lipídicas, alta modificación de moléculas proteicas, baja actividad de protección antioxidante y alta intensidad de apoptosis en todos los pacientes con GPC, con mayor gravedad entre los pacientes con patología comórbida. Los pacientes cuya SOD total estaba dentro de los valores del grupo de control, tenían su AnxA5 también dentro de los valores del grupo de control. La presencia de correlaciones entre los niveles de biomarcadores de estrés oxidativo y apoptosis, AnxA5, con los índices dentales fue más fuerte en pacientes con patología comórbida. El análisis de correlación reveló una relación positiva y estadísticamente significativa entre los niveles de SOD total, MDA y AnxA5.

Conclusión. Los resultados obtenidos indican la relación entre la intensificación del estrés oxidativo y el aumento de la intensidad de la apoptosis con el deterioro de los tejidos periodontales. Mientras que la presencia de bronquiectasias exacerba el daño del tejido periodontal en pacientes con CGP. Además, los resultados del estudio suponen que la SOD es un factor protector de la gravedad de la apoptosis en pacientes con CGP asociada y no asociada a bronquiectasias.

Palabras clave: periodontitis crónica generalizada, bronquiectasias, índices dentales, estrés oxidativo, apoptosis

Introduction

The presence of comorbid pathology is known to affect the clinical course of another somatic disease, its progression rate, and outcome. All this causes a wide interest in the study of comorbidity and the introduction of new clinical guidelines for the prevention and treatment of diseases, considering the presence of a comorbid condition[1,2].

One of the widespread inflammatory periodontal diseases, which usually has a high frequency of recorded comorbid conditions, is chronic generalized periodontitis (CGP)[3-5]. A frequently detected comorbid condition in CGP patients includes respiratory diseases. The effect of chronic obstructive pulmonary disease and bronchial asthma on the course of CGP has been widely proven and studied[6-8]. At the same time, the available literature provides no data on the features of the CGP course in patients with bronchiectasis.

The high incidence of bronchopulmonary comorbid condition in CGP patients is due to a number of anatomical and physiological features and common pathogenetic mechanisms. It is also known that bronchiectasis is a chronic relapsing disease, in which various pathogens play the main pathogenetic role. As sputum is secreted from the bronchi, pathogens come into contact with periodontal tissues and can have a negative effect thereon. The treatment of bronchiectasis provides for a long-time therapy with various groups of antibiotics, which in turn leads to dysbiosis in the oral cavity and further contributes to an increase in the number of pathogens[9]. Oxidative stress and apoptosis are among the leading mutually aggravating pathogenetic mechanisms of bronchopulmonary pathology and CGP[10-12].

One of the most studied markers of oxidative stress are malondialdehyde (MDA), which reflects an increase in the peroxidation of lipid molecules[13] and superoxide dismutase (SOD), an enzyme of the antioxidant system[14]. In recent decades, scientists and clinicians have also drawn their focus on the processes of protein peroxidation because an increased oxidative degradation of proteins, as well as lipid peroxidation, plays an important role in the structural and functional changes in the membranes of various organs and tissues. It has been proven that oxidative modification of proteins is one of the important indicators of tissue damage. One of the biomarkers of oxidative modification of proteins is AOPP - advanced oxidation protein products[15-17].

Both apoptosis and oxidative stress have complex molecular mechanisms of synergy that are still understudied. One of the markers of apoptosis is annexin A5 (AnxA5). Its source is apoptotic and destroyed cells. It can bind to negatively charged phospholipids, including phosphatidylserine, whose exposure on the cell membrane is one of the early signs of apoptosis. An important diagnostic value of AnxA5 is that cells in apoptosis bind thereto before changes in their morphology occur and their DNA hydrolysis begins[18-23].

Objective: to study the severity of oxidative stress and the level of apoptosis marker AnxA5, in relation to the dental indices in bronchiectasis-associated/non-associated chronic generalized periodontitis.

Materials and Methods

The study involved 90 CGP patients examined, which were divided into two groups: main group - patients with bronchiectasis-associated CGP, n=50, and experimental group - patients with CGP, n=40. The examined patients were monitored and underwent their comprehensive laboratory and imaging examination in the dental clinic at Astrakhan State Medical University of the Ministry of Health of Russia and in the therapeutic department of Gubin Brothers City Clinical Hospital No.2. Table 1 shows the characteristics of the examined patients.

Table 1 Patients' characteristicsn

Indicator	CGP patients (experimental group), n=40	Bronchiectasis-associated CGP patients (main group), n=50
Age, years	49.9 [40; 64]	44.1 [39; 61] p=0.122
Sex: male, n female, n	 28 persons (70%) 12 persons (30%)	 31 persons (62%) χ^2=0.13; df=1; p=0.718 19 persons (38%) χ^2=0.31; df=1; p=0.578
CGP duration, years	13.5 [4; 20]	16.4 [5; 19] p=0.145

The control group involved somatically healthy individuals with intact periodontium, n=40 (26 men (65%), 14 women (35%)). The age of control patients was 55[36,62] years.

The inclusion criteria were diagnosed CGP; diagnosed bronchiectasis; signed informed consent of the subject. The exclusion criteria were dental abnormalities and deformities; abnormal abrasion; severe concomitant disease of internal organs with functional failure; refusal of the patient to examination.

The clinical study was approved by the Regional Independent Ethical Committee (dated December 28, 2017, record No. 15).

The dental status was analyzed by a detailed survey and clinical examination of patients. In addition to a detailed survey of patients, the subjective state of the oral cavity was also assessed.

All subjects were determined their dental indices that characterize the severity of periodontal soft tissue damage: papillary-marginal alveolar index (PMA), periodontal index (PI), gingival sulcus bleeding index (Muhlemann-Cowell), and simplified hygienic index (OHI-s).

The diagnosis of bronchiectasis was verified on the basis of medical history, the presence of bronchiectasis based on the results of multislice computed tomography. Patients with bronchiectasis had a bilateral lesion, remission, respiratory failure grade 0-1. The duration of the disease was at least 5 years as it was diagnosed.

Total superoxide dismutase (SOD) of all three types (Cu/Zn-SOD + Mn-SOD + Fe-SOD) was determined by oral fluid enzyme immunoassay according to the methods recommended by the reagent manufacturers using Superoxide Dismutase Assay Kit (CaymanChemical, USA); AOPP was determined using AOPP Kit (ImmunDiagnostik, Germany); AnxA5 was determined using BenderMedSystems commercial test kit (Austria). The concentration of MDA was spectrophotometrically determined by thiobarbituric acid reaction[20,21].

The data were analyzed with STATISTICA v.11.0 (StatSoft, Inc., USA). Since the signs had a distribution different from normal, the data are presented as Me [5;95]. Numerical data of 2 unrelated groups were compared using the Mann-Whitney U-test. Differences were statistically significant at $p<0.05$. The correlation was assessed using the Spearman rank correlation coefficient, the relationship was assessed using the Chaddock score.

The first stage of the study involved the analysis of dental indices (PMA, PI, Muhlemann-Cowell, OHI-s) in the examined groups. As Table 2 shows, the patients of the main and comparison group had significantly higher dental indices than the control group.

Table 2 Dental indices

Dental indices	Somatically healthy individuals (control group), n=40	CGP patients (experimental group), n=40	Bronchiectasis-associated CGP patients (main group), n=50
PMA, %	5 [1; 7]	55 [29; 75] p_1<0.001	68 [37; 92] p_1<0.001 p_2<0.001
PI, un	0.2 [0; 0.4]	3.3 [0.5; 6.9] p_1<0.001	5.4 [1.2; 7.6] p_1<0.001 p_2=0.001
Muhlemann-Cowell, un	0.1 [0; 0.3]	1.8 [0.6; 2.6] p_1<0.001	2.5 [1; 2.9] p_1<0.001 p_2=0.001
OHI-s, un	0.5 [0.2; 0.9]	2.2 [1.6; 2.9] p_1<0.001	2.9 [1.9; 3.5] p_1<0.001 p_2<0.001

Note: p_1 - with somatically healthy individuals; p_2 - with CGP patients

The highest dental indices were found in the main group of patients. At the same time, the differences with the experimental group were statistically significant. The data indicate severe inflammatory and inflammatory-destructive processes in periodontal tissues in all examined patients with CGP, significantly more severe in patients with comorbid pathology.

Next, we studied the levels of oxidative stress markers (MDA, AOPP, total SOD) and an apoptosis marker, AnxA5. Both patients of the main and comparison groups had significantly higher MDA, AOPP, and AnxA5 compared to the control group (Table 3).

The patients of the main group had significantly higher MDA and AOPP than the comparison group. The levels of total SOD in patients of the main and comparison groups were statistically significantly lower than in the control group. At the same time, in the main group, total SOD was statistically significantly lower than in the comparison group.

The analysis of the levels of oxidative stress markers showed high peroxidation of lipid molecules, high modification of protein molecules, low activity of antioxidant protection, and high intensity of apoptosis in all CGP patients, with a greater severity among patients with comorbid pathology.

Next, we tried to analyze the incidence of patients with abnormal levels of the studied biomarkers. Table 4 presents the results of the analysis of the frequency of pathological levels of markers of oxidative stress and apoptosis in the examined groups of patients.

Table 3 Levels of the biomarkers

Biomarkers	Somatically healthy individuals (control group), n=40	CGP patients (experimental group), n=40	Bronchiectasis-associated CGP patients (main group), n=50
MDA, μmol/ml	3.88 [0.81; 5.38]	10.49 [5.1; 20.5] p_1<0.001	18.9 [7.2; 29.3] p_1<0.001 p_2<0.001
AOPP,μmol/L	52.14 [16.14; 89.65]	128.1 [56.3; 201.5] p_1<0.001	182.3 [93.4; 303.9] p_1<0.001 p_2<0.001
SOD total, U/ml	0.1602 [0.1206; 0.2267]	0.1005 [0.0883; 0.1735] p_1<0.001	0.0645 [0.0217; 0.1153] p_1<0.001 p_2<0.001
AnxA5, ng/ml	0.94 [0.68; 2.3]	14 [1.8; 29] p_1<0.001	19.8 [2.4; 41] p_1<0.001 p_2<0.001

Note: p_1 - with somatically healthy individuals; p_2 - with CGP patients

Table 4 Frequency of pathological levels of markers of oxidative stress and apoptosis

Markers	CGP patients (experimental group), n=40		Bronchiectasis-associated CGP patients (main group), n=50	
	Within the control values	**Pathologic**	**Within the control values**	**Pathologic**
MDA μmol/ml	8 persons (20%)	32 persons (80%) χ^2 Yates corrected =8.79; df=1; p_1=0.003	1 person (2%) χ^2 Yates corrected =4.81; df=1; p_2=0.028	49 persons (94%) χ^2 Yates corrected =32.12; df=1; p_1<0.001 χ^2 =0.43; df=1; p_2=0.514
AOPP,μmol/L	11 persons (27.5%)	29 persons (92.5%) χ^2 =5.52; df=1; p_1=0.019	-	50 persons (100%) χ^2 =1.04; df=1; p_2=0.307
SOD total U/ml	9 persons (22.5%)	31 persons (77.5%) χ^2 Yates corrected =7.25; df=1; p_1=0.007	4 persons (8%) χ^2 Yates corrected =1.89; df=1; p_2=0.169	46 persons (92%) χ^2 Yates corrected =23.73; df=1; p_1<0.001 χ^2 Yates corrected =0.3; df=1; p_2=0.586
AnxA5, ng/ml	8 persons (16%)	42 persons (84%) χ^2 Yates corrected =13.85; df=1; p_1<0.001	2 persons (4%) χ^2 Yates corrected =3.25; df=1; p_2=0.072	48 persons (96%) χ^2 Yates corrected =29.14; df=1; p_1<0.001 χ^2 =0.09; df=1; p_2=0.765

Note: p_1 - compared with the number of patients with indicators within control values; p_2 – compared with CGP patients

Results and discussion

According to the results, patients of both the main and experimental group had significantly more often pathological levels of the studied biomarkers found. An intergroup comparison found a higher frequency of pathological levels in patients with comorbid pathology than in CGP patients, however, without statistically significant differences. Also, we found AnxA5 within the control values both in those patients of the main (n=8) and experimental groups (n=2) who in the respective groups had total SOD within the control values.

At the last stage of the study, to assess the relationships between the intensification of oxidative stress and apoptosis processes with the value of dental indices, as well as the relationships between the levels of biomarkers of oxidative stress and apoptosis, AnxA5, a correlation analysis was carried out. Its results revealed different levels of positive relationships between the values of dental indices and the levels of MDA, AOPP, total SOD, and AnxA5 in the main and experimental groups (Fig. 1 and 2).

Figure 1

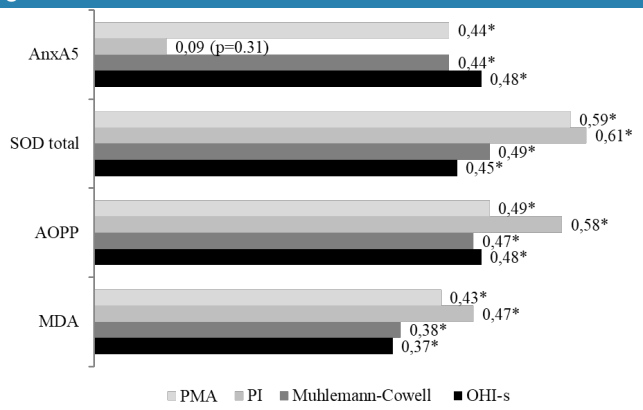
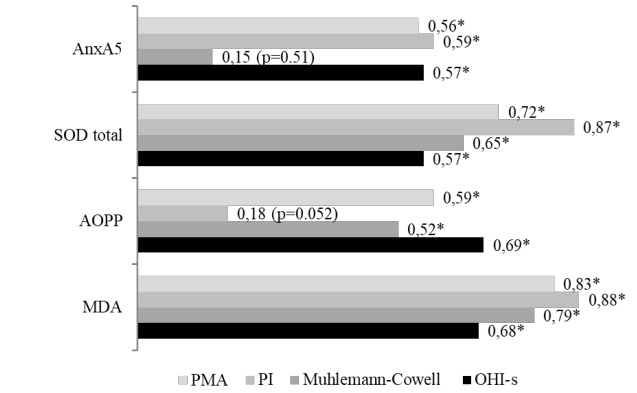


Figure 1 - Pair correlation of indicators of periodontal tissues with the levels of markers of oxidative stress and apoptosis, AnxA5, in CGP patients (experimental group). Note: *p<0.05.

The strength of relationships was greater among patients with comorbid pathology.

Figure 2 - Pair correlation of indicators of periodontal tissues with the levels of markers of oxidative stress and apoptosis, AnxA5, in bronchiectasis-associated CGP patients (main group). Note: *p<0.05.

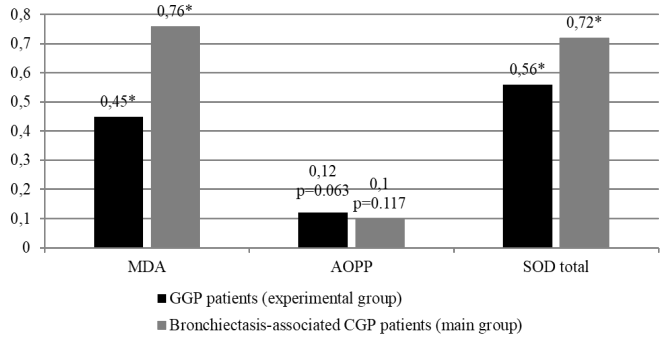
Figure 2



Positive relationships were also found between the level of AnxA5 and oxidative stress markers. The strength of relationships was greater among patients with comorbid pathology (Fig. 3).

Figure 3 - Pair correlation of AnxA5 and oxidative stress markers. Note: *p<0.05.

Figure 3



At the same time, the relationships between AOPP and AnxA5 in both groups of patients were weak and statistically insignificant. A greater relationship was observed between total SOD and AnxA5.

The results of our study indicate that CGP patients with a comorbid pathology in the form of bronchiectasis have a greater intensification of oxidative stress and an increase in the intensity of apoptosis in comparison with patients with single medical condition. The correlations between the levels of biomarkers of oxidative stress and apoptosis, AnxA5, with the dental indices of greater strength in patients with comorbid pathology indicate the relationship between the intensification of oxidative stress and the increase in the intensity of apoptosis with the deterioration of the periodontal tissues[6,8,13]. And the presence of bronchiectasis indicates exacerbation of periodontal tissue damage in CGP patients[16,17].

Conclusion

Based on the results of the current study, it can be concluded that among all the markers of oxidative stress, the strongest relationship was found between SOD and AnxA5; we also found that the patients whose total SOD was within the control values, had their AnxA5 also within the control values. That is, we can assume that the level of SOD in both bronchiectasis-associated and non-associated CGP patients is an apoptosis intensity protective factor.

Source of funding: no funding.

Conflict of interest: none.

Acknowledgments: none.

References

1. Orlova E.S., Bragin A.V. Treatment and prevention of periodontitis in patients with comorbid pathology. Academic Journal of Western Siberia. 2020; 16(4): 3-7.

2. V.A. Khaptanova, A.D. Golmenko, A.Iu. Khaptanov, D.Iu. Gamaiunov. The relationship between coronary heart disease and comorbid dental conditions // Acta Biomedica Scientifica (East Siberian Biomedical Journal). - 2021. - V. 6. - No. 4. - P. 87-99.

3. S.M. Karimov, A.S. Mirzov, A.A. Ismoilov. The results of an epidemiological analysis of the prevalence and intensity of periodontal diseases in individuals with concomitant somatic pathology // Bulletin of postgraduate education in health care. – 2020. – No. 1. - P. 39-42.

4. Tibúrcio-Machado CS, Michelon C, Zanatta FB, Gomes MS, Marin JA, Bier CA. The global prevalence of apical periodontitis: a systematic review and meta-analysis. Int Endod J. 2021;54(5):712-735. doi: 10.1111/iej.13467.

5. Jepsen S, Suvan J, Deschner J. The association of periodontal diseases with metabolic syndrome and obesity. Periodontol 2000. 2020;83(1):125-153. doi: 10.1111/prd.12326

6. A.Z. Isamulaeva, T.F. Danilina, O.A. Bashkina, D.F. Sergienko. Changes in the state of periodontal tissues in children with bronchial asthma. Astrakhan medical journal. 2011; 6(1): 70-72.

7. E.A. Volkov, A.G. Maliavin, V.Iu. Chesnokov. Periodontitis and the severity of chronic obstructive pulmonary disease. Доктор.Ру. 2014; 2(90): 33-39.

8. Hobbins S., Chapple I. L., Sapey E., Stockley R. A. Is periodontitis a comorbidity of COPD or can associations be explained by shared risk factors/behaviors? Int J Chron Obstruct Pulmon Dis. 2017. 12. 1339–1349.

9. A.I. Sinkopalnikov. COPD and bronchiectasis. Effective pharmacotherapy. 2017; 30:16-22.

10. Dabiri D., Halubai S., Layher M., Klausner C., Makhoul H., Lin G. H. ,Eckert G., Abuhussein H., Kamarajan P., Kapila Y. The Role of Apoptotic Factors in Assessing Progression of Periodontal Disease. International Journal of Dentistry and Oral Science. 2016; 3(9): 318-325. DOI: 10.19070/2377-8075-1600064

11. Chen M, Cai W, Zhao S, Shi L, Chen Y, Li X, Sun X, Mao Y, He B, Hou Y, Zhou Y, Zhou Q, Ma J, Huang S. Oxidative stress-related biomarkers in saliva and gingival crevicular fluid associated with chronic periodontitis: A systematic review and meta-analysis. J Clin Periodontol. 2019;46(6):608-622. doi: 10.1111/jcpe.13112.

12. Sczepanik FSC, Grossi ML, Casati M, Goldberg M, Glogauer M, Fine N, Tenenbaum HC. Periodontitis is an inflammatory disease of oxidative stress: We should treat it that way. Periodontol 2000. 2020;84(1):45-68. doi: 10.1111/prd.12342.

13. T.V. Kuzina, A.V. Kuzin. The use of an oxidative stress marker (MDA) and a cytogenetic marker in the system of ecological and genetic monitoring of the Northern Caspian // South of Russia: ecology, development. – 2020. – No. 1 (54). - P. 99-106.

14. Hao M, Liu R. Molecular mechanism of CAT and SOD activity change under MPA-CdTe quantum dots induced oxidative stress in the mouse primary hepatocytes. Spectrochim Acta A Mol Biomol Spectrosc. 2019 5;220:117104. doi: 10.1016/j.saa.2019.05.009

15. Gyurászová M, Kovalčíková A, Janšáková K, Šebeková K, Celec P, Tóthová Ľ. Markers of oxidative stress and antioxidant status in the plasma, urine and saliva of healthy mice. Physiol Res. 2018 18;67(6):921-934. doi: 10.33549/physiolres.933866

16. hen X, Liu W, Xiao J, Zhang Y, Chen Y, Luo C, Huang Q, Peng F, Gong W, Li S, He X, Zhuang Y, Wu N, Liu Y, Wang Y, Long H. FOXO3a accumulation and activation accelerate oxidative stress-induced podocyte injury. FASEB J. 2020;34(10):13300-13316. doi: 10.1096/fj.202000783R.

17. Sayre LM, Lin D, Yuan Q, Zhu X, Tang X. Protein adducts generated from products of lipid oxidation: focus on HNE and one. Drug Metab Rev. 2006;38(4):651-75. doi: 10.1080/03602530600959508

18. Rand J.H., Wu X.X., Lin E.Y., Griffel A., Gialanella P., Mckitrick J.C. Annexin A5 Binds to Lipopolysaccharide and Reduces Its Endotoxin Activity. MBio. 2012; 3(2): pii: e00292-11. DOI: 10.1128/mBio.00292-11

19. Hong M., Park N., Chun Y.J. Role of Annexin A5 on Mitochondria-Dependent Apoptosis Induced by Tetramethoxystilbene in Human Breast Cancer Cells. BiomolTher (Seoul). 2014; 22(6): 519-524. DOI: 10.4062/biomolther.2014.112

20. Iu.L. Osipova, S.A. Akimova, N.V. Bulkina, A.I. Nikolaev, E.N. Skvortsova. Interrelation of indicators of cell proliferation, apoptosis of periodontal tissues and cytokine imbalance of gingival fluid in patients with rapidly progressive periodontitis // Ural medical journal. – 2020. – No. 12. - P. 66-69.

21. Diaz CI, Zambrano AD, Naranjo AL, Shiguango NN, Carrasco AP, Córdova HS, Proaño CA, Diaz LC. Diabetes mellitus tipo 2 y su asociación con factores de riesgo cardiovascular en pacientes hipertensos. Diabetes Internacional. 2018;10(1):8-13.

22. Diaz CI, Calero AE, Jara AC, Fonseca MA, Quiroga SJ, Córdova VM, Sigüenza RM. Osteoartritis en pacientes con síndrome metabólico: enfoque preventivo. Sindrome Cardiometabólico. 2019;9(1):37-40.

23. Gualaquiza González R, Pérez Granja A, Tapia Caisaguano A, Legña Tibanta D, Bastidas Jiménez E, Gaibor Ortiz A, Bastidas Haro T, Allauca Yumiseba M, Bravo Bohórquez G, Miranda Buenaño F, Castañeda Morales D. Incidencia y características clínicas de lactantes menores con neumonía adquirida en la comunidadingresados en el Hospital Pediátrico “Baca Ortiz”, Ecuador. Archivos venezolanos de farmacología y terapéutica. 2020;39(4):260-3.