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Inflammatory markers of blood serum as an early predictor of chronic diseases in the rural population of Sachkhere district in Georgia

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Abstract

To identify the molecular correlates of non-communicable chronic disease risk in the territory of Upper Imereti. Practically healthy residents of the Sachkhere district were examined. In the blood serum samples of patients, the cytokines (IL-1α, IL-1β, IL-12, TNF-α, IL-6) and NOx content, as well as the total antioxidant activity of the non-enzymatic system (TAA) were determined. Study results show, that in Sareki inhabitant's blood serum levels of the IL-6, and NO increased ($p=0.004$, $p=0.05$), levels of the IL-1α tended to increase ($p=0.057$) compared to the corresponding values of the Chorvila inhabitants; in Chorvila inhabitants' indicators of blood serum TAA were lower than in Sairkhe and Sareki ($p=0.001$, $p=0.045$). The statistically significant differences in the levels of the IL-1β, IL12, and TNF-α in the blood serum of the inhabitants of Sachkhere district villages were not revealed. The alterations of the indicators of immune and oxidative status in the practically healthy populations of the Sachkhere district villages toward pro-inflammatory, and early revealed associations between population morbidity and TAA values allow us to suggest considering immune (IL6, IL-1α) and redox status deviations as markers of predisposition to chronic diseases. It should also be noted that, since each indicator belongs to the class of pleiotropic markers, only their complex can be considered as an early predictor of risk.

KEYWORDS: chronic diseases; cytokines; antioxidant activity

Introduction

In developed countries, chronic disease are the leading cause of death and disability, account in goral proximately 63% of the total socio-economic burdening each country [1]. Metabolic syndrome is common in 20-25% of the world's population; cardiovascular diseases are one of the leading causes of death (in 2015, 31% of death were due to cardiovascular diseases [2]); every year, 6% of the world's population dies from chronic respiratory diseases. Risk factors for chronic diseases include genetic, also environmental factors [3]. Oxidative stress, induced environmental pollutions is not limited to the respiratory system but is sometimes a key link in the development of endothelial damage and pathology of the cardiovascular system [4, 5, 6, 7]. Numerous studies show that environmental pollution contributes development of insulin resistance and hyperglycemia, arterial hypertension, obesity, and metabolic syndrome [8, 9, 10].

The scale of the problem

led to the need for the development of qualitatively new approaches to the assessment and management of environmental hazards - theprincipleofriskmanagementinsteadofthecurrentprincipalofthecontrolofhygienicstandards [11].

We present the results of the complex investigation of chronic diseases key cytokines, and redox balance in the blood serum of ethnically homogeneous populations of the Sachkhere region (Georgia).

The objective of the research was to identify the molecular correlates of non-communicable chronic disease risk in the territory of Upper Imereti.

Materials and Methods

Practically healthy residents of the Sachkhere district (both sexes, 50-65 years old) living in the villages of Sareki, Sairkhe, and Chorvila were examined (a total of 400 people) (Group I - residents of Sareki, 136 people (32 men, 104 women); Group II - residents of Sairkhe. 132 people (44 men, 88 women); Group III - residents of Chorvila, 132 people (20 men, 112 women).

The practically healthy persons were included in the study. Exclusion criteria were: malignant tumors, nicotine users, excessive alcohol users, severe chronic diseases (severe forms of diabetes, stage 2-3 of chronic heart failure, chronic bronchitis, etc.).

All examined persons gave written informed consent for their participation in the study; they completed a questionnaire concerning general and lifestyle characteristics (e.g. age, gender, height, weight, smoking, and drinking), as well as personal and family medical history, and provided blood samples during their health checkup.

Our study plan was approved by the Ethics Committee of Tbilisi State Medical University of Georgia.

Multi-analysis of cytokines

Collected blood samples were stored at -80°C and just before analysis thawed at 4°C in a refrigerator.

The cytokines IL-1α, IL-1β (Vector best, Russia), Il-12 (Cusabio, China), tumor necrosis factor-α (TNF-α) (Immundiagnostik, German) by the immune enzymatic ELISA method on a semi-automatic reader Stat Fax 3200. IL-6 was determined by the chemiluminescent method (Roche Diagnostics).

Measurement of Total NOx Level

The level of NOx in the blood serum samples was determined by a modified method by Miranda et al. [12] with the use of Griess Reagent. The absorbance was measured at 540 nm with a microplate reader (Multiscan GO, Thermo Fischer Scientific, Finland). The standard curve for NaNO2 was used to calculate the total NO concentration in the samples [12].

Determination of the total antioxidant activity (TAA) of blood serum

TAA was determined in deproteinized blood plasma by using the 2.2-diphenyl-1-picryl-hydrazine (DPPH)-scavenging assay, which was adapted from a study conducted by Chrzczanowicz et al. [13]. A calibration curve was built with the use of gallic acid, wherein the absorbance values (at 515 nm) were interpolated and the results were expressed as equivalents of gallic acid (%).

Statistical analysis

A student t-criterion was used to assess the statistical significance of the difference in one incidence between villages. The statistical significance of the difference between studied parameters in the population of the villages was assessed by the analysis of variance (ANOVA). The SPSS and Open BUGS software packages analyzed the data and visualized the results.

Results

Cytokines level in blood serum

The study results of cytokine levels in blood serum of practically healthy residents of the Sachkhere district living in Sareki, Sairkhe, and Chorvila are shown in Table 1 and Figure 1.

As it seems from Table 1 and Figure 1, the level of IL-6 in the blood serum of inhabitants of village Sareki is statistically significantly higher than from Chorvila ($p=0.004$) and Sairkhe ($p=0.006$) and exceeded the control values by 30% (Fiure.1A). The level of the IL-1α in the blood serum of the inhabitants of the Sareki village tended to increase compared to the corresponding values in the serum of the inhabitants from Chorvila ($p=0.057$) (Figure. 1B). The statistically significant differences in the levels of the IL-1β, IL12, and TNF-α in the blood serum of the inhabitants of Sachkhere district villages were not revealed (Figure 1). No sex-dependent differences were found in the

Table 1.
The statistical significance of the difference between the mean values of interleukins, TAA, and NO (Fisher's criterion) in village populations.

Indicator	Chorvila/Sairxe		Sairxe/Sareki		Chorvila/Sareki	
	F	*P*	*F*	*P*	*F*	*P*
IL-1α	1.96	0.174	0.17	0.680	4.89	0.057
IL-1β	0.06	0.806	0.17	0.678	0.04	0.843
TNF	0.57	0.459	0.16	0.685	0.36	0.552
IL-12	1.06	0.314	0.61	0.436	0.56	0.446
IL-6	1.65	0.211	8.59	0.006*	9.61	0.004*
TAA	32.63	0.001*	1.72	0.2	2.67	0.101
NO	1,75	0.192	0.41	0.525	3.82	0.050*

Total NOx Level in blood serum

The study results of NOx concentration in the Sachkhere district villages inhabitant's blood serum samples show that the NOx level in the blood serum of the inhabitants from the Sareki tended to increase compared to the corresponding values in the serum of the inhabitants from Chorvila (p=0.050) (Figure. 2). No sex-dependent differences were found in the values of the NOx levels (data are not shown).

The level of TAA in blood serum

The study results of the TAA of the blood serum of inhabitants from Sachkhere district villages show that the TAA level in the blood serum of the inhabitants from Sairkhe is higher compared to the corresponding values in the blood serum of the inhabitants from Chorvila (p=0.001) (Figure 3). No sex-dependent differences were found in the values of the TAA levels (data are not shown).

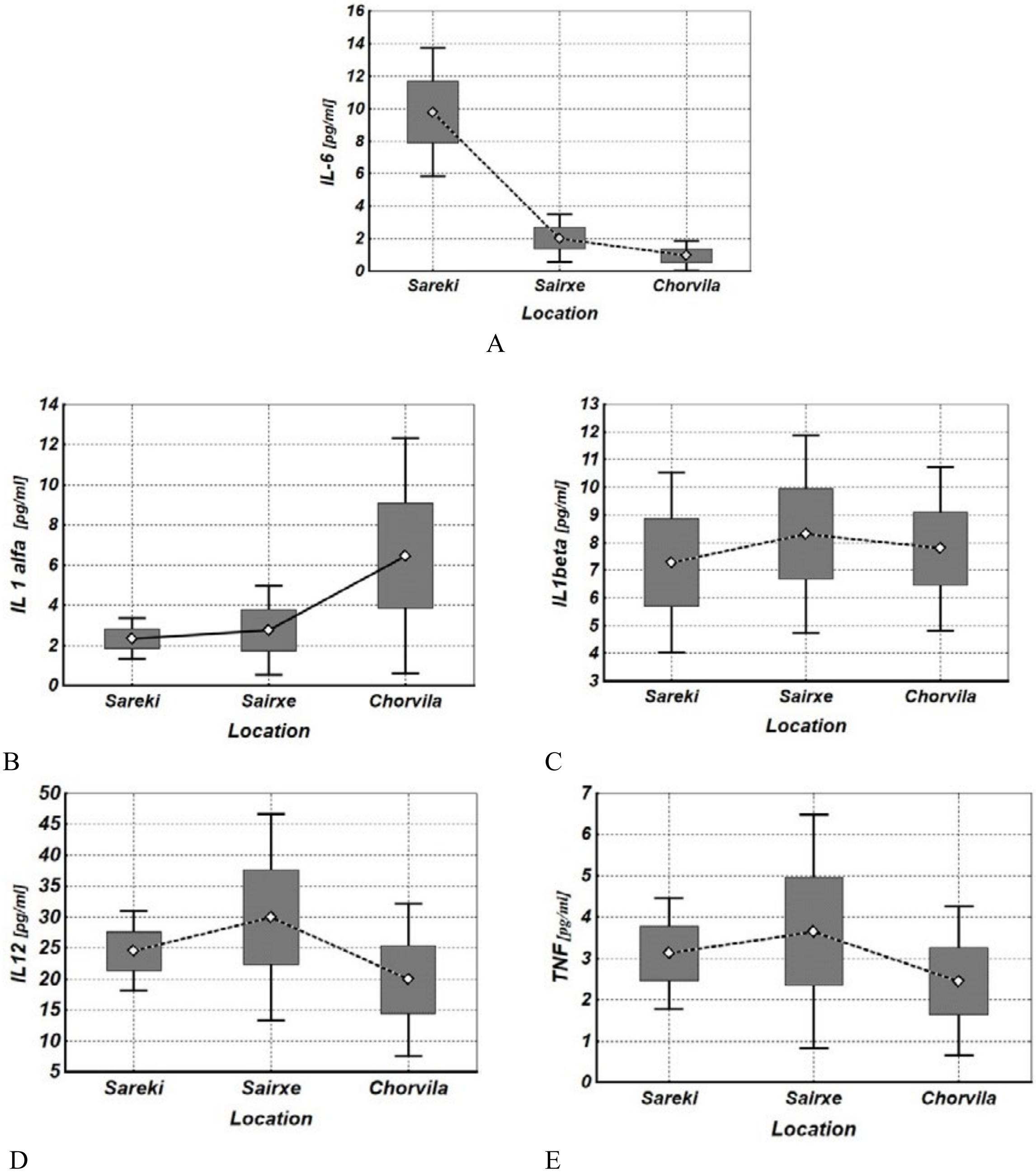

Figure 1. The mean value, standard error, and 95% confidential interval of cytokines level in blood serum of practically healthy residents from the Sachkhere district villages Sareki, Sairkhe, and Chorvila (Georgia). ◇ *- Mean,* ▩ *- Mean ± SE,* I *- Mean±95% Conf. Interval.*

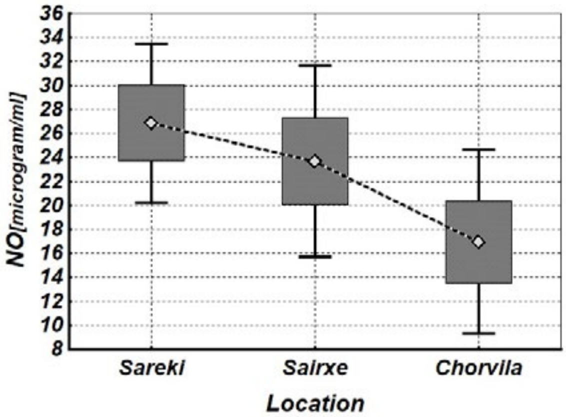

Figure. 2. The mean value, standard error, and 95% confidential interval of total NOx level in blood serum of practically healthy residents from the Sachkhere district villages Sareki, Sairkhe, and Chorvila (Georgia). ◇ *- Mean,* *- Mean ± SE,* I *- Mean±95% Conf. Interval.*

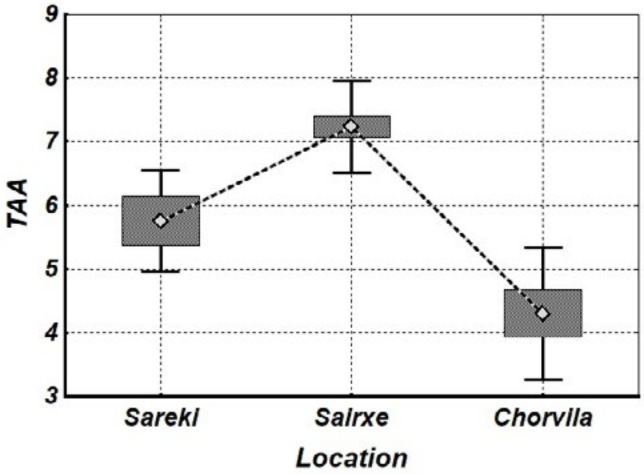

Figure 3. The mean value, standard error, and 95% confidential interval of TAA of blood serum of practically healthy residents from the Sachkhere district villages Sareki, Sairkhe, and Chorvila (Georgia). ◇ *- Mean,* *- Mean ± SE,* I *- Mean±95% Conf. Interval.*

Discussion

We present the results of the complex investigation of chronic diseases key cytokines, and redox balance in the blood serum of ethnically homogeneous populations of the Sachkhere region (Georgia). The objective of the research was to identify the molecular correlates of non-communicable chronic disease risk in the territory of Upper Imereti.

Presented study results revealed differences in the levels of IL-6, in the blood serum of the population of the Sachkhere district villages Chorvila, Sareki, and Sachkhere. In particular, it was shown, that the level of IL-6 in the blood serum of inhabitants of the village Sareki was statistically significantly higher than in Sairkhe and Chorvila and exceeded the level of control values by 30% (Fig.1A). The level of the IL-1α in the blood serum of the inhabitants of the Sareki village tended to increase compared to the corresponding values in the serum of the inhabitants from Chorvila ($p=0.057$) (Fig. 1B). There were no revealed statistically significant differences in the levels of IL-1β, Il-12, and TNF-α in the blood serum of the inhabitants of Sachkhere district villages.

IL-6 is a pleiotropic cytokine with pro- and anti-inflammatory properties. IL-6 is critical in controlling local and systemic acute inflammatory responses, particularly the level of proinflammatory cytokines, and the production of acute-phase proteins, in response to a varied inflammatory stimulus. IL-6 has a dual effect - at some levels, it reveals a protective but in chronic inflammation - rather a proinflammatory effect. When the activity of IL-6 as a proinflammatory cytokine persists, acute inflammation turns into chronic inflammation that includes immune responses [14]. IL-6 production is predominantly regulated by changes in the gene expression during inflammation by various transcription factors (e.g., transcription factor NF-kB).

The IL-1 family member, IL-1α is a pivotal proinflammatory cytokine. It is constitutively present in nearly all cell types as a bioactive mediator, and is released upon cell death or expressed by infiltrating myeloid cells within injured tissues. IL-1α binds the IL-1R1 receptor, shared with IL-1β; these cytokines induce identical proinflammatory effects: IL-1α triggers local inflammation, which rapidly escalates the secretion of IL-1β by nearby myeloid cells. IL-1α is central to the pathogenesis of numerous conditions characterized by organ or tissue inflammation: disorders of the skin, lung and upper respiratory tract, heart and pericardium, and blood vessels [15].

The presented study results testify to a shift of the cytokine balance in blood serum of Sareki village residents toward pro-inflammatory.

Inflammatory responses trigger hyperproduction of the reactive oxygen species (ROS) and intensification of oxidative stress. The oxidative stress conditions increase nitric oxide (NO) production that later can then be converted into reactive nitrogen species (RNS). Against the increased formation of ROS and RNS, there are numerous protective mechanisms in the body, including the enzymatic (SOD, Cat, peroxidases) and the non-enzymatic antioxidant system (represented by low molecular weight compounds (thiols, vitamins E, C, and A, uric acid, urate, bilirubin, estrogens, biogenic amines (dopamine, histamine, serotonin, melatonin and amino acid, tryptophan), etc.), that ensure the neutralization of free radicals [16].

During chronic inflammation, prolonged expression of excess amount of ROS and RNS induces inactivation of antioxidant enzymes, inhibits biosynthesis of macromolecules, which results in a decrease of the pool of the antioxidant enzymes, down-regulates their activity, inhibits the compensatory release of low molecular weight nonenzymatic antioxidants. Depletion of the enzymatic and non-enzymatic antioxidant protective mechanisms is described in a variety of chronic diseases. However, small ROS-scavenging molecules through a tightly coordinated system of metabolic processes are more labile (compared to the enzymatic system), respond to changes in redox homeostasis, ensure the protection of cellular structures, and macromolecules, and support total body resistance to oxidative stress. Non-enzymatic antioxidants represent a key mechanism of antiradical defense in living systems [17].

Our research results revealed alterations in the TAA and an increase of the NO content in the blood serum of the inhabitants of villages Sairkhe and Sareki compared to their levels in the blood serum of the Chorvila's inhabitants, which may be related to the intensification of ROS production, accompanied by the compensatory response of TAA and redox-induced, activation of iNOS in the inhabitants' blood serum. Under conditions of moderate oxidative stress, the compensatory activation of the non-enzymatic antioxidant system (an increase of TAA) provides regulation of redox homeostasis in the population of Sairkhe village; however, as the intensity of oxidative stress increases, NO production continuously increases, while the overload on the endogenous non-enzymatic antioxidant system causes its depletion, which is manifested in the decrease in TAA (non-compressed oxidative stress in Sareki).

In our early studies, high variability and unequal distribution in the total activity of the non-enzymatic antioxidant system of blood serum (TAA) have been revealed in the practically healthy populations of the Sachkhere and Chiatura districts villages [18, 19]. In the population of the Chiatura district villages, the correlation between the imbalance in the pro- and antioxidant systems of the body, and an increase in the level of chronic diseases (hypertension, cardiovascular diseases, chronic lung, and thyroid disease) were detected [19].

Conclusion

The alterations of the indicators of immune and oxidative status in the practically healthy populations of the Sachkhere district villages toward pro-inflammatory and early revealed associations between population morbidity and TAA values, allow us to suggest to use of immune parameters (IL6, IL-1α) and redox status deviations as markers of the predisposition of the population to chronic diseases. It should also be noted that, since each indicator belongs to the class of pleiotropic markers, only their complex can be considered as an early predictor of risk.

References

1. WHO Public Health & Environment. Global Strategy Overview. 2011. World Health Organization. http://www.who.int/en/.
2. http://www.who.int/mediacentre/factsheets/fs317/en/
3. [Adamo, K.B, Tesson F. 2008. Gene-environment interaction and the metabolic syndrome. Novartis Found Symp. 293:103-19.
4. Chuang, K.J., Chan, C.C., Su, T.C., Lee, C.T., Tang, C.S. 2007. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. American Journal of Respiratory and Critical Care Medicine. 176(4):370-376.
5. [Tonstad, S., Svendsen, M. 2005. Premature coronary heart disease, cigarette smoking, and the metabolic syndrome. The American Journal of Cardiology. 96(12):1681-1685.
6. Chen, C.C., Li T.C., Chang, P.C., et al. 2008. Association among cigarette smoking, metabolic syndrome, and its components: the metabolic syndrome study in Taiwan. Metabolism. 57(4):544–548.
7. Park, S.K., Auchincloss, A.H., O'Neill, M.S., et al. 2010. Particulate air pollution, metabolic syndrome, and heart rate variability: the multi-ethnic study of atherosclerosis (MESA) Environmental Health Perspectives. 118(10):1406-1411.
8. Cotrim, H.P., Carvalho, F., Siqueira, A.C., Lordelo, M., Rocha, R., de Freitas, L. 2005. Nonalcoholic fatty liver and insulin resistance among petrochemical workers. The Journal of the American Medical Association.294(13):1618-1620.
9. Sirit, Y., Acero, C., Bellorin, M., Portillo, R. 2008. Metabolic syndrome and other factors cardiovascular risk in workers of a plant of vinyl polychloride. Revista de SaludPublica. 10(2):239-249.
10. Mohan, V., Deepa, M., Farooq, S., Prabhakaran, D., Reddy, K.S. 2008. Surveillance for risk factors of cardiovascular disease among an industrial population in Southern India. National Medical Journal of India. 21(1):8-13.
11. Clean Air Act. US EPA, Commission of the European Communities. 1993. Council Regulation 793/93/EEC of 23 March 1993 on the evaluation and control of the risks of

existing substances. Off J Eur Communities, L84/1. http://www.epa.gov/lawsregs/laws/caa.html.

12. Miranda, K., Espey, M., Wink, D. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide: Biology and Chemistry. 5(1):62-71.
13. Chrzczanowicz, J., Gawron, A., Zwolinska, A., et al. 2008. Simple method for determining human serum 2,2-diphenyl-1-picryl-hydrazine (DPPH) radical scavenging activity - possible application in clinical studies on dietary antioxidants. Clinical Chemistry and Laboratory Medicine. 46: 342-349.
14. Gabay, C. 2006. Interleukin-6 and chronic inflammation. Arthritis Res Ther. 8 Suppl 2:S3. doi:10.1186/ar1917
15. Cavalli, G., Colafrancesco, S., Emmi, G., Imazio, M., Lopalco, G., Maggio, MC., Sota, J., Dinarello, C.A. 2021. Interleukin 1α: a comprehensive review on the role of IL-1α in the pathogenesis and treatment of autoimmune and inflammatory diseases. Autoimmunity Reviews. 20(3):102763.
16. Karki, K., Pande, D., Negi, R., et al. 2016. An Assessment of Oxidative Damage and Non-Enzymatic Antioxidants Status Alteration in Relation to Disease Progression in Breast Diseases. Med. Sci. 4(17):1-11.
17. [Robinson, C., Webb, K., Kaur A., et al. 2011. Major Role for Non-Enzymatic Antioxidant 1 Processes in the Radioresistance of Halobacterium salinarum. J Bacteriol. 93:1653-62.
18. [Sharashenidze, G., Tsimakuridze, M., Chkhikvishvili, I., et al. 2021. Bayesian modeling and inference of mixtures of distribution of blood total antiradical activity. Georgian Med News. (315):125-128.
19. Kvaratskhelia, G., Tikaradze, E., Buleishvili, M., et al. 2018. The structure and risk of chronic morbidity in some villages of the upper Imereti region of West Georgia and their molecular and cytogenetic markers. Georgian Med News. (283):97-103.Myrvold HE. Kirurgi ved refluks [Reflux surgery]. Tidsskr Nor Laegeforen. 2005 Aug 11;125(15):1988. Norwegian. PMID: 16100533