RESEARCH ARTICLE

Complex study of Cancer Morbidity and Inflammatory Markers, Presented in the Blood Serum of the Rural Population of Sachkhere District of Georgia

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Abstract

   Aim: The purpose of the study was to compare the morbidity from cancer (expressed as incidence) to the average levels of blood serum inflammatory markers in the population of the Sachkhere region (Georgia).**Methods:** healthy residents of the Sachkhere district were examined. In the blood serum samples of patients, the cytokines (IL-1α, IL-10, TGF-β, IL-12, IL-17, TNF-α, IL-6) and NOx content, as well as the total antioxidant activity of the non-enzymatic system (TAA) were determined; using light microscopy, buccal micronuclei (MnB) of epithelial cells of the oral mucosa, as indicators of chromosomal disorders, were studied. **Results:** Study results show, that cancer incidence in Sareki was statistically significantly higher as in Chorvila and Sairkhe (p=0.002; p=0.004); in Sareki inhabitant's blood serum levels of the IL-6, NO are increased (p=0.004, p=0.05), and IL-17, TGFβ, and IL-10 levels are decreased (p=0.010, p=0.001, p=0.033) in comparison to data in Chorvila; in Chorvila inhabitants' indicators of TAA of blood serum and MnB of epithelium cell levels were lower (p=0.001,p=0.045) then in Sairkhe and Sareki. **Conclusion:** The existence of statistically reliable associations between the levels of cancer incidence in the populations of the surveyed villages and the indicators of immune and oxidative status in their virtually healthy subpopulations, with a high degree of persuasiveness, allows us to assume a close causal link between them. Clarifying the reasons for the identified patterns and their significance requires more detailed studies.

   Keywords: Cancer incidence- cytokines- antioxidant activity- chromosomal disorders

Introduction

   The study of the spatial and temporal variability of the incidence of the population and its potential biomarkers is of great practical and theoretical importance. This approach is one of the main methodological approaches of environmental biomedicine (Martinez et al., 2014), which is currently being intensively developed and used to treat chronic and oncological diseases at different levels of the territorial and administrative division of the country, as well as to clarify the etiopathogenesis of these disease classes and identifying early predictors of disease risk (Hossain and Lawson 2010; Linkov et al., 2015; Kyrochristos et al., 2018).

   In the research cycle, we studied the patterns of spatial variability of the disease risk in ethnically homogeneous populations living in different ecological stress zones of the Upper Imereti region of Georgia and its correlations

with spatial distributions of potential biomarkers of disease risk in a healthy subpopulation (Kvarackhelia, et al., 2017; Sharashenidze et al., 2021; Tikaradze et al., 2021).

   The aim of the research cycle, the stage of which is the present paper, is to develop an optimal approach for Georgia for the "Health Risk Assessment in Small geographical Areas". Our approach is based on so-called "outcome-based" assessments of health risks (Martinez et al., 2014; Lioy, Smith, 2013; A Partial Review of the "London Principles", With Recommendations for Revisions and Additions, by an Expert Panel, 1999; World Health Organization, 2000; World Health Organization, Regional Office for Europe, 2000).

   Reliable recording of an increase in health risk in small geographical areas is known to be difficult due to the scarcity of research cohorts. One of the promising directions for solving the problem is a comprehensive

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study of morbidity and morbidity biomarkers.

In our early studies, high spatial heterogeneity of common oncological incidences in the Sachkhere district was revealed; for certain localizations of cancer, statistically significant high morbidity zones compared to background levels were identified. We also observed high variability in the total activity of the non-enzymatic blood serum antioxidant system (TAA) in healthy residents of rural areas of the Sachkhere district (Sharashenidze et al., 2021), accompanied by a high frequency of epithelial micro-nuclear buccal cells (Tikaradze et al., 2021).

The present study investigated the correlations of morbidity rate with distributions of blood serum inflammatory markers levels in the population.

The different extrinsic and intrinsic stimuli (e.g. ionizing radiation, UV, reactive oxygen species, viruses, microbial products, etc.) are associated with tissue damage. Cellular stress can induce the secretion of numerous pro-inflammatory cytokines from multiple cell types, including macrophages, T cells, and epithelial cells. If inflammation is unregulated, it can become chronic, inducing cells' malignant transformation in the surrounding tissue (Chen et al., 2017; Bennett et al., 2018). The inflammatory response provides the release of bioactive molecules including cytokines, growth factors, chemokines, extracellular matrix-modifying enzymes (metalloproteinases) affecting various molecular targets and signaling pathways that regulate such related to carcinogenesis processes, such as energy metabolism, immune response, genome instability, apoptosis, proliferation rate, and angiogenesis and can increase the risk of cancer (Kany et al., 2019).

Inflammation has a very strong link with various types of cancer, which is manifested by the expression of specific cytokines (Lan et al., 2021). It is known that cytokines synthesized by immune and stromal cells, such as fibroblasts and endothelial cells, regulate proliferation, cell survival, differentiation, immune cell activation, cell migration, cell-to-cell communication, and death. Depending on the microenvironment, cytokines can modulate an antitumoral response of the body against malignant cells; during chronic inflammation, their prolonged uncontrol synthesis can induce transformation and malignancy of normal cells, which is conditional on the balance of pro- and anti-inflammatory cytokines, their relative concentrations, cytokine receptor expression level, and the activation level of surrounding cells (Wang et al., 2019). It is thought that tumorigenesis and metastasis production is a process regulated by tumor cells and host cells through inflammation. Different cytokines released by innate and adaptive immune cells play different roles in the onset and resolution of inflammation, several pro-inflammatory cytokines regulate cancer cell growth and thereby contribute to tumor promotion and progression. In the present study, we investigated whether the level of onco-morbidity in the population affects the spectrum of pro- and anti-inflammatory cytokines in healthy residents. We present the results of the complex investigation of key cytokines in the blood serum of ethnically homogeneous populations of the Sachkhere region (Georgia).

The purpose of the study was to compare the morbidity from cancer (expressed as incidence) to the average levels of blood serum inflammatory markers in the population of the Sachkhere region (Georgia).

Materials and Methods

Subjects

Healthy residents of the Sachkhere district (both sexes, 50-65 years old) living in the villages of Sareki, Sairkhe, and Chorvila (total population of the districts: Sareki – 2076. Sairkhe - 2000, Chorvila - 1451) 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 healthy persons randomly were included in the study. Exclusion criteria were: malignant tumors, nicotine users, excessive alcohol users, and 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-10, transforming growth factor-β (TGF-β) (RayBio, USA), IL-1β (Vector best, Russia),Il-12 (Cusabio, China), IL-17 (Vector best, Russia), 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. (Miranda, et al., 2001) 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 (Miranda et al., 2001).

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-hidrazyne (DPPH)-scavenging assay, which was adapted from a study conducted by Chrzczanowicz et al. (Chrzczanowicz et al., 2008). 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 (%).

Registration of the chromosomal disorders

In population studies to register chromosomal disorders, a non-invasive rather informative method of determination of the level of micronuclei buccal (MnB) epithelium cells in scrapings of the oral mucosa (a scraping is taken from the inside of the cheek with a wooden spatula and placed on a glass slide) is used (Gagoshidze et al., 2005) with a modified method of H. F. Stich et al. (Stich et al., 1982)). The preparations were stained with azure-eosin and, after washing, stained with Fast green. The MnB cells were counted using a light microscope.

Epidemiological and Statistical analysis

Crude and age-standardized incidence per 100,000 population per year was used as a criterion for oncological morbidity level in a specific geographical area. The data of the oncology base of Sachkhere District Hospital-Polyclinic Association for 2011-2020 were analyzed. The 2014 General Population Census data were used for demographic data (https://www.geostat.ge/en/modules/categories/41/population). A standard error of mean age-standardized on-incidence in rural areas was calculated according to N. Keyfitz's method (Keyfitz et al., 1966).

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

According to the oncology database of Sachkhere District Hospital-Polyclinic Association for (2011-2020) in the population of the studied villages of Sachkhere district (Chorva, Sairkhe, Sareki) the following cancer incidence was observed: Chorvila – 129.1±30.40 per 100,000 population per year, Sairkhe – 130±24.57 per 100,000 population per year and Sareki – 235.4±38.96 per 100,000 population per year. Analysis of study results follows, that between the cancer incidence in Villages Chorvila – Sairxe significant difference in incidence was not revealed (p = 0.730), whereas the cancer incidence in Sareki was statistically significantly higher (by 82%) as in Chorvila and Sairkhe (p = 0.002; p = 0.004) (Table 1).

Cytokines level in blood serum

The study results of cytokine levels in blood serum of healthy residents of the Sachkhere district living in Sareki, Sairkhe, and Chorvila are shown in Table 2 and Figure 1.

As it seems from Figure 1, the level of IL-6 in the blood serum of inhabitants of village Sareki is statistically significantly higher as from Chorvila (p=0.004) and Sairkhe (p=0.006) and exceeded the normal level values by 30% (Figure 1A). In the blood serum of the inhabitants of village Sareki, the level of IL-17 was statistically significantly lower than from Chorvila (p=0.010) (but remained within the normal level (the common normal level of interleukins content in the human blood serum is

indicated in the test instructions)) (Figure 1B). The TGFβ level in the blood serum of inhabitants from Sareki was statistically significantly lower than in the blood serum of inhabitants from Chorvilia (p<0.001), and Sairkhe (p=0.002). It should be noted that in the blood serum of inhabitants from Sareki the TGFβ in the blood serum indicators were homogeneous and their mean value fluctuates within the lower limit of the normal range of TGFβ in the blood serum, while in the blood serum of inhabitants from Sairkhe and Chorvila the variability of this parameter increased significantly and its value fluctuated within the upper limit of the normal range (Figure 1C). The content of IL-10 in the blood serum of the inhabitants from the village of Sareki tended to decrease compared to the corresponding values in the serum of the inhabitants from Sairkhe (p=0.033) and Chorvila (p=0.037) (but remained within the normal range) (Figure 1D).

The statistically significant differences in the levels of the IL-1a, IL-1β, IL12, TNF-α in the blood serum of the inhabitants from Sachkhere district villages were not revealed (Fig. 1); no sex-dependent differences were found in the values of the studied interleukins levels (data are not shown).

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 village 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 of 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 village 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).

The level of the chromosomal disorders

The level of the MnB epithelial cells in the inhabitants from the village Sareki was statistically significantly higher compared to the corresponding value in the inhabitants of Chorvila (p=0.045) (Figure 4; no sex-dependent differences were found in the values of the MnB epithelium cells levels (data are not shown).

Discussion

The goal of the research was to identify a possible association between the character of cancer morbidity and early alterations in immune and redox indices of blood serum in the ethno-homogeneous population.

Analysis of the oncology database of Sachkhere District Hospital-Polyclinic Association (2011-2020) shows that there was no revealed significant difference

Table 1. Computation of Crude and Age-Standardized Incidence Rates of Cancer Incidence in Villages (2011–2020) (SDBMI, significance of difference between the means of incidence)

Location	Age-group	N	Person year risk	Age-specific Crude rate (per 10 ^{5})	Standard Georgian Population	Expected Rate in the standard population
Chorvila	0-14	0	3467.8	0	0.205	0
	15-64	14	8923.7	156.89	0.64	100.4
	65+	4	2118.5	188.81	0.152	28.7
	All	18	14510	124.05±20.02	0.997	129.1±30.40
	SDBMI in villages Chorvila – Sairxe					P = 0.730
Sairxe	0-14	0	3728.8	0	0.205	0
	15-64	13	1262.2	103	0.64	65.92
	65+	15	3589	419	0.152	63.68
	All	28	19940	140.42±23.70	0.997	130±24.57
	SDBMI in villages Sairxe-Sareki					P = 0.004
Sareki	0-14	0	2428	0	0.205	0
	15-64	21	8875	236.6	0.64	151.4
	65+	16	2896.8	552.3	0.152	84
	All	37	14200	260.56±50.61	0.997	235.4±38.96
	SDBMI in villages Chorvila – Sareki					P = 0.002

in mean onco-incidence in the population of the villages Chorvila and Sairxe, but the mean of the onco-incidence in Sareki was statistically significantly higher (by 82%) as in Chorvila and Sairkhe. The population of Sachkhere district villages differs both in the level of prevalence, also in the location of cancer in the body.

Presented study results revealed differences in the levels of IL-6, IL-17, IL-10, TGFβ 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% (Figure 1A). The levels of IL-17, TGFβ, and IL-10 in the blood serum of the inhabitants of the village Sareki were statistically significantly lower (but within the normal range) than from Sairkhe and Chorvila (Figure 1B, C, D).

IL-6 is a pleiotropic cytokine with pro- and anti-inflammatory properties, regulating the acute-phase response of inflammation. IL-6 production is predominantly regulated by changes in the gene expression during inflammation by various transcription factors (e.g., transcription factor NF-kB). Furthermore, IL-6, which is the major effector molecule of NF-kB, itself causes activation of NF-kB signaling (autocrine/paracrine) in cancer cells, which results in increased IL-6 production, enhanced IL-6 concentration in the tumor microenvironment, and constitutively activates NF-kB-s in the same or neighboring cells (Kumari et al., 2016). The expression of IL-6 can be also regulated epigenetically in breast cancer, hepatocellular carcinoma, colon cancer, prostate cancer, and lung cancer through miRNAs (Iliopoulos et al., 2009). Both autocrine and paracrine mechanisms of IL-6 signaling influence tumor progression and metastasis. IL-6 regulates nearly all hallmarks of

Table 2. The Statistical Significance of the Difference between the Mean Values of Interleukins, TAA, NO, and MnB Cells (Fisher's Criterion) in Villages 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-10	0.05	0.828	4.90	0.033*	4.68	0.033*
IL-12	1.06	0.314	0.61	0.436	0.56	0.446
IL-17	3.72	0.067	1.53	0.223	7.42	0.010*
TGF-β	2.25	0.150	11.72	0.002*	17.86	>0.001*
IL-6	1.65	0.211	8.59	0.006*	9.61	0.004*
REDOX	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*
MnB	3.57	0.083	0.57	0.456	4.42	0.045*

*, statistically significant
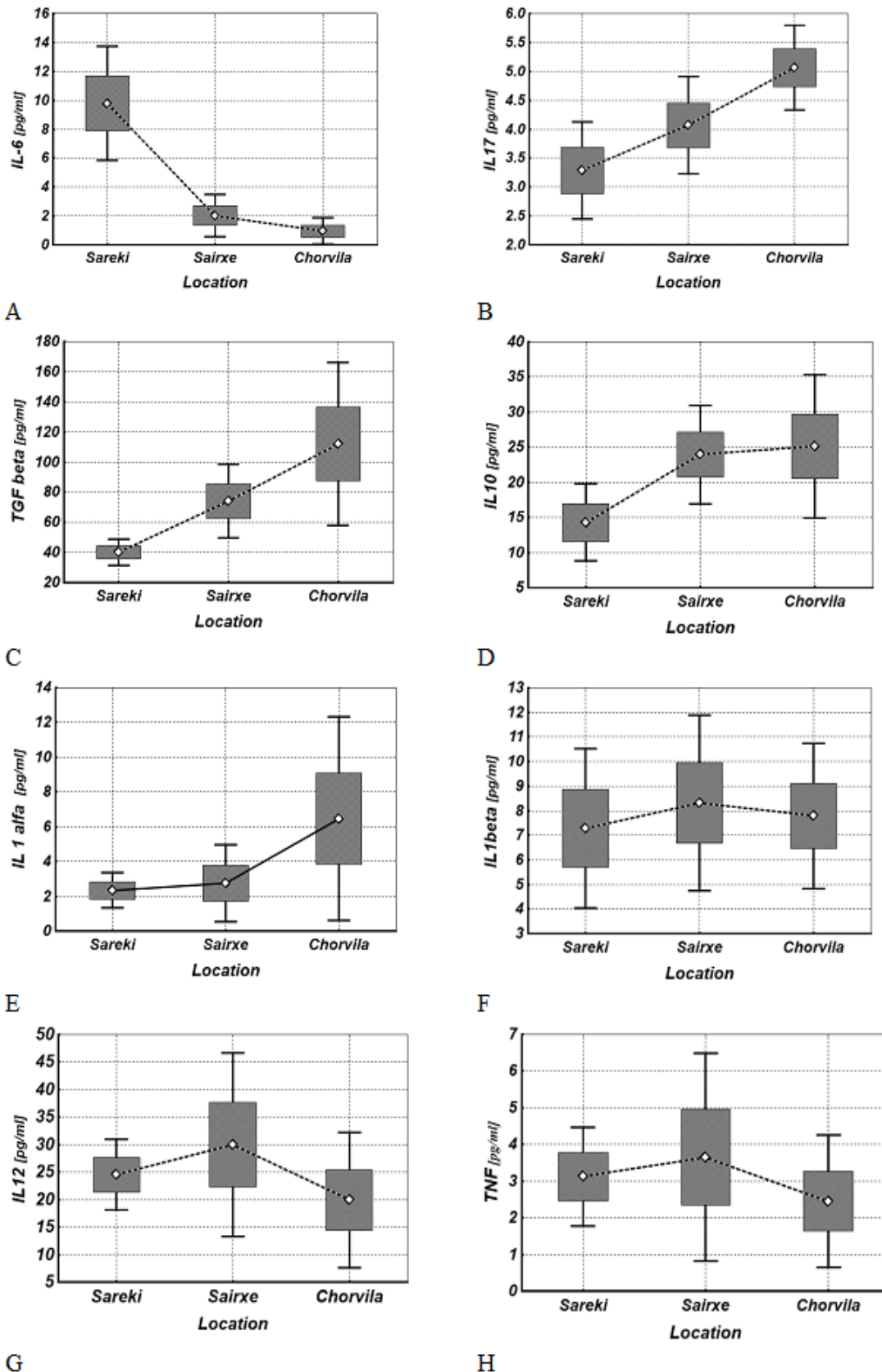
Figure 1. The Mean Value, Standard Error, and 95% Confidential Interval of Cytokines Level in Blood Serum of Healthy Residents from the Sachkhere District Villages Sareki, Sairkhe, and Chorvila (Georgia). ◊, Mean; □, Mean ± SE; **I**, Mean±95% Conf. Interval.
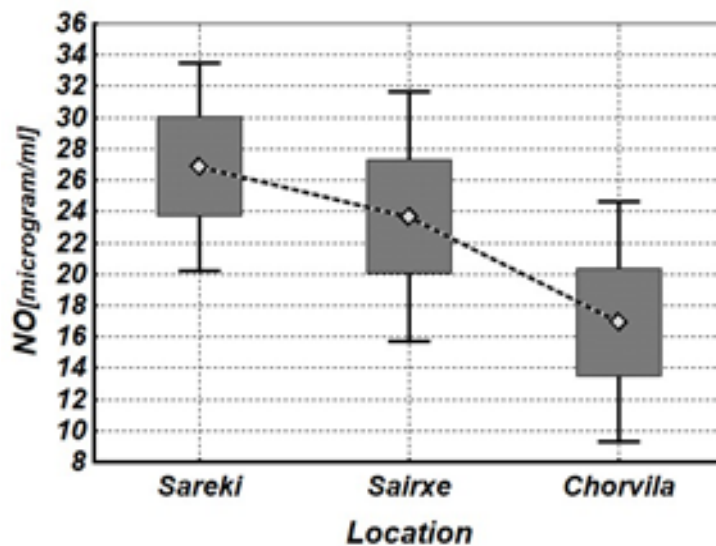
Figure 2. The Mean Value, Standard Error, and 95% Confidential Interval of Total NOx Level in Blood Serum of Healthy Residents from the Sachkhere District Villages Sareki, Sairkhe, and Chorvila (Georgia). ◊, Mean; □, Mean ± SE; **I**, Mean±95% Conf. Interval.

cancer, such as inhibition of apoptosis, promotion of survival, proliferation, angiogenesis, invasiveness, and metastasis, and is also known to regulate cancer cell metabolism (Kumari et al., 2016). IL-6 is important in the development of human cancer and activates oncogenic pathways, by hypermethylation of tumor suppressor genes (Gascheet al., 2011). Tumor cells produce IL-6 for promoting their survival and progression. Based on the foregoing, elevated levels (compared to normal levels) of IL-6 in the blood serum of village Sareki's residents may be associated with an increased risk of carcinogenesis.

IL-17 – pro-inflammatory cytokines of the CD4+ T helper 17 (Th17) cells. In addition to autoimmunity, dysregulated IL-17 is emerging as a major pathogenic factor involved in both the early and late stages of cancer development. Indeed, since its primary detection in human cancers including breast, gastric, and prostate cancer,

the role of IL-17 in oncology has been highly debated and controversial (Zhao al., 2020). Nevertheless, a few studies have also reported the anti-tumor activity of IL-17 (Fabre et al., 2016). Despite the growing evidence on the pathogenic role of IL-17 in cancer, the underlying molecular and cellular mechanisms are still not completely understood.

If inflammatory cytokines are capable of tumor induction, anti-inflammatory cytokines can limit the risk of cancer and reduce the activation of signaling pathways (Landskron et al., 2014a). TGF-β is a powerful pleiotropic cytokine, with immune-suppressing and anti-inflammatory properties. Common sources of TGF-β are cancer and stromal cells, including immune cells and fibroblasts, bone matrix. Under physiological conditions, TGF-β plays important role in embryogenesis, cell proliferation, differentiation, apoptosis, adhesion, and invasion.
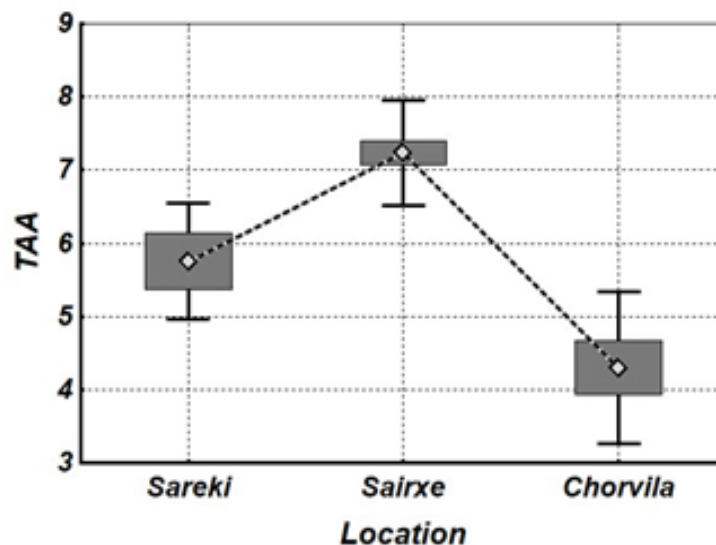

Figure 3. The Mean Value, Standard Error, and 95% Confidential Interval of TAA of Blood Serum of Healthy Residents from the Sachkhere District Villages Sareki, Sairkhe, and Chorvila (Georgia). ◊, Mean; □, Mean ± SE; **I**, Mean±95% Conf. Interval.
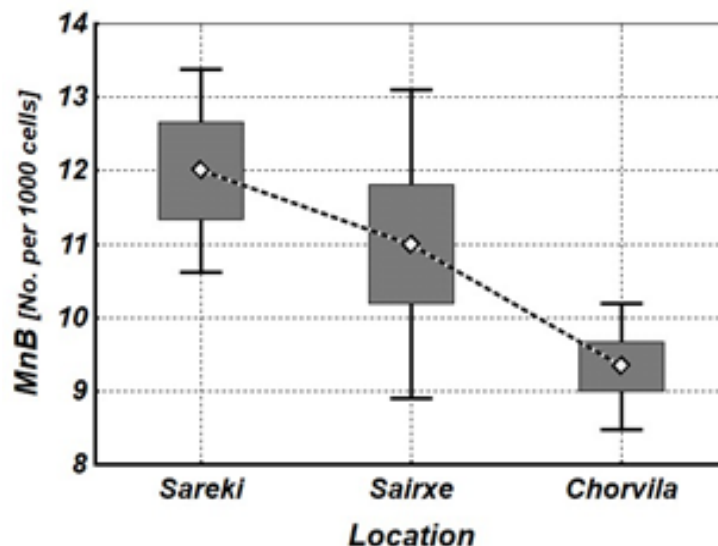
Figure 4. The Mean Value, Standard Error, and 95% Confidential Interval of Level of MnB Epithelial Cells of Healthy Residents of the Sachkhere District Villages Sareki, Sairkhe, and Chorvila of (Georgia). ◊, Mean; □, Mean ± SE; **I**, Mean±95% Conf. Interval.

Differential phosphorylation of its serine and threonine-rich linker region contributes to the manifestation of various cellular functions, including cytostatic effects, cell growth, invasion, extracellular matrix synthesis, cell cycle arrest, and migration (Massagué, 2008; Matsuzaki, 2013). In tumors, TGF-β can be either a proto-oncogene or a tumor suppressor, depending on the cell environment and tumor stage. As a potent inhibitor of cell proliferation, in normal cells, TGF-β acts as a tumor suppressor. TGF-β activation can be induced by different factors (retinoic acid and fibroblast growth factor-2 (FGF-2), endotoxin and bleomycin, matrix metalloproteases (MMP-2, MMP-9), also reactive oxygen species (ROS), acidic environment (Kubiczkova, et al., 2012). The role of TGF-β is complex and paradoxical, varying by cell type and physiological conditions (Massagué et al., 2000). From the results of our study, it follows that TGFβ levels in village Sareki fluctuate within the lower, while in Chorvila - within the upper limit of the normal range, which anticorrelates with the onco-incidence in these villages and may be due to the anticarcinogenic activity of this cytokine.

Interleukin 10 (IL-10) is known to be a potent anti-inflammatory cytokine. Almost all immune cells, including T cells, B cells, monocytes, macrophages, mast cells, granulocytes, dendritic cells, and keratinocytes, produce IL-10. Several studies have indicated that IL-10 has both pro- and antitumoral effects. IL-10 inhibits NF-ₖB signaling; therefore, it can downregulate proinflammatory cytokine expression and act as an antitumoral cytokine. Due to its immunosuppressive effect on dendritic cells and macrophages, IL-10 can dampen antigen presentation, cell maturation, and differentiation, allowing tumor cells to evade immune surveillance mechanisms (Iyer and Cheng, 2012; Gonzalez-Garza et al., 2020).

The presented study results of the immune status (cytokines) of the population of Sachkhere district's villages testify to a shift of the cytokine balance in blood serum of Sareki village residents towards pro-inflammatory.

Considering the critical role of the cytokines in the regulators of the inflammatory response and the important role of the inflammation in the interaction between the tumor cells and host cells and in the coordination of the tumor progression (Kubiczkova et al., 2012), it can be assumed that there is a causal relationship between the relatively high level of the onco-incidences in village Sareki and the shift of the cytokine balance in the blood serum of this village's population towards pro-inflammatory.

Inflammatory response triggers activation of epithelial and immune cells, accompanied by hyperproduction of the superoxide anion ($O_2-•$) and nitric oxide (NO), which can then be converted into reactive nitrogen species (RNS) (such as nitrogen dioxide ($NO_2•$), peroxynitrite ($ONOO−$), and dinitrogen trioxide (N_2O_3)) and reactive oxygen species (ROS) (hydrogen peroxide (H_2O_2), and hydroxyl radical (HO·). ROS and RNS are involved in cancer development through various mechanisms - they cause cellular oxidative stress and damage to lipids, proteins, and DNA, thereby causing damage to cells and the genome. (Landskron et al., 2014b).

Against the increased formation of ROS in the body, there are numerous protective mechanisms, 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 (Karki et al., 2016). The ability of cells of a living organism to prevent oxidative damage is a key factor in the survival mechanism.

Depleted levels of enzymatic and non-enzymatic antioxidant protective mechanisms have been documented in a wide variety of chronic diseases including malignancies (Karki et al., 2016). During chronic inflammation, prolonged expression of excess amount of ROS induces inactivation of antioxidant enzymes,

inhibits biosynthesis of macromolecules, which slows down replenishment of the pool of the antioxidant enzymes activity, inhibits the compensatory release of low molecular weight nonenzymatic antioxidants. However, these small ROS-scavenging molecules through a tightly coordinated system of metabolic processes, more labile (compared to the enzyme system) respond to changes in redox homeostasis and ensure the protection of cellular structures and macromolecules and body resistance to oxidative stress. Non-enzymatic antioxidants represent a key mechanism of antiradical defense in living systems. (Robinson et al., 2011).

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 shown in the healthy populations of the Sachkhere district villages (Chorvila, Sarek, Sairkhe) (Sharashenidze et al., 2021), accompanied with the changes in the number of MnB epithelial cells (Tikaradze et al., 2021).

The presented research's results revealed alterations in the TAA and increase of the NO content in the blood serum of the inhabitants from 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 blood of the population. Under conditions of moderate oxidative stress, the compensatory activation of the non-enzymatic antioxidant system (growth of TAA) provides regulation of redox homeostasis in the residents' bodies (Sairkhe village); however, as the intensity of oxidative stress increases, NO production continues to increase, while the overload of the endogenous non-enzymatic antioxidant system causes its depletion, which is manifested in the decrease in TAA (non-compressed oxidative stress in Sareki). With an increase in the intensity of oxidative stress, the number of MnB epithelial cells increases in the studied population (especially in Sareki). The especially high level of the MnB epithelial cells in inhabitants from the village Sareki indicates the chromosomal damage in populations, possibly resulting from the intensification of the oxidative stress (Martinez et al., 2005; Minicucci et al., 2005; Bhatt et al., 2010; Farhadi et al., 2016).

In conclusion, the existence of statistically reliable associations between the levels of cancer incidence in the populations of the surveyed villages and the indicators of immune and oxidative status in their virtually healthy subpopulations, with a high degree of persuasiveness, allows us to assume a close causal link between them. From these positions, deviations in immune and redox status can quite rightly be considered markers of pre-morbidity. 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 oncological risk.

Evaluation of the clinical informativeness of such complex predictors of carcinogenic risk is the subject of further longitudinal studies in the investigated populations, however, already at this stage of the research are clear the prospects of the obtained results in terms of developing an approach based on the synthesis of evidence of health risk in small geographical areas. Clarifying the reasons for the identified patterns and their significance requires more detailed studies.

References

A Partial Review of the "London Principles", With Recommendations for Revisions and Additions, by an Expert Panel. Epidemiology in hazard and risk assessment. 1999. http://www.fedfocus.org/finalreport.pdf

Bennett J, Reeves G, Billman G, Sturmberg J (2018). Inflammation–Nature's Way to Efficiently Respond to All Types of Challenges: Implications for Understanding and Managing "the Epidemic" of Chronic Diseases. *Front Med*, **5**, 316.

Bhatt A, Mathur R, Farooque A, et al (2017). Cancer biomarkers - Current perspectives, Indian J Med Res. 2010;132:129-149.

Chen L, Deng H, Cui H, et al (2017). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*, **9**, 7204–18.

Chrzczanowicz J, Gawron A, Zwolinska A, et al (2008). Simple method for determining human serum 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity - possible application in clinical studies on dietary antioxidants. *Clin Chem Lab Med*, **46**, 342–9.

Fabre J, Giustiniani J, Garbar C, et al (2016). Targeting the Tumor Microenvironment: The Protumor Effects of IL-17 Related to Cancer Type. *Int J Mol Sci*, **17**, 1433.

Farhadi S, Sadri D, Sarshar S (2016). Micronucleus assay of Buccal mucosa: a Useful noninvasive approach in Screening of Genotoxic nuclear damage. *Adv Biores*, **7**, 20-9.

Gagoshidze M, Antelava M, Zedginidze A, et al (2005). Dependence of child health on the ecology. *Georgian Med News*, **118**, 49-52.

Gasche J, Hoffmann J, Boland C, Goel A (2011). Interleukin-6 promotes tumorigenesis by altering DNA methylation in oral cancer cells. *Int J Cancer*, **129**, 1053-63.

Gonzalez-Garza M, Cruz-Vega D, Maldonado-Bernal C (2021). IL10 as Cancer Biomarker. In: Translational Research in Cancer. Editted by Sundaresan S, Gu Y. 2021.

Hossain Md, Lawson A (2010). Space-time Bayesian small area disease risk models: development and evaluation with a focus on cluster detection. *Environ Ecol Stat*, **17**, 73–95.

Iliopoulos D, Hirsch H, Struhl K (2009). An epigenetic switch involving NF-kappaB, Lin28, Let-7 microRNA, and IL6 links inflammation to cell transformation. *Cell*, **139**, 693–706.

Iyer S, Cheng G (2012). Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease. *Crit Rev Immunol*, **32**, 23–63.

Kany S, Vollrath J, Relja B (2019). Cytokines in Inflammatory Disease. *Int J Mol Sci*, **20**, 6008.

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**, 1-11.

Keyfitz N (1966). Sampling Variance of Standardized Mortality Rates. *Hum Biol*, **38**, 309-17.

Kubiczkova L, Sedlarikova L, Hajek R, Sevcikova S (2012). TGF-β – an excellent servant but a bad master. *J Transl Med*, **10**, 183.

Kumari N, Dwarakanath B, Das A, Bhatt A (2016). Role of interleukin-6 in cancer progression and therapeutic resistance. *Tumor Biol*, **35**, 11553-72.

Kvarackhelia G, Kverenchkhiladze R, Buleishvili M, et al (2017). Screening level environmental health risk assessment

by health data in small areas of upper Imereti (Chiatura district). *Georgian Med News,* **270**, 145-52.

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.

Kyrochristos I, Ziogas D, Lykoudis E, Roukos D (2018). Breast cancer genome analysis in time and space: biomarker development strategy. *Biomark Med*, **2018**, 547–50.

Lan T, Chen L, Wei X (2021). Inflammatory Cytokines in Cancer: Comprehensive Understanding and Clinical Progress in Gene Therapy. *Cells*, **10**, 100.

Landskron G, De la Fuente M, Thuwajit P, et al (2014a). Chronic Inflammation and Cytokines in the Tumor Microenvironment. *J Immunol Res*, **2014a**, 149185.

Landskron G, De la Fuente M, Thuwajit P, et al (2014b). Chronic Inflammation and Cytokines in the Tumor Microenvironment. *J Immunol Res*, **2014**, 149185.

Linkov I, Massey O, Keisler J, et al (2015). From "Weight of Evidence" to Quantitative Data Integration using multi criteria Decision Analysis and Bayesian Methods. *ALTEX*, **32**, 3–8.

Lioy P, Smith K (2013). A Discussion of Exposure Science in the 21st Century: A Vision and a Strategy. *Environ Health Perspect*, **121**, 405-9.

Martinez E, Achcar J (2014). Trends in epidemiology in the 21st century: time to adopt Bayesian methods. *Saude Publica*, **30**, 703-14.

Martínez V, Creus A, Venegas W, et al (2005). Micronuclei assessment in buccal cells of people environmentally exposed to arsenic in northern Chile. *Toxicol Lett*, **155**, 319-27.

Massagué J, Blain S, Lo R (2000). TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell*, **103**, 295-309.

Massagué J (2008). TGFbeta in cancer. *Cell*, **134**, 215-30.

Matsuzaki K. Smad phosphor isoforms direct contextdependent TGF-β signaling. *Cytokine Growth Factor Rev*, **24**, 385-99.

Minicucci E, Kowalski L, Maia M, et al (2005). Cytogenetic damage in circulating lymphocytes and buccal mucosa cells of head-and-neck cancer patients undergoing radiotherapy. *J Radiat Res*, **46**, 135-42.

Miranda, K, Espey M, Wink D (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide Biol Chem*, **5**, 62–71.

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.

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**, 125-8.

Stich H, Stich W, Parida B (1982). Elevated of micro nucleated cells in the buccal mucosa. *Cancer Lett*, **17**, 125-34.

Tikaradze E, Bakradze L, Tsimakuridze M, et al (2021). Bayesian modeling and inference of mixtures of distribution of micronuclear buccal cells in the population of Sachkhere district's villages. *Georgian Med News*, (**316-317**), 154-8.

Wang J, Li D, Cang H, Guo B (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. *Cancer Med*, **8**, 4709–21.

World Health Organization (2000). Methodology for assessment of environmental burden of disease. https://www.who.int/publicatio.ns/i/item/WHO-SDE-WSH-00.7.

World Health Organization, Regional Office for Europe (2000). Evaluation and use of epidemiological evidence for environmental health risk assessment.

Zhao J, Chen X, Herjan T, Li X (2020). The role of interleukin-17 in tumor development and progression. *J Exp Med*, **217**, e20190297.