RESEARCH ARTICLE

Editorial Process: Submission:07/27/2023 Acceptance:02/11/2024

Possible Markers for Distinguishing benign and Malignant Thyroid Tumors and Predicting Malignancy in Patients with Genetic Predisposition to Cancer

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Abstract

Background: We hypothesized that mutations in several genes disrupt oxidative metabolism, increasing the risk of developing tumors and their malignancy in patients with a family predisposition to cancer. The purpose of our study was to assess the characteristics of oxidative metabolism in patients with malignant and benign tumor with and without a family history of cancer and identify the marker predicting the likelihood of malignancy. **Methods:** We conducted a study on patients with thyroid pathology (thyrotoxicosis, benign tumor pathology of the thyroid gland, and thyroid cancer) who underwent treatment at LLC "Oncology Scientific Research Center" in Tbilisi, Georgia between 2020-2021. In patients' blood the thyroid hormones content, the oxidative metabolism parameters (activity of nonenzymatic antioxidant system (TAA), malondialdehyde (MDA) content), geometrical and rheological (deformability index (EDI), membrane proteins content) characteristics of erythrocytes were determined. **Results:** in the patient's blood serum with benign tumor (47 patients) MDA exceeded ($p<0.005$) and TAA decreased ($p<0.005$) in comparison to the control level; in patients with thyroid cancer (35 patients), MDA also exceeded ($p<0.005$), while TAA increased ($p<0.005$) up to the control level. In patients with benign and malignant tumors, the size of erythrocytes increased compared to the control indicators ($p<0.005$); in patients with thyroid cancer and benign tumors with a family history of cancer (29 patients) EDI increased ($p<0.005$), content of GLUT1 in erythrocyte membranes decreased ($p<0.005$) compared to the control level. **Conclusions:** Alterations in redox metabolism play a crucial role in tumor formation; an imbalance between anti-/pro-oxidant systems may contribute to tumor formation and support its progression into a more malignant state. Thyroid cancer is characterized by a reduction in erythrocyte deformability, related to TSH levels. These alterations are less detectable in patients with benign thyroid tumors with a family history of cancer.

Keywords: Thyroid tumor- cancer- family history of cancer- oxidative stress- erythrocyte deformability- *GLUT1- TSH*

Asian Pac J Cancer Prev, **25 (2)**, 465-472

Introduction

The second leading cause of death worldwide is oncological diseases [1]. Among endocrine tumors, thyroid tumors are the most common. In Georgia, malignant tumors are the second most prevalent, according to the National Center for Disease Control and Public Health of the Ministry of Labor, Health and Social Protection [2]. The rise in thyroid cancer cases may be due to worsening environmental conditions, such as an increase in radiation exposure. This growing global problem makes cancer treatment and prevention one of the most significant public health challenges of the 21st century.

For diagnosing neoplasms in the thyroid gland, the following examinations are given priority - ultrasound examination, fine-needle aspiration biopsy and cytological examination, and determining the level of thyroid hormones in the blood serum. Cytological examination plays an important role, but it only correctly verifies the disease in 77% of cases [1]. This may lead to diagnostic errors, which can interfere with choosing the correct treatment tactics [3, 4].

Determining the type of neoplasia is the most challenging diagnostic issue [5, 6]. Reactive oxygen and nitrogen species (ROS, RNS) as an unavoidable product of cellular metabolism, are potentially harmful species, nevertheless, they are also intracellular signaling molecules [7]. Cellular enzymatic and nonenzymatic antioxidant systems limit the formation of ROS/RNS or detoxify the reactive metabolites. An increase in ROS can induce DNA damage, also, through the regulation of different cellular signaling pathways and nuclear factors, support

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the enhanced proliferation of transformed cells increase cellular growth, contribute to genomic instability, initiate tumorigenesis, and the development of cancer [8]. The balance between ROS and antioxidants is maintained by activating antioxidant systems or upregulating antioxidant gene expression [9, 10]. There is increasing evidence to suggest that the tumor suppressor genes are involved in the regulation of the cell cycle and other important cellular process, such as DNA reparation, replication, transcription [11, 12], and the regulation of oxidative stress through the upregulation of antioxidant response [13, 14]. This upregulation leads to an increase in the expression of several genes, including glutathione S-transferases, which decrease the levels of ROS [15, 16], and mutation of these genes contributes to the intensification of oxidative stress and further malignant transformation of the tumor cells.

The purpose of our study was to assess the characteristics of oxidative metabolism in patients with malignant and benign tumors with and without a family history of cancer and identify the marker predicting the likelihood of malignancy.

Materials and Methods

We conducted a study on 117 patients with thyroid pathology, including thyrotoxicosis, benign tumor pathology of the thyroid gland (adenoma, colloid gout), and thyroid cancer. These patients underwent treatment at LLC "Oncology Scientific Research Center" in Tbilisi, Georgia between 2020 and 2021.

The diagnosis of thyroid pathology was based on a physical exam, ultrasound examination, fine-needle aspiration biopsy, and determination of thyroid hormone levels in the blood serum. The inclusion criteria for the study were adenomatous nodules, colloidal nodes, and low-differentiated, undifferentiated, and highly differentiated cancer of the thyroid gland, as determined by cytological examination. Exclusion criteria in the study: multiple tumors, autoimmune thyroiditis without nodular transformation, or other thyroid gland pathology that did not include a nodular transformation in the thyroid gland.The patients included in the study are divided into 4 groups: Group 1 (control) - 10 healthy people, Group 2 - thyrotoxicosis (25 patients), Group 3 - benign tumor pathology of the thyroid gland - (57 patients), Group 4 - Thyroid cancer (35 patients). According to anamnesis data, the patients of Group III were divided into 2 subgroups: Group 3a – without a family history of cancer (28 patients) and Group 3b - with a family history of cancer (29 patients).

Our study plan was approved by the Ethics Committee of Tbilisi State Medical University of Georgia. 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, alcohol consumption), as well as personal and family medical history of cancer, and provided blood samples during their health checkup. Collected blood samples immediately were used for analysis in clinical settings. In patients' blood the thyroid hormones content (free Triiodothyronine (fFT3), free Thyroxine (fFT4), bound Triiodothyronine (FT3), bound Thyroxine (FT4), Thyroid-stimulating hormone (TSH)), the intensity of oxidative stress and NOx content, geometrical characteristics (volume, thickness, surface area) and rheological characteristics of erythrocytes (erythrocytes deformability index (EDI), membrane proteins content) were determined.

Oxidative stress intensity

The oxidative stress intensity in the blood samples was evaluated according to the total activity of the nonenzymatic antioxidant system (TAA) and the lipid peroxidation product, malondialdehyde (MDA), content in the blood serum.

Total antioxidant activity (TAA) of blood serum

TAA was determined in deproteinized blood serum by using the 2.2-diphenyl-1-picryl-hydrazine (DPPH)-scavenging assay, adapted by Chrzczanowicz et al. [17]. Blood serum samples (1ml) were deproteinized by adding 3 ml of acetonitrile and centrifuging them for 10 min (4°C, 9,500g). A supernatant was immediately collected and transferred (1 ml) to a tube, subsequently, 3 ml of DPPH was added, and the resultant solution's absorbance was determined at 515nm. A calibration curve was built with the use of Gallic acid, wherein the absorbance values were interpolated and the results were expressed as Gallic acid equivalents (%).

MDA content in blood serum.

A Thiobarbituric acid assay determined MDA content in blood serum [18].

Total NOx content in blood serum

The level of NOx in the blood serum samples was determined by a modified method by Miranda et al. [19]. As the first step, sample deproteinization was achieved by adding an equal volume of 0.3M NaOH to 100µl of blood serum. The sample was mixed well and incubated at room temperature for 5 min. Then 100µl of 5% $ZnSO_4$ was added, mixed well, and incubated for an additional 5 min at room temperature. After the incubation, the mixture was centrifuged at 3,000 rpm, 4°C for 15 min. An aliquot of 100µl of the clear supernatant was mixed with 200µl of Griess Reagent, which was prepared just before the assay and contained 0.25% VCl3, 0.1% sulfanilamide, and 0.05% N-(1-Naphthyl)-ethylenediamine (NEDD) in 0.5 M HCl. The reagent blank was the same but contained 100µl of distilled water instead of the sample. The mixture was incubated for 30 min at 37°C and absorbance was measured at 540nm with a microplate reader (Multiscan GO, Thermo Fischer Scientific, Finland). The standard curve for $NaNO_2$ was used to calculate the total NO concentration in the samples.

Geometrical characteristics of Erythrocytes

Erythrocyte membrane isolation was performed by the Hast method [20].

Volume of erythrocytes

The volume of erythrocytes was calculated from

the ratio between hematocrit and the number of erythrocytes per unit volume according to Welker formula: V= Ht/RBC, where Ht is hematocrit (the volumetric mass of erythrocytes in 1 mm^3 of blood); RBC - the number of erythrocytes in 1 mm^3 of blood.

Thickness of erythrocyte

The thickness of erythrocytes was calculated according to the Boros formula, taking the geometry of the erythrocyte as a cylindrical body: $T = V/\pi R^2$, where V is the volume (μm^3), R - average radius of an erythrocyte (μm).

The surface area of erythrocytes

The surface area of erythrocytes was calculated according to the formula of A. Hurtado: $S = 2v/T + \pi RT$, where V is the volume (μm^3), T - thickness, μm; R is the average radius of an erythrocyte (μm).

Protein content in erythrocytes' membrane
Isolation of erythrocyte membrane by the Hast method

Blood samples, collected in tubes containing anticoagulants were centrifuged at 3,000g for 15 min. The obtained erythrocyte sediment was washed 3 times with a 1: 4 volume of solution A, containing 130 μM KCl, and 20 μM Tris-HCl (pH-7.4). For hemolysis of the obtained erythrocyte sediment, the 1:10 volume of solution B, containing 5 μM Tris-HCl, and 1 mm EDTA, was added and the resulting mixture was left all night (for about 15 hours). The next day the suspension was centrifuged at 12,000 g for 20 min. The obtained precipitate was washed again with solution "B" 2-3 times before bleaching. The precipitate was washed again with an "A" solution in volume 1:10.

Determination of protein content (Protein analytical electrophoresis under dissociated conditions)

The membrane protein content was quantified using the DC (detergent-compatible) protein assay for protein solubilized in Laemmli buffer [21]. Protein analytical electrophoresis was performed under dissociated conditions in a 12.5% gradient polyacrylamide gel with 1 mm thick and 6 ml volume with 0.1% sodium dodecyl sulfate (SDS), by heating the samples at 100◦C for 10 min and loading 20 μg of membrane proteins on an 8% gel for protein staining by colloidal 0.2% Coomassie Blue G-250 [22]. A set of standard proteins (kDa) as electrophoresis markers were used.

The protein content in the erythrocyte membranes' samples was determined by evaluating the area of the corresponding area on the electrophoretic picture by use of the special analytical device (TAS plus, Leitz, Germany).

Statistical analysis

An analysis of variance (ANOVA) (SPSS-12 for Windows) was used to compare the data.

Results

Determination of the Hormonal status in the patients' blood

The alterations in the thyroid hormone status of patients with thyrotoxicosis (group 2), benign (without and with a family history of cancer (group 3a, b)), and malignant tumors (group 4) of the thyroid gland are shown (Table 1): in patients with thyrotoxicosis, the content of free FT4 and FT3 in the blood increased by 60% and 71%, and the content of the bound form of these hormones increased by 100% and 64%, respectively; TSH values were reduced by 70% compared to the control rate. In the case of benign (without and with a family history of cancer) and malignant tumors of the thyroid gland, the content of free and bound forms of FT4 and FT3 did not differ, while the content of TSH increased by 69% and 84%, respectively, compared to the control level (Table 1). It is worth noting here that in all studied patients, the level of thyroid hormones in the blood did not exceed the limits of clinically established norms (Table 1).

Pro-, antioxidant status, and NOx content inpatients' blood

The investigation of the lipid peroxidation product, MDA, nitric oxide metabolites (nitrites and nitrates) – total NOx content, as well as the activity of the total non-enzymatic antioxidant system activity (TAA) in the blood serum of the studied patients, showed, that in the patients with thyrotoxicosis, the MDA content increased by 4 fold, NOx content by 32%, and TAA decreased by 69%, compared to the control values; in patients with benign tumor pathology TAA decreased by 78%, while NOx content increased by 22%, and in patients with a malignant tumor of the thyroid gland TAA and NOx content didn't change in comparison to the control level. In a group of patients with benign tumor pathology and family history of cancer MDA content increased by 6 fold, in patients without a family history of cancer - by 3 fold, and in patients with a malignant tumor of the thyroid gland

Table 1. Hormones Level in the Blood of Healthy Donors and Patients with Thyrotoxicosis, benign Tumor Pathology of the Thyroid Gland, and Thyroid Cancer

Parameters	Group 1	Group 2	Group 3		Group 4	Clinical norm
			3a	3b		
FT4 (mmol/l)	15.0±5.2	24,2±2,5	14,1±2,4	14,0±2,7	16,2±1,5	10 – 22
T4 (nmol/l)	70.2±14.6	140.3±32.0*	67.2±18.3	68.1±19.0	69.4±6.9	59 -160
TSH (mU/l)	1.3±0.5	0.4±0.2	2.3±0.7*	2.2±0.9*	2.4±0.9*	0.4 – 4
FT3 (pmol/l)	3.5±1.0	6.0±0.2*	3.1±0.6	3.0±0.5	4.0±0.4	2.6 – 5.6
T3 (nmol/l)	1.7±0,2	2.8±0,1*	1.8±0,6	1.9±0,4	2.0±0,2	1.3 – 2.7

*, statistically significant changes compared to the control group level (P<0.005)

Table 2. The MD, NOx Content TAA in the Blood Serum of the Studied Patients

Group		MDA	TAA (%)	NOx (μM/l)
Group I		1.1±.0.8	25±3%	10,8±0.8
Group 2		4.6±0.7*	7.8±2,6%*	14.3±1.2*
Group 3	3a	3.7±1.1*	5,6±2,6%*	13.5±0.9*
	3b	6.2±1.1* **	6.0±2,2%*	13.2±0.8
Group 4		5.8±2.8* ***	28±5.3%***	11.4±1.3

*, statistically significant change compared to the control group level (P<0.005); **, statistically significant change between 3a and 3b groups levels (P<0.005); ***, statistically significant change between 4 and 3 groups levels (P<0.005)

Table 3. The Geometric Characteristics (Volume, Thickness, Surface Area) of Blood Erythrocytes of Healthy Donors and the Studied Patients with Thyrotoxicosis, benign Tumor Pathology of the Thyroid Gland, and Thyroid Cancer.

	Group 1	Group 2	Group 3		Group 4
			3a	3b	
The volume of erythrocytes (μm³)	7.1 ± 0.20	8.0 ± 0.40	8.0 ± 0.50	8.1 ± 0.40	9.2 ± 0,20*
The thickness of erythrocytes (μm)	2.00 ± 0.40	2.05 ± 0.20	2.04 ± 0.29	2.08 ± 0.40	2.30 ± 0.50*
The surface area of erythrocytes (μm²)	86.0 ± 10.0	105.0 ± 12.0	117.0 ± 8.0*	118.0 ± 8.9*	140.0 ± 8.9* **

*, statistically significant change compared to the control group level (P<0.005); **, statistically significant change between 4 and 3 groups levels (P<0.005)

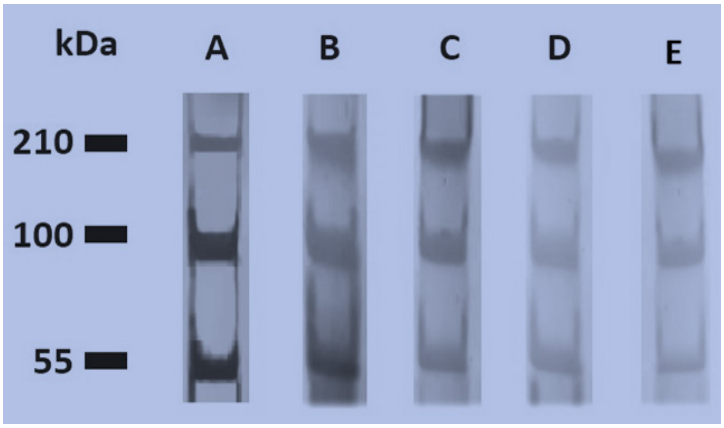

Figure 1. The Sodium Dodecyl Sulfate (SDS)-Polyacrylamide Gel Electrophoresis Data Related Erythrocytes Membranes from Healthy Volunteers (A) and patients with thyrotoxicosis (B), benign (C- without a family history of cancer, D – with a family history of cancer), and malignant (E) tumors of the thyroid gland.

-5 fold, when compared to the control level (Table 2).

Structural and functional properties of erythrocytes

It follows from the results of the study that in patients with thyrotoxicosis the size of erythrocytes (volume, thickness, surface area) didn't importantly change compared to the control characteristics. In patients with benign tumors of the thyroid gland (without and with a family history of cancer), the volume and thickness of erythrocytes didn't change compared to the control indicators, while the surface area of erythrocytes increased compared to the control indicators in both subgroups (3a, b) (Table 3). In patients with thyroid cancer, the size of erythrocytes (volume, thickness, surface area) statistically significantly increased compared to the indicators of healthy individuals and patients with benign tumors (Table 3).

In patients with thyrotoxicosis and benign tumors without a family history of cancer erythrocyte deformability index (EDI) didn't change in comparison to the control level; in patients with malignant tumors of the thyroid gland and benign tumors with a family history of cancer,

Table 4. Erythrocyte Deformability (EDI) in Control and Patients with Thyrotoxicosis, benign Tumor Pathology of the Thyroid Gland, and Thyroid Cancer

	Group 1	Group 2	Group 3		Group 4
			3a	3b	
EDI	1.23±0.05	1.23±0.06	1.18±0.05	1.12±0.05*	1.13±0.05* **

*, statistically significant change compared to the control group level (P<0.005); **, statistically significant change between 4 and 3a groups levels (P<0.005)

Table 5. Membrane Proteins (210 kDa, 100kDa 55kDa) Content in Erythrocytes from Healthy Volunteers (A) and Patients with Thyrotoxicosis (B), benign (C, D), and Malignant (E) Tumors of the Thyroid Gland.

KDa	Group 1	Group 2	Group 3		Group 4
			3a	3b	
on 210	18.0±3.5	20.2±4.8	18.1±3.8	18/3±5.2	18.2±4.9
on 100	40.0±5.4	48.0±6.2	38.3±5.9	38,7±6.1	36.4±5.2
on 55	44.3±5.7	40.5±4.7	30.4±3.7*	26.2±4.3*	20.4±4.1* **

*, statistically significant change compared to the control group level (P<0.005);** - statistically significant change between 4 and 3a groups levels (P<0005)

EDI indicator statistically significant decreased by 10% in comparison to the control level (Table 4).

The proteins (210 kDa, 100kDa, 55kDa) content (determined by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis) in erythrocytes membrane from healthy volunteers (A), patients with thyrotoxicosis (B), benign (C, D), and malignant (E) tumors of the thyroid gland was determined (Table 5, Figure 1). These proteins of erythrocyte membranes were referred to as the ankyrin (210 kDa), Band 3 protein (B3p) (100kDa), and Band 4.5 (GLUT1) protein (55kDa) [17, 23].

As seems from the results of the study (Table 5 and Figure 1), the content of ankyrin and B3p in the erythrocytes membranes from patients with thyrotoxicosis, benign, and malignant tumors statistically significantly did not differ from their level in erythrocytes mmbranes from healthy volunteers. The content of the protein with molecular mass 55kDa (GLUT1) in erythrocyte membranes from patients with benign and malignant tumors of the thyroid gland was decreased compared to the control level, especially in patients with thyroid cancer, and benign tumors of the thyroid gland with a family history of cancer.

Discussion

In patients with benign and malignant tumors, any changes in their thyroid hormone levels (T4 and T3) were not detected. However, the level of TSH increased by 64% and 84%, respectively. TSH can increase the production of H_2O_2 through the TSH receptor (TSHR), leading to a high level of oxidative stress in patients with thyroid malignancy. Many studies have shown that high TSH levels are linked to an increased risk of malignancy in thyroid nodules [24]. In patients with malignant tumors of the thyroid gland and benign tumors with a family history of cancer, the level of MDA was found to be especially high (significantly higher than in patients without a family history of cancer). These findings align with previous studies that have demonstrated a high level of ROS in cancer cells, particularly in thyroid cancer cells as they progress towards a more de-differentiated phenotype [25-27]. The disruption of redox homeostasis may be related to the mutation of tumor suppressor genes, can be mediated by oncogene-stimulated mitochondria, suppression of antioxidant enzymes' gene expression, or post-translational modifications of the enzymes such as the acetylation of SOD or other factors.

According to the results of our investigation, the TAA level was low in patients with benign nodules (with and without a family history of cancer), but increased in patients with thyroid cancer against the background of a high level of lipid peroxidation process.

ROSs are pro-tumorigenic, and through the regulation of different cellular signaling pathways and nuclear factors, they support the enhanced proliferation of transformed cells increase cellular growth, and cell survival, contribute to genomic instability, initiate tumorigenesis, and the development of cancer [28]. To optimize ROS-driven proliferation, tumor cells are restructuring the redox potential (from the reduced to the oxidized), possibly by decreasing the activity of the antioxidant system, and are adapted to live under moderate oxidative stress conditions. However, as high ROS levels can also stimulate senescence or apoptosis of neoplastic cells [8], to support the proliferative benefits of high ROS levels while mitigating the risk of senescence/apoptosis, cancer cells can upregulate transcription factors and reprogram metabolism to increase the de novo synthesis of antioxidants. Antioxidant status is commonly increased in tumor tissue, presumably to combat the high ROS burden (and this is associated with poor prognosis) [29].

Therefore, the antioxidant system can play a critical role in tumorigenesis, and antioxidant/oxidant imbalance may contribute to the formation of benign tumors and support its further malignant transformation. Our study results show a significant increase in erythrocyte volume, thickness, and surface area in patients with thyroid tumors (especially with malignant tumors) in comparison to the erythrocyte characteristics in healthy individuals. The regulation of erythrocyte volume involves cell hydration/dehydration mechanisms, regulated by signaling pathways and transmembrane proteins (Na+/K+-ATPase, calcium pumps, Na+/K+/2Cl--cotransporter, K+/Cl--cotransporter, Na+/H+-exchanger, Cl--HCO3--exchanger, Gardos channel, and others transporters) that mediate the flow of ions across the cell membrane [30-34].

The activity of these proteins can be modulated by hormones and xenobiotics [32-34]. TSH through membrane functional receptor TSHR through regulation of erythrocytes' oxygenation state can regulate their ATP content [35], and therefore, modify the conformation of Na+/K+-ATPase [36, 37] and its pumping capacity [36, 38]. In turn, the ATP molecules can be sequestered by the membrane complex of band 3 protein with cytoskeletal proteins (β-spectrin, ankyrin) binding to the Na+/K+-ATPase [39, 23], which represents the one of the mechanisms of the regulation of the Na+/K+-

ATPase activity [40]. Excess ATP, as a main fuel required for function ATP-ases, may suppress glucose uptake by erythrocytes via GLUT1, which interacts with various proteins (band 3 dimer, dematin, adducin, and proteins 4.1 and 4.2) of membrane junctional complexes attached to the cytoskeleton's spectrin by F-actin filaments [41, 42]. During intensification of glucose transport, GLUT1 forms monomeric clusters (55 kDa) [42], which represents the result of the negative feedback regulation mechanism.

The results of our study using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis show that the content of GLUT1 monomer (55 kDa) was significantly reduced in the erythrocyte membranes of patients with benign thyroid tumors (especially with a family history of cancer) and thyroid carcinoma. However, the content of ankyrin (210 kDa) and Band 3 protein (100 kDa) did not change in all studied groups of patients. This suggests a decrease in glucose supply, ATP synthesis, and reduced activity of the Na+/K+-ATPase pump, which leads to disturbances in the osmotic balance, hydration, and swelling of the erythrocyte. These factors, along with the fluidity of the plasma membrane, hemoglobin content, metabolism rate, adaptive responses, etc., determine erythrocytes' deformability and their rheological properties [43, 44] and are critical in microcirculation and tissue oxygenation. In our study, erythrocyte deformability in patients with malignant tumors of the thyroid gland and benign tumors with a family history of cancer significantly decreased.

Na+/K+-ATPase, through interaction with TSHR and surrounding membrane proteins, mediates signaling pathways affecting the erythrocyte's volume and membrane frigidity. A strong association between an indicator of the heterogeneity of erythrocyte volume, erythrocyte distribution wide (EDW), and thyroid function (TSH content) was revealed [37, 45, 35, 40]. It should be noted that although we detected a significant increase in the MDA and NO content, as well as a decrease in TAA in the blood serum of patients with hyperthyroidism against the background of increased expression of thyroid hormones (free and bound forms of T4 and T3, but not TSH). These changes should be related to oxidative stress and usually cause complications in target tissues. No significant changes in the shape, size, membrane-structural or functional characteristics of erythrocytes were detected.

The results of our studies allow us to conclude that during thyroid gland malignant transformation, the development of membrane-structural or functional alterations in erythrocytes involves other mechanisms, in addition to oxidative ones. Taken together, we can conclude that a decrease in erythrocyte deformability in patients with thyroid tumors may be associated with TSH-induced conformational changes in erythrocyte's membrane proteins and protein complexes, a subsequent decrease in the intensity of their energetical metabolism, further hydration and swelling.

The literature data testify to the alterations in erythrocyte Na+/K+-ATPase enzyme functional activity, a significant lowering of erythrocyte deformability in patients with differentiated thyroid cancers than in healthy individuals that were not modified even after radioiodine treatment. Additionally, the alterations of erythrocytes' membrane structure, deformability, and rheological properties have been detected in men with prostate cancer.

In conclusion, due to the small number of patients, we cannot make convincing conclusions, but the existence of statistically reliable associations between the levels of oxidative stress alterations of hormonal status and erythrocyte deformability in patients of studied groups, allows us to assume a close causal link between them the role of the oxidative stress in the tumor formation and support its progression into a more malignant state.

From these positions, deviations in erythrocyte deformability can quite rightly be considered markers of tumor malignant transformation risk.

Evaluation of the clinical informativeness of such predictors of carcinogenic risk is the subject of further more detailed studies. Alterations in redox metabolism play a crucial role in tumor formation. An imbalance between anti- and pro-oxidant systems may contribute to tumor formation and support its progression into a more malignant state.

Thyroid cancer is characterized by a reduction in erythrocyte deformability, related to the changes in membrane proteins and protein complexes affected by TSH levels. These changes lead to a decrease in the intensity of their energetic metabolism, further hydration, and swelling. These alterations are less detectable in patients with benign thyroid tumors who have a family history of cancer, and non-detectable in patients without a family history of cancer.

List of abbreviations

fFT3 - free Triiodothyronine
fFT4 - free Thyroxine,
FT3 - bound Triiodothyronine
FT4 - bound Thyroxine
TSH - Thyroid-stimulating hormone
EDI - erythrocytes deformability index
TAA - total antioxidant activity
OS - ocidative stress
SOD - superoxide dismutase
GP - glutathione peroxidase
ROS - Reactive oxygen species
RNS - Reactive nitrogen species
MDA - malondialdehyde

Author Contribution Statement

Ivane Javakhishvili - concept and design of research; Konstantine Mardaleishvili - clinical studies; Maia Mantskava - experimental studies; Eka Shekiladze - experimental studies; Magda Tortladze - literature review; Sophio Kalmakhelidze data analysis; Tamar Sanikidze - manuscript preparation, manuscript editing, and manuscript review.

Acknowledgements

All authors of this research paper have directly participated in the planning, execution, or analysis of this study; All authors of this paper have read and approved

the final version of the manuscript.

Ethical Approval

The study was approved by the Internal Review Board of the Biomedical Research Ethics Committee of Tbilisi State Medical University (TSMU REC); Decision Number: N3-2020/80 on 20th May 2020 year.

Since 2015 TSMU REC has been registered in the database of the Office for Human Research Protections (OHRP), which is part of the Office of the Assistant Secretary for Health in the Office of the Secretary of U.S. Department of Health and Human Services (HHS). TSMU REC has also obtained Federal-wide Assurances (FWA) the same year.

Funding statement

This study was approved by the Quality Assurance Policy of Tbilisi State Medical University (Georgia) and financed in the frames of the PhD program.

Availability of data

The information about the data of the research can be received from the corresponding author.

Competing interests

Authors have no competing interests of a financial or personal nature or other interests that might be perceived to influence the results and/or discussion reported in this paper.

References

1. Abu-Seadah SS, Attiah SM, Ali MY, Shams El-Din M, El-Kholy MA. Immunohistochemical expression of hbme-1 and trop-2 in some follicular-derived thyroid lesions. Asian Pac J Cancer Prev. 2023;24(7):2305-11. https://doi.org/10.31557/apjcp.2023.24.7.2305.
2. Ademoğlu E, Gökkuşu C, Yarman S, Azizlerli H. The effect of methimazole on the oxidant and antioxidant system in patients with hyperthyroidism. Pharmacol Res. 1998;38(2):93-6. https://doi.org/10.1006/phrs.1998.0336.
3. McHenry CR, Phitayakorn R. Follicular adenoma and carcinoma of the thyroid gland. Oncologist. 2011;16(5):585-93. https://doi.org/10.1634/theoncologist.2010-0405.
4. Badulescu CI, Piciu D, Apostu D, Badan M, Piciu A. Follicular thyroid carcinoma - clinical and diagnostic findings in a 20-year follow up study. Acta Endocrinol (Buchar). 2020;16(2):170-7. https://doi.org/10.4183/aeb.2020.170.
5. Miskad UA, Aswidah A, Dahlan H, Ikram D, Cangara MH, Djimahit T, et al. The role of claudin-1 expression in follicular and papillary thyroid neoplasm. Asian Pac J Cancer Prev. 2022;23(12):4023-7. https://doi.org/10.31557/apjcp.2022.23.12.4023.
6. Harahap WA, Tofrizal T, Oktahermoniza O. Relationship between the expression of braf v600e and ki-67 with the recurrence of well-differentiated thyroid cancer. Asian Pac J Cancer Prev. 2022;23(11):3617-22. https://doi.org/10.31557/apjcp.2022.23.11.3617.
7. Halliwell B, Gutteridge J. Free radicals in biology and medicine. 2015.
8. Moloney JN, Cotter TG. Ros signalling in the biology of cancer. Semin Cell Dev Biol. 2018;80:50-64. https://doi.org/10.1016/j.semcdb.2017.05.023.
9. Sakamoto K, Iwasaki K, Sugiyama H, Tsuji Y. Role of the tumor suppressor pten in antioxidant responsive element-mediated transcription and associated histone modifications. Mol Biol Cell. 2009;20(6):1606-17. https://doi.org/10.1091/mbc.e08-07-0762.
10. van der Reest J, Lilla S, Zheng L, Zanivan S, Gottlieb E. Proteome-wide analysis of cysteine oxidation reveals metabolic sensitivity to redox stress. Nat Commun. 2018;9(1):1581. https://doi.org/10.1038/s41467-018-04003-3.
11. Foulkes WD. Inherited susceptibility to common cancers. N Engl J Med. 2008;359(20):2143-53. https://doi.org/10.1056/NEJMra0802968.
12. Koen K, Robin P, Eline N. Chek2 mutations and papillary thyroid cancer: Correlation or coincidence? Hered Cancer Clin Pract. 2022;20(1):5. https://doi.org/10.1186/s13053-022-00211-7.
13. Mahmood A, Srivastava R. Etiology of cancer in understanding cancer, elsevier inc. 2022.
14. Bandargal S, Rajab M, Forest VI, Pusztaszeri MP, Hier MP, da Silva SD, et al. Characteristics of pten mutation in thyroid tumours: A retrospective chart review. Cancers (Basel). 2023;15(5). https://doi.org/10.3390/cancers15051575.
15. Bae I, Fan S, Meng Q, Rih JK, Kim HJ, Kang HJ, et al. Brca1 induces antioxidant gene expression and resistance to oxidative stress. Cancer Res. 2004;64(21):7893-909. https://doi.org/10.1158/0008-5472.Can-04-1119.
16. Saha T, Rih JK, Rosen EM. Brca1 down-regulates cellular levels of reactive oxygen species. FEBS Lett. 2009;583(9):1535-43. https://doi.org/10.1016/j.febslet.2009.04.005.
17. Chrzczanowicz J, Gawron A, Zwolinska A, de Graft-Johnson J, Krajewski W, Krol M, et al. 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. 2008;46(3):342-9. https://doi.org/10.1515/cclm.2008.062.
18. Khoubnasabjafari M, Ansarin K, Jouyban A. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. Bioimpacts. 2015;5(3):123-7. https://doi.org/10.15171/bi.2015.20.
19. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 2001;5(1):62-71. https://doi.org/10.1006/niox.2000.0319.
20. Mas-Oliva J. Effect of lead on the erythrocyte (ca2+,mg2+)-atpase activity calmodulin involvement. Mol Cell Biochem. 1989;89(1):87-93. https://doi.org/10.1007/BF00228283.
21. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage t4. Nature. 1970;227(5259):680-5. https://doi.org/10.1038/227680a0.
22. Barbero G, Carta F, Giribaldi G, Mandili G, Crobu S, Ceruti C, et al. Protein/rna coextraction and small two-dimensional polyacrylamide gel electrophoresis for proteomic/gene expression analysis of renal cancer biopsies. Anal Biochem. 2006;349(1):62-71. https://doi.org/10.1016/j.ab.2005.10.020.
23. Davis JQ, Bennett V. The anion exchanger and na+k(+)-atpase interact with distinct sites on ankyrin in in vitro assays. J Biol Chem. 1990;265(28):17252-6.
24. Ney GM, Yang KB, Ng V, Liu L, Zhao M, Kuk W, et al. Oncogenic n-ras mitigates oxidative stress-induced apoptosis of hematopoietic stem cells. Cancer Res. 2021;81(5):1240-51. https://doi.org/10.1158/0008-5472.Can-20-0118.
25. Faam B, Ghadiri AA, Ghaffari MA, Totonchi M, Khorsandi L. Comparing oxidative stress status among iranian males and females with malignant and non-malignant thyroid nodules. Int J Endocrinol Metab. 2021;19(1):e105669. https://doi.org/10.5812/ijem.105669.

26. Sadani GR, Nadkarni GD. Role of tissue antioxidant defence in thyroid cancers. Cancer Lett. 1996;109(1-2):231-5. https://doi.org/10.1016/s0304-3835(96)04484-9.
27. Laatikainen LE, Castellone MD, Hebrant A, Hoste C, Cantisani MC, Laurila JP, et al. Extracellular superoxide dismutase is a thyroid differentiation marker down-regulated in cancer. Endocr Relat Cancer. 2010;17(3):785-96. https://doi.org/10.1677/erc-10-0021.
28. Sies H. Oxidative stress: A concept in redox biology and medicine. Redox Biol. 2015;4:180-3. https://doi.org/10.1016/j.redox.2015.01.002.
29. Benhar M, Shytaj IL, Stamler JS, Savarino A. Dual targeting of the thioredoxin and glutathione systems in cancer and hiv. J Clin Invest. 2016;126(5):1630-9. https://doi.org/10.1172/jci85339.
30. Sugie J, Intaglietta M, Sung LA. Water transport and homeostasis as a major function of erythrocytes. Am J Physiol Heart Circ Physiol. 2018;314(5):H1098-h107. https://doi.org/10.1152/ajpheart.00263.2017.
31. Thomas SL, Bouyer G, Cueff A, Egée S, Glogowska E, Ollivaux C. Ion channels in human red blood cell membrane: Actors or relics? Blood Cells Mol Dis. 2011;46(4):261-5. https://doi.org/10.1016/j.bcmd.2011.02.007.
32. Mishra N, Rizvi SI. Quercetin modulates na(+)/k(+) atpase and sodium hydrogen exchanger in type 2 diabetic erythrocytes. Cell Mol Biol (Noisy-le-grand). 2012;58(1):148-52.
33. Omar AK, Ahmed KA, Helmi NM, Abdullah KT, Qarii MH, Hasan HE, et al. The sensitivity of na(+), k(+) atpase as an indicator of blood diseases. Afr Health Sci. 2017;17(1):262-9. https://doi.org/10.4314/ahs.v17i1.32.
34. Tekin N, Akyüz F, Temel HE. No levels in diabetes mellitus: Effects of l-name and insulin on lcat, na(+)/k(+) atpase activity and lipid profile. Diabetes Metab Syndr. 2011;5(4):191-5. https://doi.org/10.1016/j.dsx.2010.12.004.
35. Balzan S, Carpi A, Evangelista M, Nicolini G, Pollastri A, Bottoni A, et al. Acute effect of tsh on oxygenation state and volume of erythrocytes from subjects thyroidectomized for differentiated thyroid carcinoma. Biomed Pharmacother. 2011;65(5):381-4. https://doi.org/10.1016/j.biopha.2011.03.002.
36. Balzan S, Nicolini G, Forini F, Boni G, Del Carratore R, Nicolini A, et al. Presence of a functional tsh receptor on human erythrocytes. Biomed Pharmacother. 2007;61(8):463-7. https://doi.org/10.1016/j.biopha.2007.04.009.
37. Balzan S, Del Carratore R, Nicolini G, Forini F, Lubrano V, Simili M, et al. Tsh induces co-localization of tsh receptor and na/k-atpase in human erythrocytes. Cell Biochem Funct. 2009;27(5):259-63. https://doi.org/10.1002/cbf.1567.
38. DeLuise M, Flier JS. Status of the red cell na,k-pump in hyper- and hypothyroidism. Metabolism. 1983;32(1):25-30. https://doi.org/10.1016/0026-0495(83)90151-8.
39. Nelson WJ, Veshnock PJ. Ankyrin binding to (na+ + k+) atpase and implications for the organization of membrane domains in polarized cells. Nature. 1987;328(6130):533-6. https://doi.org/10.1038/328533a0.
40. Reis E, Nobre C, Artur PC, Miranda-Alves L, Berto Junior C. Effects of tsh on erythrocyte osmotic fragility: Signaling pathway. Int J Blood Res Disord. 2020;7(1). https://doi.org/10.23937/2469-5696/1410048.
41. Guizouarn H, Allegrini B. Erythroid glucose transport in health and disease. Pflugers Arch. 2020;472(9):1371-83. https://doi.org/10.1007/s00424-020-02406-0.
42. Yan Q, Lu Y, Zhou L, Chen J, Xu H, Cai M, et al. Mechanistic insights into glut1 activation and clustering revealed by super-resolution imaging. Proc Natl Acad Sci U S A. 2018;115(27):7033-8. https://doi.org/10.1073/pnas.1803859115.
43. Huisjes R, Bogdanova A, van Solinge WW, Schiffelers RM, Kaestner L, van Wijk R. Squeezing for life - properties of red blood cell deformability. Front Physiol. 2018;9:656. https://doi.org/10.3389/fphys.2018.00656.
44. Kuck L, Grau M, Bloch W, Simmonds MJ. Shear stress ameliorates superoxide impairment to erythrocyte deformability with concurrent nitric oxide synthase activation. Front Physiol. 2019;10:36. https://doi.org/10.3389/fphys.2019.00036.
45. Zhou G, Ai Y, Guo S, Chen Q, Feng X, Xu K, et al. Association between red blood cell distribution width and thyroid function. Front Endocrinol (Lausanne). 2021;12:807482. https://doi.org/10.3389/fendo.2021.807482.