INFLAMMATION IN CHILDHOOD EPILEPSY SYNDROMES

Bakhtadze S., Geladze N., Khachapuridze N.

Tbilisi State Medical University, Department of Paediatric Neurology, Georgia

Epilepsy is one of the most common neurological disorder affecting up to 1% of the world population [4]. It is a heterogeneous disorder and includes genetic, structural, metabolic causes, sometimes reason is unknown. Epileptic encephalopathies are the group of epilepsies when seizure itself can cause severe cognitive and behavioral abnormalities. Mostly they are considered as a drug resistant epilepsy [1]. Despite the adequate treatment about 30% of patients will continue to experience seizures which are defined as resistant epilepsies [11]. The role of inflammation in central nervous system (CNS) has already been investigated in the recent years. This theory arises from the fact that corticosteroids can successfully treat severe epileptic encephalopathy like West syndrome, also there is a link between fever and seizures in Dravet syndrome which leads to the hypothesis that inflammation can trigger epileptogenesis. Inflammation has to be assessed by inflammatory mediators like cytokines [14]. 51 different inflammatory mediators including IL-1ra, IL-1β, IL-6 and CXCL8/IL-8, IFN-γand TNF-α were found in serum, cerebrospinal fluid and brain tissues of the patients with epilepsies. Most of them were elevated in all three media while some of them only in serum or CSF. Inflammation contributes to the breakdown of blood brain barrier (BBB) through the upregulation of inflammatory mediators. It is proved that TNF-α and IL-6 increase BBB permeability and are implicated in seizure generation and severity. The binding of cytokines to receptors located in brain vasculature can cause the production of molecules, such as endothelial cell adhesion molecules, prostaglandins and chemokines that may further compromise the integrity of BBB. However research into this mechanism related to epilepsy is sparse. Spontaneous recurrent seizures lead to chronic expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) and its upregulation may contribute to BBB permeability, neuroinflammation and epileptogenesis in experimental models. Using a mouse model of epilepsy Fabene and colleagues [3] showed that seizures induce elevated expression of vascular cell adhesion molecules and enhanced leukocyte rolling and arrest in brain vessels mediated by the leukocyte mucin P-selectin glycoprotein ligand-1 (PSGL-1, encoded by Selplg) and leukocyte integrins a4b1 and aLb2. Inhibition of leukocyte-vascular interactions, either with blocking antibodies or by genetically interfering with PSGL-1 function in mice, markedly reduced seizures. Treatment with blocking antibodies after acute seizures prevented the development of epilepsy. Neutrophil depletion also inhibited acute seizure induction and chronic spontaneous recurrent seizures. Blood-brain barrier (BBB) leakage, which is known to enhance neuronal excitability, was induced by acute seizure activity but was prevented by blockade of leukocyte-vascular adhesion, suggesting a pathogenetic link between leukocytevascular interactions, BBB damage and seizure generation. Consistent with the potential leukocyte involvement in epilepsy in humans, leukocytes were more abundant in brains of individuals with epilepsy than in controls. As for prostaglandins they are secreted mainly by astrocytes and microglia. Prostaglandin E2 (PGE2) is coupled with its receptors including EP3 located on astrocytes, causes astrocytic glutamate release, hyper excitability and neuronal cell death in experimental models. Although the function of PGE2 in epileptogenesis have been studied for a considerable amount of time there still exists bidirectional data about it [3,12]. Another important group of chemokines CCL2, CCL3, CCL4 and CCL11 can alter neuronal physiology through the modulation of voltage dependent channels, activation of G-protein-gated potassium influx channels and increased release of certain neurotransmitters including glutamate. Chemokines have been detected in DNA microarray analysis of surgically removed hippocampus in experimental model with epilepsy. Furthermore it was suggested that they can be related with resistance of seizures in experimental models which is still disputable [13,15]. Reported studies are based on experimental evidences and they need clinical confirmation. Furthermore we do not know if there is a link between expression of these substances and the rate of resistance against the antiepileptic medication in various forms of paediatric epilepsies.

Study aims to evaluate the VCAM-1 and MIP (CCL2, CCL3, CCL11) concentrations in the controls and study group (drug resistant and resolved epilepsies).

Material and methods. We have examined 56 patients from 0-16 years of age of both gender. Study was done at G. Zhvania Academic Clinic of Paediatry, Tbilisi State Medical University. Group 1 was identified as controls and consists from 20 subjects. The study group was divided in 2 subgroups: First subgroup (Group 2A) involved 20 patients with resolved seizures and second subgroup (Group 2B) - 16 -patients with drug resistant epilepsy. Patients in study group (Group 2A and Group 2B)- Group 2A included the patients with proved epilepsy by EEG and clinical assessment who achieved seizures control. Group 2B are those subjects who have diagnosis of drug-resistant epileptic encephalopathies. All patients underwent a) Clinical and neurologic assessment. B) Electroencephalograpic (EEG) assessment. In controls it will help to exclude so called non convulsive forms or epilepsy and in target group it will help to clarify seizure type. C) Establishing the difference in the concentrations of cytokines in both groups. In case of different concentrations , it should be established which cytokine is present in a different quantitative concentration in both groups. Also it should be found if there is a correlation between concentrations of cytokines and frequency of the seizures. In case of this correlation it should be measured quantitatively relationship between the concentration of cytokines and the frequency of the seizures. The received results should undergo statistical analysis with in terms of patients' data. The patients were divided as controls (Group1) and study groups (patients with resolved seizures (Group 2A) and drug resistant seizures (Group 2B). For this clinical history, history of the seizures and neurological check-up should be done. The existing literature should be reviewed. Patients' database should be created. On the next stage, EEG or Video EEG should be performed and types of the seizure should be identified. On the following stage, the blood samples should be taken, they have to be prepared and sent to the lab. After taking the blood samples the results should be evaluated and a coherence should be found with the seizure type and relapses in cases of resistant epilepsy. The primary results should be published. On the last stage statistical analysis of the results has to be done and a correlation with age, sex, seizure type and relapse frequency should be found. The final database, final results and practical recommendation as well as scientific paper should be done. The statistical analysis should be done by SPSS (IBM SPSS Statistics, version 21.0, Armonk, NY).

Results and discussion. We have assessed VCAM-1, CCL2, CCL3, CCL11 in controls as well as in both study groups. VCAM-1 and CCL2, CCL3 were within normal range in controls and both study groups ($p < 0.05$). As for CCL 11 it was increased in group with resistant seizures while was normal in controls and in the group with well controlled seizures. The increase range for CCL11 was within 1000-2000 pg/ml and was strongly correlated with frequency and repetition rate of seizures (R^2-0.78). The correlation between the increased level of CCL11 and age and gender was not found (R^2-0.35).

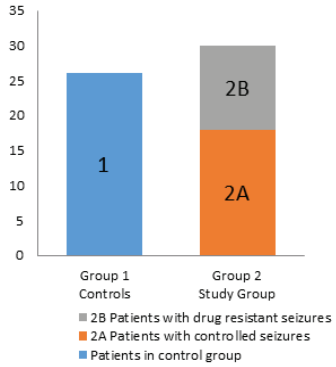

Fig.1. Methods: Control and study groups included 56 patients from 0 -16 years of age of both gender

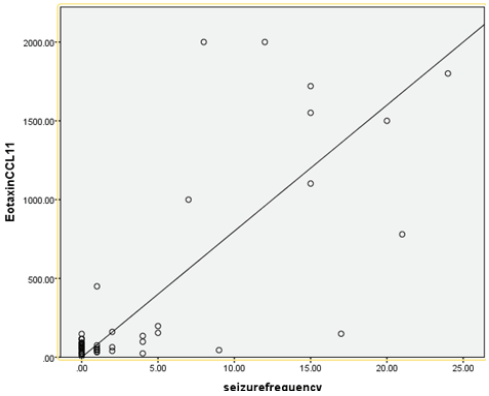

Fig. 2. Correlation between Eotaxin CCL11 and frequency and repetition rate of seizures

Table. 1. Correlation between Eotaxin CCL11 and frequency and repetition rate of seizures

Correlations

		EotaxinCCL11	Gender
EotaxinCCL11	Pearson Correlation	1	0.34
	Sig. (2-tailed)		.218
	N	56	56
Gender	Pearson Correlation	0.34	1
	Sig. (2-tailed)	.218	
	N	56	56

Table 2. Eotaxin CCL11 level in patients with resistant seizures and in the normal control groups

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
EotaxinCCL11	Resistant	16	921.6688	749.30364	187.32591
	Controlled Seizures	20	63.1250	24.89914	5.56762

Table 3. Eotaxin CCL11 level in patients with resistant seizures and in the group with well controlled seizures

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
EotaxinCCL11	Equal variances assumed	88.865	.000	5.139	34	.000	858.54375	167.04896	519.05942	1198.02808
	Equal variances not assumed			4.581	15.027	.000	858.54375	187.40863	459.15306	1257.93444

Table 4, Correlation between EotaxinCCL11 and gender

Correlations

		EotaxinCCL11	Gender
EotaxinCCL11	Pearson Correlation	1	0.34
	Sig. (2-tailed)		.218
	N	56	56
Gender	Pearson Correlation	0.34	1
	Sig. (2-tailed)	.218	
	N	56	56

Table 5- Correlation between Eotaxin CCL11 and gender

Correlations

		EotaxinCCL11	Gender
EotaxinCCL11	Pearson Correlation	1	0.34
	Sig. (2-tailed)		.218
	N	56	56
age	Pearson Correlation	0.34	1
	Sig. (2-tailed)	.218	
	N	56	56

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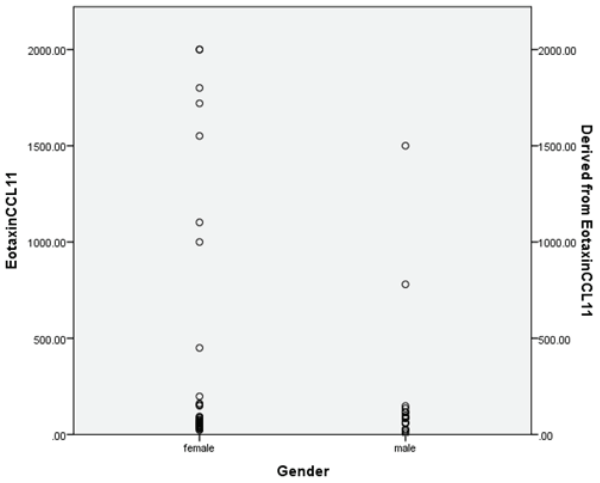

Fig. 3. Correlation between EotaxinCCL11 and gender

Thus our findings support the hypothesis that epilepsy could be triggered by inflammation as excessive expression of CCL 11 was found in children with drug resistant seizures. CCL11 has to be produced by perivascular glial cells, astrocytes, infiltrated leukocytes as they have ability to modulate neuronal activity by means of voltage gated ion channels (Potassium, Sodium Calcium); G protein activated inward rectifier potassium current and modulation of excretion of neurotransmitters (GABA, Glutamate and Dopamine [6,7]. The significant increase of cytokine level could be related with increased permeability of BBB and attraction of leukocytes in epileptogenic area of the brain. Thus our result showing the increase of CCL11 is in accordance with scientific data proving the impairment of BBB as a pathogenic factor in etiology of epilepsy. Pro inflammatory cytokines can rapidly alter the function of classical neurotransmitters by modulating their receptor assembly and phosphorylation at neuronal membranes [5]. Inflammatory mediators can also increase vascular permeability and promote angiogenesis [10].Thus their overexpression in perivascular astrocytes and endothelial cells after epileptogenic challenges may affect BBB properties, consequently promoting excitability in surrounding neurons. Inflammatory mediators are also critically involved in several different cascades mediating cell death and neurogenesis as well as synaptic reorganization that are concomitant phenomena of the epileptogenic process in several animal models and human conditions including post-traumatic epilepsy [2,8]. The cytokines could decrease the seizure threshold in long-term possibly mediated by transcriptional activation of genes contributing to molecular and cellular plasticity [9]. Pharmacological targeting of these proinflammatory pathways using selective receptor antagonists or the use of transgenic mice with perturbed cell signalling demonstrated that activation of IL-1R and TLR 4 by endogenous IL-1β and HMGB1 is implicated in the precipitation and recurrence of experimentally induced seizures in rodents. This evidence highlights a new target system for pharmacological intervention to inhibit seizures by interfering with mechanisms involved in their genesis and recurrence. It had been observed that IL-1Ra which was a naturally occurring antagonist to IL-1β inhibited IL-1β expression in mice astrocytes and decreased seizures in mice. The anti-inflammatory cytokines were associated with reduction of neuronal cell loss, decreased microglia activation and less BBB leakage. Their proconvulsant activity is hypothesized to be mediated by increasing glutamatergic neurotransmission [16].

Conclusions. Thus our study confirms all abovementioned hypothesis done in experimental models that pro-inflammatory cytokines could have proconvulsive action. Although the level of VCAM-1, CCL2 and CCL3 were identically normal in all three groups the increased level of CCL 11 was found in blood serum of the subjects with intractable seizures supposing its role in epileptogenesis.

This finding is very important in clinical epileptology as increased expression of inflammatory cytokine in drug resistant epilepsy compared with controls and those subjects with controlled seizures could prove the leading role of inflammation in the mechanism of epileptic encephalopathy. Besides the elevated level of cytokines was correlated with repetition rate of seizures supposing that inflammation could affect on resistance of seizures to antiepileptic medication and could predict the outcome of seizure severity. This result could help pharmaceutical industry to consider anti-inflammatory drugs as an add on therapy for suppressing epileptogenesis. As an add on therapy they will improve the results achieved by antiepileptic drugs and could significantly decrease the resistance of seizures especially in epileptic encephalopathies when resistant seizures lead to physical and even more mental delay of affected children thereby worsening their quality of life.

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SUMMARY

INFLAMMATION IN CHILDHOOD EPILEPSY SYNDROMES

Bakhtadze S., Geladze N., Khachapuridze N.

Tbilisi State Medical University, Department of Paediatric Neurology, Georgia

Epilepsy is one of the most common neurological disorder affecting up to 1% of the world population. It is a heterogeneous disorder and includes genetic, structural, metabolic causes, sometimes reason is unknown. In recent 20 years inflammation has been considered as a possible etiologic factor in angiogenesis and epileptogenesis in experimental models but there is still lack of evidence if inflammation could be seen in clinical cases of children with different forms of epilepsy. Epileptic encephalopathies are the group of epilepsies when seizure itself can cause severe cognitive and behavioral abnormalities. Besides seizures occurring in epileptic encephalopathies prone to be highly resistant to medication. Thus any etiological factor contributing to epileptogenesis could have high clinical relevance in modern epileptology. The aim of our research was to study the pro-inflammatory cytokines in different forms of epilepsy in children.

We have assessed 56 children from 0-16 years of age. 20 were included in control group (Group 1), 20 children with resolved seizures were involved in study group (Group 2a) and 16 children with resistant seizures were identified as group 2b. The concentration of the following pro-inflammatory cytokines was assessed in blood serum: VCAM-1, CCL2, CCL3, CCL11 as well as a correlation between concentration and seizure repetition rate was also studied. All pro-inflammatory markers were within normal range in controls as well as in both study groups except CCL11. The concentration of CCL11 was elevated in group 2b. Thus we could hypothesize that inflammation could contribute to etiology of resistant epilepsies including epileptic encephalopathies. This evidence could serve as very significant information for pharmaceutical industry for future development of anti-inflammatory medicines as add on therapy with antiepileptic drugs for treatment of drug resistant epilepsies.

Keywords: Inflammation, seizures, resistant epilepsy, cytokines, epileptic encephalopathies.

РЕЗЮМЕ

ВОСПАЛЕНИЕ И ДЕТСКАЯ ЭПИЛЕПСИЯ

Бахтадзе С.З., Геладзе Н.М., Хачапуридзе Н.С.

Тбилисский государственный медицинский университет, департамент детской неврологии, Грузия

Эпилепсия является распространенным неврологическим заболеванием, которое отмечается у 1% населения мира. Этиология эпилепсии гетерогенна и содержит в себе генетические, структурные и метаболические причины, хотя в некоторых случаях ее патогенез неизвестен. По сей день ведутся активные исследования в плане рассмотрения воспаления как возможной причины развития эпилепсии. Исследованиями, проведенными на экспериментальных моделях, установлена роль воспаления в ангиогенезе и эпилептогенезе, хотя в клинической эпилептологии исследования в этом направлении крайне малочисленны. В педиатрической эпилептологии значительная доля приходится на эпилептические энцефалопатии, поскольку развившиеся судороги значительное негативное влияние оказывают не только на моторную функцию, но и когнитивное развитие. Терапия судорог, развившихся при эпилептической энцефалопатии, значительно затруднена, так как характеризуется высокой резистентностью к антиэпилептическому лечению. Отсюда следует, что все этиологические факторы, участвующие в развитии эпилептической энцефалопатии требуют тщательного изучения.

Целью исследования явилось определение роли проинфламаторных цитокинов при различных формах эпилепсии у детей.

Исследовано 56 детей с эпилепсией в возрасте от 0 до 16 лет, из них 20 детей - контрольная группа, 20 - с судорогами, купированными антиконвульсантами и 16 - с резистентной эпилепсией. В плазме крови исследована концентрация проинфламаторных цитокинов VCAM-1, CCL2, CCL3, CCL11 и определена связь их концентрации с частотой рецидивов судорог. Установлено, что как в контрольной, так и в группе с судорогами, купируемыми антиконвульсантами, концентрация всех перечисленных цитокинов находилась в пределах нормы. Хотя концентрация CCL11 в двух вышеперечисленных группах была нормальной, в группе детей с резистентной эпилепсией ее уровень был значительно повышен. Таким образом, наличие проинфламаторных цитокинов возможно рассматривать как значительные этиологические факторы в развитии резистентной эпилепсии, в том числе и эпилептических энцефалопатий. Вышеуказанное возможно станет основанием для разработки новой дополнительной терапии эпилепсии в комбинации с антиэпилептическими препаратами.

რეზიუმე

ანთება და ბავშვთა ასაკის ეპილეფსიები

ს.ბახტაძე, ნ.გელაძე, ნ.ხაჭაპურიძე

თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ბავშვთა ნევროლოგიის დეპარტამენტი, საქართველო

ეპილეფსია წარმოადგენს ერთ-ერთ ყველაზე გავრცელებულ ნევროლოგიურ დაავადებას, რომელიც

აღნიშნება მსოფლიო მოსახლეობის 1%-ს. მისი ეტიოლოგია ჰეტეროგენურია და მოიცავს გენეტიკურ, სტრუქტურულ და მეტაბოლურ მიზეზებს, თუმცა ზოგ შემთხვევებში მისი პათოგენეზი უცნობია. უკანასკნელი 20 წლის მანძილზე აქტიურად მიმდინარეობს კვლევები ანთების, როგორც ეპილეფსიის შესაძლო მექანიზმის განხილვის შესახებ. ჩატარებული ექსპერიმენტული მოდელებით დამტკიცდა ანთების როლი ანგიოგენეზსა და ეპილეპტოგენეზში, თუმცა კლინიკურ ეპილეპტოლოგიაში ამ თვალსაზრისით კვლევები საკმაოდ მცირეა. პედიატრიული ეპილეფტოლოგიის განსაკუთრებით მნიშვნელოვან ნაწილს წარმოადგენს ეპილეფსიური ენცეფალოპათიები, რადგან ამ დროს განვითარებული გულყრები მნიშვნელოვან ნეგატიურ ზეგავლენას ახდენს ბავშვის არა მარტო მოტორულ, არამედ კოგნიტურ განვითარებაზეც. გარდა ამისა, ეპილეფსიური ენცეფალოპათიების დროს აღმოცენებული გულყრების მართვა ძალზე ძნელია, რადგან ისინი ხასიათდება მაღალი რეზისტენტობით ანტიეპილეფსიური მკურნალობისადმი. შესაბამისად, ყველა ეტიოლოგიური ფაქტორი, რომელიც შესაძლოა მონაწილეობდეს ეპილეფსიური ენცეფალოპათიის განვითარებაში საჭიროებს მნიშვნელოვან შესწავლას.

კვლევის მიზანს წარმოადგენდა პროინფლამატორული ციტოკინების შესწავლა სხვადასხვა სახის ეპილეფსიის დროს ბავშვებში.

შესწავლილია 0-დან 16 წლამდე ასაკის 56 ბავშვი: 20 - საკონტროლო ჯგუფიდან, 20 - წამალდაქვემდებარებული გულყრებით, ხოლო 16 - რეზისტენტული ეპილეფსიით. სისხლის შრატში განისაზღვრა შემდეგი პროინფლამატორული ციტოკინების კონცენტრაცია: VCAM-1, CCL2, CCL3, CCL11 და მათი კონცენტრაციის კავშირი გულყრების განმეორების სიხშირესთან. დადგინდა, რომ როგორც საკონტროლო, ისე საკვლევი ჯგუფის იმ პირებში, რომელთაც აღნიშნებოდათ წამალდაქვემდებარებული გულყრები, ყველა მათგანის კონცენტრაცია იყო ნორმის ფარგლებში, ხოლო CCL11 იყო ნორმული კონცენტრაციით საკონტროლო ჯგუფსა და წამალდაქვემდებარებულ პირებში, მისი კონცენტრაცია კი მნიშვნელოვნად იყო მომატებული ბავშვებში რეზისტენტული ეპილეფსიით. შესაბამისად, პროინფლამატორული ციტოკინები შესაძლოა განვიხილოთ, როგორც მნიშვნელოვანი ეტიოლოგიური ფაქტორი რეზისტენტული ეპილეფსიების, მათ შორის ეპილეფსიური ენცეფალოპათიების დროს. აღნიშნული შესაძლოა გახდეს ახალი, დამხმარე თერაპიული საშუალებების შექმნის საფუძველი ანტიეპილეფსიურ პრეპარატებთან ერთად.

DISTRIBUTION OF STEM CELLS IN DIFFERENT THYROID LESIONS IN PATIENTS OF REPRODUCTIVE, MENOPAUSAL AND POST-MENOPAUSAL AGE

Muzashvili T., Gachechiladze M., Burkadze G.

Tbilisi State Medical University

Several lines of evidence show that cancer stem cells (CMC) play the major role in the progression and therapy resistance in various tumor types [1]. CSCs are characterised with the similarity to normal stem cells, including their ability for self-renewal and differentiation, which gives rise to heterogeneous cancer cells [1]. There are many different markers which are associated with CSCs, including CD44, which represents one of the important markers of CSCs. CD44 is the glycoprotein which is encoded by CD44 gene [2]. CD44 is widely distributed in normal adult and foetal tissues. In normal tissues CD44 regulates the hyaluronic metabolism, wound healing and keratinocyte proliferation [2]. In vitro studies also have shown that CD44 causes the increase of metastatic potential of different cell lines. However, the role of CD44 in the development of metastases in human malignancies is still under investigation [2]. In addition, there is less known about the distribution of CD44 in different types of inflammatory, premalignant and malignant lesions, including the lesions of thyroid gland.

Thyroid carcinoma represents the fifth most frequent cancer in the world [3]. The frequency of thyroid cancer is higher in women between 20-55 years old. Several studies indicate that oestrogen might play and important role in the development of thyroid cancer [4], from which papillary thyroid carcinoma (PTC) represents the most frequent subtype [5]. Frequently, PTC is found in association with Hashimoto's thyroiditis [6]. However, the causal link between Hashimoto's thyroiditis and PTC is not yet clear.

The aim of our study was to investigate the distribution of CSCs, marked by CD44 in different types of thyroid lesions, in different age groups, including reproductive, menopausal and post-menopausal women. In addition, we wanted to compare the expression of CD44 with other markers of malignancy, including proliferation marker – Ki67, apoptotic marker – Bcl2 and other markers such as CK19, CD56 and ER.

Material and methods. Study included 200 formalin-fixed and paraffin-embedded tissue material from the teaching, research and diagnostic laboratory of Tbilisi State Medical University. Study material was divided into following histopathological groups: normal thyroid gland (45 cases), Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFTP) (n=34), Hashimoto's thyroiditis (50 cases), classic papillary carcinoma (n=42), and the co-occurrence of Hashimoto's thyroiditis and papillary carcinoma (n=29). In addition, each group was divided into following three age groups: reproductive age (15-44 y), menopausal age (45-55 y) and post-menopausal age (>55 y) (according to WHO Women Health, Fact Sheet №334, Updated September 2013). The detailed distribution of patient numbers into each group is given in Table 1.