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Expression of Zinc transporter 8 in thyroid tissues from patient with immune and non-immune thyroid diseases

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INTRODUCTION

Zn homeostasis is regulated by ZnT (SLC30A gene family) and Zip (SLC39A gene family) zinc transporters.
* **Zinc transporter 8** is localized in insulin containing secretory granule membrane and transports zinc from the cytosol into the vesicles. **ZnT8** conteins six transmembrane domains and a histidine-rich loop between transmembrane domains 4 & 5, which is the putative zinc binding domain.
* **Human SLC30A8 gene** is located to **chromosome 8** at the position **q24.11** which contains 8 exons and encoding a 369 amino acid ZnT8 protein.
* **Zn** is important for the generation of proinflammatory cytokines: IL-6, TNF-α after LPS stimulations and development of T cells.
* **Zinc deficiency** induces thymic atrophy, lymphopenia, supression of cytolitic T cell responses & NK activity.
* **Zinc protects** pancreatic B-cells from cytokine-induced destruction, which is observed in patients with DT1 & DT2.

AIM

We studied the expression of ZnT8 transporter in thyroid tissues from patients with immune and non-immune thyroid diseases.

METHOD

The study was performed in thyroid tissues after thyroidectomy from patients with thyroid nodular goiter (n=12, mean age 16.5 years ± 4) and cases with Graves' disease (n=13, mean age 14.6 years±2.5)

Method: The ZnT8 expression protein was evaluated **using immunohistochemistry**. The specimens were paraffin embedded tissues, derived from the pediatric patients, who had thyroid nodular goiter or GD. The antibody against ZnT8 was goat polyclonal antibody (Santa Cruz Biotechnology USA; sc-98243). The antigen was retrieval was done using high pH (PTLink DAKO) and antibody was incubated in 4°C overnight in 1:50 dilution. The patients with pancreatitis were as a positive controls. The intensity and the proportion of stained cells were determined by examining the entire slide and section as: + (low staining intensity in less than 10% cells in the section); ++ (moderate staining intensity in 10-40% cells in the section); +++ (high and diffuse staining intensity in more than 50% cells in the section).

Protein extraction and Western Blot: The samples were homogenized in ice-cold RIPA (radioimmunoprecipitation assay) buffer containing protease and phosphatase inhibitors (Roche Diagnostics GmbH, Mannheim, Germany) for 1 min at 4°C. The homogenates were then incubated on the ice for 40 minutes. Next, homogenates were spin for 30 min at 10000 g at 4°C. After centrifugation the supernatant was collected and transferred into new eppendorf tubes. Total protein concentration was determined using BCA protein assay kit with BSA (bovine serum albumin) as a standard. Samples were boiled at 95°C for 10 minutes in sample buffer containing 2-mercaptoethanol. Protein (40 µg) was subjected to 10% SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred into nitrocellulose membranes. After blocking membranes in TTBS buffer (50 mM Tris-HCl, 130 mM NaCl and 0,05 % Tween-20) containing 5% nonfat dry milk for 90 min at room temperature membranes were incubated overnight at 4°C with the corresponding antibodies at a dilution of 1:500. Primary antibodies (Zn-T8 and GAPDH) were purchased from Santa Cruz Biotechnology. Thereafter the membranes were incubated with anti-rabbit IgG (for Zn-T8) and anti-mouse IgG (for GAPDH) horseradish peroxidase-conjugated secondary antibody (1:3000; Santa Cruz Biotechnology, USA). Immunoreactive protein bands were visualized by using an enhanced chemiluminescence substrate (Thermo Scientific, USA) and quantified by densitometry (Biorad, USA). Equal protein concentrations were loaded in each lane as confirmed by Ponceau staining on the blot membrane. Protein expression was normalized to GAPDH and expressed in arbitrary units.

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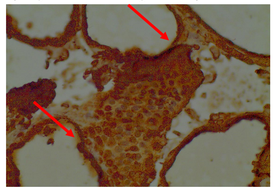
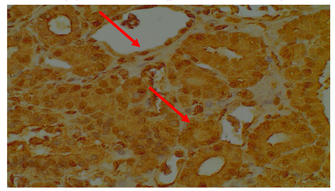
RESULTS

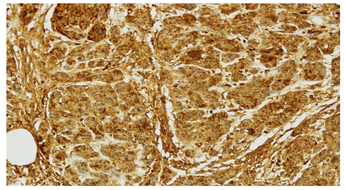
Fig. Identification of ZnT8 transporter in thyroid tissues by immunohistochemistry in patients with: a) thyroid nodular goiter, b) Graves' disease & c) pancreatitis (positive control)



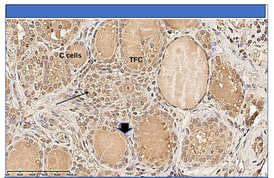
a) ZnT8 (++)



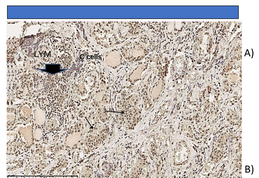
b) ZnT8 (+++)



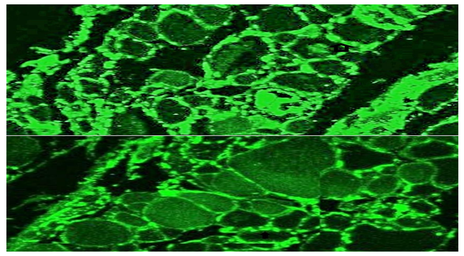
c) ZnT8 (+++)



ZnT8 expression within the cytoplasm and cytoplasmic membrane in follicular cells (thick arrow) and in C cells (membrane-cytoplasmic reaction) with proliferation features (thin arrow)



ZnT8 expression in the lymphoid cells nuclei (thick arrow) and in C cell groups (thin arrow)



Expression of ZnT8 in thyroid follicular cells from Graves' patient (a) and TNG (b) identified by IF

	Graves' disease	TNG	*p
Weight(kg)	54±5.28	58.2±7.8	p<0.01
Height (cm)	158.6±4.3	156±8	NS
fT4 (ng/dl)	1.9±0.63	1.2±0.46	p<0.01
fT3 (ng/dl)	5.78±0.5	3.2±0.38	p<0.01
TSH (µU/ml)	0.87±2.37	3.04±0.72	p<0.01
TRAb (U/l)	12.5±0.31	0.4±0.22	p<0.0001
anti-TGAb (IU/mL)	620.98±240.34	98.6±40.6	p<0.001
anti-TPOAb (IU/mL)	482.2±62.43	56±32.3	p<0.01
treatment	methimazole	L-thyroxine	

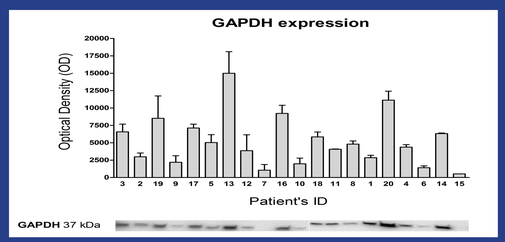


Fig 2a.Expression of GAPDH by WB

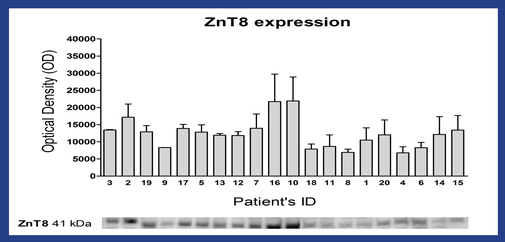


Fig 2b.Expression of ZnT8 in thyroid tissue by WB

CONCLUSIONS

1.Expression of ZnT8 transporter was identified by immunohistochemistry in the thyroid tissues from pediatric patients with Graves' disease (on +++) and nontoxic nodular goiter (on ++).

2. ZnT8 transporter expression was found both in thyroid follicular cells(within the cytoplasm and cytoplasmic membrane in follicular cells) and C cells (membrane-cytoplasmic reaction) in fluorescence.

3. Predominant expression of ZnT8 in band 41 kDa (cases:2,3,5,7,10,14,15,16,17,19) in immune than in non-immune (cases: 1,4,6,8,9,18) thyroid disorders (in Fig.2b) may suggest potential role of ZnT8 as a new thyroid autoanitgen but it requires further study on a larger cohort.