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ORIGINAL ARTICLE

The Thyroid Gland's Glycosylation: Aspect of Glycoprotein Function in Thyrocyte Physiology and Thyroid Disorders

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ABSTRACT

Objective: The focus of this study is on the role of glycoproteins in normal thyrocyte activity and pathologies of the thyroid.
Study Design: Observational study
Place and Duration: The study was carried at Pak International Medical College and Hospital from November 2021 to January 2022
Methods: There were 38 patients of both genders were presented in this study. Detailed demographics of enrolled cases included age, sex and body mass index were recorded after taking informed written consent. 19 patients had benign diffuse goiter in were included in group I and 19 (controls) patients were include in group II. Using a nonspecific proteomic strategy, such as this double difference (2D-DIGE) electrophoresis and lattice laser desorption/ionization time-of-flight masses spectrometry, we were able to determine which proteins were present in thyroid tissue (MALDI-TOF-MS).
Results: Among the 92 proteins significantly different between BDG and the control group, 47 were found to be elevated and 45 were found to be lowered (1.5-fold change, ANOVA, p 0.05). Bioinformatics analysis of Ingenuity Pathway Analysis has connected the dysregulation of ERK1/2, Glutathione peroxidase, and NADPH oxidase signaling pathways to tissue damage, endocrine system dysfunction, and cancer (IPA).
Conclusion: Low levels of thyroglobulin, dysregulated glycolysis, and an uptick in pro-oxidant peroxidase enzymes were found in the thyroid tissue proteome of patients with BDG. There was also a finding of dysregulation in the thyrocyte cytoskeletal proteins, redox proteins, and glycolytic pathways.
Keywords: Thyroid Gland's, Benign Diffuse Goiter, Proteins, Glycosylation

INTRODUCTION

Hormones secreted by the thyroid gland, specifically triiodothyronine (T3) and thyroxine (T4), play a critical role in controlling metabolic rate, cellular differentiation, and overall body size and growth [1]. The molecular mechanism in the thyroid follicles responsible for iodine uptake, concentration, oxidation, and integration into thyroglobulin (Tg) is unique [2, 3]. Tg is a structural protein that is released by thyrocytes into the thyroid colloid [3,]. It is required for the biosynthesis of thyroid hormones. Thyroid peroxidase, also known as thyroperoxidase (TPO), needs iron in order to function properly since the ectodomain of this enzyme includes a heme group [4]. TPO is a catalysis enzyme that accomplishes iodine oxidation, factors that causes of Tg, and the external synthesis of mono- and consumers are faced at the apical surface of thyrocytes. These processes all take place outside of the cell. T3 is a hybrid of MIT and DIT [3,5], while T4 is made up of two DITs that are connected to one another. Thyroxine (t4 (TSH) from the pituitary gland binds to the thyroid stimulating hormone receptor (TSHR) on the membrane of the follicular cells to regulate the generation of thyroid hormone [6]. Before becoming N-glycosylated, glycoproteins are unable to perform their functions as intended [7-9]. This is true for all of the thyroid-related proteins, including Tg, TPO, and TSHR, as well as the glycoproteins that make up these thyroid-related proteins (TSH).

Different from nodular goiters, which only affect select nodules, diffuse goiters impact the whole thyroid gland. There are a variety of clinical manifestations, including euthyroidism, hypothyroidism, and hyperthyroidism, as well as variations in goiter size and location. Simple nontoxic goiters, which can be either endemic or sporadic, are the most often observed kind of goiter in clinical practice and are characterized by the absence of inflammation and thyroid dysfunction. While endemic goiter brought on by an inadequate supply of iodine is largely to blame for the epidemic growth of the condition over the world, this is not true of every case of goiter. An increasing number of sporadic occurrences of goiter have been recorded due to the widespread presence of goitrogens and endocrine disruptors. [10] On the other side, an overabundance of iodine might stop the thyroid from reacting to TSH and potentially lead to the death of thyroid cells. [11]

Thyroid epithelial cells proliferate globally as a result of an iodine deficiency or other goitrogenic stimuli, resulting in the formation of a goiter. Thyroid follicular cells form a benign goiter when they overreact to the thyroid stimulating hormone in response to a stimulation that decreases their ability to generate TH (TSH). Inducing both proliferative and mitotic effects, TSH rise leads to increases in thyroid volume. [12] It is hypothesized that persistent goiters contribute to the formation of thyroid nodules through the differential activation of thyroid follicles. One percent of participants in the Framingham trial acquired a nodule per year, which equates to a 5- to 10-percent lifetime risk. There is a correlation between central obesity and the development of goiter and thyroid nodules, with the risk increasing by a factor of 1.6 [13]. Thyroid nodule growth is accelerated by hyperinsulinemia, which is typical in the obese population. Under these circumstances, the mitogenic activity of insulin in addition to the greater availability of incretin growth factor-1 may contribute to the development to cystic hyperplasia. In the past, we made use of MALDI imaging to locate peptides that showed significant variation between individuals with goiter who were underweight and those who were overweight. [14]

Proteomics, the high-throughput analysis of cellular protein content and the discovery of the underlying processes of biological activities, is one of the most important research fields in biology. Proteomic approaches based on mass spectrometry can characterize all the protein in a cell, organ, or biological organelle without bias. Proteome profiling is one use, while another is comparing disease-specific expression levels. Few studies have examined plasma proteome changes in people with hyperthyroidism and hyperthyroidism before to and during treatment, and even fewer have focused on the variations in these populations. However, a few studies have compared benign nodules and cancer [15] or thyroid tissue and tumors to see whether there are significant variations in respective proteomic profiles.

MATERIAL AND METHODS

This observation study was conducted at Pak International Medical College and Hospital from November 2021 to January 2022 and

comprised of 38 patients. Detailed demographics of enrolled cases included age, sex and body mass index were recorded after taking informed written consent.

Included patients were aged between 5-20 years. Patients who presented to the ambulatory endocrine breast complaining of benign goiter were matched for age and sex with individuals who were identified with BDG (n=19) in order to collect thyroid tissue at the moment of surgery. Each patient with BDG had a total thyroidectomy, and tissue samples were taken at that time. Our partnering surgeon obtained the control samples from patients who were having benign adenoma surgery on the healthy thyroid lobe. Thyroid tissue, totaling 100 milligrammes (mg), was removed and immediately frozen in liquid nitrogen for further study. After each patient had fasted for 10 hours, blood was drawn via venipuncture into plain tubes for standard metabolic assays and thyroid profile analysis. The analysis in this biochemical investigation was performed using a Siemens Healthcare -Dimension Xpand Plus [15] integrated auto analyzer. Using the non-linear dynamics statistical programme Progenesis Same Spots, a power analysis was performed to establish the appropriate sample size.

Liquid lysate (0.5 ml, pH 8.8, 30 mM) was used to homogenise thyroid tissue samples. Proteins were extracted in RIPA buffer (Tris-HCl, 7 M uria, 2 M anhydride, 2% CHAPS, and 1x protease mix) on ice using a computerized ULTRA TURRAX T25 homogenizer. The suspension was sonicated after being agitated for a hour at a room temperature. After dithiothreitol was added to the protein extracts, they were agitated at 4 degrees Celsius, 20,000 grammes, and for forty minutes (DTT, Fifty mM). Supernatants were cleaned according to manufacturer specifications using a 2D clean-up kit.

RESULTS

Among 38 presented cases, majority were males 25 (65.8%) and 13 (34.2%) were females. Mean age of the patients was 15.8±11.24 years and had mean BMI 26.02±8.41 kg/m^2 (table 1)

Table-1: Baseline characteristics of enrolled cases

Variables	Frequency	Percentage
Mean age (years)	15.8±11.24	
Mean BMI (kg/m^2)	26.02±8.41	
Gender		
Male	25	65.8
Female	13	34.2

We found no evidence of elevated thyroid antibodies or any other changes to the thyroid profile in any of our patients with benign diffuse goiter (BDG). Large hyperplastic thyroid follicles containing copious colloid were found histologically in samples taken after a complete thyroidectomy. (table 2)

Table-2: Patients with biochemical characteristics

Biochemical Characteristics	Group I	Group II
Urea (mmol/L)	5.8±6.37	5.2±4.17
Creatinine (mmol/L)	75.02±6.25	71.17±6.52
TSH (mIU/L)	1.3±5.25	1.8±5.11
FT4 (pmol/L)	13.8±11.9	16.7±6.34
Triglycerides(mmol/L)	1.9±5.22	1.4±6.25
LDL (mmol/L)	1.13±9.15	1.15±4.27
HDL (mmol/L)	3.5±6.41	4.7±7.22

In order to analyses gel images, we used Pyogenesis Similar Spots v3.3 (). Using AI, we identified 1150 spots on the gels; of these, 152 demonstrated statistical significance when comparing BDG to use across (ANOVA, P 0.05; fold-change 1.5). An exemplary 2D-DIGE overlay of Cy3/Cy5/Cy2 images and fluorescent protein patterns from BDG specimens labelled with Cy3, positive control marked with Cy5, a pooled internal regulatory labelled with Cy2, and so on is shown in Figure 1. Fifteen hundred and twenty-two spots on the gels showed at least a 1.5-fold difference between the positive control and the BDG specimens (ANOVA, P 0.05). (See Figure 2) Because they had the same spot patterns, all 14 picture gels could be compared and analyzed together. Cy2-labeled reference protein were used as a standard to ensure reproducible quantification across gel sets and for internal standardization. Proteins were isolated by cutting them from the 152 locations on the sample preparation gel where differences between the Overall accuracy and controls were statistically significant.

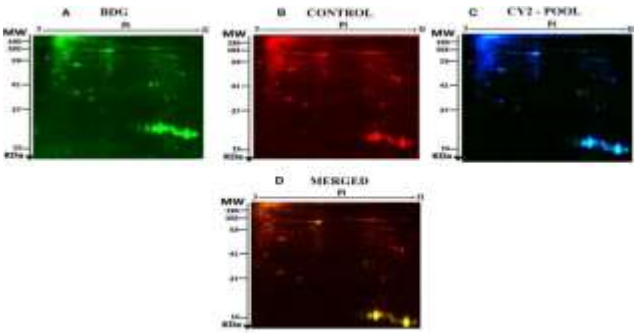

Figure-1: Tissue samples from individuals with BDG and labelled with Cy3 to produce a fluorescent protein profile for a 2D-DIGE

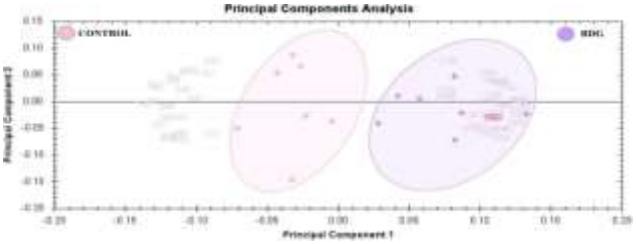

Figure-2: PCA plots the two principal components. They explained 82% of the spot's variability. Gels are represented by colorful dots and numbers.

As with LH, FSH, and hCG, TSH is a glycoprotein hormone [30]. In contrast to the common chain found in other glycoprotein hormones, the TSH component is exclusive to TSH. These TSH subunit genes (and their counterparts) are located on chromosomes 6 and 1, respectively. Activating TSHR, TSH prompts thyroid cells to produce thyroid hormones. Glycosylation accounts about 15-25% of the weight of human TSH, which is a 28-30 kDa glycoprotein. In hTSH, you'll find three potential N-glycosylation sites. Two in, one out (Asn23). Complex N-glycans like these oligosaccharides. (Figure 3)

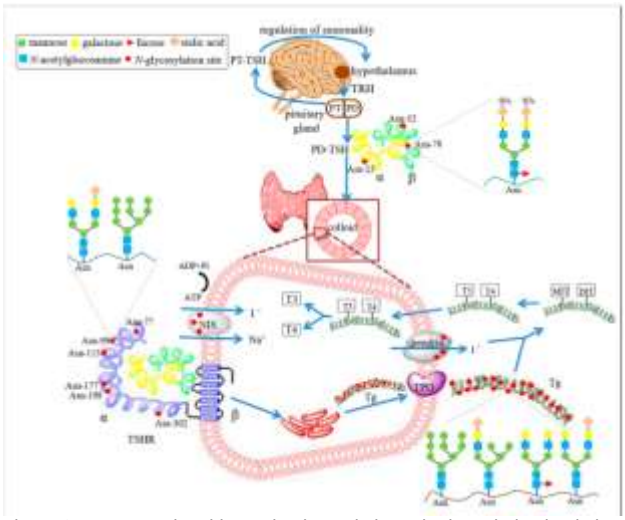

Figure-3: Important thyroid protein glycosylation. The hypothalamic-pituitary-thyroid axis controls the production of thyroid hormones.

The pituitary gland's thyroid-stimulating hormone (TSH) and thyrocytes' TSH receptors may both include N-glycans. The protein thyroglobulin (Tg) may be N-glycosylated at up to 20 different locations. Tg is delivered by N-oligosaccharides to both the follicular lumen (where thyroid hormones are synthesized) and the thyrocytes (where hyposialylated Tg is degraded). The N-glycans on sodium/iodide cotransporter and pendrin are necessary for iodide transport. Abnormalities in glycosylation lead to thyroid dysfunction as well as a slower clearance rate for hormones. Changes in glycan structures have been linked to the development of thyroid carcinoma and autoimmune diseases. Thyroid cancer is associated with alterations in a number of glycan modifications, including sialylation and fucosylation, 1,6-branching, poly-LacNAc chain abundance and structure, and O-GlcNAcylation. Thyroid autoimmunity is linked to alterations in sialylation and fucosylation. (figure 3)

DISCUSSION

Differential protein expression was found between BDG and normal thyroid tissue in a proteomic analysis. Thyroid enlargement in the absence of nodules and hyperthyroidism is known as a diffuse nontoxic goiter. It is called a "simple goiter" or a "colloid goitre" since there are no nodules present. [16] By expressing itself in a variety of proteins, BDG tissues provide insight into its possible pathogenesis. Our results demonstrate that BDG tissues include higher levels of chaperone proteins, which control protein folding and glycosylation, and proteins that improve thyrocyte redox state. This is borne out in the pathway analysis that has been conducted. In the IPA analysis, NADPH oxidase, glutathione peroxidase, and ERK1/2 signalling were the most centrally linked nodes. Additionally, thyrocyte cytoskeletal proteins and glycolysis were revealed to be dysregulated. [17]

Proteomic examination of thyroid tissue from BDG patients revealed an increased number of enzymes involved in regulating the redox status of thyrocytes compared to thyroid tissue from control patients. Superoxide dismutase (SOD), paraoxonase (RAB14), peroxiredoxins (1-3), and peroxiredoxin (1-4). (SOD). The manufacture of thyroid hormone (TH) requires the enzyme thyroid peroxidase, which acts as a catalyst the oxidizing of iodide, factors that causes of tyrosine's, and binding of iodothyronines to tyrosine hydroxylase (Tg). [18] H2O2 is produced in the thyroid due to the cooperation of nitric oxide synthase and superoxide dismutase. In our studies, superoxide was reduced to hydrogen peroxide by a reaction catalyzed by superoxide dismutase (Cu-Zn), commonly known as dismutase 1 (SOD1). [19] Patients with BDG may have increased levels of this enzyme because their thyrocytes are making an attempt to increase TH synthesis. There is substantial evidence that Son of sevenless homologue 2 (SOS2) and RAB14 interact with the NOX system and raise intracellular levels of H2O2 and ROS. In cell signaling, the guanine nucleotide interaction factor SOS2 increases Ras-GTPases, which directly trigger NOX enzymes. Uncertainty still [20] about how Ras controls ROS.

Both hypothyroidism and hyperthyroidism are thyroid diseases caused by hormonal imbalances. Problems in the hypothalamic, pituitary, or peripheral may be to fault in exceedingly rare cases. Thyroid autoimmune disorders such as Thyroid disease thyroiditis and Grave' disease are common. Auto immune disease (AITD) is characterized by an immune response to the thyroid's three primary antigen (Tg, TSHR, or TPO) [22]. Anti-Tg IgG is N-glycosylated, which is one of the few effects of AITD glycosylation. In individuals with HT, increased anti-Tg serum are the most obvious sign of AITD. Anti-Tg levels in people with HT, GD, and PTC were evaluated for N-glycosylation using enzyme-linked immunosorbent assay lectin assays. Hashimoto's anti-Tg had the lowest fucosylation level. Both AITD and PTC had similar levels of galactosylation and SA content in their anti-Tg IgG [23]. More Man, final SA, central Fuc, and Gal1,4GlcNAc1,2Man glycoprotein were found in anti-Tg IgG from HT patients than in IgG from healthy persons [24]. Recent data analysis of three European cohorts revealed that patients with AITD tended to have lower concentrations of IgG core advanced state. Anti-TPO levels correlated inversely with the degree in which the IgG component was fucosylated. Using a things (UEA I) unique for 1,2-linked Fuc, lower amounts of Fuc were found in the PBMCs of HT patients. There are no shared ancestors between fuc. 1 and AITD [25].

The glycosylation pattern of hTSH in the sera of patients with hypothyroidism differs from that of healthy donors. Increased levels of terminal Gal and SA in TSH are seen in both subclinical and overt primary hypothyroidisms. The blood TSH levels of individuals with asymptomatic and overt hypothyroidism were shown to be raised following treatment with pharmacological dosages of thyrotropin-releasing hormones (TRH) [26]. But neither the sialylated nor the terminally galactosylated variants of TSH showed any change in concentration in response to TRH therapy. Analysis [27] showed that TSH glycan core fucosylation was decreased in the serum of hypothyroid patients compared to healthy donors. Final SA and Gal were found to be elevated to abnormally high levels in individuals with resistance to tsh T3, a rare inherited illness induced by a defect of the tsh receptor (TR1). Because of a lack of the T3-binding region of TR1, hypothyroidism develops when thyroid hormone is unable to connect with the TR1 receptor. Thyroid hormones, namely T3 and T4, regulate these processes, along with growth, metabolism, and development. Among the more than 2,000 genes found in the liver, T3 has been proven to both positively and negatively control 55 genes. Thyroid hormone treatment in an animal model of hypothyroidism resulted in the downregulation of 41 liver gene, including those producing sialyl transferases 2,6 and 2,3[28].

CONCLUSION

Low levels of thyroglobulin, dysregulated glycolysis, and an uptick in pro-oxidant peroxidase enzymes were found in the thyroid tissue proteome of patients with BDG. There was also a finding of dysregulation in the thyrocyte cytoskeletal proteins, redox proteins, and glycolytic pathways.

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