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Histological Changes of Thyroid Tissues in Patients with Liver Cirrhosis

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Abstract. Alterations in thyroid hormone regulation and metabolism, such as low serum T3 and T4 with normal TSH, are frequently noted in liver cirrhosis, but few morphological studies have ever been done on thyroid tissue in liver cirrhosis. In this study we analyzed the histological changes of thyroid tissue in the patients with liver cirrhosis. Specimens of thyroid gland obtained from autopsies in 16 cirrhotic patients were examined, and compared with those of two control groups. Control group I consisted of 7 patients with diabetes mellitus and control group II, 12 patients who died from sudden onset of cardiovascular problems. We measured follicular diameter and epithelial width on light micrographs of the central portion of thyroid specimens. We graded the degree of colloid vacuole, lipofuscin deposition in follicular epithelia, regenerative reaction and perivascular fibrosis in 10 consecutive light microscopic fields of the specimen. In the cirrhotic group, mean follicular diameter and epithelial width were significantly shorter and thinner, respectively, than those in the two control groups. Perivascular fibrosis was more prominent in the cirrhosis group than in the controls. These findings suggest that thyroid glands in patients with liver cirrhosis have the characteristic features of hypoactivity.

Key Words: Thyroid tissues, Liver cirrhosis, Lipofuscin, Fibrosis

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ALTERATIONS in thyroid hormone regulation and metabolism are frequently observed in patients with liver cirrhosis, with the decreased serum T3 concentration probably being due to impaired conversion of T4 to T3 in liver [1]. In severe cases, low T4 concentrations are also observed [2]. Serum TSH levels are usually within the normal range or slightly elevated [1, 2], while circadian rhythm of serum TSH may be altered. TSH surge, which normally occurs at midnight in healthy subjects, is absent [3] or found in the late afternoon [4] in these patients.

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Hegedus [5] found a significant reduction in thyroid volume measured by ultrasonography in patients with alcoholic liver cirrhosis compared to healthy controls. Since patients with non-alcoholic liver cirrhosis had normal thyroid volume [6], Hegedus postulated the direct effect of alcohol on the thyroid gland. In contrast, Bianchi *et al.* [7] reported that thyroid volume in 118 patients with liver cirrhosis was significantly increased compared to that of 48 control subjects. In their study, no significant difference in thyroid volume was found in terms of the severity of liver dysfunction or the etiology of liver cirrhosis.

No detailed studies of the morphological changes of thyroid tissues associated with liver cirrhosis have been reported. To our knowledge, there is only one report which describes prominent fibrotic changes in thyroid glands in patients with alcoholic liver cirrho-

sis [6]. We examined the thyroid tissues of 16 patients with liver cirrhosis and found the characteristic features of atrophic follicular changes with prominent fibrosis of the thyroid gland.

Materials and Methods

Thyroid glands

Specimens of thyroid glands obtained from autopsies in 16 male patients with liver cirrhosis (mean age 56±14 years, mean±SD) were examined, and compared with those of two control groups. Control group I consisted of 7 male patients with diabetes mellitus (59±11 years) and group II, 12 patients who died from sudden onset of cardiovascular problems (61±15 years, 10 males and 2 females). Subjects who had definite thyroid diseases such as multinodular goiter, chronic thyroiditis and carcinoma were excluded from the study. None of them had history of minocycline treatment. Liver cirrhosis was diagnosed based on clinical findings and histological examinations of postmortem liver specimens. Etiology of liver cirrhosis included viral hepatitis in 11 patients (5 with type B and 6 with type C), alcohol in 3 and unknown in 2 patients. Histological examination ruled out the diagnosis of primary biliary cirrhosis in these 2 patients. Duration of liver cirrhosis was from 4 to 16 years. Twelve of 16 patients had hepatocellular carcinoma (HCC). The HCC patients were treated with transarterial embolization and anti-cancer drug administration in hepatic artery, but did not receive systemic administration of anti-cancer drugs. In the control group I with diabetes mellitus, 4 patients died from cerebrovascular disease, 2 from pneumonia and one from acute myocardial infarction. Duration of diabetes mellitus was from 14 to 30 years. Histological features of liver specimens in the diabetic patients included mild atrophy, mild fatty change, congestion and mild necrosis in one person, respectively. None of them revealed liver fibrosis. In control group II with cardiovascular disease, 6 patients died of rupture of aortic aneurysm, 4 from acute myocardial infarction and one from subarachnoidal hemorrhage. One patient was dead on arrival due to unknown cause. Duration of cardiovascular diseases varied from a few hours to several months. Age distribution of patients was not significantly different among the groups (Table 1).

Thyroid tissues were obtained at autopsy, fixed in 10% formalin, prepared in 3- to 5-μm paraffin sections and stained with hematoxylin-eosin for morphological analyses. Schmorl's staining [8] and immunostaining with anti-Ki67 antibody (Immunotech) and anti-thyroglobulin antibody [9] were also performed to detect lipofuscin deposition, regenerative follicular cells and thyroglobulin content, respectively.

Morphological analyses of every thyroid specimen were performed by two persons, a pathologist and a physician, in blind fashion to avoid any preconception.

Measurement of thyroid follicle diameter

Light micrographs of thyroid specimens were obtained at a final magnification of ×200. Central portions of the thyroid tissues were investigated and were assumed to be representative of the whole thyroid gland [10]. Maximal diameters of 50 follicles were measured on 5 to 8 light micrographs. Mean values±SD were obtained for every thyroid specimen.

Measurement of epithelial cell width

Thyroid specimens were examined by light micrography at a final magnification of ×830. Central portions of the thyroid tissues were used for the study. Average widths of three epithelia were measured in one follicle and the mean values were obtained by analyzing 30 follicles using 5 to 8 light micrographs in every specimen.

Colloid vacuoles

Colloid vacuoles in the periphery of the follicular lumen showed micropinocytosis. The degree of colloid vacuoles was evaluated in 10 consecutive fields on the light micrographs at a magnification of ×25. Their grading was defined as follows: grade 1, scarce (detected in less than 25% of total follicles examined); grade 2, a few (25 to 50%); grade 3, moderate (50 to 75%); grade 4, many (more than 75%).

Lipofuscin granules

Many brown granules of irregular shapes and various sizes were observed in the cytoplasm of the follicular epithelial cells. These granules stained positive for Schmorl reaction (Fig. 1), which is a specific histochemical identification for lipofuscin pigments [8, 11]. Lipofuscin deposit was evaluated in 10 consecutive light micrographic fields at a magnification of $\times 50$. The grading of lipofuscin deposit was defined as follows: grade 1, scarce (detected in less than 25% of total epithelia examined); grade 2, a few (25 to 50%); grade 3, moderate (50 to 75%); grade 4, many (more than 75%).

Regenerative thyroid follicular cells

In some cases, follicular cells with clear cytoplasm and large nucleus formed small follicles (Fig. 2A). These cells were often positive for Ki67 antigen (Fig. 2B), a human nuclear antigen expressed in proliferating cells [12]. To detect Ki67 antigen, a monoclonal antibody Ki67 which is of mouse IgG1 subclass raised against a human recombinant Ki67 fragment (Immunotech, Marseilles, France), was used [9]. After boiling the paraffin-embedded tissue sections in citric acid buffer (0.01 mol, pH 6.0) for 15 min in a microwave oven for antigen retrieval, the sections were immunostained with the antibody by a labeled streptavidin biotin (LSAB) technique [13, 14]. The cells regarded as regenerative were counted in 10 consecutive fields at a magnification of $\times 50$. The grading system for regenerative follicular cells was as follows: grade 1, scarce (less than 5% in total follicular cells examined); grade 2, a few (5 to 10%); grade 3, moderate (10 to 15%); grade 4, many (more than 15%). Fig. 2A shows representative examples of grade 4 specimens.

Fibrosis

Fibrous tissues were examined in 10 consecutive light micrographic fields at a magnification of $\times 10$. The grading of fibrosis was defined as follows: grade 1, no fibrous bands; grade 2, localized around the vessels; grade 3, elongated from perivascular area to surrounding follicles; grade 4, fibrous bands separating follicles into small nodules. Figs. 3A and B show representative examples of grades 1 and 4, respectively.

Immunostaining with anti-thyroglobulin antibody

Paraffin-embedded tissue sections were immunostained with prediluted anti-thyroglobulin rabbit antibody (Dako, Kyoto, Japan) using LSAB technique.

Table 1. Histological parameters of the thyroid tissue from patients with liver cirrhosis, diabetes mellitus and cardiovascular diseases.

Group	Liver cirrhosis	Diabetes mellitus (Control group I)	Cardiovascular disease (Control group II)
Number	16	7	12
Age (years)	56±14	59±11	61±15
Follicular diameter (μm)[1)]	122±26[bc]	195±44	159±44
Epithelial width (μm)[2)]	2.4±0.5[bd]	3.1±0.5	3.1±0.7
Colloid vacuole[3)]	2.1±0.6	1.6±0.5	2.1±0.8
Lipofuscin granule[3)]	2.7±0.8[c]	2.9±0.9[c]	2.0±0.6
Regenerative cell[3)]	1.9±0.9[a]	2.9±0.7	2.6±1.4
Fibrosis[3)]	3.0±1.0[ad]	1.9±0.7	1.6±0.9

1) mean value±SD for maximal diameter of 50 follicles
2) mean value±SD for epithelial width of 30 follicles
3) mean value±SD for grading; see Materials and Methods
a: $p<0.05$ vs control group I
b: $p<0.01$ vs control group I
c: $p<0.05$ vs control group II
d: $p<0.01$ vs control group II

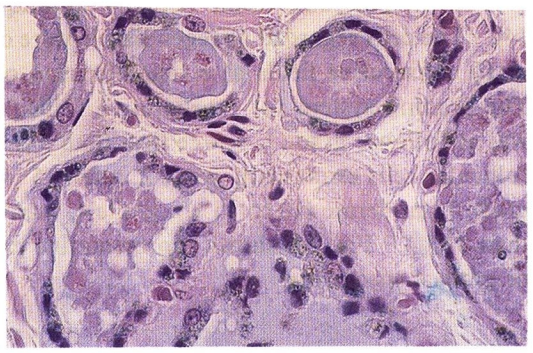

Fig. 1

Fig. 1. Lipofuscin deposits. Numerous brown granules are seen in the cytoplasm of follicular epithelia. The granules were verified as lipofuscin deposits by Schmorl's staining (×100).

Fig. 2. Regenerative cells. A: Many regenerative small follicles composed of cuboidal epithelia having light cytoplasm and large euchromatic nuclei (Grade 4) are seen. H.E. staining (×50). B: These cells are positively immunostained with anti-Ki67 antibody, which is a marker of proliferating cells (×100).

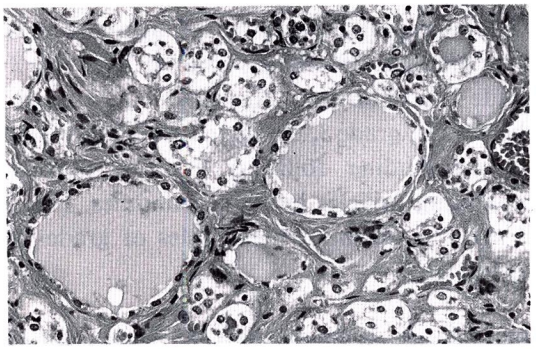

Fig. 2A

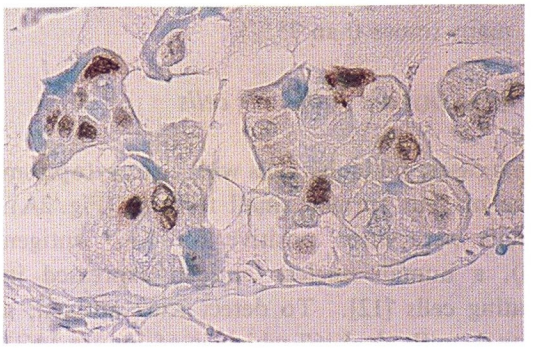

Fig. 2B

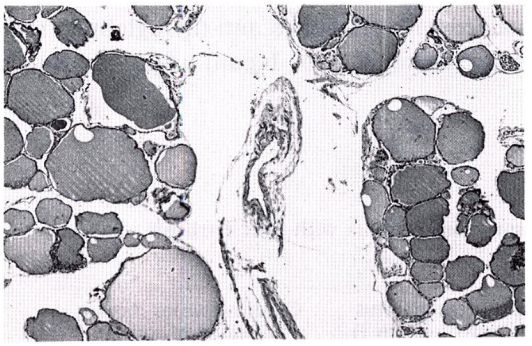

Fig. 3A

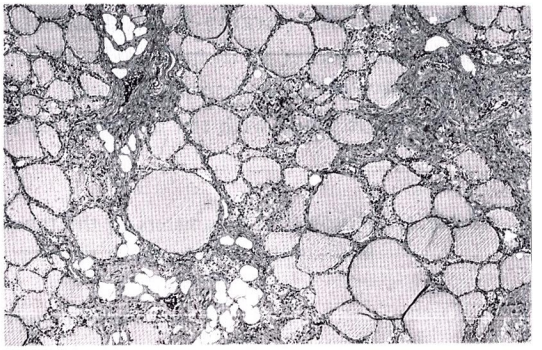

Fig. 3B

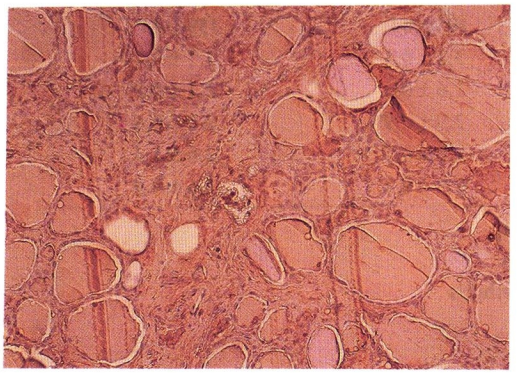

Fig. 5A

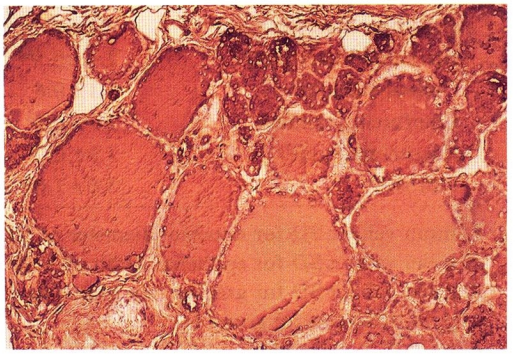

Fig. 5B

Statistical analysis

Statistical differences in all parameters between the cirrhosis group and each control group were evaluated by Mann-Whitney's U test. Differences in etiologic subgroups of liver cirrhosis and those with or without HCC were assessed by Kruskal-Wallis test and Mann-Whitney's U test, respectively. The difference in the grade of lipofuscin deposit was also assessed between control group I and II by Mann-Whitney's U test.

Results

The histological changes of thyroid tissues are summarized in Table 1 and Fig. 4. The mean values for maximal diameters of 50 follicles and for epithelial widths of 30 follicles were significantly smaller and thinner, respectively, in the cirrhosis group than in the control groups. In most of these relatively small follicles with thin epithelia, follicular lumen and epithelia showed faint reactivity to anti-thyroglobulin immunostaining (Fig. 5A). Although the grade of colloid vacuoles was similar (Fig. 4A), peri-

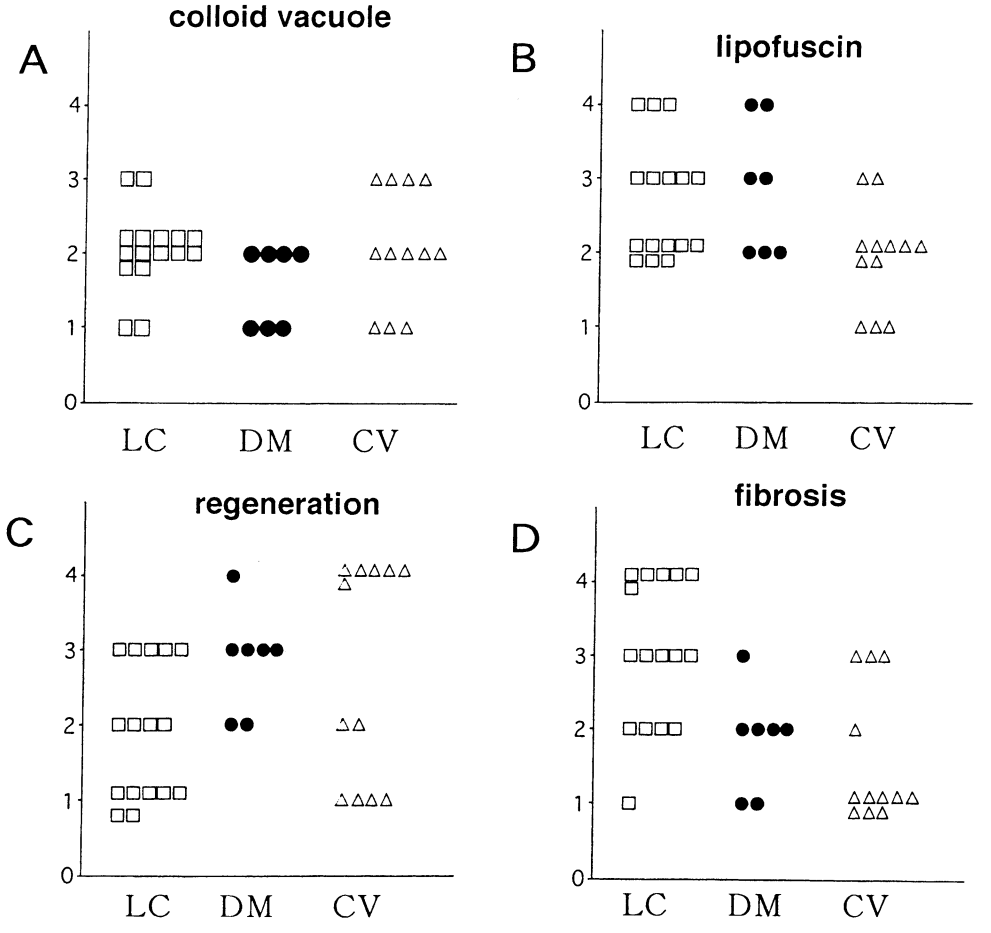

Fig. 4. Grading for colloid vacuoles (panel A), lipofuscin deposit (panel B), regenerative cells (panel C) and fibrosis (panel D) in liver cirrhosis group (LC), control group I of diabetes mellitus (DM) and control group II of cardiovascular diseases (CV) (see Materials and Methods and Table 1).

Fig. 3. Fibrosis. H.E. staining (×10). A: No increase in fibrous components is seen around the blood vessel (Grade 1). B: Thick fibrous bands around the vessels extend to the interfollicular area showing multiple small nodules (Grade 4).

Fig. 5. Thyroglobulin immunostaining. A: Small follicles with thin epithelia are scarcely immunostained with anti-thyroglobulin antibody. In a few follicles only follicular lumen are weakly stained. B: Regenerative follicles showed strong reactivity to anti-thyroglobulin antibody in both follicular lumen and epithelial cells (×25).

vascular fibrosis was more prominent (Fig. 4D) in the cirrhosis group compared with those of the two control groups. Fibrous tissues extended from the perivascular to the inter follicular area, forming small nodular structures in the cirrhosis group. In one cirrhotic patient with no increase in thyroid fibrosis, the duration of cirrhosis was 4 years, which was relatively short compared to 7 to 16 years in other cirrhotic patients. Although fibrosis was prominent, no inflammatory cells or fibroblasts were detected in the thyroid tissues in liver cirrhosis. As for the lipofuscin deposition, the grade of the cirrhosis group was significantly higher than that of the control group II, but was not significantly different from that of control group I (Fig. 4B). In the cirrhosis group, the regenerative follicles showed strong reactivity to anti-thyroglobulin immunostaining (Fig. 5B), and were significantly reduced compared to control group I, and tended to be more reduced than that in the control group II (Fig. 4C).

No correlation was observed between the ages of the patients and the morphological changes of thyroid tissues which included follicle diameter, epithelial width, grade of colloid vacuole, lipofuscin deposit, number of regenerative cells and fibrosis (data not shown). Etiologic subtypes of liver cirrhosis did not seem to affect the morphological changes of thyroid tissues. The coexistence of HCC did not affect differences in thyroid tissues.

Discussion

In the present study, we observed the small follicles and thin epithelia in thyroid tissues of patients with liver cirrhosis. Although the size of thyroid follicles and width of epithelia are dependent on TSH [15], the small thyroid follicles and thin epithelia in cirrhosis group did not appear to be caused by thyrotropin dysfunction. During TSH stimulation, small follicular lumina with tall epithelial cells are observed, while TSH deficiency induces large lumina with low cuboidal or flat epithelial cells [15]. The changes in thyroid tissues of cirrhotic patients were different from "cold" follicles found in aging mouse and adult marmoset thyroid. "Cold" follicles have large lumina with flat epithelia and fail to iodinate thyroglobulin because of impaired endocytosis [16–19].

Compared to the diabetes mellitus control group with similar long duration of disease, the liver cirrhosis group showed a reduced number of regenerative follicular cells and more prominent perivascular fibrosis. Since no fibroblasts or inflammatory cells were observed in the thyroid tissues, the depleted follicles might gradually be replaced with fibrous connective tissues, instead of increased fibrogenesis.

Lipofuscin granules in the thyroid gland has been reported in adult marmoset [17], aged mice [20], an autopsy of a 73-year-old man [21], and patients with chronic minocycline administration [22]. Lipofuscin, which has been shown to arise from peroxidative destruction of polyunsaturated lipid membranes, is considered to be derived from lysosomes [23]. In most tissues, lysosomes hydrolyze degenerating materials in the cell. In the thyroid gland, lysosomes are important for the hydrolysis of thyroglobulin releasing thyroid hormones. Lipofuscin in the thyroid gland is considered to be deposits of secondary lysosomes produced by reabsorbed colloid droplets fused with primary lysosomes, or a lysosomal residual body derived from autolysosomes [23]. However, there is no report concerning lipofuscin deposits in the thyroid gland in the cases with nonthyroidal illness. The prominent lipofuscin deposit in follicular epithelia in the liver cirrhosis group would suggest an impaired lysosomal metabolism in the epithelia and a reduced proteolytic process of thyroglobulin. Since lipofuscin deposits were also observed in the diabetic control group, it is possible that lysosomal metabolism is impaired in so-called chronic diseases such as liver cirrhosis or diabetes mellitus.

The morphological findings in this study suggest that thyroid glands in liver cirrhosis are hypoactive, with poor regeneration and prominent fibrosis. Since we did not obtain similar findings in the thyroid tissues of long-time diabetes patients, the morphological features observed in liver cirrhosis seem not to be nonspecific changes caused by the chronic illness. However, atrophic changes in liver cirrhosis may not be thyroid specific, since we observed disappearance of the lipid-filled zona fasciculata and smaller diameter of adrenal cortex in the cirrhosis group as compared with the other groups [data not shown]. Although 12 of 16 patients had HCC, none received systemic anti-cancer drug administration, hence the direct influence of anti-cancer drugs to thyroid tissues can be ruled out.

Follicular cell proliferation is modulated by various growth factors including insulin-like growth factors (IGF) and transforming growth factor (TGF)-β [24]. IGF-1 stimulates the growth of thyroid cells and enhances TSH effects [24]. The liver is the major source of circulating IGF-I and its serum concentration decreases with the progress of liver cirrhosis [25]. TGF-β, on the other hand, inhibits thyroid cell function and proliferation [26] and is important for fibrogenesis in the liver [27]. High serum concentrations of TGF-β have been reported in cirrhotic patients [28]. Although we did not measure serum IGF-I and TGF-β concentrations in cirrhotic patients, it is conceivable that decreased IGF-I and increased TGF-β suppress follicular cell proliferation and regeneration. In addition, sustained hypoxia, frequently seen in liver cirrhosis [29, 30], might also affect thyroid tissues. In summary, we found the characteristic morphological features of hypoactivity in thyroid tissues in patients with liver cirrhosis.

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