

CASE REPORT

Postmortem Diagnosis of Anorexia Nervosa : An Endocrinological and Immunohistochemical Approach

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Abstract : A female in her 30s was found dead after a fire. She was severely emaciated and had been diagnosed with anorexia nervosa (AN) about 5 years ago, but had not been treated recently. Therefore, we investigated not only her cause of death but also her condition of AN. Some of her organs weighed less than normal although no clear lesions were observed. In the pituitary gland, the number of follicle-stimulating hormone-immunopositive cells was markedly decreased although a normal number of thyroid-stimulating hormone-positive cells were detected. A histological examination of the ovary suggested that she had been suffering from amenorrhea. The thyroid gland was atrophic, and marked variations in follicle size were observed. Because we could not obtain enough volume of her blood for endocrinological examinations, we tried to investigate her endocrinological condition by immunohistochemistry. Immunohistochemical staining detected decreased triiodothyronine immunoreactivity and normal thyroxine immunoreactivity. The adrenal glands were also atrophic. Based on these findings, it was considered that she had been suffering from AN at the time of her death. The autopsy and other findings revealed that she had died of burning with carbon monoxide intoxication. *J. Med. Invest.* 63 : 305-309, August, 2016

Keywords : anorexia nervosa, endocrine disturbance, immunohistochemistry, postmortem diagnosis, forensic pathology

1. INTRODUCTION

Anorexia nervosa (AN) is the one of main types of eating disorder. The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, (DSM-V) defined the diagnostic criteria for AN as follows : 1) energy intake restriction relative to the appropriate nutritional requirements, leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health ; 2) an intense fear of gaining weight or becoming fat, even at a significantly low weight ; 3) a disturbance in the way in which one's body weight or shape is experienced, an undue influence of body weight or shape on self-evaluation, or a persistent lack of recognition of the seriousness of one's current low body weight (1).

Here, we report a forensic autopsy case of AN, in which a body was found after a fire, together with an investigation of the pathological findings. The aim of this study was to confirm that the subject was suffering from AN at the time of her death.

2. CASE REPORT

A fire broke out a house early in the morning in December, and a female in her 30s was found dead after the fire.

Medical history : She had been emotionally unstable for 7 years after delivering her first child and had been diagnosed with AN 5 years ago. However, she had not been treated for AN within the past 6 months and had refused to consume anything except yogurt and liquids.

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2.1 Autopsy findings

The subject was severely emaciated (height : 158 cm ; weight : 27 kg, body mass index : 10.8), and her head and face were covered in burns. Her left limbs and the left side of her trunk were also burnt (Fig. 1a). Apart from the burn injuries, no other obvious wounds were observed on her body. There was a large amount of soot in her trachea and bronchus (Fig. 1b). A small amount of soot was also found in the esophagus and stomach. No thermal damage was seen in the laryngopharynx or glottis. Her heart was significantly small, weight ; 126 g, brain and other organs had no obvious lesions. However, various organs weighed less than normal. In particular, the adrenal glands, uterus, and ovaries had shrunk markedly (Table 1) (2). Her stomach was empty, and a small amount of a gray-yellow substance was found in the intestines.

Toxicological examinations : The saturation level of carbon monoxide (CO)-hemoglobin in her left cardiac blood was 82%. No other drugs or substances such as ethanol were detected.

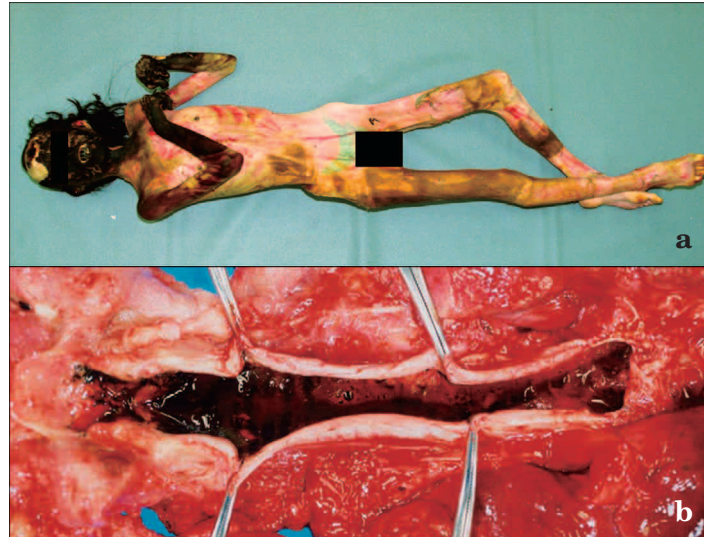
Endocrinological examinations : Unfortunately, we could not obtain enough volume of her blood for examination.

2.2 Histopathological findings

Tissue samples were fixed with phosphate-buffered formalin, embedded in paraffin, and sectioned at 5 µm. The sections were stained with conventional stains such as hematoxylin-eosin (HE).

The myocardial fibers in her heart had thinned (Fig. 2). No marked reduction in cell number or changes in cell structure were seen in the pituitary gland (Fig. 3a). A histological examination of the ovaries did not reveal any vesicular ovarian follicles or second ovarian follicles. A corpus luteum and a few primary ovarian follicles were observed. Marked variations in follicle size were observed in the thyroid (Fig. 4a). The adrenal gland seemed to be morphologically normal.

No significant changes were seen in her brain. The lungs, spleen, liver, and kidneys were congested.

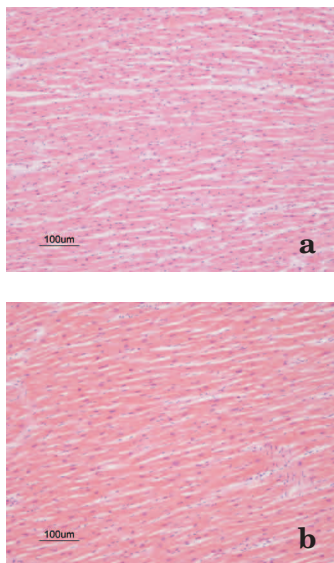


a ; outer view of the burn injuries, b ; the soot in the subject's airway

Fig. 1 Macroscopic findings of the cadaver
a : outer view of the burn injuries, b : the soot in her airway

Table 1 The weights of the subject's organs (comparison with the mean weights of normal Japanese organs)

Organ	Weight	Mean ± SD	Organ	Weight	Mean ± SD	
Heart	126	277 ± 60	Pituitary gland	0.48	0.61 ± 0.20	
Lung	left	465	397 ± 133	Thyroid	7.8	15.3 ± 4.9
	right	300	459 ± 146	Adrenal gland		
Kidney	left	52	133 ± 28	left	2.7	5.8 ± 1.8
	right	62	126 ± 28	right	3.3	5.2 ± 1.5
Liver	680	1343 ± 326	Uterus with adnexa	33	124 ± 44	
Pancreas	29	93 ± 27	Spleen	29	96 ± 44	
			Thymus	2.7	26 ± 13	



a ; this case.
b ; 38 y.o., female. The cause of death was drowning. The myocardial fibers had thinned in this case.

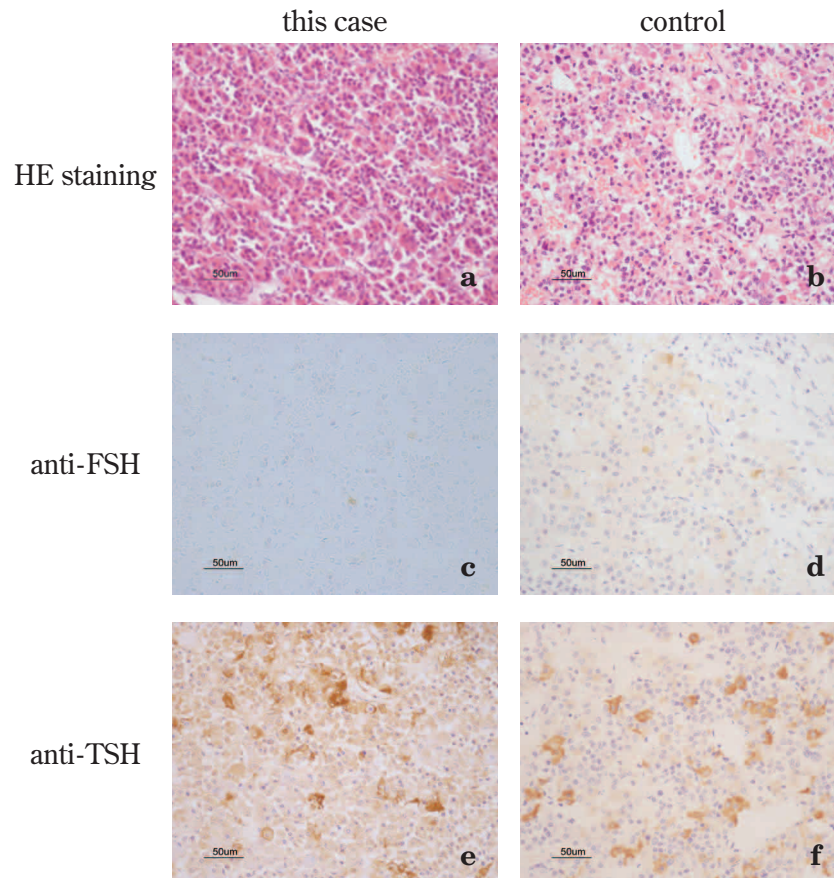
Fig. 2 Microscopic findings of the left ventricle (HE staining)
The myocardial fibers had thinned.

2.3 Immunohistochemical findings

Immunostaining was performed with antibodies against follicle-stimulating hormone (FSH, 1 : 50, Cell Marque, USA), thyroid-stimulating hormone (TSH, 1 : 200, Cell Marque, USA), triiodothyronine (T3, 1 : 200, Novus Biologicals, USA), thyroxine (T4, 1 : 200, Novus Biologicals, USA), and cortisol (1 : 100, Chemicon, USA). The immunostaining was carried out using the VECTASTAIN universal quick kit (Vector Laboratories, USA) according to the manufacturer's instructions. The immunostaining was visualized by incubating the tissue slices with 0.02% 3,3'-diaminobenzidine (DAB) and 0.03% hydrogen peroxide in Tris-buffered saline. A coverslip was placed on each glass slide, and then the slides were examined and photographed. Negative controls, which were used to determine the specificity of the immunohistochemical reactions, were performed by omitting the primary antibody.

The immunohistochemical findings were examined separately by two pathologists and were then screened.

An immunohistochemical study of the pituitary gland detected a decreased number of FSH-positive cells, but normal levels of TSH immunoreactivity (Fig. 3c and 3e). In the immunohistochemical examination of thyroid hormone expression, T3 immunoreactivity was decreased and immunoreactivity for T4 was stronger than T3 (Fig. 4c and 4e). Normal levels of cortisol immunoreactivity were observed.



a, c, e ; this case.
b, d, f ; 19 y.o., female. The cause of death was drowning.
A number of FSH-positive cells were decreased, but normal levels of TSH immunoreactivity.

Fig. 3 Immunohistochemical findings of the pituitary gland
a, b : HE staining, c, d : anti-FSH staining, e, f : anti-TSH immunostaining

3. DISCUSSION

AN is an eating disorder and has a prevalence of about 0.048% in Japanese women (3). Patients with AN exhibit various physical findings and psychiatric disorders as complications (Table 2) (1, 4, 5).

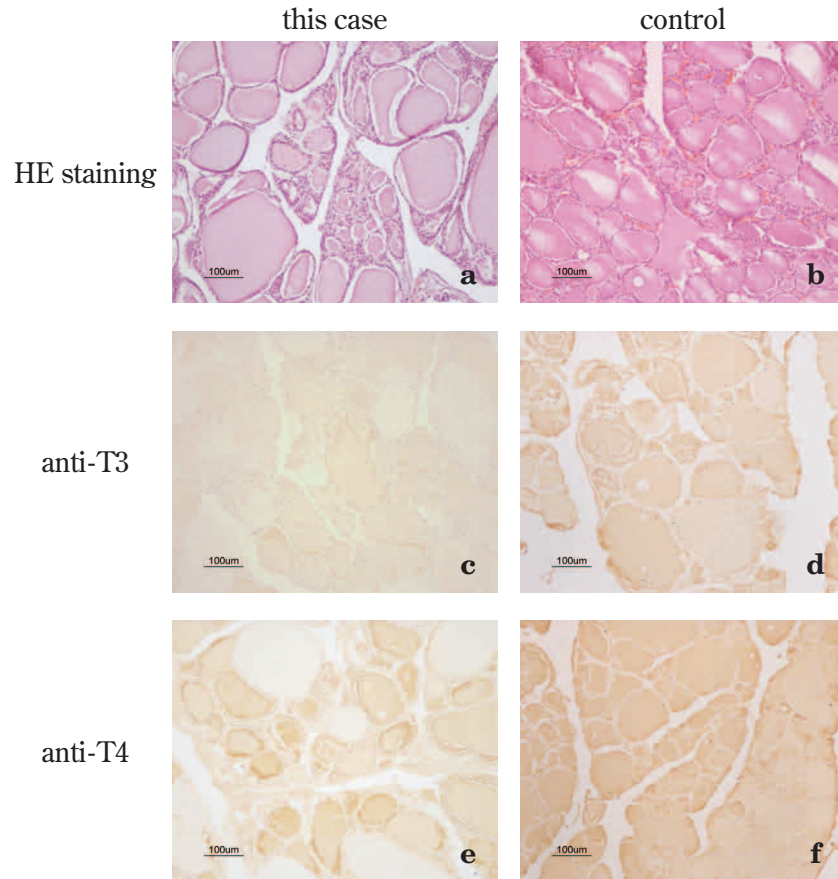
In the present case, almost all of her organs had atrophied and were light. Thus, the subject seemed to have been severely malnourished. Her heart weighed less than half the weight of a normal heart and contained little adipose tissue. In addition, the myocardial fibers had thinned and atrophied. Thus, it was suspected that her heart had failed.

Endocrine and metabolic disturbances are known to occur in AN (5, 6). Severe caloric restriction suppresses the hypothalamic-pituitary axis, and patients with AN exhibit hypogonadotropic-hypogonadism of hypothalamic origin (7). Clinical diagnosis of AN is partly based on endocrinological examinations. However, in our forensic autopsy case, we could not obtain enough blood for those examination. So, we tried to investigate her endocrinological condition by immunohistochemistry. Histological examination did not detect any obvious abnormalities in the pituitary gland although it weighed about half of its normal weight. However, an immunohistochemical examination showed that the number of FSH-positive cells was decreased. Therefore, it was considered that FSH secretion was downregulated.

A corpus luteum, but no secondary follicles or mature follicles, was detected in the ovaries, although the weights of the ovaries were within the normal range. The subject seemed to have been suffering from amenorrhea. Amenorrhea, which is one of the criteria used to diagnose AN in the DSM-IV (8), is common in patients with AN (1). This finding was in concordance with her antemortem situation ; i.e., she had been diagnosed with AN but had stopped receiving treatment.

Due to abnormalities of the hypothalamic-pituitary-thyroid axis, patients with AN present with a condition called 'low T3 syndrome', which is characterized by low T3 levels, normal or low T4 levels, and normal TSH levels (9). In the present case, normal TSH immunoreactivity was detected in the pituitary gland. In the thyroid, variations in follicle size, decreased T3 immunoreactivity were observed, but T4 immunoreactivity was stronger than that of T3. These findings suggest that the subject was suffering from thyroid dysfunction.

As for the function of the hypothalamic-pituitary-adrenal axis in AN, AN patients often exhibit elevated peripheral cortisol levels. Hypercortisolism in AN is considered to be caused by both increased cortisol secretion and decreased cortisol metabolism, and AN patients display normal cortisol production (6). In the present case, the weight of each adrenal gland had decreased to the bottom of the normal range. In addition, a histological examination did not detect any obvious abnormalities, and normal cortisol immunoreactivity



a, c, e ; this case.

b, d, f ; 40 y.o., female. The cause of death was pulmonary thromboembolism.

Marked variations in follicle size were observed. T3 immunoreactivity was decreased and T4 immunoreactivity was slightly decreased.

Fig. 4 Immunohistochemical findings of the thyroid gland

a, b : HE staining, c, d : anti-T3 immunostaining, e, f : anti-T4 immunostaining

Table 2 Various symptoms and findings that can develop as complications of AN

1. Mental symptoms
depressed mood, social withdrawal, irritability, insomnia, diminished interest in sex
2. Abnormal behavior
dietary intake restriction : skipping meals, cutting portion sizes
purging : over-exercising ; self-induced vomiting ; misuse of laxatives, diuretics, or enemas
self-mutilation, suicidal intent, abuse of drugs or alcohol
3. Physical findings
weight loss and malnutrition
hypothermia, edema
leukopenia, anemia, thrombocytopenia
metabolic alkalosis, hypokalemia, hypochloremia
low blood pressure, pulse, or ECG voltage ; sinus bradycardia ; prolonged QTc interval
hormonal changes : low serum levels of triiodothyronine (T3) or estrogen
low-normal serum levels of thyroxine (T4)
amenorrhea
osteoporosis, fractures, muscle wasting, diminished deep tendon reflex

was observed in the adrenal gland. Cortisol levels could not be measured in this case, but the above results suggest that her cortisol production was normal. These pathological findings indicate that she had been suffering with AN until her death.

A recent study revealed that the fronto-temporo-parietal cortices of AN patients had atrophied (10). In the present case, no obvious

brain atrophication was observed. However, the heat generated by the fire might have affected the structure of the cerebrum.

AN has one of the highest mortality rates among psychiatric disorders (11). Fatal cases of AN can involve various causes of death, including suicide ; alcoholism ; gastric rupturing ; and the complications of AN (Table 2) such as inanition, dehydration, and

heart failure (12-14). In our case, it was considered that not the suicide but the accidental fire brought her death with a criminal investigation by police. Autopsy revealed that her head, face, left limbs and the left side of her trunk were burnt. And the saturation level of CO-hemoglobin in her blood was 82%, and soot had adhered to her airway and the upper gastrointestinal tract. No toxicants except CO were detected, nor was alcohol. Therefore, it was considered that her cause of death was burning with CO intoxication.

4. CONCLUSION

Based on the pathological findings of the present subject's endocrine organs, immunohistochemical examinations with antibodies against related hormones might provide information that is useful for diagnosing AN, even if the endocrinological examination from blood cannot be carried out.

5. CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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