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Serum Zinc Levels in Heterozygous Carriers of the Gene for Acrodermatitis Enteropathica

Identifikation of a Carrier State is Not Possible

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Summary. Three cases of acrodermatitis enteropathica (a.e.) from two non-related families are described. Two siblings had characteristic symptoms of a.e. in childhood. Both survived to adulthood without treatment, at which time the clinical picture became uncharacteristic of a.e. Even so, the serum zinc levels confirmed the diagnosis in both cases. The third case showed classic symptoms of a.e.; the patient had a greatly reduced serum zinc level and responded at once to treatment with zinc sulphate.

Heterozygous carriers of the gene for a.e. have often had slightly reduced serum zinc levels. The value of this test could probably be improved by correcting the normal range of serum zinc for factors known to influence this, such as the patient's age and serum albumin level. The normal range ought also to be corrected for diurnal and postprandial variations of serum zinc.

Introduction

Acrodermatitis enteropathica (a.e.) was first described in detail by Brandt (1936), and was given its name by Danbolt and Closs (1943).

Formerly, the prognosis was thought to be very poor, as the children usually died of complicating infectious diseases. In the literature six cases of untreated a.e. are described in which the patients reached adult age (Guy, 1927; Vedder et al., 1956; Piper, 1957; Wells and Winkelmann, 1961; Lindström, 1963; Tompkins and Livingood, 1969). However, manuals of genetic medicine still state that the disease is fatal. Roberts (1970) writes 'death at an early age is almost invariable if untreated'. Bergsma (1973) writes 'untreated, outlook is death at an early age'.

The prognosis improved when Dillaha et al. (1953) found that diiodohydroxyquin was able to control the disease. Sufficiently high doses could usually control most symptoms, but at the same time the risk of developing neurotoxic symptoms, especially opticus neuritis, threatened.

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Moynahan and Barnes (1973) showed that patients with a.e. had severely reduced serum zinc levels and that treatment with small doses of zinc sulphate would free the patients of all symptoms. Since then no cases of a.e. have been reported that could not be controlled by zinc sulphate. Lombeck et al. (1975) found reduced zinc absorption in patients with a.e., while the urinary excretion of zinc was normal. The authors thus assumed that patients with a.e. had a zinc absorption defect and found it to be similar in extent to that of primary hypomagnesiaemia. The symptoms could be controlled by a supplement of 5—20 times the ordinary content of zinc in the food.

A.e. is regarded as having an autosomal recessive mode of inheritance (Roberts, 1970; Bergsma, 1973). Several cases are reported in which healthy parents have had one or more affected children (Guy, 1927; Schultz, 1935; Brandt, 1936; Danbolt and Closs, 1943; Vedder et al., 1956; Tompkins and Livingood, 1969), and in some cases the parents were consanguineous (Guy, 1927; Bloom and Sobel, 1955; Piper, 1957). No cases have been reported in direct lines within two or more generations.

The following report is based on the hypothesis that serum zinc could be used in detecting heterozygous carriers of the gene for a.e., as the autosomal recessive gene might cause a reduced serum zinc level in carriers.

Author's Own Investigations

Method

The nearest relations of the patients were interviewed and examined by the author, and at the same time blood samples were taken to determine their serum zinc levels. The pedigree information (Figs. 1 and 2) was obtained from death certificates and church registers. The blood samples were collected in specially cleaned glass tubes, and within a few hours they were centrifuged and the serum was removed. The sample was examined in a Perkin-Elmer atomic absorption spectrophotometer and the result was given as micromoles per liter. The normal ranges of serum zinc given by Medicinsk Laboratorium A/S, Copenhagen, were 11.4—18.9

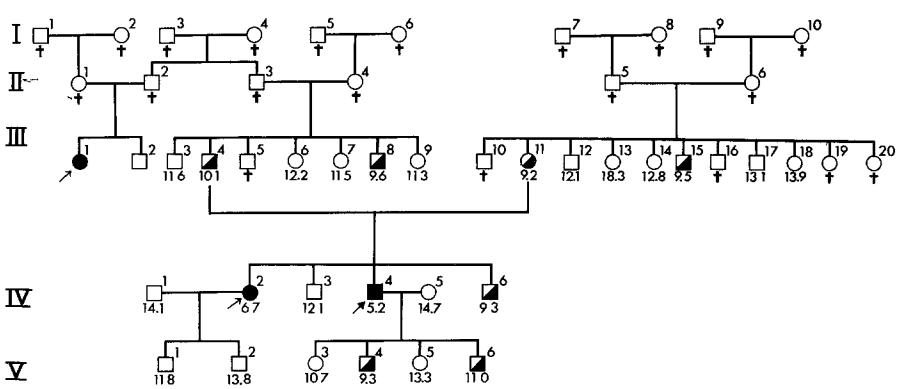

Fig. 1. Pedigree of Family 1. Serum zinc levels are stated below family members examined. ■ acrodermatitis enteropathica; ◪ slightly reduced serum zinc; ↗ patient described in text; † dead

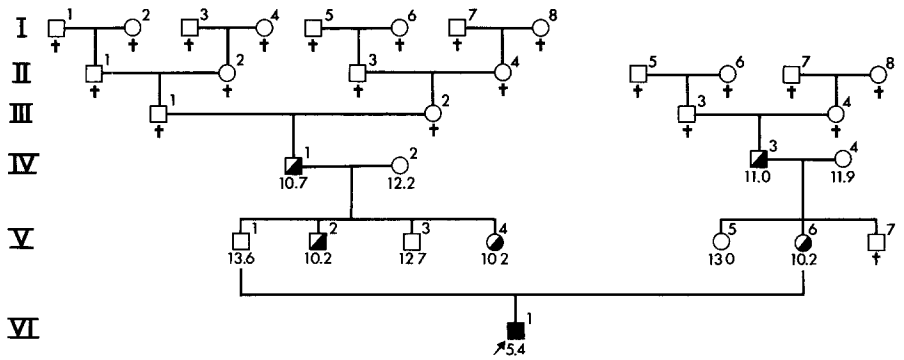

Fig. 2. Pedigree of Family 2. Serum zinc levels are stated below examined family members. ■ acrodermatitis enteropathica; ◪ slightly reduced serum zinc; ↗ patient described in text; † dead

μmol/l for men, and 10.6—17.7 μmol/l for women. The normal range was based on 300 analyses of adults who did not suffer from diseases known to influence the serum zinc levels. The standard deviation for the single analysis was 0.4 μmol/l. The controls were non-fasting and an interval of more than 1 h since the last meal was aimed at. No adjustments were made for age or serum albumin.

Family 1

Case 1

A 33-year-old married gardener was referred to the Dermatological Department, Odense University Hospital, because of impetigo (Fig. 3). His mother informed us that pregnancy and delivery were uncomplicated. She does not remember for how long the patient was breast-fed, but the disease first appeared when he was a few months old. The skin lesions occurred at intervals of 1—3 weeks, and during most of his childhood they were localized to the perioral skin, hands, feet, and elbow and knee regions. During the days preceding the outbreaks there was a prickly feeling in the affected regions, which then became bullous and, for a few days, weeping and painful. During healing the skin became hyperkeratotic in the affected regions. The attacks were often accompanied by numerous acne-like papules and pustules on the face and the trunk. In adulthood his skin changes were mainly localized around the mouth and on the feet. There were never any intestinal symptoms, or growth retardation. The hair has always been stiff and lustreless but there has been no great hair loss or involvement of the nails. He has four children. The second and third suffer from atopic dermatitis. None of the children suffers from any congenital malformations.

Physical Examination. On admission the patient weighed 68.9 kg, was 187 cm in height, and leptosomic in build. His hair was medium blond, dense but lustreless. Periorally there were numerous papules, pustules and closely placed deep scars. The front and back of the trunk showed inflammatory papules, pustules, and scars with a distribution resembling that of acne vulgaris, but no comedones were seen. The skin lesions on the feet had a very sharp boundary proximally just above the ankles. On admission the skin was eroded, profusely weeping, and had an impetigo-like appearance, but no bacteria were cultured from the lesions. Flaccid bullae were seen along the margin of the affected areas. All toes showed paronychial inflammation, but both the finger- and the toenails were normal.

Laboratory Investigations. On admission serum zinc was 5.2 μmol/l, alkaline phosphatase 30 U/l (normal range 29—88 U/l), serum IgE 720 U/ml (normal range less than 500 U/ml in adults), se-

Table 1. HLA types, distribution of T and B lymphocytes and lymphocyte transformation test in three patients suffering from acrodermatitis enteropathica

	HLA Types	Rosettes*				Lymphocyte transformation tests**				
		T[a]			B[b]	PHA[c]	PWM[d]	Con A[e]	PPD[f]	MLC[g] ($R - P_x$)
		≥3	2	1						
Case 1	A 2,3 B W15, W40 C W3	65±2	2±1	6±1	22±3	12,192± 3	3634±107	457±39	3419± 56	8524±294
Case 2	A 2,3 B W15 C W3,4	59±2	4±1	8±1	16±1	11,433± 66	4476±322	109±69	2077±149	8158±754
Case 3	A 2 B W15 C W3	58±4	3±1	9±0	19±1	6532±1042	—	—	62± 25	15,743±248

* Rosette formation tests for [a] T and [b] B lymphocytes. T cells with ≥3, 2, and 1 sheep erythrocyte(s) were counted separately. For method and normal range see Birkeland (1975)

** Lymphocytes were stimulated to blast transformation with plant mitogens, phytohaemagglutinin (PHA)[c], pokeweed mitogen (PWM)[d] and concanavalin A (Con A)[e], specific antigen, tuberculin-purified protein derivative (PPD)[f], and in mixed lymphocyte cultures (MLC)[g], between responder cells (R) and a pool of irradiated stimulating cells ($P_x = A_x + B_x + C_x$, where A, B and C are HLA-different). For method and normal range see Birkeland (1976)

Numbers are given as means of triplicates ± 1 SD. All values are within normal ranges

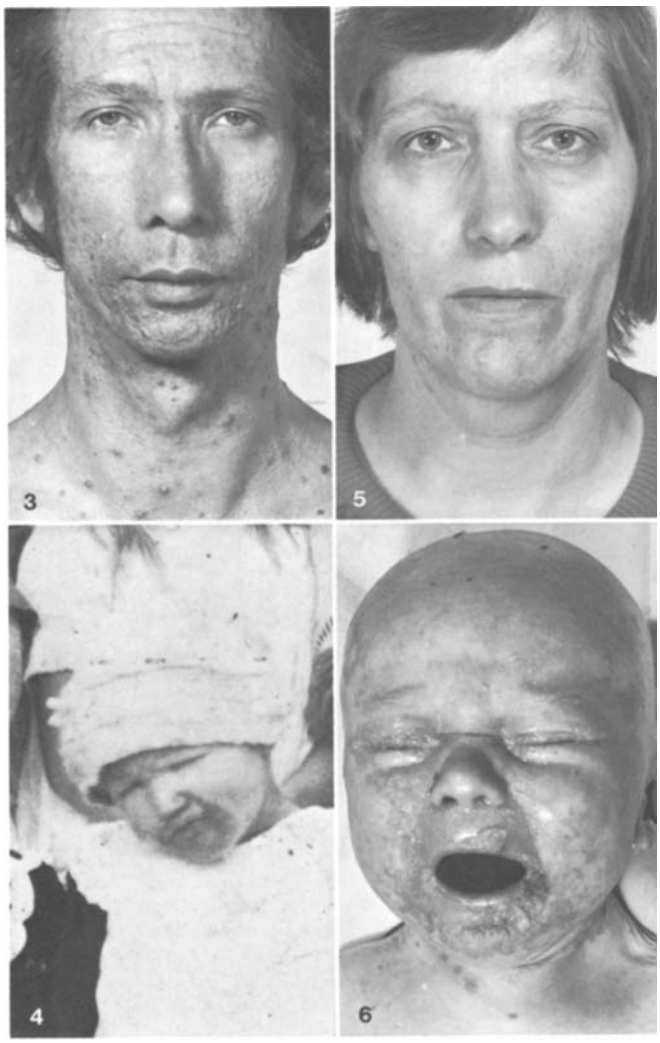

Fig. 3. Case 1. Before treatment there were papules, pustules and closely placed deep scars periorally, and scattered, inflamed pustules on the neck

Fig. 4. An enlarged section of a school photo of 1920 showing a child (Family 1, individual III,1) with dermatitis around the mouth and photophobia, signs suggestive of a.e.

Fig. 5. Case 2. There are no cutaneous signs of a.e., but there is a Parkinsonoid oligomimia

Fig. 6. Case 3. Few days after admission to the Dermatological Department

rum IgG 18.2 g/l (normal range 6.2—13.3 g/l), serum IgA 2.64 g/l (normal range 0.40—3.43 g/l), serum IgM 0.33 g/l (normal range 0.18—1.30 g/l), serum albumin 44 g/l (normal range 36—46 g/l). After 4 months of peroral zinc sulphate treatment (200 mg four times daily) the alkaline phosphatase reached 82 U/l, serum IgE dropped to 130 U/ml and serum IgG to 14.4 g/l, while serum IgA and serum IgM did not change. Table 1 shows the HLA types, the T and B lymphocyte distribution, and the results of lymphocyte transformation tests. After treatment with 200 mg zinc sulphate four times daily for 2 weeks the skin became normal, though somewhat hyperaemic on the feet. Four months after the start of treatment the serum zinc normalized and the dose was reduced to 200 mg three times daily; this dose was given for the next 15 months. During this period there has been no relapse and the general condition and working condition of the patient have improved. The growth of hair and beard has been stronger, darker, and more lustrous.

Case 1 is the third of four children. The oldest child (Case 2) has a.e. The other two siblings are healthy. The parents are healthy and presumably not related, as consanguinity was not to be found in three earlier generations. Among the father's relatives there is a predisposition to asthma bronchiale, and the grandfather's cousin (Fig. 1, III,1) died in 1923, at the age of 11 years, of pneumonia. From the description this child suffered from photophobia, alopecia, constant eczema around the mouth, and inflammation of the nails. An enlarged school photo of 1920 (Fig. 4), together with the other information, indicates that the child suffered from a.e.

A paternal uncle died of cancer coli ('III,5'), a maternal uncle ('III,10') died of cancer ventriculi, another ('III,16') of lymphosarcoma, a maternal aunt ('III,19') of cancer colli uteri, another ('III,20') of blood poisoning at the age of 18.

Case 2

A 40-year-old woman (Fig. 5) developed her first symptoms at the age of 18 months in the form of recurrent periods of diarrhoea accompanied by skin changes of the same nature as in her brother. The changes were mainly localized around the mouth and on the hands, feet, and flexor sides of the knees. Apart from early childhood there have been no intestinal symptoms, but throughout her life the patient has suffered from repeated attacks of depression, which have incapacitated her for long periods. At the age of 21 the patient became pregnant and throughout the pregnancy there was pronounced exacerbation of the skin disease. After the birth of a healthy son all skin symptoms disappeared and the patient was free of symptoms for the next 2 years, when she again became pregnant at the age of 23. After this, the skin disease recurred. Again the patient gave birth to a healthy boy, and again the skin symptoms disappeared. Two subsequent pregnancies involved renewed exacerbations of the skin disease; in both cases abortion was performed and subsequently sterilization. Since then the patient has had a healthy skin.

At the age of 37 the patient developed Parkinsonism. After treatment with zinc sulphate (200 mg three times daily) for 1 year there has been some improvement in the patient's depression but no changes have been observed in the Parkinsonism.

Before treatment with zinc sulphate was started the serum zinc was 6.7 µmol/l. Further findings were: serum albumin 56 g/l, serum alkaline phosphatase 30 U/l increasing to 47 U/l during zinc sulphate treatment. Table 1 shows HLA types, T and B lymphocyte distribution, and the results of lymphocyte transformation tests.

Family 2

The patient (Case 3) is now a 14-month-old boy. The pregnancy was normal. Because of an affected heart sound the patient was delivered by sectio caesaria. His weight at birth was 3600 g, his length 53 cm. The child presented no abnormalities immediately after birth.

He was breast-fed during the first week after birth and then fed with a mother's milk substitute. The disease started when the child was one month old, with erythematous, scaling skin changes on the face, accompanied by pustules. The skin lesions spread to the genital region and to the backs of his thighs. At the age of 2 months he was admitted to the Dermatological

Department with suspected dermatitis seborrhoica (Fig. 6). In a few days he developed flat bullae followed by large suppurating erosions on the buttocks, the backs of the thighs, and hands and feet. As the suspicion arose that the child was suffering from a.e., serum zinc was determined and found to be 5.4 µmol/l. After this the patient was fed with mother's milk and given 50 mg zinc sulphate twice a day. After a few days the general condition improved, and after one week almost all skin lesions were healed. After normalization of serum zinc the patient continued on a dose of 50 mg zinc sulphate daily for about 1 year. During this period there has been no sign of relapse and his development has been normal. During treatment with zinc sulphate the alkaline phosphatase rose from 34 U/l to a maximum of 516 U/l, then dropped to 288 U/l. Table 1 shows HLA type, distribution of T and B lymphocytes, and the results of lymphocyte transformation tests.

Case 3 is the only child of non-consanguineous parents (consanguinity not found in four earlier generations). No relationship with Family 1 could be demonstrated. The family of the father is predisposed to psoriasis. On the whole the father and other examined members of the family were healthy. The mother has had asthma bronchiale since the age of 16 and for the past two years, including the period of pregnancy, she has been treated with 0.25 mg betamethason daily. Menstruation did not start in the mother till the age of 18, and since then she has had oligomenorrhoea with about two menstrual periods yearly. There are no hereditary diseases in her family and the relatives examined are generally healthy.

Discussion

It has become very important to be able to diagnose a.e. now that substitution treatment with zinc sulphate apparently terminates all symptoms and, unlike treatment with diiodohydroxyquin, does not seem to involve any risk (Ølholm-Larsen et al., 1976).

While the diagnosis is easy when a.e. presents classic symptoms involving the intestine and skin, it becomes more difficult in forms with a mild course, and especially if the patient survives to adulthood. A.e. without intestinal symptoms has been reported (Portnoy and Marsden, 1961), and was found in Case 1. It is uncertain whether skin symptoms can be absent or be present in an abortive form, since the skin symptoms have always been the decisive factor in diagnosis. It is now possible to solve this problem by determining the serum zinc, as it has become clear that a.e. is a zinc deficiency syndrome, presumably the result of defective intestinal absorption of zinc (Lombeck et al., 1975). Although low serum zinc levels have been described in several conditions, e.g., ulcus cruris (Ølholm-Larsen et al., 1976), chronic liver disease, uraemia and malabsorption (Halsted and Smith, 1970), severely reduced levels of serum zinc have only been described in a.e., in the zinc depletion syndrome during prolonged parenteral feeding (Weismann et al., 1976), and in nutritional dwarfism (Halsted et al., 1972).

Case 2 has shown that serum zinc may stay at extremely low levels in spite of the disappearance of intestinal and cutaneous symptoms. It is probable, therefore, that serum zinc will be very useful in the differential diagnosis of mild cases of a.e.

Family studies and investigations of the zinc balance in patients indicate that a.e. has an autosomal recessive mode of inheritance and is a zinc absorption defect. According to this, slightly reduced serum zinc levels might be expected in all children of a person affected by a.e., in 50% of the siblings of the patient, in

both parents, and in 50% of the parents' siblings. Two children of Case 1 had serum zinc levels within the normal range for adults and the same applies to both children of Case 2 (Fig. 1). The father of Case 3 also has a normal serum zinc level (Fig. 2).

These results should be considered in the light of the reliability of the zinc analysis and the normal range. In the present study, the normal range is based on an examination of 300 adults who did not suffer from diseases known to influence the serum zinc levels. The samples were taken between 8 a.m. and 2 p.m. It is known that the serum zinc level is 12% higher in a fasting person and 10% lower 1—2 h after a meal (Davies et al., 1968). Of the zinc in serum, 85% is loosely bound to serum albumin and will therefore vary according to serum albumin levels (Boyett and Sullivan, 1970). Serum zinc shows a downward trend with age (Lindeman et al., 1971). In the above-mentioned normal range these conditions were not taken into consideration. Burr (1973) has shown an ephemeral variation in serum zinc, with highest levels in the morning and lowest levels late in the afternoon.

During examination of six healthy women aged 25—40 years, a fall in serum zinc from a mean of 13.4 µmol/l at noon to 12.7 µmol/l at 4 p.m. was found. All samples were taken more than 2 h after the last meal. The difference was not statistically significant (author's own investigations, not published).

In a previous investigation (Ølholm-Larsen et al., 1976) concerning 42 patients suffering from venous leg ulcers, significantly lower serum zinc levels were found in patients with slowly healing wounds ($\bar{x} = 13.6$ µmol/l) than in patients with fast healing wounds ($\bar{x} = 16.3$ µmol/l). No serum zinc levels were found to be below the normal range of the laboratory, which was the same as that used for the present investigation.

Even though many of the blood samples in this investigation were taken late in the afternoon, and might therefore be in the lower range, it is unlikely that this alone would lead to levels below the normal range of serum zinc.

The normal range of serum zinc for children is not known with certainty, but Berfenstam (1952) found that serum zinc in children aged 1—5 years is about 20% higher than that of adults. If this is the case, the serum zinc levels of the two children of Case 1, which were within the normal range of adults, might be low for their ages. However, Hirsh et al. (1976) found that siblings of patients with a.e., who were most probably heterozygotes, had serum zinc values on a level with those of the parents, which were depressed 25% below the lowest normal range.

In contrast to expectations, the two children of Case 2, aged 19 and 20 respectively, and the father of Case 3 had normal serum zinc levels. Falsely high serum zinc levels can appear in the presence of zinc contamination and in haemolysis. We tried to avoid the first by using cleaned tubes and the latter was not seen.

Immunological defects in patients with a.e. have been found in several cases (Julius et al., 1973; Moynahan, 1975). Similar defects have been found in cattle with Adema disease, a condition very similar to a.e. in man (Andresen et al., 1973). Case 1 has increased serum IgE and IgG. During treatment IgE normalized and IgG was reduced. In all three cases the T and B cell distribution in

peripheral blood was normal, as were the results of the lymphocyte transformation tests.

To my knowledge, HLA typing of patients with a.e. has not been done. The two siblings (Case 1 and 2) had HLA type A 2, B W15 and C W3 identical with Case 3. It would be interesting to compare this finding with the HLA types of other cases with a.e.

Cases 1 and 2 show that the disease may have a mild course and a late onset, and that a.e. is not necessarily fatal as was formerly presumed. Because of this, the disease may be supposed to be more frequent than previous publications suggest.

A.e. is a clear illustration that a purely hereditary disease can be treated successfully although the molecular pathology is unknown.

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