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Element Concentrations in Serum, Erythrocytes, Hair and Urine of Alopecia Patients

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The trace element concentrations of Se, Rb, Zn, Fe, Co, Cs, Mg, Ca, F, Cu, Cr and Ag in serum and of Se, Rb, Zn, Fe, Co and Cs in red cells of Finnish alopecia patients were determined. In addition the Cu and Zn content in 24 h urine and Cu, Zn, Cd, Cr and Se concentrations in the hair of these patients were studied. No differences in element concentrations of the samples mentioned above as compared to those of the normal population could be found. In addition, there was no tendency of excesses or deficiences of elements analysed in the samples. Statistically significant difference was found between the copper content of serum in alopecia areata and alopecia universalis patients and also between the copper content of serum in alopecia areata plus alopecia totalis and alopecia universalis patients. The selenium concentration in serum samples of a few patients was low, but this is in agreement with the fact that the selenium content in the Finnish population is low due to the scanty content of selenium in food. *Key words: Elements; Alopecia.* (Received September 3, 1985.)

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The etiology of alopecia areata is still unknown. It has been speculated that it is a disease involving immune mechanisms (1). Different kinds of treatments have been tried with variable results for alopecia areata, i.e., corticosteroids, photochemotherapy, topical irritants and allergens. Reports have also been published concerning oral zinc sulphate therapy (2, 3, 4), with encouraging results in some cases of both alopecia areata and the male type alopecia (4, 5).

A great majority of the trace elements serve chiefly as key components in enzyme systems connected to vital protein synthesis. If the metal ion is removed, the protein usually loses its functional capacity. Particularly zinc, iron, magnesium and selenium are known to be important for maintaining immunological competence (6). Thus, it could be expected that these elements may be involved in the pathogenesis of alopecia areata. In sheep the copper and selenium deficiencies have markedly changed the protein structure of wool (7, 8). The zinc deficiency has caused the shedding of hair in rats (9).

In this study the trace element concentration in serum, erythrocytes, hair and urine samples of alopecia areata patients were determined to find out whether these persons have differences in element concentrations in the samples mentioned above as compared to those in the normal Finnish population. Special concern was attached to the zinc, iron, magnesium and selenium concentrations.

MATERIAL AND METHODS

1. *Sample donors*

The series consisted of 27 patients examined at the Department of Dermatology, University Central Hospital, Helsinki. Blood, 24 h urine and hair samples were obtained and the patients were inter-

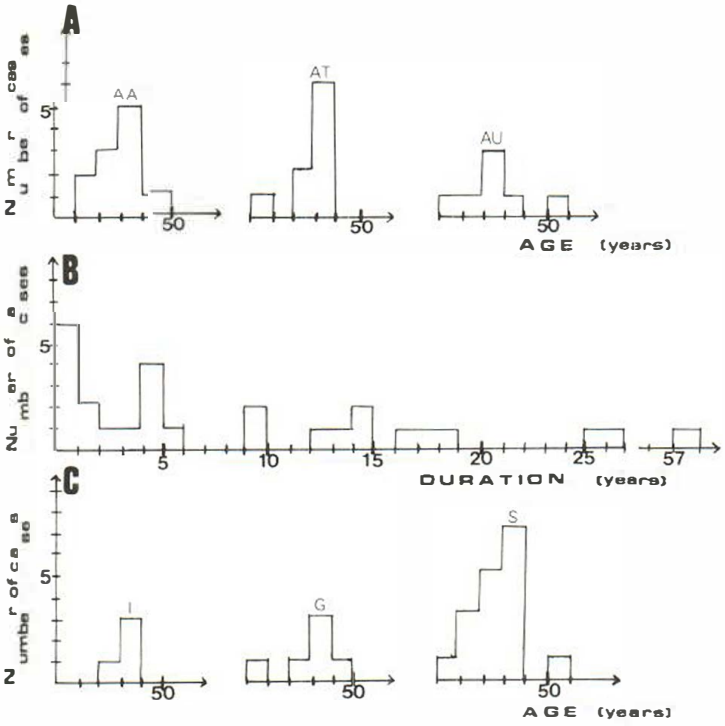

Fig. 1. (A) The distribution of age in the three groups of alopecia areata (*AA*), alopecia totalis (*AT*) and alopecia universalis (*AU*). (B) The duration time of alopecia. (C) The distribution of age in the alopecia patients being in initiation state (*I*), growth state (*G*) and static state (*S*).

viewed on their life history, smoking and drinking habits, on their occupation, on the possibility of occupational exposure, on their shampoo brand etc.

Nineteen of the patients were female while eight were male. The mean age of the patients was 29±11 years (SD) with a range from 8.5 to 63 years; median 32 years. Eleven patients had alopecia areata, nine had alopecia totalis and seven had alopecia universalis. The duration of alopecia varied from 6 months to 57 years, median 6 years, mean (SD) was 10.5 (12.2) years (Fig. 1). None of the patients had received any treatment for alopecia within three months preceding the investigation.

2. *Sample collection*

The blood samples were drawn with a plastic teflon catheter (Venflon®, Viggo cannula, Sweden) to minimize the metal contamination. No anticoagulant was used. The samples were taken after overnight fasting. The serum was separated from the erythrocytes by centrifuging, and both the erythrocytes and the serum were used for the analysis. To avoid contamination from the reagents no washing procedure was carried out for the erythrocytes. Five alopecia areata and two alopecia totalis cases delivered a 24 h urine sample which was collected into acid-washed plastic vials. In addition, hair samples were taken whenever possible (in 9 cases), with stainless steel scissors. The hair sample from the seven alopecia areata patients was taken from the small area behind the ear and the 2–3 cm piece of hair near the scalp was analysed. In the case of alopecia totalis (2 cases) the hair sample was collected from the single hairs found round the scalp and the whole hair was analysed.

The serum, erythrocyte and urine samples were stored in a deep-freezer (−20°C). For the instrumental neutron activation analysis determinations the samples were freeze-dryed. All plastic ware used for the storage of the samples were acid-washed with HNO_3 and deionized water.

3. *Analytical methods*

Atomic absorption spectrometry (AAS, Perkin Elmer 5000) and instrumental neutron activation analysis (INAA) were used in the trace element determinations. The fourteen serum samples first gathered were analysed by the INAA method (4 alopecia areata, 8 alopecia totalis and 2 alopecia universalis patients).

The Zn, Cu, Mg and Ca concentrations of all serum samples were determined by flame atomic absorption spectrometry using the method developed by Salmela & Vuori (10). The selenium and chromium concentrations in serum were analysed by the method described by Alfthan & Kumpu-

lainen (11) and Kumpulainen et al. (12), respectively. The fluoride concentration was measured by an ion-selective electrode with the method modified by Fry & Taves (13) while the iron concentration in serum samples was measured by the colorimetric technique (14). Instrumental neutron activation analysis (15) was used for the determination of Zn, Se, Rb, Fe, Co, Cs and Ag in serum and red cells of 14 patients.

The hair samples were handled by means of the method published by Salmela et al. (16). The basic of the method was rinsing with hexane and washing with sodium lauryl sulfate. The Cd, Cr, Zn, Cu and Se contents in hair were analysed after wet ashing with conc. HNO_3 (*supra pur*, Merck) by flameless atomic absorption spectrometry. The Zn and Cu contents in urine were analysed by flame AAS.

The accuracy and precision of the analytical methods were checked with commercial standard reference materials Seronorm® batch no. 157 (Nyegaard & Co, Oslo) and Lanonorm® Lot No. B 62 5201 (Behring, Marburg). In addition, standard reference materials distributed by the National Bureau of Standards (NBS), bovine liver SRM 1577 and the International Atomic Energy Agency (IAEA), animal muscle H-4 were also used. The results are given in Table I.

The statistical analysis was performed with Student's *t*-test.

RESULTS AND DISCUSSION

The mean content of the element analysis of the samples are given in Tables II and III. All the single results obtained by AAS were in excellent agreement with those determined by INAA.

Reference values for the Finnish population were available only for a few trace elements (Table II). Thus, we have here compared those results without 'normal Finnish values' with the results reported by Iyengar et al. (17) obtained as a synthesis of literature values. The reference values reported by Vuori et al. (18, 19) were determined earlier in our Laboratory (in the Department of Public Health Science) with the same method as used in

Table I. *Element concentrations in control samples by AAS and INAA*

	NBS bovine liver (μg/g)			IAEA muscle H-4 (μg/g)		Seronorm® (mg/l)	
Element	AAS	INAA	Certified (±SD)	INAA	Certified	AAS	Certified (range)
Se	1.2	1.02	1.1±0.1	0.26	0.28	0.086[a]	0.082–0.096[a]
Rb		17.7	18.3±1.0	19	19		
Zn	140	136	130±13	85	86	0.88	0.72–0.92
Cu	200		193±10			1.12	1.11
Fe		253	268±8	46	49	1.66[b]	1.39–1.69
Co		0.20	0.18[d]				
Cs		0.012	0.013[e]	0.12	0.12		
Ag		0.06–0.02	0.06[d]				
Mg						23.6	23.0–26.0
Ca						109	106–113
F						0.88[c]	0.88–0.96[c]
Cr	0.10		0.088±0.012			0.0042[c]	0.0034–0.0051[c]
Cd	0.28		0.27±0.04				

[a] Selenium standard for serum was distributed by the National Institute of Public Health, Helsinki, Finland

[b] By the colorimetric method.

[c] Lanonorm®: fluoride concentration determined by the ion-specific electrode.

[d] Noncertified values given by NBS.

[e] Gladney (22).

Table II. *Element concentrations of serum and erythrocyte samples in this study and literature*

Sample	Method		Concentration (μg/g wet weight) Se	Rb	Zn	Fe	Co
This study							
Serum	INAA[a]	Mean	0.067	0.19	0.86	ND[b]	<0.007[i]
		(SD)	(0.011)	(0.10)	(0.16)		
	AAS[a]	Mean	0.068		0.92	1.43	
		(SD)	(0.014)		(0.13)	(0.38)	
		Range	0.046–0.098		0.70–1.14	0.69–2.12	
Erythrocytes	INAA	Mean	0.103	4.87	9.35	807	0.01
		(SD)	(0.027)	(1.05)	(1.27)	(108)	
Literature							
Serum[c]		Mean	0.122	0.20	1.15	1.09	
		Range	0.098–0.327	0.04–0.58	0.67–1.83	0.87–1.87	0.0008–0.00185
Erythrocytes[c]		Range	0.071–0.238	4.18	7.6–16.1	985–1 140	0.00016–0.0125
Serum/Finland		Mean	0.055				
		(SD)	(0.015)[e]				
		Males			0.88		
		(SD)			(0.13)[f]		
		Females			0.76		
		(SD)			(0.08)[f]		

[a] INAA 14 cases. AAS 27 cases. [b] ND = not detected. [c] Iyengar et al. (17). [d] Ion selective electrode, 13 cases. [e] Salonen et al. (20). [f] Björksten et al. (23); in mg/l. [g] Hanhijärvi (24); in mg/l. [h] Vuori et al. (18). [i] Minimum detectable level.

this study. The selenium and chromium analysing methods were the same in our study as those used by Salonen et al. (20) and Kumpulainen et al. (21) in their work, respectively. In intercomparison carried out between these two laboratories mentioned above and our laboratory a good agreement was obtained.

The concentration of trace elements varies in many cases according to age and sex. In this study a statistical difference was found only between the calcium content of males and females and copper content of males and females ($p \leq 0.01$). No tendency of deficiences or excesses of trace elements analysed in the samples of alopecia patients could be found as compared to the normal Finnish population. Statistically significant differences (at the 5% level) were found neither in the groups classified according to the state of alopecia, i.e., initiation, growth or static state of alopecia. The mean content of copper in serum of female alopecia areata patients was 1.05±0.11 μg/g, of female alopecia totalis 1.08±0.15 μg/g and of female alopecia universalis 1.30±0.28 μg/g. Statistically significant difference excisted between the copper value of the alopecia areata and alopecia universalis patients ($p=0.05$) and the copper value of the alopecia areata plus the alopecia totalis and alopecia universalis patients ($p=0.02$). The selenium concentration in the serum samples of alopecia patients was low in a few cases. This is, however, in agreement with the fact that the serum concentration of selenium in Finnish people usually is also low due to the scanty content of selenium in Finnish foodstuffs. Only in one person a measurable amount of silver could be found in the serum sample.

Cs	Mg	Ca	F	Cu	Cr
0.001 (0.001)					
	21 (1) 18.0– 22.2	92 (4) 83– 98	0.021 (0.006)[d] 0.017– 0.036	1.06 (0.19) 0.82– 1.41	0.09 (0.05) 0.03– 0.20
0.006 (0.002)					
0.00133– 0.06	21.7 19.9– 27.5	97 92– 109	0.019– 0.035	1.19 0.97– 1.64	
			0.013 (0.002)[g]		
				0.96 (0.17)[h] 1.10 (0.32)[h]	

Table III. *Element concentrations in hair samples (µg/g dry weight) and content of 24 h collection urine (µg/day)*

Sample		Element				
		Cu	Zn	Cd	Cr	Se
This study						
Hair[a]	Mean (SD)	29 (19)	142 (50)	0.6 (1.4)	0.18 (0.24)	7.9 (11.1)
	Range	9.2–61.9	49.9–195.3	0.01–4.38	0.03–0.81	0.8–32.7
	Median	34	159	0.07	0.10	2.9
Urine[b]	Mean (SD)	136 (251)	357 (201)			
	Range	30–706	180–630			
	Median	34	393			
Literature						
Hair[c]	Mean	19	218			1.88
	Range	11–34	99–450	0.24–2.7	0.13–3.65	0.64–2.53
Urine[c]	Mean	48.8	444			
	Range	11–325	42–1250			
Hair/Finland	Median (SD)	23 (18.5)[d]	199 (64)[d]	0.4 (0.3)[d]	0.15 (0.08)[e]	

[a] Number of cases 9. [b] Number of cases 5. [c] Iyengar et al. (17). [d] Vuori et al. (19). [e] Kumpulainen et al. (21).

There was a great variance to be seen in the trace element concentrations in hair samples, e.g. the selenium concentration in the hair of alopecia areata patients ranged from 0.8 to 32.7 μg/g and from 1.7 to 20.0 μg/g in the alopecia totalis cases. The cadmium concentration of the alopecia areata patients ranged from 0.01 to 0.35 μg/g, whereas it was from 0.40 to 4.4 μg/g in the alopecia totalis cases (Table III). Exceptionally high content of chromium, selenium and cadmium in hair were found in an 8.5 year-old-boy having alopecia totalis.

Similarly, the urine's copper and zinc content in alopecia patients varied significantly, from 30 to 706 μg/day for copper and from 180 to 630 μg/day for zinc (Table III). In the case of alopecia areata the ranges were from 30 to 52 μg/day for copper and 180 to 536 μg/day for zinc. On the other hand, the high concentration of selenium in two cases of hair samples (20.0 and 32.7 μg/g) may be due to a contamination to selenium when using shampoos containing selenium.

Thus, the present study did not indicate any statistical differences in the element concentration between alopecia patients as compared with the normal population. Unfortunately the number of alopecia cases was small in the study, because this disease is fairly rare in Finland. However, there was a significant difference to be noticed in the serum's copper content between different alopecia groups. This will call for further studies.

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