TRACE ELEMENTS ANALYSIS IN THE NAILS OF PEMFIGUS VULGARIS PATIENTS DETERMINED BY THE ELECTRONIC MICROPROBE

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Summary

Trace elements varied in humans with sex, age, heredity, occupation, environment as well as disease and therapy. We have determined trace elements in human nails because we consider to be less influenced by any variables and for its possible implications in medical purposes. Detection and quantitative determination of trace elements were performed by the electronic microprobe (emission of X-rays by electronic excitation) technique in 6 normal subjects (12 determinations) and 5 patients with pemfigus vulgaris (11 determinations). About 9-11 elements (Si, S, Cl, K, Ca, Mn, Fe, Mg, Al, Zn) have been detected in human nail samples, taken from both hands and feet separately (parts per million-ppm). The study showed a constant high content of S in the fingernails of normal subjects (40,000 ppm), and in pemfigus vulgaris patients too. Other constant trace elements faced were, Cl, K, Ca, with a wide range of values probably influenced by various factors (nutrition, environment, therapy). Si, Mn, Cu, Fe were inconsistantly present while Mg, Al and Zn were totally absent with this method, as was Na.

In conclusion, we believe that trace elements, content of human nails, are under the influence of the environment, disease process and therapy and that the electronic microprobe technique could be a useful tool for various studies.

Keywords: *trace elements, human nail, electronic microprobe, pemfigus vulgaris.*

Rezumat

Microelementele (oligoelementele) variază la om în funcție de sex, vârstă, ereditate, ocupație, mediu lînconjurător atât cât și afecțiuni și tratamente. Am studiat prezența lor la nivelul unghiilor pentru că noi considerăm că sunt mai puțin influențate de către orice variabile și pentru posibilele implicații în scopuri medicale. Detectarea și determinarea cantitativă a microelementelor au fost efectuate cu ajutorul microsondei electronice (emisie de raze X prin excitație electronică) tehnice la 6 indivizi normali (12 determinări) și 5 pacienți cu pemfigus vulgar (11 determinări). Aproximativ 9-11 microelemente (Si, S, Cl, K, Ca, Mn, Fe, Mg, Al, Zn) au fost detectate în probe unghiale luate de la nivelul ambelor mâini și al picioarelor separat (valoarea exprimată în părți pe milion = ppm). Studiul a arătat un conținut înalt constant de sulf la nivelul unghiilor subiecților normali (40000 ppm) atât cât și cei cu pemfigus vulgar. Alte oligoelemente constant găsite au fost Cl, K, Ca cu valori variabile probabil influențate de factori diverși (alimentație, mediul înconjurător, tratamente). Și, Mn, Cu, Fe au fost semnalate prezente, în timp ce Mg, Al, și Zn au fost total absente prin această metodă la fel ca și Na.

În concluzie oligoelementele aflate la nivelul unghiilor se află sub influența mediului a afecțiunilor și a tratamentelor și că tehnica microsondei electronice ar putea fi o unealtă folositoare multor studii.

Cuvinte cheie: *oligoelemente, unghii, microsonda electronică, pemfigus vulgar.*

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In the organism, some mineral elements are found in very large quantities, others in very small quantities/amounts. Na, K, Ca, Mg belong to the first category, and to the second wide range of elements, oligoelements, belong the mineral elements which do not exceed 0.05% of the body weight. Some of them have a compulsory role in the good display of biological processes, Fe, Zn, Se, Mn, Co, Cu, Mo, Cr and F being named essential, while others are found in the organism, simply by its contamination, Al, Bi, Pb, and they do not have a biological role.

Microelements are present in the organism in extremely reduced quantities, sometimes only in traces. These are grouped into:

Invariable (indispensable) microelements-present in all living organisms. Among them are included: Cu, Co, Zn, F, I, Mo, etc.

Variable microelements-present only in certain organisms. Such elements are: Si, B, Ni, Se.Oligoelements have especially a structural and energetic role. Microelements have a preponderant catalytic role.

Living beings are real biochemical plants, whose functionality can be achieved in an optimum way only in the presence of certain adequate quantities of mineral substances. Many of the functions of vitamins, enzymes and hormones are dependent on the presence of mineral substances, some of them with an activating role of the metabolic processes (Ca, Mg), others with an inhibitory role (Cu).

A special mention is made of the fact that there are many organic biomolecules which contain METALS. Although these have been known in biochemistry for a long time, only after the 1970s the subject connected with these has been investigated more thoroughly. These researches have pointed out the role of the metal-organic compounds in biochemistry, thus outlining a new field of inorganic biochemistry or of bioinorganic chemistry with deep implications in the field of molecular biology, biochemical pathology, food and ecology [1, 2, 3].

The interest for the study of cutaneous annexes has increased lately, especially referring to the content in oligo and microelements as well as their medical implications. The aim of this paper is to determine the trace elements of human nails in pemfigus vulgaris patients by means of electronic microprobe analysis and their implication in the nail dystrophies [2, 3].

Material and method

Human material

There were 5 patients with pemfigus vulgaris. The diagnosis was established on clinical and paraclinical features (Tzanck cytodiagnosis, serical immunoglobulins and complement C3 fraction and histopathologic examination stained haematoxilin-eosin). The patients were aged between 35 and 60 years of age and the female/male ratio was 4:1. The patients were in our observation for many years and under corticotherapy alone or in association with azathioprin. One patient had an association with a centro-facial granuloma. 3 patients had successive determination of trace elements during a flare of pemfigus vulgaris as shown in table I and II.

Table I. Trace elements values, in ppm, in fingernails from pemfigus vulgaris patients

Obs.	Det	Si	S	Cl	K	Ca	Mn	Cu	Fe	Mg	Al	Zn	Total
F.R.	1	20	23260	210	270	11420	0	0	0	0	0	0	35190
	2	0	36096	1495	456	1117	0	0	0	0	0	0	39100
	3	30	40066	1673	116	1882	0	0	0	0	0	0	42831
N.S.	1	40	32820	2660	3000	1642	0	0	0	0	0	0	40170
	2	6	39969	3334	1334	855	0	0	0	0	0	0	45498
	3	16	42918	2135	537	40	0	0	0	0	0	0	46141
R.S.	1	430	38840	1970	580	490	0	0	0	0	0	0	43300
	2	26	48233	2129	427	4771	0	0	0	0	0	0	51575
	3												
L.	1	4	36123	0		1334	514	12	8	185	0	0	0	38178
G.	1	176	37536	0		583	1360	46	95	992	0	0	0	40788

Table II. Trace elements values, in ppm, in toenails from pemfigus vulgaris patients

Obs.	Det	Si	S	Cl	K	Ca	Mn	Cu	Fe	Mg	Al	Zn	Total
F.R.	1	0	25070	8310	12910	370	0	0	0	0	0	0	46660
	2	15	38276	1927	537	3088	14	0	0	0	0	0	43857
	3	10	39629	2735	298	640	60	0	0	0	0	0	43590
N.S.	1	40	35680	4930	16520	450	100	0	0	0	0	0	57720
	2	0	30136	3341	4769	2756	0	0	0	0	0	0	41002
	3	35	42489	5211	6440	532	0	0	0	0	0	0	54768
R.S.	1	49	41231	2000	3316	1021	16	0	0	0	0	0	47633
	2												
L.	1	0	38968	0		472	3692	0	304	739	0	0	0	44175

The normal subjects were 6, with age between 33 and 58 years; the female/male ratio was 4/2 and there was performed only one determination of trace elements in the nails (table III and IV).

Table III. Trace elements values, in ppm, in subjects' fingernails

Age	Si	S	Cl	K	Ca	Mn	Cu	Fe	Al	Zn	Mg	Total
36	27	42737	3973	1022	725	0	0	0	2	0	62	48538
33	26	43750	1536	199	448	53	0	0	5	0	66	46083
38	17	45656	1933	500	1637	13	0	13	0	0	0	49769
44	83	43618	1038	230	596	16	0	45	0	0	0	45626
55	4	41179	2169	201	414	0	0	4	0	0	0	43971
58	20	41143	1646	212	313	39	0	47	0	0	0	43420

Table IV. Trace elements values, in ppm, in subjects' toenails

Age	Si	S	Cl	K	Ca	Mn	Cu	Fe	Mg	Total
36	21	40432	3457	2167	1922	77	0	229	0	48478
33	2	38857	2669	2439	447	0	0	13	0	44428
38	82	40872	5114	6419	2625	0	0	24	0	55136
44	30	42326	2557	1220	603	0	0	3	0	46738
55	0	44499	3486	554	868	41	0	0	0	49448
58	16	39888	1969	215	2795	0	0	12	0	44895

Method

The samples used for analyses have been prepared for examination in the following way:

a) nail fragments from subjects' hands and respectively feet having a breadth of at least 1 mm, have been cut out;

b) they were included in a mass of acrylic resin;

c) they were processed with abrasive paper and than polished with diamond spray in order to obtain a perfect plane surface;

d) a layer of carbon was laid down by vaporization in vacuum to ensure an electro-conductor film on the nail sample surface necessary in the case of electronic sampler examinations to eliminate the electrons accumulating on nonconductor materials [4, 5].

For effecting the analyses on nail samples, the Japanese JCXA-50A electronic microsampler has been used, an operation system of the computer, in which the working conditions were: the diameter of the electronic spot: 20-50 um, the intensity of the electronic beam 9.10-8, acceleration tension as SkV. For effecting the chemical quantitative analyses, there was a measuring time of 15 seconds on the spectral line with the repetition of three times in the same condition, and working out of the average. All analyses both quantitative and qualitative have been made in an automatic system, being controled by the American computer PDP-8/m, using adequate programmes of analysis [3, 4, 6].

The samples prepared were qualitatively and quantitatively analysed by means of the computer. The results obtained, following the quantitative analysis could not be corrected (ZAF for example) because the concentrations of heavy elements (with a number of order $Z > 11$) did not exceed 3-5% altogether. The rest of the elements present in samples were of organic nature, probably containing C, H, O and N, elements which could not be determined experimentally with the apparatus. Therefore, the results obtained have a semiquantitative character and are useful when comparisons among samples are made.

The values obtained by us for trace elements of human nails in healthy subjects do not show significant variations depending on age or sex [3, 4, 7].

Results and discussions

Our technique has pointed out 11 elements and specified them semiquantitatively in ppm. The authors remark the compositional qualitative similarities of the elements contained in hair and nail, respectively (Zn, Cu). These compositional qualitative and quantitative similarities seem to be determined by the fact that they contain significant quantities of keratin, which would explain certain affinities for the metabolists in the blood.

The determinations have been effected from fragments obtained from the free edge of the nail (hand, foot), in 6 volunteers (irrespective of sex-therefore, we will not bring a discussion dependent on this factor) but with ages, as you can also notice in the table, between 33 and 58 (age correlated or close to those of the subjects included in the study) [4, 5, 8].

Only a single determination has been registered for each subject. Generally, the volunteers were not supposed to have other affections or to be under the influence of a chronic treatment. Eleven elements have been made evident by the electronic microprobe: Si, S, Cl, K,

Ca, Mn, Cu, Fe, Al, Zn, Mg, in the fragments drawn from thethe free edge of the finger nail less Al and Zn which could not be found in the fragments taken from the feet. Their sum is found between 43.000 and 48.500 ppm but with exceptions regarding the superior limit.

We have divided the 11 elements according to their presence or absence, respectively to the value level determinants (parts per million/ppm) in several categories:

- the predominant element with the biggest concentration (the basic element) is S, which represent approximately 85% of the total (40.000-43.000 ppm) [9].

S is the basic constituent of scleroproteins-the keratins (AA with S).

The aspect of a hair in electronic microscopy (ME), that of scanning, renders evident a cuticule covered by flattened cells and an unelevated longitudinal zone. These hairs have been analyzed in *Energy Dispersive X-ray Analysis*, and by using the relations between S and Al, it has been established that the concentration of S in the hair was more reduced (3.645%). At the same time, the hair has also been analysed through X-ray diffraction and it has been established the presence of a content in S of 6,12 compared to witness = 6.52. We have taken fragments from the nail affected by koilonychia which have been analysed by X-ray diffraction. A value of 2.17 has been noticed compared to the witness, who presented a value of 3.31. It seems that the S variations are rather strict (within narrow limits), its reduction under a certain value being incompatible with a normal nails and under recovery phase the S content is normalising as well as the nail morphology [9, 10, 11].

- the elements constantly present: Cl, K, Ca (500-3000 ppm) but with great variations concerning the behaviour of concentration, being probably under the influence of several factors (internal, external). We mention and underline the fact that in our determining Na, the most prevalent extracelullar element, is not to be found, perhaps just in order not to influence the reserve of the organism. An equally constant element by its presence but at much more reduced values than those mentioned above is Si (0-100 ppm); it is not a common element of the human cell, it might have originated in the processing technique of the nail fragments [4, 5].

- Fe, Al, Mn and Mg appear with an inconstant character in our determinings at values between 0-100 ppm and with a different behaviour in subjects respectively pemfigus vulgaris patients.

By the method used, we have obtained the following values: Al was not determined in subjects and in pemfigus vulgaris patients had a value of 0; Si had values 2-83 ppm in subjects, 6-430 ppm in pemfigus vulgaris, Cu and Zn are absent in normal subjects, appearing by way of exception in some determinings, though Zn was 0 Cu had values of 8-304 ppm in pemfigus vulgaris patients (12). It seems to be a difference between hand and foot in favour of the first [13].

The first remark which must be made consists in the fact that in order to have a diagnostic aim, the analysis of the nail oligo and trace elements must recognize any of their inhomogeneity from one nail to the other in the same person. We must make a remark here that in our study there have not been made any distinctions among the different fingers or toes; they have been analysed as a whole. The results of some authors show the fact that some elements can vary from one finger to the other while others have a relatively fixed concentration. These variations can be determined by external or internal causes. Other authors suggest that nail fragments from all fingers/toes should achieve a pool in order to obtain average values [14]. We have found the following methods used in literature: atomic absorbtion, SSMS (spark source mass spectrometry), emission spectroscopy, neutronic activation (a variant being also the technique used by us), spectrophotometry, fluorometry [6, 7, 15, 16].

These methods vary regarding accuracy and precision in the possibility of making evident the human nail trace elements. Thus, SSMS can appreciate about 30 trace elements at a level of parts per million (ppm).

By the SSMS method a wide range of trace elements can be determined and the qualitative results thus obtained can be specified with the help of atomic absorbtion [17].

It seems interesting the fact that the elements noticed in the human nail are the same with those noticed in the human hair, suggesting similarities

in the qualitative composition of these two epidermic tissues. Even the quantitative composition shows similarities between hair and nail. Thus Zn 14-280 ug/g in hair and respectively 62-360 ug/g in nail, Cu between 31-98 ug/g in hair and 17-64 ug/g in nail. The high values obtained for Si (SILICON) reflect probably the inclusion of the fragments in quartz. The Al value was also increased compared to the values obtained in hair, without being able to determine a source of contamination [6].

Comparisons with data from literature

Other methods of determining oligo and trace elements from the nail have also been mentioned above, the discussion being made on 5 elements for which there are sufficient data of comparison [15, 16].

Thus SSMS gave the value of 62-360 ug/g for Zn, while spectroscopy by atomic absorbtion gave the value 130-391 ug/g; Zn was absent in our study. The above data are at the inferior limit of those obtained by emission spectrography and beyond the value limits obtained through the analysis of activating the neutrons. The scarcity of data might be a reason for these discordances. Still, Cu and Fe are compared favourably by SSMS methods, neutronic activation, atomic absorbtion, emission spectrography. Spectrophotometry has given results at the inferior limit of those above. In our study Fe was 3-229 ppm in subjects nails [12].

Mg gave values of 11-170 ug/g (excepting 270-380 ug/g values given by renal patients) by SSMS method; Mg was absent in subjects. It was determined only in the fingernails of two subjects (62 respectively 66 ppm). Ca has given results comparable by SSMS method and atomic absorbtion but they superimpose only at the inferior limit of those determined by fluorimetric method or emission spectrography. In our study Ca had a great fluctuation of 313-2795 ppm in the nail of witnesses, and 40-3692 in the nail of pemfigus patients.

Chronological variations

A complete study effected by Jervsi and Perkons on human hair, pointed out the fact that the concentration of elements is linked to sex, diet, heredity, occupation and possibly other factors such as age and season. Taking into account that hair and nail are somehow similar chemically, it can be concluded that the concentration of elements in the nail can also be linked to the above factors [15, 16].

A study effected over a period of 12 months on a reduced number of donators and analysed in approximately identical conditions by SSMS, from on sample taking to another, did not point out any systematic tendency in the variation of Fe and of Ca; while for Zn, P, K, Mg and Na, the curve obtained, emphasized a spike at every 3-4 months, however without being equivalent among them (probably a spike for a certain steady state/a certain state of equilibrium). Nevertheless, these conditions seem to be characteristic for certain subjects as other studies do not confirm their systematic appearance [12, 15].

Possible inter-element relations

An element may depend on or may be linked to another element to exercise its function; its effect can be dependent on the presence oe even on the concentration of another element. This possible interdependence can help us understand the function of these trace elements. The study of a particular element can help us understand another element it is related to. Therefore, it is interesting to follow if an element in the nail is dependent on the concentration of another element of the same fragment. This fact has been determined by calculating the ration between two elements following the individual variation of the elements over the same given period (K/Na, Zn/P). The previous reports presented the most constant behaviour over the tested period. If this is more than a coincidence, is left to be defined more accurately by an extensive study [12].

The content in trace elements of the human nails do not vary with age or sex but there is a difference between fingernails and toes. The major component S is influenced by the pathologic process in pemfigus vulgaris and with therapy too; low levels of S are not compatible with a normal nail; normalising of the S content during recovery phase of pemfigus vulgaris contributes to a morphologic normal nail [1, 2, 11].

The method of electronic microprobe is recommended in some ecological studies,

influence of the environment, disease and therapy over the human organism, and even in the forensic analysis.

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