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Prospective Controlled Study Evaluating Pull Test with Correlation Serum Iron, Ferritin, Tranferin, Vitamin B12, Folic Acid in Patients with Alopecias in The Czech Republic

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Abstract

Hair has not only socio-communication function, but it is also a barometer of the human psyche and the internal environment of the body. Hair may very well reflect the long-term condition of mental frame of mind, but also the general health status of the individuals. The main objective of the study was to identify possible links between pull test and some pathological, biochemical and haematological parameters – serum iron, ferritin, tranferin, vitamin B12, folic acid in patients with alopecia areata, telogen deffluvium and androgenetic alopecias in The Czech Republic.

Keywords: Hair loss; Alopecia; Pull test; Iron Folate; Vitamin B12; Ferritin

Introduction

Hair loss occurs at any age in both sexes. Most often it takes a progressive thinning hair after puberty with genetic and hormonal influence and the progressive miniaturization of hair follicles with the consequent loss of hair in a typical localization. There are two types of mechanisms for the exchange of the hair. In what is both different and what unites them In humans, each hair follicle is undergoing an independent hair cycle hair follicles on the adjacent. It means that each hair follicle goes through an individual cycle. One hair is in the anagen, telogen in katagen and the other is ready to strike. In summary it can be said that the growth of hair and hair loss at the human is mosaic and asynchronous. Synchronous growth and the loss of hair we shortly find postnatally between the 4th-6th months of age of the child. Furthermore, it was shown that in pregnant women was appearing synchronization of hair growth and in the period of lactation there was synchronic loss of hair [1].

Alopecias, as the clinical unit, are relatively marginal or flawed area in dermatology. Yet there are several reasons that lead to this position: the diagnosis of alopecia is difficult differential diagnosis of alopecia is quite diverse, yet there are confusing differences in terminology and various clinical entities; dermatologists, the physicians of the primary contact and out-patient specialists always have not performed a biopsy of each alopecia bearings for histological verification of diagnosis, any doctor cannot correctly perform exploratory excision of alopecia bearings, each pathologist can't read the removed histological preparation of alopecia bearings, interdisciplinary cooperation are still fairly limited therapeutic options are limited, forecast of alopecia is indeterminate, the basis of success to diagnosis and management therapy is the close cooperation of the doctor-patient.

For the purposes of diagnosis and clinical practice were, over time, created different scaling and grading systems, which assess the hair loss in men and women. For the diagnosis of alopecia is important the exact medical history, clinical and laboratory examination. For the determination of therapy and forecasts, accurate diagnosis is important. The correct key for the determination of diagnosis is the understanding of the consequences, which led to the disruption of the balance of the hair cycle and the loss of the hair. One of the most important sources of valid information is a detailed medical history, when the doctor follows up with a sick the first diagnostic and therapeutic contact [2]. The relationship is based on the trust and cooperation of the patient, which leads not only to success in the diagnosis, but and chosen and guided therapy too. We use both in trichology – direct history taking (the data we get from the patient himself: alopecia, deffluvia and their possible causes), as well as an indirect history taking (we get most often by relatives or acquaintances of the patient, especially in the diagnosis of trichotillomania in children). In clinical trichology is appropriate to work with so called primary and secondary working diagnosis. After their evaluation we confirm the final diagnosis.

Examination methods in clinical trichology can be divided into non-invasive, semi-invasive, invasive, and additional. In our work we used a non-invasive method of pull test and method of the invasive, among which is included the donation of blood from a vein to biochemical and haematological examination.

Pull test (traction test) is a test based on the concept of a mild stroke of hair scalp. The purpose of the test is the assessment of the extent and location of hair loss in curls, lunge. A positive test result is in cases of anagen deffluvium, the telogen and loose anagen syndrome and in the early stage of alopecia areata. The result of the pull test will negatively affect the washing of the hair, combing and brushing of the hair before the test. Among the benefits of the pull test is included a quick test that can be performed on an outpatient basis and repeatedly without local anesthesia. It's financially unexpensive method that helps to estimate

the location and intensity of hair in curls lunge. The disadvantages of pull test are that it is a simple method that is appropriate only for the acute stage of the disease strike of hair. Negative pull test does not rule out telogen deffluvium. The actual method is based on the assumption that the gentle stroke of hair occurs telogen hair release. There is, of course, inter-individual variability of the test and also the possibility of subjective assessment by the examiner.

Selected Haematological and Biochemical Indicators

Iron

Iron in the human body is found bound in haemoglobin, myoglobin, ferritin, transferrin, haemosiderin (siderofilin), peroxidases, catalases, cytochromes (e.g. cytochrome-c, cytochrome-c-function oxidase), plasma, enzymes (e.g., xanthine oxidase, lactate dehydrogenase). The largest storehouse of iron in the body is haemoglobin (56-68%), ferritin and hemosiderin, myoglobin (4.0-9.0%) [3]. The best absorbed is iron anorganic and from haemoglobin. Absorption takes place in the upper part of the small intestine, where it is from iron bound to the hem absorbed about 23%. From unbound iron it is only 3%, this proportion increases to 8% at the diet reach on ascorbic acid and meat. After absorption from the intestine is needed the bind to the mucosal apoferritin, when becomes the oxidation of Fe2+ to Fe3+. Transport from the intestines into the stocks of iron and from there to the marrow ensures transferrin (β-globulin) [4]. Inadequate supply of iron is caused by inappropriate diet composition with a low share of the meat [5]. Most of iron in the diet is found in meat, liver (25-30% iron), eggs and vegetables (5% iron). When the preponderance of vegetable diet reduces the absorption of iron formation of phosphate and phytate absorbable heavily [5]. Negative balance iron is reflected by sideropeny which has 3 levels – prelatent, latent and manifest.

Ferritin

Ferritin is present in all cells of the body, body fluids, but most is stored in the liver, spleen, bone marrow, in skeletal and cardiac muscles. In pregnancy is present also in the placenta. Circulating in the blood ferritin is a mixture of serum ferritin (glycosylated ferritin, regarded as a normal secretory product of the cell) and tissue ferritin, which is released from damaged cells. Serum ferritin is a protein free of iron. It is found in low concentrations in comparison with the tissue ferritin. When it is in balance with the tissue ferritin, its concentration correlates with the overall stocks of iron [6]. In healthy individuals of working age, the serum ferritin is different in both men and women. In menopausal women with its concentration approaching similar values for men. Ferritin levels in children are lower than in adults. Women with a lack of iron in serum have a higher risk of developing deffluvia with lunge telogen hair. In women without systemic inflammation or other internal disorders, serum ferritin is below 30 ng/ml strongly associated with telogen hair loss and diffuse telogen deffluvia [7]. The extent of optimal serum levels of ferritin in women suffering from hair loss without the presence of systemic inflammation was set to a value between 30 and 70 ng/ml [8].

Transferin

The molecule of transferrin binds two atoms of trivalent iron Fe3+. Daily turnover occurs about 25 mg of iron in the blood [9]. In the body there is commonly 2.0-3.6 g/l for transferrin, with only one-third of it is saturated with iron. The remainder consists of free transferrin ready to bind iron, when the requirements of the organism increase to its transport (after absorption from food or release from depots stocks) [10]. Under physiological conditions its capacity is saturated from 1/3. The rest of the 2/3 represents free binding capacity of transferrin. The decline of transferrin saturation is one of the primary incentives for drop of hepcidin and release of iron from the hepatocytes. The decline in the concentration of transferrin is present in the acute phase reaction, hepatic insufficiency, malignant and chronic diseases, renal losses or malnutrition. The package works, dedicated to the relationship of tranferin hair loss is not discussed much. Transferrin is evaluated in the context of the level of iron and ferritin in serum. One of the summary of work which deals with this matter is Trosta et al. [11]. The review of transferin receptor expression is sufficient to regulate the content of iron in many keratinocytes in the epidermis. The increase of ferritin in the epidermis in transgenic animals means that the epidermal response to increase intracellular iron is physiological, and even if that it is not the cause of the increase. Ferritin in human skin is usually detected in the basal cells of the epidermis, but after exposure by to UV light it is easily detectable in all layers of the epidermis [12].

Folate

The absorption of folic acid depends on the normal function of the intestinal mucosa, that stores in the liver. The highest concentrations are in winter and the lowest in summer. It is found in fresh fruits and vegetables, nuts, beans, kidney and liver. Folic acid deficiency results from its low dietary intake, taking possession of the irrational, like as crash diets, when her impaired absorption of intestinal mucous membrane, tropical sprue, celiac disease, during pregnancy and alcoholism and the use of antispastic drugs. The main and most frequent manifestation of folate deficiency is megaloblastic anemia. Also the relationship of a slight deficit of folate was detected cardiovascular diseases. In fact, its deficiency leads to an increase in the concentration of homocystein [4]. Because it is slow regeneration of methionin from homocystein. To the assessment of the effect of folate on [4] hair loss, telogen deffluvium, alopecia areata or androgenetic alopecia is dedicated several works. However, the results are not ambiguous. Some of the authors have confirmed the effect of low levels of folate to increase hair loss, others eliminated it of course. From the works dealing with not only the assessment of the levels of folate but vitamin B12 too, but I mention the work of Thompson et al. [13] or the work of Cheung et al., who studied the effects of vitamins and minerals (vitamin D, ferritin, vitamin B12, folate, zinc) in the telogen deffluvium. The authors found that the telogen deffluvium is related to reduced levels of ferritin, vitamin D and zinc. Blood levels of folate were in standard and vitamin B12 deficiency was 2.6% [14].

Vitamin B12

Vitamin B12 is needed for the metabolism of DNA, further it biochemically claims at recycling of folate coenzymes and the metabolism of valin [4]. It is included and represented in meat, liver, milk, milk products and eggs. This vitamin in nature is synthesized by microorganisms, but the man is not able to synthesize it naturally in sufficient quantities. Vitamin B12 requires the synthesis of the internal factor of parietal cells of the stomach due to absorption through the mucosa terminal ileum. In the blood it is bound to the transport protein transkobalamin I (TC I) and transkobalamin II (TC-II, it is an acute phase protein). The failure of DNA replication in the absence of

kobalamin or folic acid leads to the breakdown of cell growth and division and leads to the crowding of the cells in the S-phase of the cell cycle [5]. In the work of Ozturk et al. [15] were observed levels of vitamin B12 in serum, folate, TSH, ferritin and zinc in patients with anxiety, telogen alopecia and trychodynia. The authors found no significant differences in serum levels of vitamin B12, folic acid, TSH, ferritin and zinc in patients with control groups. Gönül et al. haven't found a link between the serum iron, ferritin, vitamin B12, folate, and alopecia areata activity [16].

Material and Methods

In the range of 4 years, from September 2011 to April 2015 were on The Department of Dermatology in Hospital Přerov o.z., Stredomoravska nemocnicni a.s. totally examined 275 patients with hair loss. The number of patients at the end of prospectively evaluated sample was 244 people. A total of 31 patients were discarded due to poor cooperation or had different diseases of the hair, for example tinea capitis, pediculosis capitis, canities or folliculitis decalvans capilitii. We did not assess patients with cicatrical alopecias. The sample of patients comprised a total of 215 women and 29 men. No patient from the file was not infected with viral hepatitis, syphilis or HIV.

Filed patients (n=244) we have divided into two basic groups, women with hair loss (n=197) and women healthy/controls (n=18), men with hair loss (n=24), men healthy/controls (n=5). Another division of patients into groups was carried out according to the ICD-10 classification, XIIth Chapter: diseases of the dermis and subcutaneous tissue, subchapter: diseases of dermal adnexa.

Patients were divided into three groups according to the ICD-10 classification:

- Alopecia areata; Dg.: L63.8. Our abbreviation: AA
- Androgenic alopecia; Dg.: L64.8. Our abbreviation: FAGA (female), MAGA (male)
- Other non-cicatrical hair loss; Dg. L65.8. Our abbreviation: NCHL.

Due to the higher incidence filled patients with hypothyreosis, we divided them into the other two groups. First group of patients with disease of the thyroid gland (TG) and second group of patients without hypothyreosis (nTG). To the referenced groups have been assigned healthy controls of the patients.

Diagnoses	Code of ICD-10	Men	Women	Total
alopecia areata	L63.8	7	10	17
alopecia areata and hypothyreosis	L63.8 + nTG	5	3	8
FAGA, MAGA	L64.8	9	30	39
FAGA, MAGA and hypothyreosis	L64.8 + nTG	3	5	8
non-cicatrical hair loss and telogen deffluvium	L65.8	x	113	113
non-cicatrical hair loss and telogen deffluvium and hypothyreosis	L65.8 + nTG	x	36	36
all diagnoses total:		24	197	221
healthy people/controls:		5	18	23
total number of patients: (diagnoses and controls)		29	215	244

Table 1: Tested diagnoses and number of registered patients.

In the sample, 244 patients (n=215 women; men n=29) were evaluated:

- Pull test before treatment and after treatment for all patients.
- The detailed evaluation of selected haematological and biochemical parameters in relation to the charge of hair
- features: iron
- proteins: ferritin, transferin
- vitamins: folat, vitamin B12

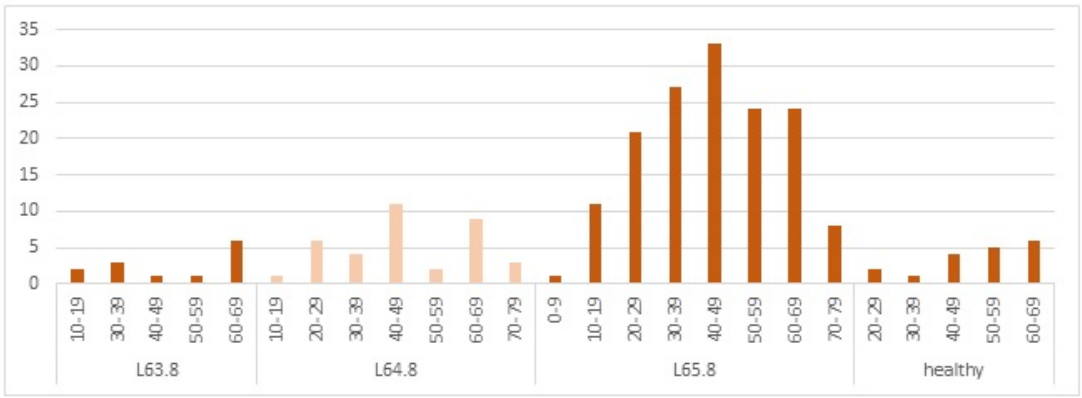
Preparation of the patient prior to the examination included

- an entrance interview of a patient with the doctor
- patient inclusion into one of the tested groups
- getting to know the patient's diagnosis: clinical and laboratory examination plan
- the introduction of a patient with therapy
- the introduction a patient with a frequency of checks after treatment

The group of patients had a wide age range. The youngest patient in our group was 8 years old. The oldest patient was 79 years old.

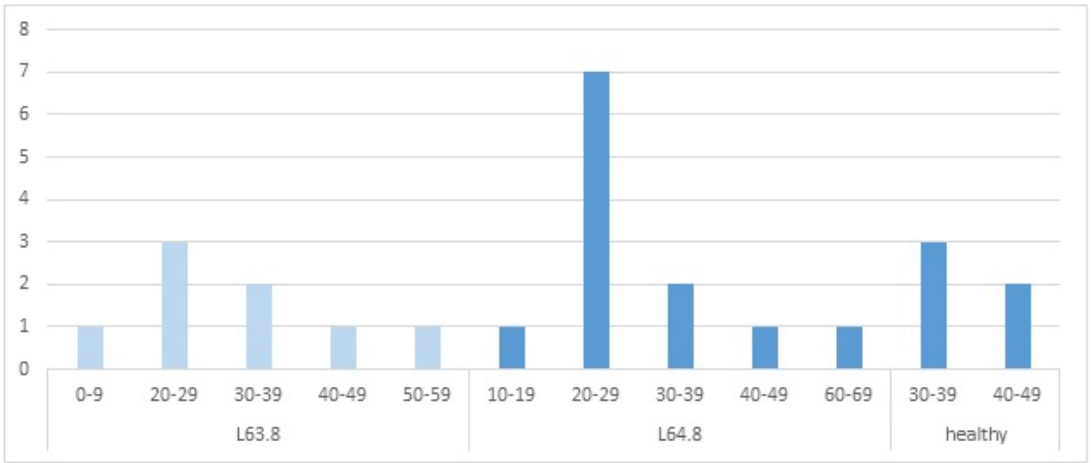
The age distribution of patients represented for researched diagnoses L63.8, L65.8, L64.8, and healthy controls (n=215) is assessed in the graph 1. The largest representation of women is for the diagnosis of deffluvia, chronic diffuse telogenic hair loss. In the chart you can see

the highest peak for age from 40 to 49 years. For the diagnosis of female androgenetic alopecia is the age peak in women the same as in the group of women with telogenic deffluvium. The second peak age is the age from 60 to 69 years for the diagnosis of female androgenetic alopecia. For the diagnosis of alopecia areata among women is the peak age from 60 to 69 years. In the group of women has not been confirmed any statistical dependency between the hair loss and pre or post-menopausal changes. Further, we did not examine this dependency of hair loss in pre or post-menopausal changes.



Graph 1: The age distribution of women patients and the diagnosis (n=215).

The age distribution of patients represented for researched diagnoses L63.8, L64.8 and healthy controls (n=29) is evaluated in graph 2. In the group of alopecia areata, the most numerous age peak in men is the age from 20 to 29 years. For a group diagnosis of male androgenetic alopecia is the age from 20 to 29 years too. From the above we can see that the men most often visit dermatological clinic with hair loss at the age from 20 to 29 years.



Graph 2: The age distribution of men patients and the diagnosis (n=29).

Pull test

Perform pull test: 2 days before the test, the patient may not wash his/her hair. When examined in rubber glove grips the investigating between the thumb, index finger and middle finger 20-60 (sometimes up to 100) of hair nearest to the scalp skin and mild, permanent (not energetic) fixed by pulling outward from the hair pulls out hair. The hair is being pulled from several opposite areas. Hair is divided into four quadrants and bitemporal area. In our case, we lunge for the diagnosis of chronic diffuse hair telogenic deffluvia (L65.8) pulled the

hair of the 4 areas, frontal, parietal, occipital and bitemporal. In the diagnosis of androgenetic alopecia (L64.8) and alopecia areata (L63.8) we followed as well, but in the diagnosis of alopecia areata we pulled hair from the edges of the alopetic area. The test is positive if it is from the four areas of scalp hair pulled out more than 10. Mostly it is the telogen hair, which is going to know by a macroscopic scale on one of their end there is reminiscent of the "small header PIN." If we pull the test less than 5 hairs pull test result we rate as negative [17-19].

Laboratory test

Laboratory biochemical tests of blood donations has been carried out in an accredited laboratory of Clinical Biochemistry and Clinical Haematology in Hospital Prerov, o.z. Stredomoravska nemocnicni a.s.. Specialized laboratory biochemical tests of blood donations has been carried out in an accredited Laboratory of Clinical Biochemistry and Clinical Haematology in Hospital Prostejov and Novy Jicin. Reviews of selected blood parameters was investigated in relation to the reference intervals at that time, the measuring methods used. Biological material was serum, blood sampling was carried out in a test tube when precipitation and separation gel or granulate, LH lithium heparin, EDTA. Taking a blood sample from a vein was made with the written consent of the patient in its documentation. Blood serum tests include those rated blood parameters: iron, ferritin, transferrin, folate, vitamin B12. The value of the reference limits in detail analyzed blood parameters in relation to the hair loss are listed in Table 2 [4].

Assessed heamatological parameter	Reference value	Base value
iron	9,20-33,70	µmol/l
ferritin	5,00-148,00	µg/l
transferin	2,00-3,60	g/l
folate	5,40-17,00	µg/l
vitamin B12	211-911	ng/l

Table 2: Observed laboratory parameters.

Results

Pull test results

Results of pull test for women: Summary of table 3 for women shows a comparison of the results of the pull tests before treatment and after treatment. The diameter of the pull test (Table 6) in a group with all the patients together (n=198) across diagnoses before treatment and after treatment was 30.92 amounted to 28.17. Improvement test for the diagnosis of the most numerous pull L65.8 (n=149) it was about 8.71% for the diagnosis of L64.8 (n=36) there was improvement up to 10.28%, and for the diagnosis of L63.8 (n=13) was 7.44% improvement.

Pull test	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	198	Before treatment	30,92	-2,75	-8,90%	0,00
		After treatment	28,17			
Diagnosis L65.8	149	Before treatment	31,60	-2,75	-8,71%	0,00
		After treatment	28,85			
Diagnosis L64.8	36	Before treatment	29,19	-3,00	-10,28%	0,00
		After treatment	26,19			
Diagnosis L63.8	13	Before treatment	27,92	-2,08	-7,44%	0,00
		After treatment	25,85			

Table 3: Comparison of pull tests in women before/after treatment.

Improvement in all the patients together across diagnoses (n=198) it was about 8.90%. In the table there are groups with a statistically significant improvement in the red. Collectively, it can be said that the combination therapy is not only clinically, but statistically significantly contributed in the group of women on the improvement of the value of the pull test and reduction of strike at the investigational diagnoses, and that is in an average of 2.75.

Results of pull test for women with hypothyreosis

Pull test, women with hypothyreosis	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
Diagnosis L65.8	36	Before treatment	21,31	1,92	9,00%	0,56
		After treatment	23,22			

Table 4: Comparison of pull tests in women with hypothyreosis before/after treatment.

From Table 4 for the value of the pull test shows that, for the diagnosis of L65.8 (chronic diffuse telogen deffluvium) in women with thyroid disease occurred after treatment of even slight worsening of unequal hair. In the table they are indicated by a statistically insignificant results in blue (p greater than 0.05). The average value of the pull test in the Group of patients with thyroid disease together (n=36) before treatment and after treatment was 21.31 amounted to 23.22. Deterioration of hair in women began by an average of 9%, i.e. 1.92. In summary one can say that seed therapy to improve the outcome of the test and reduce the dash pull hair in the diagnosis of chronic diffuse telogen hair/sortie deffluvium in patients with thyroid disease was not significantly involved.

Results of pull test for men

Pull test, women with hypothyreosis	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	20	Before treatment	22,10	-6,80	-30,77%	0,10
		After treatment	15,30			

Table 5: Comparison of pull tests in men before/after treatment.

From Table 5 for the value of the pull test in men, it appears that for all rated diagnoses together (L63.8 and L64.8) is the result of the pull test values after treatment significantly declined (improved). The diameter of the pull test in the group together in all patients (n=20) across the two diagnoses before treatment and after treatment was 20.10 amounted to 15.30. Improvement in all patients (n=20) was 30.77%, this is 6.80 hair. In the table are the group with a statistically significant improvement in red and statistically nonsignificant results are marked in blue (p greater than 0.05). In summary one can say that seed therapy to improve the result values of pull test in men and reducing the unequal hair, was not statistically involved (p greater than 0.05), even though there was a clinically significant improvement of the value of 30.77% pull test.

Comparison of the serum iron level in women

For serum iron (Table 6), we conclude that for all the diagnoses together (n=121) as well as individually with the result of serum iron after the treatment increased (improved).

Serum iron	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	121	Before treatment	16,48	0,51	3,10%	0,65
		After treatment	16,99			
Diagnosis L65.8	86	Before treatment	16,20	0,15	0,93%	0,92
		After treatment	16,35			
Diagnosis L64.8	27	Before treatment	18,44	1,62	8,80%	0,23
		After treatment	20,06			
Diagnosis L63.8	8	Before treatment	12,88	0,64	4,95%	0,82
		After treatment	13,51			

Table 6: Comparison of serum iron level in women before/after treatment.

In Table 6 percentage difference is marked in green, which is the largest (L64.8). Increased levels of serum iron in all the patients together across diagnoses were 3.10%. The value of the statistical significance of p is greater than 0.05. For all diagnoses collectively p value is 0.65, which means that the serum iron in women to change the unequal hair statistically is not taking part.

Comparison of the serum folate level in women

For the levels of folate (Table 7) for women shows that, for all the diagnoses together (n=24) as well as individually with the result of the folate in serum treatment significantly increased (improved). In the table there are the groups with a statistically significant change marked in red. Increasing folate for all the patients together across diagnoses was about 90%. For the diagnosis of L65.8 is improvement of 87.99%. For the diagnosis of L64.8 there was improvement of 87.78% and for the diagnosis of L63.8 was improvement even of 116.77%.

Serum folate	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	124	Before treatment	7,54	6,78	90,00%	0,00
		After treatment	14,32			
Diagnosis L65.8	87	Before treatment	7,62	6,71	87,99%	0,00
		After treatment	14,33			
Diagnosis L64.8	28	Before treatment	7,30	6,41	87,78%	0,00
		After treatment	13,71			
Diagnosis L63.8	9	Before treatment	7,42	8,67	116,77%	0,03
		After treatment	16,08			

Table 7: Comparison of serum folate level in women before/after treatment.

Comparison of the serum vitamin B12 level in women

From the summary Table 8 for serum vitamin B12 levels in women suggests that for all diagnoses together (n=124) is the result of a vitamin B12 following treatment statistically significantly increased (improved). In the table there are groups with a statistically significant change marked in red. Increased levels of vitamin B12 deficiency in all the patients together across diagnoses were 31.15%. For the diagnosis of L65.8 is an improvement of 31.85%. For the diagnoses of L63.8 and L64.8 did not start improvement. The value of p is greater than 0.05. In our case, for the diagnoses of L64.8 (p=0.25) and L63.8 (p=0.52), thus it is statistically insignificant.

Serum vitamin B12	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	124	Before treatment	283,59	88,35	31,15%	0,00
		After treatment	371,94			
Diagnosis L65.8	87	Before treatment	279,18	88,93	31,85%	0,00
		After treatment	368,11			
Diagnosis L64.8	28	Before treatment	285,93	94,90	33,19%	0,25
		After treatment	380,83			
Diagnosis L63.8	9	Before treatment	318,89	62,33	19,55%	0,52
		After treatment	381,22			

Table 8: Comparison of serum level of vitamin B12 in women before/after treatment.

Comparison of the serum ferritin level in women

From the summary of Table 9 for serum ferritin levels in women follows that for all diagnoses together (n=118) is the result of the ferritin levels following treatment statistically significantly changed (improved). In the table there are the groups with a statistically significant change marked in red, but the changes have occurred in all patients. For the diagnosis of L63.8 (n=8), the result is statistically significant, the value of the standard deviation of p is less than 0.05. Zoom (improvement) occurred about 120.66%. For the diagnosis of L64.8 and L65.8 did not start improvement. The value of p is greater than 0.05. In our case, for the diagnosis of L65.8 p=0.52, and for L64.8 it is p=0.54, thus statistically insignificant.

Serum ferritin	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	118	Before treatment	74,72	4,75	6,36%	0,56
		After treatment	79,48			
Diagnosis L65.8	84	Before treatment	75,42	5,83	7,73%	0,52
		After treatment	81,25			
Diagnosis L64.8	26	Before treatment	81,82	-13,75	-16,81%	0,54
		After treatment	68,07			
Diagnosis L63.8	8	Before treatment	44,40	53,58	120,66%	0,04
		After treatment	97,98			

Table 9: Comparison of serum level of ferritin in women before/after treatment.

The value of statistical significance for all diagnoses collectively is 0.56. As a result, this means that the levels of ferritin in serum for women to change the unequal hair reduction does not participate significantly. For the diagnosis of L63.8 it is statistically significant, but not generally draw conclusions for clinical use because the data collection was conducted on a small sample of patients.

Comparison of the serum transferin level in women

From table 10 for serum transferrin level in women suggests that for all diagnoses together (n=122) the result of the transferrin levels following treatment statistically significantly changed (improved). In the table there are the groups with a statistically significant change marked in red. For each diagnosis L63.8 (n=8), L64.8 (n=28) and L65.8 (n=86) the result is statistically significant. The value of the standard deviation of p is greater than 0.05.

Transferin	Number of patients in the group	The average in the group		Difference	Difference (%)	Statistical significance p
All diagnoses	122	Before treatment	2,81	-0,07	-2,51%	0,03
		After treatment	2,74			
Diagnosis L65.8	86	Before treatment	2,80	-0,07	-2,32%	0,06
		After treatment	2,73			
Diagnosis L64.8	28	Before treatment	2,91	-0,10	-3,36%	0,22
		After treatment	2,82			
Diagnosis L63.8	8	Before treatment	2,55	-0,03	-1,28%	0,85
		After treatment	2,52			

Table 10: Comparison of serum level of transferin in women before/after treatment.

Conclusions

In the years from September 2011 to April 2015 there were on The Department of Dermatology in Hospital Prerov o.z., Stredomoravska nemocnicni a.s. examined a total of 244 patients with hair loss. There were a total number of 215 women and 29 men. Sick women with hair loss, a total of 197 discharge and 18 healthy women have been assigned as a control sample. The sick men with hair loss were 24 and 5 healthy men were assigned to them as a control sample. File patients comprised a total of 88% of women and 12% of men, including 80.74% were women with hair loss and 9.84% of the patients were men with hair loss. We confirmed that the ambulance of dermatologists is visited by the hair for a substantially higher percentage of thrust women than men. In the sample of population, we have found that it is about 8 times more trichologically ill women than men.

Improvement of the result of the pull test in women without hypothyreosis after treatment for clinical diagnosis telogen deffluvium/ chronic diffuse hair loss was about 8.71%, for the diagnosis of female androgenic alopecia it was 10.28%, and for the diagnosis of alopecia areata it was 7.44%. Improvement of the value of the pull test for all the patients together was about 8.90%. From the above values it shows that the overall deployment and local combined therapy in a group of women without the disease of the thyroid gland did not only clinically,

but statistically significantly contribute on the change of the outcome of the pull test and reduce of the dash pull hair in investigational diagnoses, and that is in the average of 2.75 hair.

The results of the pull test for the diagnosis of chronic diffuse hair loss/telogen deffluvium in women with hypothyreosis after treatment show that there was a slight deterioration in the strong loss of the hair. The diameter of the pull test in the group of patients with hypothyreosis together before and after treatment was 21.31 amounted to 23.22. The deterioration of the hair loss in women with hypothyreosis occurred on average about 9%, this is 1.92 hair. This result shows that you cannot treat all patients at a flat rate, but first and foremost it is necessary to focus on the treatment of specific identified pathology, such as hypothyreosis or thyreopathy.

From the result of pull test in men it shows that the diameter of the pull test in the group all patients before and after treatment were 20.10 amounted to 15.30. Improvement of pull test in men was 30.77%, this is 6.80 hair. From this follows that assumed combination therapy for men to improve the outcome of the pull test and reduce the dash pull hair did not statistically participate (p greater than 0.05). Even though there was a clinically significant improvement of the pull test and the value was 30.77%. An evaluated sample of men was little with comparison of women, and therefore cannot draw definite conclusions.

Comparison of serum folate levels before and after treatment in women for all diagnoses together and individually, showed that serum folate are following treatment statistically significantly improved. Increased levels of folate for all patients was about 90%. For the diagnosis of telogen deffluvium/chronic diffuse hair loss was an improvement on the 87.99%, for the diagnosis of female androgenic alopecia there was improvement of 87.78% and for the diagnosis of alopecia areata was an improvement even on 116.77%. From these values shows that in the group of women, there was a statistically significant improvement in serum folate values after treatment, and therefore, the examination of the levels of folate supplementation we consider to be fundamental.

The value of serum vitamin B12 levels before and after treatment in women suggest that for all the diagnoses together with the result of the serum vitamin B12 levels following treatment statistically significantly improved. Increased levels of vitamin B12 for all patients were 31.15%. For the diagnosis of chronic diffuse hair loss/telogen deffluvium was the improvement at 31.85%. For the diagnoses of alopecia areata and androgenetic alopecia in women there was not statistically significantly improvement. The determination of the levels of vitamin B12 deficiency and its supplementation has proven as very significant.

A comparison of the level of serum ferritin in women before and after treatment it is apparent that for all the diagnoses together with the result of the levels of ferritin after treatment has not changed significantly. For the diagnosis of alopecia areata, the result is statistically significant (p greater than 0.05). There was improvement of 120.66%. For the diagnosis of androgenetic alopecia and chronic diffuse hair loss did not materialise a statistically significant improvement. From our clinical experience and comparing each value means that when reaching average values of serum ferritin around 90 µg/l, patients subjectively indicate a reduction of hair loss.

For serum iron level in women the result statistically insignificantly improved after treatmen. Increased levels of serum iron reached about 3.10%. We found that the serum iron does not affect hair.

From a comparison of the level of transferrin values before and after treatment it shows that for all the diagnoses result values together with the transferrin levels following treatment statistically significantly improved. For each diagnosis - alopecia areata, androgenetic alopecia, chronic diffuse hair loss/telogen deffluvium, is the result statistically significant. In recommendations for practice, it is important to perform a comparison of the values of the levels of transferrin, ferritin, serum iron and blood counts with the clinical status of the patient, difficulties with the hair loss and the type of alopecia. This evaluation has been used for monitoring the effect of the therapy in the substitution of iron and for the determination of a dosis and duration of therapy.

In a comparison of the results of the present authors of the current scientific works dedicated to the unequal relationship of the hair and mikronutrients [20], we found that individual research results are different. In the work of Park [21] that is devoted to the role of iron, ferritin, iron binding capacity and hemoglobin in relation to the charge of hair was made statistical evaluation on 210 patients (113 women and 97 men) with female and male androgenetic alopecia. It was found that in patients with female androgenic alopecia, compared to healthy controls, lower serum ferritin level occurred. Ferritin levels in men was also lower, but did not decrease after 70 µg/l in comparison with healthy controls. Our research shows that an increase in serum iron with all the patients together across diagnoses was 3.10%. The value of the statistical significance of p is greater than 0.05, which means that the serum iron in women and men on the change of the unequal hair did not statistically significantly participate. Not even a significant change in serum iron after the treatment appear, which lasted 14 weeks and all patients with hair loss have taken ferrosi sulfate pill at a dose of 1 pill daily. In correlation with the pull test, we uncovered the link levels of serum iron with a lunge of the hair.

When designing a treatment procedure in the study we looked for the available and currently published scientific knowledge existing at the time of the start of work on the study [22-34] after reviewing scientific papers and expert advice [22-34]. We developed a custom for a therapeutic procedure for topical and systemic treatment. This medical procedure we applied to clinical practice. We compared the effect of the therapy with the clinical status of the patient, the intensity of the loss of hair, pull test and each laboratory values (folate, vitamin B12, ferritin, transferrin, iron). The treatment of the hair loss should be initiated very early. The correct aim of therapy is to address the specific inducing cause and essence of the hair loss. As an available folate supplementation proved advantageous (Acidum folicum® Pharmaceuticals pill per os 1-0-0, 2 times per week for 15 weeks) and vitamin B12 (Milgamma N® injection solution, 1 times in 2 weeks intramuscular application 1 ampulla after 10 weeks), even if a specific role played the external application of therapy (for women Alpicort F® dermatologic solution, after 5 weeks, Belohair® 2% dermatologic solution after 15 weeks, for men Alpicort® dermatologic solution after 5 weeks, the Belohair® 5% dermatologic solution after 15 weeks). Long-term administration of the preparation containing ferrosi sulfate (Sorbifer Durules® pill per os 1-0-0, daily for 14 weeks) and spironolacton (Verospiron® 25 mg pill per os 1-0-0 after 25 weeks) had no effect on the patients hair loss. It is also necessary to treat, respectively, to ensure the treatment of a specific identified pathology like anaemia, malnutrition, hormone imbalance, hypothyroidism, total infections, cancer and so on.

Develop and establish a clear consensus diagnostic and therapeutic for patients with diffuse hair loss, male and female androgenetic

alopecia or alopecia areata is very difficult and responsible task. On this opinion works worldwide, several scientific institutions and research teams. The results of published studies are different. The benefit of our work was to reflect the connection between the hair and the selected discharge haematological and biochemical parameters and compare these values with the pull test. We confirmed that it is appropriate, in addition to the basic biochemical parameters, trace elements, folate, iron and vitamin B12 in particular to track levels of ferritin, transferrin, pull test and evaluate them in the context of medical history, clinical status, and type of alopecia in each patient. Thus, it follows from the above that for further research, statistical evaluation and successful shift in the treatment of alopecia will need much larger sample of male and female patients. It is necessary to carry out more detailed studies that will address each of the diagnoses of hair loss (cicatrical, non-cicatrical alopecias).

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