Oxidative Stress- related Markers and Alopecia Areata Through Latex Turbidimetric Immunoassay Method

SIMONA ROXANA GEORGESCU[1], CORINA DANIELA ENE[2]*, MIRCEA TAMPA[1], CLARA MATEI[1], VASILE BENEA[3], ILINCA NICOLAE[3]

[1] Carol Davila University of Medicine and Pharmacy, Department of Dermatology and Venereology. 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[2] Carol Davila Nephrology Hospital, 17 Apolodor Str., 050741, Bucharest, Romania

[3] Victor Babes Hospital for Infectious and Tropical Diseases, 283 Sos. Mihai Bravu, 030303, Bucharest, Romania

Alopecia areata is the most important inflammatory alopecia, caused by immunological factors, genetic factors, emotional factors, endocrine dysfunctions, infectious foci, nutritional deficiencies, and the harmful action of various environmental stimuli. The use of aggressive shampoos, of inadequate cosmetic and styling products, hair dyeing and waving, external pollutants might participate in the physiopathology of alopecia by sustaining inflammation, disturbing the local microcirculation, promoting oxidative stress. The aim of this study is to verify the hypothesis that oxidative stress might influence the progression of alopecia areata. Regulating the possible identified imbalances in alopecia areata might represent a means of preventing or improving this disorder.

Keywords: toxic products, oxidative stress, antioxidant status, alopecia areata

A series of experimental proof support the conception that the etiopathogenesis of alopecia areata is multifactorial, with immunological factors, genetic factors, neuroendocrine factors, oxidants, toxins being involved [1-4].

Cleaning products, care products of beauty products for hair (shampoos, balms, lotions, gels, creams, masks, serums, dyes) contain, in their composition, beside valuable ingredients, a series of compounds with harmful effects for the scalp and the hair. Ingredients with good action (glycerine, oils from various seeds, sodium citrate or citric acid, panthenol) act as a protection barrier for the hair, offer a shiny aspect, assure an adequate pH, stimulate growth, hydration and nourishment of the hair. The accelerated development of plastic material industry leads to the identification of synthetic compounds which prolong the shelf life of haircare products and influence their commercialisation. Ingredients which ensure the removal of protective oils from the hairs and scalp, disrupt the hormonal equilibrium of the organism, influence the penetration of chemical cleansing agents in the hair, modify the superficial tension of liquids, are included in almost all categories of cleansing products, haircare products and beauty products. A series of ingredients from the composition of haircare products affect the hormonal equilibrium, cause asthma, eye and skin irritation, hair fall, redness, allergic reactions, eczema, cancer. As a consequence, the use of formaldehyde, dioxans, oxidants, ethoxylated alkylphenols, sulphates, propylene glycol, parabens represents a major criteria in choosing the haircare products. The use of products with an adequate chemical structure ensure an appropriate structure of the hair medulla, cortex and cuticle and an unaltered going of the fundamental biochemical processes (growth, hydration, nourishment, lubrication, pigment genesis, keratinisation) [5-8].

Keratin and melanin are the two major components of human hair. Keratin has large amounts of cystine, a diamino acid containing sulphur, which binds keratin polypeptide chains producing disulphide bonds with a high degree of stability [5]. Secondary bonds such as Van der Waals interactions, hydrogen bonds and salt links are also presents but they are very weak and they can be very easily broken [6]. Melanins are biological pigments which determine the colour of skin, hair and eyes. Melanins have a protective role as they can immobilize free radicals from oxidative stress [5, 7].

Hair bleaching and hair dyes are a form of oxidative stress. Demi-permanent dyes contain 2% hydrogen-peroxide (H_2O_2) and an alkalizing agent. Permanent dyes contain 6% H_2O_2 and ammonia as an alkalizing agent while bleaches contain H_2O_2, ammonia and persulfates. During hair dyeing and hair bleaching the peroxide attacks certain amino acids, especially the molecules which contain sulphur, leading to oxidative degradation products. The melanin is also altered in the process and therefore it cannot protect hair proteins from the effect of reactive oxygen species. Methionine and cystine are mostly affected by reactive oxygen species and the disulphide bonds break. Therefore, the hair becomes weaker and it breaks more easily [5, 8]. On the other hand oxidative hair dyes and bleaching products can cause allergic reactions and irritation, due to the presence of H_2O_2 and ammonia [8].

Alopecia areata is a non-scarring alopecia affecting approximately 0.1-0.2% of population. It is characterized by round or oval patches of hair loss, with e*xclamation point* hairs at the periphery. It is usually the size of a quarter, but patients can lose the whole hair on their scalp (alopecia totalis) or even the whole scalp and body hair (alopecia universalis). Genetic and environmental factors are incriminated in the aetiology [9].

Oxidative stress seems to also have a role in the occurrence of alopecia areata, the disequilibrium between oxygen reactive species and antioxidants leading to oxidative damage in cellular molecules. Since lipids are more sensitive to the harmful effects of free radicals several authors aimed, and succeeded, to prove that lipid peroxidation products, such as malondialdehyde (MDA), are higher in patients with alopecia areata than in healthy controls while the antioxidant activity of superoxide dismutase, which plays a very important role in protection against oxidative stress, is defective. In other studies the

* email: koranik85@yahoo.com

total oxidant capacity (TOC) and the total antioxidant capacity (TAC) were measured in patients with alopecia areata and the authors demonstrated the presence of oxidative stress [10, 11].

Recent studies show that oxidative stress has an essential role in the aetiology and pathogeny of alopecia areata [1,12-15]. Reactive oxygen species are generated by a multitude of endogenous (cellular metabolism, mitochondrial respiration) and exogenous triggers (UV radiation, pollutants, chemical oxidants). The free radicals produced induce inflammation and affect cellular structures through oxidation of nucleic acids, proteins, carbohydrates and lipids [16-22]. The organism possesses endogenous defense mechanisms (enzymatic, non-enzymatic), which have the capacity to reduce or neutralize the free radicals formed. The serum components with antioxidant activity are represented by bilirubin, uric acid, albumin, transferrin, vitamins A, D, E, ubiquinone, glutathione, various oligo elements. The enzymatic antioxidant complexes present in the plasma are: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GRs), glutathione S-transferase (GSTs), catalase, peroxiredoxins, thioredoxin [12,13,16,22].

Presently, the determination of serum global oxidant capacity can be performed through several standardized tests, such as: the total oxidant status (TOS), the total peroxide (TP), the serum oxidation activity (SOA), the reactive oxygen metabolites (ROM). The global antioxidant capacity can be appreciated through different methods, of which we note: the ferric reducing ability of plasma (FRAP), the total equivalent antioxidant capacity (TEAC), the cupric reducing antioxidant capacity (CUPRAC), the biological antioxidant potential (BAP), the 2,2- diphenyl-picrylhydrazyl (DPPH), the total antioxidant status (TAS), the 2,2-azobis (2-amidinopropane) dichlorohydrate (AAPH), the total radical trapping parameter (TRAP), the oxygen radical antioxidant capacity (ORAC), the oxy-adsorbent test (OXY) [1,13, 20-22]. Based on these considerations, the present work has investigated comparative analysis of serum oxidative and antioxidative status parameters in alopecia areata patients and healthy persons.

Experimental part

Materials and methods

In this prospective-observational study, performed over a period of 2 years, two groups were monitored:

Group A- included 49 patients diagnosed with alopecia areata, 27 women and 22 men, aged between 19 and 48 years;

Group B- included 49 healthy persons, 29 women and 20 men, aged between 20 and 48 years;

Selection criteria: adults, adequate nutritional status, normal weight, non-smokers.

Exclusion criteria: pregnancy, alcoholism, drug abuse, systemic treatment, dietary supplements, other afflictions. All study participants signed a written consent for the use of their biological samples in the present study. The study protocol was approved by the Ethics Committee of the Hospital.

Biological sample processing: blood samples are harvested with the holder-vacutainer system, in basal conditions, before any diagnostic and therapeutic procedures.

Venous blood harvested on anticoagulant (K3EDTA) is used for haematological determinations. Blood serum obtained from venous blood harvested in vacutainer without anticoagulant is used for biochemical, serological, immunological determinations and determinations exclusively destined for research. The samples are immediately analysed or frozen at minus 60 degrees Celsius. The haemolysed, lactescent, icteric specimens are rejected.

Usual haematological, biochemical (including bilirubin, uric acid albumin), serological immunological determinations were performed through standardized analysis methods, using automatic work systems.

C-reactive protein (CRP) was determined through latex turbidimetric immunoassay method. The results were expressed in mg/dL serum, grouped as follows: normal range (0.0-0.60 mg/dL serum), low grade inflammation (0.60-1.0mg/dL serum), moderate grade inflammation (1.0-3.0 mg/dL serum).

The total oxidative status (TOS) in serum is performed by the reaction of a peroxidase with peroxides in the sample followed by the conversion of TMB (tetramethyl-benzidine). The coloured product are measured at 450 nm in a microtiter plate reader. The results were expressed in umol/L serum, grouped as follows: low oxidative stress (<180nmoli/L ser), moderate oxidative stress (180-310nmoli/L ser), high oxidative stress (>310nmoli/L ser)

The determination of the total antioxidative status (TAS) is performed by the reaction of antioxidants with hydrogen peroxide followed by the conversion of TMB. The samples are measured at 450nm in a microtiter reader. The results were expressed in nmol/L serum and were grouped as follows: low antioxidative capacity (<280nmoli/L, midle antioxidative capacity (280-320nmoli/L), high antioxidative capacity (>320nmoli/L).

The data was presented using the mean value and mean standard deviation, using the program SPSS. To compare the variables determined in this study we used statistical tests which are adequate for biomedical research. The significance level accepted was 0.05. Correlations between variables were established through linear regression, using the Pearson correlation coefficient.

Results and discussions

Basal characteristics of patients with alopecia areata and control, regarding the haematological parameters, blood glucose level, serum concentrations of urea, creatinine, lipids, triglycerides, cholesterol, amino-transferases, presented no significant differences between the two groups. C-reactive protein (CRP) serum level presented statistically significant differences between the two monitored groups. Assessment of the total oxidative status (TOS), individual serum components with an antioxidant potential (uric acid, bilirubin, albumin) determined in patients with alopecia areata and controls and statistical analysis of the data are represented in table 1.

CRP concentration was significantly higher in patients with alopecia areata as compared to the controls (table 1 and 2). Basal levels of CRP in patients with alopecia areata were situated in the reference domain in 34.5% of cases, in 39% of cases levels between 0.60 and 1.0 mg/dL serum were recorded for CRP, interval defining the presence of a low intensity inflammatory process, and, in 26.5% of cases CRP values were situated between 1.0 and 2.3 mg/dL serum, interval corresponding to a moderate intensity inflammatory process. In controls, 95% of values are situated in the reference domain established by the manufacturer of the analysis kit.

For serum bilirubin we obtained comparable values in patients with alopecia areata and controls (0.17-0.31 mg bilirubin/dL serum versus 0.18-0.61 mg bilirubin/ dL serum, $p>0.05$g). In 40% of cases with alopecia areata normal vales were obtained for serum albumin (over 3.5 g/dL serum), and in 60% of cases low values were obtained

Parameters	Alopecia areata(n)	Control	p significance
Gender (Male/Female)	22/27	20/29	0.484
Body mass index (kg/m2)	22.7+-1.7	23.0+-2.1	0.603
Blood pressure systolic (mm Hg)	125+-10	128+-11	0.715
Blood pressure diastolic (mm Hg)	67+-8	70+-9	0.266
Bilirubin (mg/dl)	0.22+-0.03	0.39+-0.18	0.109
Uric acid (mg/dl)	3.6+-0.9	3.9+-0.6	0.622
Albumin (g/dl)	3.68+-0.66	4.00+-0.45	0.217
CRP (mg/dl)	0.87+-0.53	0.16+-0.18	0.002
TOS (umoli/l)	341+-179	213+-82	0.001
TAS (nmoli/l)	288.1+-16.3	299.2+-21.5	0.053

Table 1
GENERAL COMPARISON BETWEEN ALOPECIA AREATA GROUP AND CONTROL GROUP

CRP: C-reactive protein; TOS: total oxidative status; TAS: total antioxidative status

Table 2
COMPARISON OF SERUM CRP IN ALOPECIA AREATA AND CONTROL

Significance	CRP (mg/dl)	Alopecia areata (number patients)	Control (number subjects)
Normal range	<0.60	17	47
Low grade inflammation	0.60 – 1.0	19	2
Moderate grade inflammation	>1.0	13	0

Tabelul 3
COMPARISON OF SERUM TOS IN ALOPECIA AREATA AND CONTROL

Significance	TOS(umoli/l)	Alopecia areata (number patients)	Control (number subjects)
Low oxidative stress	<180	2	8
Moderate oxidative stress	180- 310	14	39
High oxidative stress	>310	33	2

Significance	TAS (nmoli/l)	Alopecia areata (number patients)	Control (number subjects)
Low antioxidative capacity	<280	10	4
Midle antioxidative capacity	280 - 320	38	43
High antioxidative capacity	>320	1	2

Tabelul 4
COMPARISON OF SERUM TAS IN ALOPECIA AREATA AND CONTROL

(under 3.5 g/dL serum). In controls, over 95% of cases presented normal values for serum albumin and 5.0% presented low values. Even though the mean values of serum albumin were different in the two analysed groups, no statistically significant values were obtained between alopecia areata and controls. In 15% of cases with alopecia areata we obtained low levels of uric acid (under 3.0 mg/dL serum) and in 85% normal levels (over 3.0 mg/dL serum). In controls, 98% of analysed cases presented normal serum values for uric acid. No statistically significant differences were achieved for uric acid between patients with alopecia areata and controls (table 1).

Presently, there is no consensus regarding the optimal levels of TOS and TAS. For the analysis of experimental

Parameters	Alopecia areata		Control	
	R (coefficient of correlation)	P (statistic significance)	R (coefficient of correlation)	P (statistic significance)
TOS versus TAS	-0.47	0.04	-0.04	0.31
TOS versus CRP	0.42	0.00	0.06	0.87
TAS versus CRP	-0.21	0.06	-0.11	0.67
TOS versus Albumin	-0.34	0.01	0.00	1.00
TAS versus Albumin	0.15	0.05	0.07	0.93
TOS versus Uric acid	-0.09	0.20	0.00	0.78
TAS versus Uric acid	0.00	1.00	0.00	0.58
TOS versus Bilirubin	0.13	0.23	0.05	0.72
TAS versus Bilirubin	0.06	0.31	0.00	0.95

Table 5
COMPARISON OF SERUM OXIDATIVE AND ANTIOXIDATIVE STATUS PARAMETERS IN STUDY GROUPS

data, population samples were taken in this study, from certain geographic areas, comparable with regard to the demographic particularities and nutritional status. This approach allowed the establishment of control values, presented in table 1, without having the pretension that this is the optimal interval for the population's health status. In patients with alopecia areata a superproduction of TOS was identified, as compared to the controls ($p<0.05$) (table 1, 3). More than 67.0% of patients with alopecia areata included in the study presented high TOS levels (over 350 umol/l serum). Only 4.0% of patients with alopecia areata monitored presented a low serum level of TOS (under 180 umol/l serum), and 29.0% presented levels comparable with the control's (180-350 umol/L). In 21% of cases with alopecia areata low levels of TAS were identified (<280 nmol/L serum), in 77% optimal values (280-320nmol/l serum) and in 2% high levels (table 4). In controls, over 87% of cases presented values between 280 and 320 nmol TAS/L serum. Statistically, no significant differences were achieved for TAS between monitored groups (table 1).

The results obtained in this study show an increase in the production of free radicals (TOS) and a moderate reduction in the serum antioxidative capacity (TAS) in patients with alopecia areata as compared to controls. This finding is also supported by the statistically significant correlations, established between serum values of TOS and TAS ($r= -0.47$ $p<0.005$), between TOS and CRP ($r= -0.42$, $p<0.05$), between TOS and albumin ($r= -0.34$, $p<0.05$), between TAS and albumin ($r = 0.15$, $p=0.05$). In controls, no statistically significant relations were obtained between the studied chemical mediators (table 5).

This study shows that free radical production increases in patients with alopecia areata, while endogenous antioxidative mechanisms decrease. This disequilibrium leads to progressive deterioration of cell structures and hair growth disturbances. Our observations support the concept that the cell redox status plays an important role in the adjustment of hair growth. The interference of oxidative stress in alopecia areata might be due to the fact that oxygen reactive species and other free radicals disturb the regulation of gene transcription. It was reported that cells respond to oxidative stress through activation of transcription factors [4, 23 - 25]. These transcription factors lead to stimulation of antioxidative endogenous mechanisms synthesis, capable of fighting against free radicals, oxidants, toxins. Reestablishment of a balance between production of oxidants and antioxidants plays a crucial role in cutaneous homeostasis [26-28].

The results of this study regarding the identification of possible molecular imbalances involved in the physiopathology of alopecia might be useful in the discovery of efficacious therapeutic means with minimal adverse events for the treatment of patients with this pathology.

Conclusions

The authors appreciate that the results of this study, concretized in the super expression of oxidants and decrease of endogenous antioxidant mechanisms in patients with alopecia areata, might be involved in the promotion and progression of this disease. The identification of therapeutic means of normalizing oxidative stress might represent useful tools in the prevention of alopecia areata.

Acknowledgement: This work was possible partially with the financial support of Young Researchers Grant from the Carol Davila University of Medicine and Pharmacy, no. 33884 / 11.11.2014 and 33897 / 11.11.2004.

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Manuscript received: 12.01.2016