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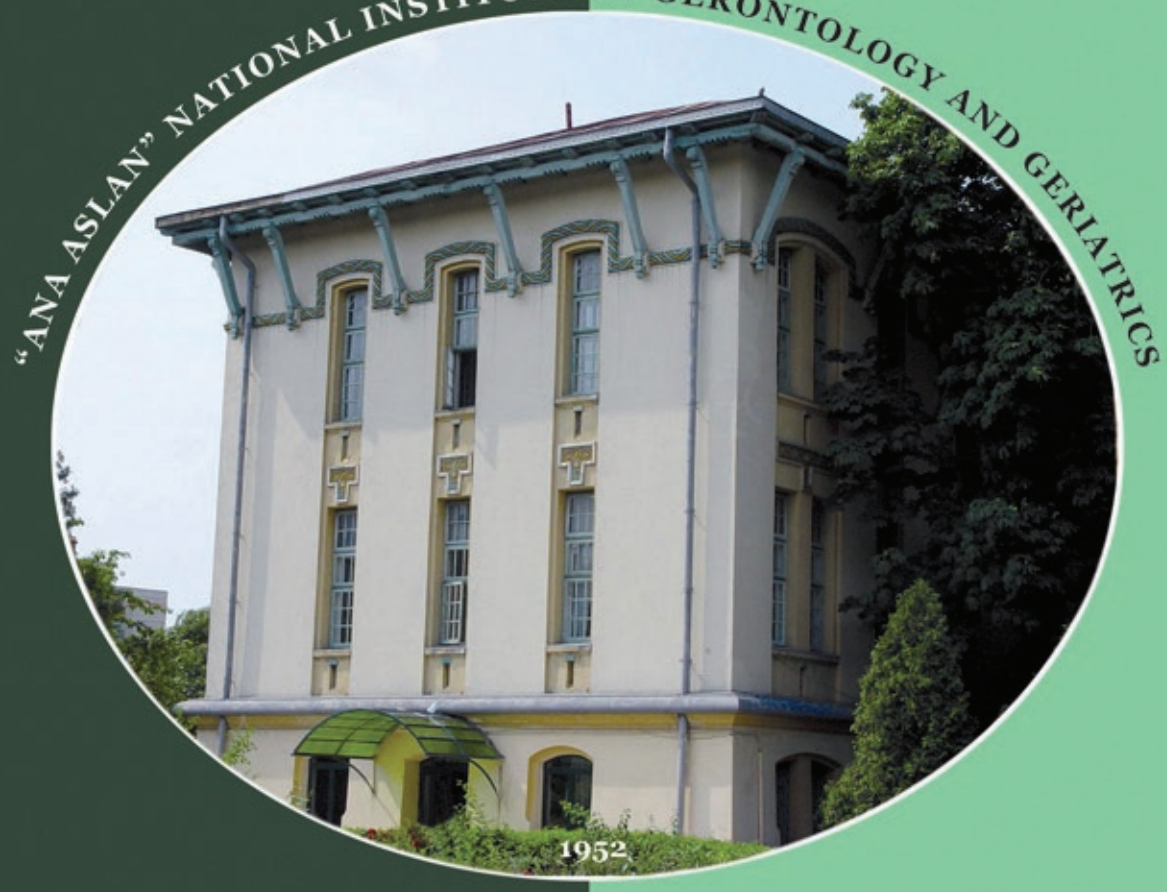

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Contents

Cuprins

THE LIFE STYLE AND HEALTH STATUS CONNECTED TO ELEMENTS OF THE PROTEIN PROFILE

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Abstract. During the last decade, chronic kidney disease became a worldwide public health problem. Actually, this is the result of pre-existing epidemics of diabetes mellitus, obesity and cardiovascular diseases. Nephrologists emphasize the importance of early diseases detection in order to enable therapeutic measures. The work studies some links between the protein profile and both overall health status (physical & psycho-social aspects) and lifestyle aspects. 199 patients, mostly women (average age X=69.21 years), are evaluated through socio-medical tests concerning functionality and nutritional status. Diagnoses and biological investigations were collected from clinical records. The work emphasizes the link between nitrogen retention products (especially creatinine and urea) with three groups of chronic kidney disease risk factors (as taken from Romanian Society of Nephrology Guidelines). Correlations with lifestyle elements were also calculated. If we present the correlations of the risk factors solely with creatinine and order the resulting coefficients by strength, we can conclude that kidney health depends upon: We can conclude that kidney health depends upon: 1)fat-free diet (r=.254/p.=.000), 2)diabetes mellitus (r=.199/p=.005), 3)metabolic syndrome (r=.177/p=.013), 4)dyslipidemia, 5)more sustained physical activity, 6)smoking, 7)age. Ordering the correlations by strength, the importance of certain factors becomes prominent: lifestyle (especially nutrition), risks which initiate kidney damage (diabetes, metabolic syndrome), risk factors which lead to the worsening of the disease (dyslipidemia, smoking) and risks which increase susceptibility to disease (age). Thus, our study confirms data collected from current domain research.
Key words: lifestyle, global health, protein profile

LEGĂTURA DINTRE STILUL DE VIAŢĂ, STAREA DE SĂNĂTATE GLOBALĂ ŞI PROFILUL PROTIDIC

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Rezumat. În ultimul deceniu, boala cronică renală a devenit o problemă de sănătate publică. Practic, ea este consecinţa epidemiilor de diabet, obezitate şi boli cardiovasculare preexistente. Nefrologii subliniază importanţa depistării precoce a afecţiunii, pentru măsuri terapeutice. Lucrarea prezintă unele legături dintre profilul protidic, atât cu starea de sănătate globală (fizică şi psiho-socială), cât şi cu stilul de viaţă. 199 pacienţi, în majoritate femei (vârsta medie X=69,21ani) sunt evaluaţi prin teste medico-sociale privind funcţionalitatea şi statusul nutriţional. Diagnosticele şi investigaţiile biologice sunt culese din clinică. Datele relevă legătura dintre produşii de retenţie azotată (în principal, creatinina şi ureea) cu trei grupe de factori de risc ai bolii cronice renale (preluaţi din Ghidul Societăţii Române de Nefrologie). De asemenea s-au efectuat şi corelaţii cu aspecte ale stilului de viaţă. Daca prezentăm ordonarea factorilor de risc corelaţi doar cu creatinina, în funcţie de intensitatea coeficienţilor corelaţionali, putem spune că sănatatea rinichilor depinde de: 1)alimentaţia fără grăsimi (r=.254/p.=.000), 2)diabetul zaharat (r=.199/p=.005), 3)sindromul dismetabolic (r=.177/p=.013), 4)sindromul dislipidemic, 5)activitatea fizică mai susţinută, 6)fumat, 7)vârstă. Această ordonare a corelaţiilor semnificative

subliniază importanţa factorilor: stil de viată (mai ales a alimentaţiei), riscuri ce iniţiază leziunile renale (diabet, sindrom metabolic), riscuri care determină progresia bolii (dislipidemii, fumat) şi factori de risc ce cresc susceptibilitatea la boală (vârsta). Astfel, studiul nostru a confirmat date recente din literatura de specialitate.
Cuvinte cheie: stil de viaţă, sănătăte globală, profil protidic

INTRODUCTION

In the last 20 years, chronic kidney disease (CKD) has become one of the major public health problems of the XXI century. About 8% of Europe's population suffers from CKD to some extent. The figures grow gradually and, if this trend continues, the number of affected people will double in the next decade. In the US it is estimated that 11% of the population is affected by the disease and another 20 million people are at risk of developing it [1]. At European level, patients with Stage 5 CKD have a 10 times higher risk of death than the general population. In our country, chronic kidney disease has a prevalence of about 11.4% [2]. The CKD silent epidemic is a problem of national health systems: if dialysis treatment alone reaches 2% of national health budgets, the cost of implementing prevention strategies for CKD can be modest. More specifically, 97% of kidney disease allocations are intended for disease treatment, while expenses with preventive measures represent only 3%) [3].

The first study that enables accurate assessment of the prevalence of CKD in Romania is the National Study PREDATORR, started in 2013 and carried out by the *Romanian Society of Nephrology* in partnership with the *Romanian Society of Diabetes, Nutrition and Metabolic Diseases*. PREDATORR gives a clear picture of the situation of diabetes mellitus, chronic kidney disease and related comorbidities (metabolic syndrome, dyslipidemia, overweight, obesity, hypertension, hyperuricemia [4].

Chronic kidney disease is defined by: (1) reduction in glomerular filtration rate (GFR) below 60ml/min/1.73m2, for a minimum duration of three months, or: (2) kidney impairment for a period longer than three months. Kidney impairment can be diagnosed without knowing the etiology; it consists of kidney structural or functional abnormalities, reflected by: •abnormalities of blood tests (nitrogen retention, dyselectrolytemia, metabolic acidosis); •abnormal urine tests (proteinuria, albuminuria, hematuria, leucocyturia); •abnormal renal imaging and renal biopsy results.

At the moment, all the major medical societies have issued guidelines which state the obligation to calculate creatinine clearance in order to assess kidney function [5].

These international guidelines provide a classification of CKD risk factors. Ordered by relevance, they are: diabetes, hypertension, heart disease, smoking, obesity, family history of kidney disease, age. The first three diseases are the most important and must be monitored in order to detect early signs of kidney damage [6].

Therefore, we study the links between patients' protein profile (e.g. urea, creatinine, uric acid) with both elderly health and lifestyle elements in order to establish the risk of developing kidney disease, by socio-medical tests.

MATERIALS AND METHODS

Our sample consists of 199 patients from INGG (27 men and 172 women) aged between 45 and 92 years (X = 69.21 years), evaluated through global socio-medical tests concerning:

- Lifestyle: *Simple Lifestyle Indicator Questionnaire* [7];
- Nutritional status: MNA-long form (Mini Nutritional Assessment) [8] and Waist-Hip Index;
- Physical functionality: Hand Grip Test, ADL, IADL;
- Groningen Frailty Index (GFI) [9].

Diagnoses and biological investigations are collected from the clinical records.

RESULTS AND DISCUSSIONS

1. The prevalence of chronic conditions

The prevalence of chronic pathology is shown in Fig. 1, by age groups, ordered by the prevalence values for the older group (70-95 years). Cardiovascular diseases (CVD) are the most common in our sample. As regarding CVD, the difference between the two age groups is exceptionally high (124%). Percentage differences are also noted in the neurological diseases: nearly 50% higher percentages for the older ones, a fact explained by atherosclerotic pathology which increases with age. The large differences between the age groups for cardiovascular and neurological diseases suggest that young subjects tend to have certain health risk behaviors that cause the development of circulatory cardiac and neurological diseases in later life. As regarding the endocrine-metabolic pathology, the younger group outperforms the elders' group by 57.5%.

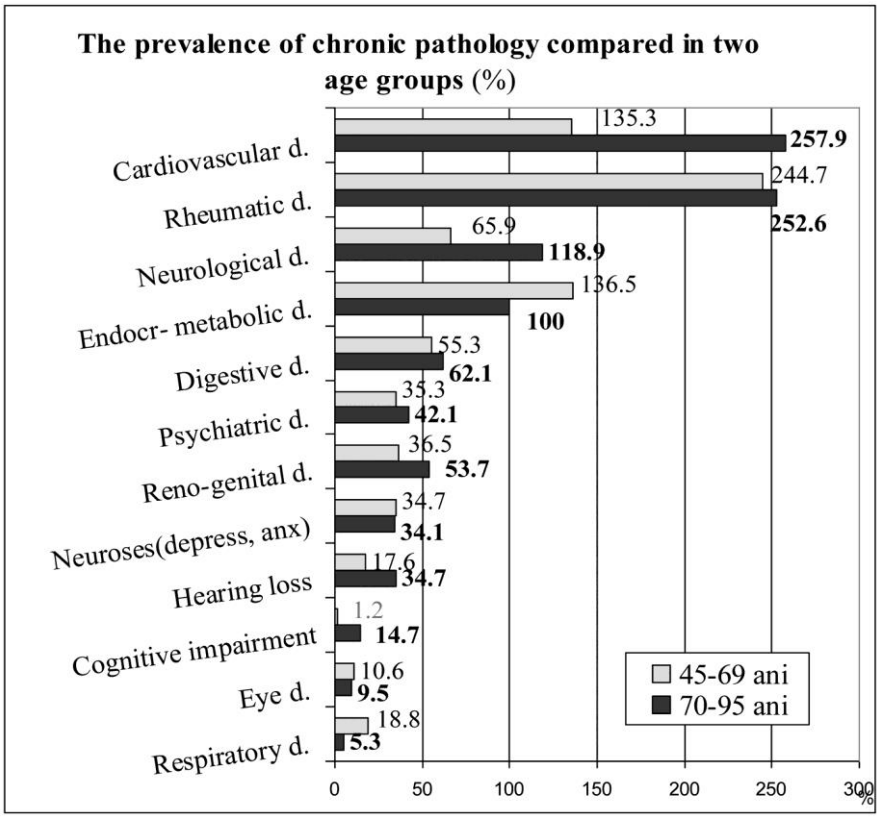

Fig.1 The prevalence of chronic pathology compared in two age groups

Tab.I Percentage values of some serum substances of nitrogen retention (n=199)

	Urea	Creatinine	Uric acid
	%	%	%
Normal values	80.4	**86.9**	74.9
Higher values	**19.6**	**13.1**	**25.1**
Total	100.0	100.0	100.0

Tab. II Percentage distribution of the sample in glomerular filtration rate categories, under the KDIGO Guidelines-2012

		%
G1 (normal or raised GFR)	90 - 189 ml/min/1.73 m2	20.1
G2 (slight decline GFR)	60 - 89 ml/min/1.73 m2	52.3
G3a (slight to moderate decline)	45 - 59 ml/min/1.73 m2	20.6
G3b (moderate to severe decline)	30 - 44 ml/min/1.73 m2	7.0
G4 (severe decline)	15 - 29 ml/min/1.73 m2	0
G5 (kidney failure)	< 15 ml/min/1.73 m2	0
Total		100.0

GFR- glomerular filtration rate; creatinine clearance was calculated by Cockroft-Gault formula

Comparing the percentage of normal creatinine patients (86.9%) with those also having normal GFR (only 20.1% from the subjects), we sought an explanation: the elderly or those with reduced muscle mass have lower creatinine level, and it may conceal for a long time the developing of a chronic renal failure (Tab. I and Tab. II). Creatinine clearance more accurately describes the renal function, because it take into account the patients' age and weight. We learned from data processing that 69.4% subjects are aged 65 years and over, while the share of obesity in the sample is 41.12% and overweight is 37.6%.

The epidemiology of CKD is still under evaluation. The *Romanian Society of Nephrology: "Medical practice guides - Chronic kidney disease"* [5] describes a classification of CKD risk factors, grouped in three categories: factors that may increase susceptibility to kidney damage, others that can cause the onset of the kidney disease and still others that can cause the worsening of the disease.

2. Factors that may increase kidney disease susceptibility

Factors that may increase kidney disease susceptibility

- Progression to the end-stage of CKD is usually seen in the elderly, because the aging process is associated with renal senescence. An exception, however, is type 1 diabetes, where the terminal phase occurs at much younger ages [2]. The link between kidney disease and age was verified in our study by correlations with creatinine and urea. The link with urea is stronger because often elderly kidneys cannot adequately concentrate urine and thus urea increases. Instead, creatinine values in the elderly may be small due to low muscle mass, masking the renal failure.
- In general, susceptibility to disease can be connected to a lifestyle marked by poverty, depending on socioeconomic factors as: educational level, income, marital status. The correlations from Tab. III prove these assertions. Widowhood has a high percentage, 48.3%. Generally, revenues are small, the average pension in the sample being 1019 lei, about 100 lei higher than the average pension in the economy (890 lei).

Tab. III Factors that may increase kidney disease susceptibility

		Creatinine	Creatinine clearance	Urea
Age > 65 years		r =.154/p =.030		r = .301/p = .000
Low socio-economic level:	Marital status		r =.272/p =.004	r = .248/p = .008
	Household income			r = -.301/p =.001
	High school graduate			r = -.226/p = .016

Chronic conditions that may initiate kidney damage

- Diabetes and hypertension are the leading causes of CKD. Therefore, careful assessment and monitoring are necessary to detect early signs of kidney damage (Tab.IV).
- Hypertension is almost constantly present (90%) in patients with chronic kidney disease. It may represent either a cause or an effect of renal disease. The higher the blood pressure values, the higher the risk of chronic kidney disease progression [10].
- The total percentage for CVD is 78.9%. The percents for the most common CVD diseases are: hypertension-66.83%, ischemic heart disease-61.31% and venous circulatory failure-61.31%. The total share of neurological diseases is 59.8% and the percentage of vertiginous syndrome + vertebro-basilar circulatory disorders is 40.7%.

Tab.IV Conditions that may initiate kidney damage

		Creatinine	Creatinine clearance	Urea
►Diabetes mellitus				
	Global sample (n=199)	r = .199/p =.005		r=.301/p=.000
	Prefrails, frails (n=87)	r = -.415/p=.000		
► Metabolic syndrome				
	The healthy (n=199)	r = .177/p = .013	r=-.151/p=.033	r=.230/p=.001
► Atherosclerotic disease: (coronary, cerebral or peripheral)				
	→ Hypertension *(n=199)*			r=.301/p=.001
	→ Ischemic heart disease		r= -.146/p=.046	r=.151/p=.033
	→ Heart failure			r=.142/p=.046
	→ Venous circulatory failure		r= -.169/p=.017	
	→ Neurological diseases		r= -.157/p =.027	
	→ Carotid atheroma		r= -.173/p=.014	
	→ Vertiginous syndrome+ vertebrobasilar circulatory disorders		r= -.239/p=.001	r=.251/p=.000
	→ Polyneuropathy *Prefrails, frails(n=87)*	r = .316/p = .003	r= -.215/p=.046	r=.139/p=.050
► Urinary tract obstructions				
	→ Urinary stones		r=-.232/p=.001	
	→ Urinary infection		r=-.139/p=.050	
	→ Prostate adenoma		r=-.218/p=.021	

Long-term treatments that may initiate kidney damage

Tab. V Long-term treatments with potentially nephrotoxic drugs

Inhibit. angiotensin converting enzyme (IACE); Angiotensin receptor blockers (ARBs); Nonsteroidal anti-inflammatory drugs (NSAIDs) Antineoplastic (e.g. methotrexate)	They are drugs commonly used in clinical medicine(!) their toxicity is sugested by significant correlation between serum urea and MNA-h item (over 3 different drugs/day) r =.146 /p= .040

Due to high pathological load (9.2 diseases per patient), our subjects receive a large number of drugs. The MNA-h item shows the consumption of more than three medicines daily.

The correlation between serum urea and MNA-h item suggests a toxic effect of medication, in general. According to nephrologists' belief, nephrotoxic effect of the therapies can lead to chronic kidney disease. And drug classes listed in Tab.V (IACE, ARBs and NSAIDs) are very commonly used to treat hypertension and rheumatism.

Factors that may accelerate kidney functional decline

Tab. VI Factors that can accelerate renal functional decline in patients with confirmed CKD

	Creatinine	Creatinine clearance	Urea	Uric acid
→ Hypertension	(See Tab. IV)			r = .310/p = .000
→ Poorly controlled blood glucose				
→Dyslipidemia:				
Triglycerides * (n=87)	r = .219 /p = .042			r = .154/p = .030
HDL-Chol ** (n=112)	r = -.203 /p = .031			
→ Obesity		r = -.335 /p = .000		
→Abdominal obesity		r = -.249 / p = .000	r = .250 / p = .000	r = .182/p = .010
→ Smoking Score (n=199)	r = .140/p = .049			
→ Number of years of smoking	r =.203/p = .032			r = .177/p = .013

*According to GFI frilty scale, the study sample (n=199) is divided in: * the "frail + prefrail" group (n=87) and ** "the healthy" group (n =112)*

3. Complications of chronic kidney disease

The correlations between nitrogen retention and CVD were already noted in Tab. IV. Also, PAD correlated significantly with creatinine clearance: r=.194/p=.014. Anemia is important in the disease symptomatology, but is reversible through treatment. Anemia significantly and directly correlates with serum creatinine: r=.142/p =.045.

The metabolic bone disease progresses with decreasing kidney function. It includes CKD Mineral and Bone Disorders (CKD-MBD) and comprises two entities: (a) renal osteodystrophy and (b) vascular calcification in bone mineral metabolism disorders. For (a.) *renal osteodystrophy*, we found two significant links with kidney disease expressed by the correlations: osteoporosis - serum creatinine (r =.182/p=.010), and polyarthrosis - blood urea (r=.240/p=.014). For (b.) *vascular calcification*, we point out the correlation of carotid atheroma with serum urea (r =.173/p=.014).

4. Prevention of chronic kidney disease

Romanian Society of Nephrology recommends eight rules to prevent CKD or to slow down its progression. Romanian Society of Nephrology recommends eight rules to prevent CKD or to slow down its progression. They are represented by: keeping normal limits for >blood sugar and cholesterol, >blood pressure, >body weight; also, >balanced diet (>sufficient liquid consumption, >reducing salt intake), >avoiding sedentary and > avoiding smoking. The same lifestyle recommendations are contained by "European Charter on Heart Health"(2007), but the latter includes additional one: stress avoiding. Our data prove the link between stress and chronic renal pathology, by highlighting an almost constant link between stress (from depressive neurosis and anxiety) and byproducts of nitrogen retention. Thus, in the full sample (199 subjects), we denote the correlation between these neuroses with creatinine level (r=.175 / p=.013) and with the creatinine clearance (r= -.171/p=0.016). In the subgroup of the ills (pre-frails + frails), these correlations are more powerful. The values of correlation coefficients for neurotic syndromes are: with creatinine, r=.391/p=.000, with creatinine clearance, r=.376/p=.000, and with serum urea, r=.267/p=.013.

Tab. VII Correlations of protein profile with elements of lifestyle in healthy patients (n=112)

(a).Healthy patients (n=112)			
	Creatinine	Creatinine clearance	Urea
Average compressive strength		r=.460/p=.000	
Physical exercises score	r= -.141/p=.047	r=.195/p=.039	r=-.256/p=.006
Consumption of fatty foods, rich in cholesterol	r= .220/p=.020		
Liquid consumption (MNA-m)		r=.266/p=.005	
(**b.) Frails + prefrails patients** (n=87)			
Average compressive strength		r=.322/ =.003	
Consumption of fatty foods rich in cholesterol	r=.323/p=.002		r=.280/p=.009

Tab. VII highlights the links between products of nitrogen retention and lifestyle elements: level of physical activity, fat-free diet and adequate consumption of liquids.
As expected, the muscle strength of the frails correlates poorly with creatinine clearance, compared to the "healthy" subjects. In contrast, consumption of fatty foods has a weaker connection with creatinine among "the healthy" ones. And, as a proof for the better health, among "the healthy" patients the relation between creatinine clearance and consumption of liquids has a stronger correlation value (r=.266 /p=.005).

CONCLUSIONS

Worldwide, dramatic changes of the environmental and human behavior brought an epidemic of diabetes type 2 and obesity. Both conditions contribute to others two epidemics: one represented by CVD and the other by CKD. The nephrologists underline the importance of early detection, since initial stages, to impose a treatment plan that would halt the evolution and complications.
The significant correlations revealed the presence of risk factors: some that may increase susceptibility to disease, some that initiate the disease and others that can produce the worsening of the CKD. On the other hand, correlations with lifestyle

elements have pointed out that prevention is possible through healthy eating (avoiding fats, consuming enough liquids), sustained physical activity, avoiding smoking and stress.

In fact, the care of these patients requires a multidisciplinary team. It should include besides nephrologists, general practitioners, nutritionists, cardiologists and... psychologists. Also, active involvement from patients is essential in prevention programs, and in ensuring adherence to a healthy lifestyle.

Acknowledgment

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PROGERIA – A SYNDROME OF CELLULAR ENERGY DEFICIT?

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Abstract. A critical analysis of the accelerated aging syndromes may explain what aging is, but also why some tissues and organs age at accelerated rates. Syndromes of accelerated aging (progeria) are caused by a recessive autosomal mutation with a frequency of 1 in 10 millions, which affects a helicase involved in DNA repair. The Hutchinson-Gilford progeria syndrome (HGPS), child's progeria, has an even lower frequency of occurrrence. In most cases, it is caused by a point mutation of a gene encoding a protein of the nuclear envelope, lamin A. Lamin A mutations occur in other progeroid syndromes too, causing various organ degenerations (dystrophies). In HGPS, aging symptoms appear in early years of life, and death occurs at very young ages, 13-15 years due to cardiovascular diseases. HGPS may provide valuable insights into the aging process. In patients with HGPS, dementia and cancer are not frequent, and most degenerative processes occurrring in these patients may be attributed to extended fibrosis. Fibrosis accompanies a plethora of degenerative disorders associated or not with aging. Understanding mechanisms of fibrosis may help better explain HGPS and some important aspects of aging. In this paper, we have shown HGPS and accelerated aging syndromes' particularities, in the light of the biochemical hypothesis of aging we advanced. According to this hypothesis, aging results from functioning of the organism and some reactions are less stimulated and diminishing in time, affecting not only specific cellular biochemical processes such as protein synthesis, but also cell energy. Possible therapeutic strategies for these conditions are also presented.
Key words: progeria, aging, Werner syndrome, Hutchinson-Gilford progeria, the biochemical hypothesis of aging

PROGERIA- SINDROM AL DEFICITULUI DE ENERGIE CELULARĂ?

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Rezumat. O analiză critică a literaturii privind sindroamele îmbătrânirii accelerate, poate explica ce reprezintă îmbătrânirea dar şi de ce unele ţesuturi şi organe „îmbătrânesc" accelerat. Sindroamele îmbătrânirii accelerate (progeria) sunt cauzate de o mutaţie recesivă autozomală, cu frecvenţa 1 la 10 milioane, care afectează o helicază implicată în repararea ADN. Sindromul progeroid Hutchinson-Gilford (HGPS) la copii, are o frecvenţă mai mică de apariţie. În cele mai multe cazuri este cauzat de o mutaţie punctiformă la nivelul genei care codifică lamina A (înveliş nuclear). Mutaţiile laminei A apar şi în alte sindroame progeroide şi determină degenerarea organelor (distrofii). În HGPS, semnele îmbătrânirii apar în anii timpurii de viaţă, iar decesul apare la vârste foarte tinere (13-15 ani) din cauza bolii cardiovasculare. Sindromul progeroid HGPS poate oferi aspecte aprofundate, valoroase privind procesul îmbătrânirii. La pacienţii cu HGPS, demenţă şi cancerul nu sunt frecvente şi cele mai multe dintre procesele degenerative în cazul acestora pot fi în relaţie cu fibroza extinsă. Fibroza se asociază cu o mulţime de tulburări degenerative, la rândul lor, asociate sau nu cu îmbătrânirea. Înţelegerea mecanismelor fibrozei poate ajuta la explicarea corectă a sindromului HGPS şi a unor aspecte importante ale îmbătrânirii. În această lucrare am arătat particularităţile sindromului HGPS şi ale sindromelor de îmbătrânire accelerată prin

intermediul ipotezei biochimice a îmbătrânirii pe care am avansat-o. Conform acesteia, îmbătrânirea este rezultatul funcţiilor realizate în organism, unele reacţii fiind mai puţin stimulate, diminuând în timp şi afectând nu numai procesele biochimice celulare cum este sinteza proteinelor, dar şi energia celulei. Posibile strategii terapeutice sunt de asemenea prezentate.
Cuvinte cheie: progeria, îmbătrânire, sindromul Werner, progeria Hutchinson-Guilford, ipoteza biochimică a îmbătrânirii

INTRODUCTION

A critical analysis of the accelerated aging syndromes may explain what aging is, but also why some tissues and organs age at accelerated rates. If we are to understand aging, our first look would be at syndromes of premature, accelerated aging. Some of these under the generic term of progeria, are extremely rare diseases during which aging occurs at accelerated rates, with onsets in childhood. Patients with syndromes of premature aging hardly reach the adult age. The Werner syndrome and the Hutchinson-Gilford progeria (HGPS) have been the most studied progeroid syndromes. The word “progeria” comes from the Greeek words "pro" (πρό), “ahead” or “premature” and "gēras" ((γῆρας) “advanced age”.

In the present work we have attempted to analyze existing data of specialized literature with regard to accelerated aging known under the generic term of “progeria”. Also, we aim at analyzing these syndromes in relation with the biochemical hypothesis on aging we advanced [1]. According to the above hypothesis, aging would be the result of alterations of specific chemical reactions that occur as a consequence of diminishment of cellular signaling.

In aging specific cellular reactions, of which those involved in cellular differentiation but also those in relation with repair of the genetic material and protein processing could take place at increasingly lower rates. Hence, aging could express itself in two ways : 1. as affecting cell differentiation; 2. as affecting cell energy and these two ways having influence on each other. In the following sections we have atempted to analyze the syndromes of accelerated aging taking into account cellular differentiation and cell energy.

INTRIGUING FACTS ABOUT PROGERIA

The accelerated (premature) aging syndromes are genetic diseases most often caused by mutations in enzymes involved in repair and structuring of the genetic material, thus affecting integrity of the later mentioned [2]. The most well known such diseases are the Werner syndrome, the Cockayne syndrome, xeroderma pigmentosum, ataxia telangiectasia, which are all caused by genetic alterations of certain enzymes.

The Werner syndrome also known as “adult progeria” is an autosomal recessive disease with a frequency of 10 in a million of births and more frequent though, in certain populations. The gene involved with this syndrome encodes a helicase, the Werner syndrome ATP-dependent helicase, also known as type 3 RecQ-like, which is an important enzyme for DNA structuring, with roles in replication and recombination but probably as well in telomere maintenance [3]. Subjects with the Werner syndrome are born normal but the syndrome starts to manifest itself in the second decade of life when there are onsets of diseases associated with aging such as cardiovascular, osteoporosis, cancer the type of mesenchymal neoplasia (sarcomas), alopecia, type 2 diabetes, cataract, hypogonadism. Subject’s death generally occurs in the fourth decade of life due to cardiovascular diseases or cancer. It is interesting that fibroblasts removed from patients with the Werner syndrome and studied “in vitro” afterwards, are

characterized by an accelerated senescence, meaning that these fibroblasts have a more reduced capacity to divide in cell cultures than those removed from normal subjects [4].

The "Werner syndrome ATP-dependent helicase", so the WRN protein (an enzyme involved in DNA unwinding and elongation in view of transcription) could have complex cellular functions being related also with p53, which due to its involvement in cell division regulation, affects the risk of malignancy [4]. Also, recent data have shown that in the case of the Werner syndrome there is alteration of heterochromatin (non-information DNA sequence). Differentiation of WRN-null human embryonic stem cells to mesenchymal stem cells recapitulates changes in heterochromatin architecture, features of premature cellular aging, and also a specific pattern of metylation associated with cellular aging. The WRN protein as taken into account in the study by Zhang [5], could play the role of preserving heterochromatin's integrity, which is crucial in aging.

In cellular senescence loss of telomeres is a loss of heterochromatin. This phenomenon could be part of a broader view on heterochromatin disintegration at least for "in vitro" cellular aging. A supplementary amount of an enzyme that is involved in histones' methylation prevents losses of heterochromatin in WRN-null stem cells [5]. Hence, methylation might protect heterochromatin. On the other hand, methylation reactions could reflect the cell energy status. Methylations of DNA and histones (with likely formation of heterochromatin) lead to homocysteine formation and hyperhomocysteteinemia has been as well associated with progeria [6].

Homocysteine might be re-methylated to methionine by folic acid through the methylenetetrahydrofolate reductase MTHFR pathway. Methylenetetrahydro-folate reductase is dependent on NAD(P)H. On the other hand, NAD has an interesting action in aging. Under the influence of mitogenic factors, NAD increases in cells removed from young subjects, but not in those from old ones. Increases in NAD levels with age, were shown, and this fact could be related to the constant protein/NAD ratio of cells, that is also higher in old cells. Cellular NAD level was found out much higher as well in patients with HGPS [7].

The link between NAD and sirtuins that are involved in maintenance of DNA architecture and prevention of recombination is much debated in relation with aging. "Sirtuins" SIRT are a family of protein deacetylases/ ADP ribosyltrans-ferases NAD dependent. Sirtuins' deacetylasing activities are similar to methylations that as epigenetic changes play the same role of inactivating genes. In mammals, SIRT2, SIRT1 orthologues coordinate metabolic reactions as a response to presence of available nutrients in various tissues [8]. Furthermore, it appears that both NAD and a limiting enzyme for NAD synthesis might have circadian variations that in turn have influence on transcription of genes the expression of which is also circadian. Laboratory rats subjected to caloric restriction had higher WRN levels and at the same time SIRT1 levels were increased under caloric restriction. In mice lacking sirt-1, the WRN levels were reduced. These results showed that deacetylation by sirtuins might determine constant WRN levels [9].

In the disease model of the Cockayne syndrome a lipid and NAD rich diet changed the transcriptomic, metabolomic and behavioral characteristics of mice [10]. The Cockayne syndrome is a syndrome of accelerated aging characterized by neurodegeneration and in which enzymes involved with DNA repair are affected. Administration of a NAD precursor such as nicotinamide riboside in 24 months old mice (very old) led to a good muscle regeneration but also life span increase [11]. Both lipids (fatty acids) and NAD are

reactants for the mitochondrial oxidative phosphorylation. Actually, these reactants stimulate the most important source of cellular energy, namely the mitochondrial oxidation.

Information we presented above support the observation that there may be a deficit of cellular energy in accelerated aging syndromes. This deficit would reflect itself in processes of synthesis but as well heterochromatin maintenance, both in the Werner syndrome and the Cockayne syndrome. Also, a cellular energy deficit might have implications on transcription, capacity to differentiate and cell survival. The "Werner syndrome ATP-dependent helicase" -WRN protein itself is dependent on ATP and so, dependent on cellular energy.

The Werner syndrome seems accelerated aging in which aging related changes at the level of the DNA, occur earlier than normal. Although the cellular energy deficit appears as evident in the Werner syndrome, it is interesting whether diminished signaling also plays a role in this syndrome.

In the Werner syndrome, defective transcription could "catalyze" the latter mentioned processes. Helicases are involved not only in replication, repair and recombination but also transcription. It is interesting that the onset of the Werner syndrome is in the second life decade. Probably accumulation of mutations through defective replication for a sufficiently increased number of times and recombination could determine the aging start. Actually, mutations and recombination are noxious because finally they affect cell survival and cellular processes. In patients with this syndrome, due to accelerated telomere loss, critically short telomeres prevent cell division, suggesting the relationship with organism aging. Cells from individuals with Werner syndrome undergo rapid cellular senescence, probably caused by accelerated telomere loss. Actually, what strikes in patients with the Werner syndrome is that the "adolescent growth spurt" is not noticeable and this fact leads to diagnosing the syndrome [12].

According to the biochemical hypothesis on aging, defective transcription is related with diminished signaling which could be causing start of aging. This prediction can be illustrated experimentally through alterations of transcription factors but also by stimulating specific cellular reactions.

However, the genetic defect in the most spectacular form of progeria, the Hutchinson-Gilford Progeria Syndrome (HGPS) also called "the child's progeria", in which the aging rate is 8-10 times higher than normal, is expressed by proteins of the nuclear membrane known as lamins A and B-type. Lamin A and lamin B are the main proteins forming the nuclear lamina, a filamentous meshwork (proteins of 20-50 nm in mammals) as interface between the inner nuclear membrane and chromatin. A point mutation of the gene encoding lamin A leads to defective processing of this protein and accumulation of lamin A in the nuclear membrane, which presents blebbings [13].

It is interesting that in the case of the Hutchinson-Gilford progeria, the risk for cancer and respectively, senile dementia is not increased as it is the case of the Werner syndrome. Although patients with this rare syndrome hardly reach the age of twenty years old, their intelligence quotients are slightly higher than the average [14]. This syndrome is extremely rare, at birth children are apparently normal, but with a thinner skin and increased visibility vessels. In the months following birth, these children have some symptoms, among which no weight gain localized scleroderma-like symptoms meaning skin thickening and creasing, especially at the extremities. Between ages of 18 and 24 months, specific changes appear such as slow- growing, generalized alopecia and a specific look with a small face, a retracted maxilla and a convex nasal profile. As the child grows older, he/she has signs of aging and diseases associated with aging.

The skin wrinkles and cardiovascular diseases, atherosclerosis, loss of vision and renal failure occur. Next muscle, skeletal degeneration, hip dislocation and fat loss occur and joints become rigid. Generally, deaths of patients with HGPS are caused by cardiovascular complications.

Relations of blebbings of the nuclear membrane with aging related changes are not known at present. Could lamin A be related with processes occurring in HGPS? As lamin A is linked to the nucleo-skeleton and this fact affecting transcription, a conclusion drawn would be that transcription reactions could be hence limited. Abnormal interactions of nuclear lamina's proteins might lead to phenotypes of progeroid syndromes and progeria. Probably, nuclear's membrane blebbings impede hormones and nuclear receptors functions in view of signaling transcription. So, transcription might be overall affected in the case of the Hutchinson-Gilford progeria.

WHICH COULD BE THE CAUSE OF THE HUTCHINSON-GILFORD'S MUTATION?

In the history of studying the above syndrome, at first HGPS was considered a recessive autosomal disorder as it appeared in a larger proportion in families with consangvinization [15]. Genetic studies showed that 90 % percent of patients with HGPS had a point mutation, which subsequently appeared in some patients as a "*de novo*" mutation through paternal line. Sporadic mutations of paternal alleles occur also in other syndromes such as achondroplasia, the Apert, the Crouzon, the Pfeiffer syndromes and were attributed to fathers' advanced ages (same with the Hutchinson-Gilford progeria). Advanced ages of fathers could be correlated with higher mutation rates in sperm's genetic material. Nonetheless, in the case of achondroplasia, the above hypothesis was rejected [14].

The mutation responsible for Hutchinson-Gilford progeria is a cytosine-thymine substitution at codone 608 in cytosine guanine CG dinucleotides' -rich area (isochore) in which methylations are frequent. It is even considered that a methylated cytosine could easily undergo deamination followed by an erroneous transcription. So, these reactions would take place in an area predisposed to mutations but also epigenetic changes, which in our opinion, could be a subsequent source for other mutations.

The mutation that occurs in the Hutchinson-Gilford progeria and affects the pre-lamin A protein impedes removal of a farnesyl group (aliphatic rest as a cholesterol precursor). In absence of the above post-translational change, an abnormal lamin A, namely progerin, permanently attached to the nuclear rim is found out. Progerin leads to abnormal formation of the nuclear lamina. In absence of this mutation, the farnesyl group is removed from the normal lamin A protein, which reaches the nuclear envelope. On the contrary, these processes do not occur in the case of progerin that is lamin A protein from which the farnesyl group was not removed.

Drugs used in an attempt to treat the Hutchinson-Gilford were those utilized in cancer such as lonafarnib targeting the farnesylation reaction, [16] that was also subject of a clinical trial [17] but also statins [18] or zolendronat used to treat hypercalcemia. In this sense, results of the clinical study on lonafarnib showed some moderate benefits.

If the mutant variation of lamin A protein also occurs in "in vitro" cultivated cells from young and healthy subjects, after several successive multiplications, while lamin A accumulates in the nucleus in old healthy subjects without an increase in mRNA [19], these facts could be attributed to defective protein processing responsible for the above accumulation. In other words, we can presume that abnormal processing and functioning of other proteins might

lead to this phenotype of aging. The good question, if we take into account this hypothesis, would be whether there are subjects carrying this mutation but not developing the syndrome, or healthy siblings of patients with the HGPS syndrome, carry this mutation.

Facts were more complicated when lamin A mutations were found out in the heterozygote parents of a child with progeria also having symptoms of other progeroid syndromes [20]. This child's parents, who were carriers of different mutations, had 6% lymphocytes with abnormal nuclei. Abnormalities of the nuclei both in the child (36% abnormalities of the nuclei) and his parents were different from those taken into account as classical (nuclear membrane's blebbings) for the Hutchinson-Gilford progeria. In this case, abnormal lamin A was not accumulated to the nuclear periphery, but was leading to other nuclear abnormalities, hive shape and blebbings in which lamin B (another protein of the nuclear envelope) was lacking. More than that, inhibitors of farnesylation did not lead to reversal of the cellular phenotype as in the case of the Hutchinson-Gilford progeria, but to other nuclear abnormalities including accumulation of pre-lamin A at the peripheral nuclear lamin.

These results suggested that the terminal C area of pre-lamin A is very important for its function, but also that accumulation of this protein would not be a decisive harmful factor for the symptoms [20].

Other studies showed that the expression of lamin A is only 25% in lymphocytes from patients with Hutchinson-Gilford progeria. But the most interesting fact is that there are patients with both HGPS and the Werner syndrome who do not have any of the mutations mentioned above (approximately 10%). However, a study showed that all patients with these syndromes had various methylation patterns compared with age-matched healthy subjects. Both for the HGPS and the Werner syndromes methylation patterns are different in case mutations are present compared with the case of absent mutations. More than that, a case was reported for a healthy subject with a methylation pattern identical with that of children having HGPS, but no LMNA mutation. This case was that of the father of a little girl with HGPS.

In this context we may suggest cases of cellular signaling that might lead to certain methylation patterns (epigenetic changes), which could determine „de novo" mutations in CG-rich areas. This is how the LMNA mutation might occur in a large number of patients with HGPS (90%), as a very likely phenomenon occurring in case epigenetic changes were present.

LOW CELL ENERGY AND FIBROSIS

The majority of the Hutchinson-Gilford syndrome symptoms can be taken into account as related to extended fibrosis. Fibrosis is at the origin of most of alterations of the HGPS and implicated in heart diseases such as atherosclerosis [14]. Hyper-proliferation followed by apoptosis was noted in fibroblasts from patients with HGPS [21].

Lamin A could play roles in replication, transcription and DNA repair. Through interactions with retinoblastoma pRb proteins, lamin A would be implicated in cell proliferation and differentiation. Proteins the type of lamin A play roles in signaling pathways involving beta-1 tumor growth factor (TGF beta-1) and these pathways could explain the relationships between a cellular accelerated turn-over and premature aging. Lamins of type A could modulate production of collagen through TGF beta-1induced signaling. In lamin A protein knockout mice, the production of collagen is increased and this fact would explain increased fibrosis in murine animals of experimental models of Hutchinson-Gilford progeria and patients with laminopathies [20].

It is well-known that located sites, at which there are large condensations of protein

fibers, are very much affected by aging. For example, joints tear very fast and free radical attacks, glycosylations and cross-linking are enumerated as mechanisms involved with joint degenerations as shown by numerous data. According to these latter mentioned, above reactions lead to degradation and loss of functions of proteins. But a rapid protein turn-over could prevent the degradation process. Caloric restriction which increases average and maximum life-spans in many phylogenetically distant species also increases protein turn-over and autophagy. In patients with HGPS, rapamycin increased progerin degradation, restored blebbings of the nuclear membrane and slowed cellular senescence. The formation of insoluble aggregates of progerin also decreased under rapamycin, which induces clearance through autophagic mechanisms in normal fibroblasts [19]. These data have been in support of the hypothesis according to which in the case of progeria, enzymes involved in protein degradation are as well affected.

We consider that in the case of progeria and progeroid syndromes aside from mutations in lamin A, it is very likely that other proteins, which are involved with protein processing, cell proliferation and differentiation, might play important roles. If there are subjects with mutations in lamin A located at key sites, but nonetheless healthy, the above assertion about important roles of other proteins is endorsed. The fact that a phenotype of progeria was found out in families with consangvinization can advance for a lack of enzymes to counteract mutations in lamin A at key sites. For such a phenotype, the low probability to be found out might result from the fact that aside from mutations whose probabilities to occur are low anyway, enzymatic deficiencies may contribute to a phenotype of progeria.

Taking into account that few cells mean decreased cell division and increased cell differentiation in association with a decreased insulin/IGF1 (insulin growth factor 1) signaling pathway, fibrosis could be related to cellular energy deficits [22]. Recent data have suggested as well that cellular energy is indeed affected in HGPS. The question would be whether mitochondrial functions are affected by lamin A expression or mitochondrial dysfunction is related to increased expression of progerin. In fibroblasts from patients with Hutchinson-Gilford syndrome a downregulation of mitochondrial oxidative phosphorylation proteins accompanied by mitochondrial dysfunction, was noticed. Mitochondrial dysfunctions were also found in fibroblasts from adult progeroid mice expressing progerin (*Lmna*$^{G609G/G609G}$ knock-in mice) or prelamin A (*Zmpste24*-null mice). Analysis of tissues from these mouse models revealed that the damaging effect of these proteins on mitochondrial function is time- and dose-dependent. Mitochondrial alterations were not observed in the brain, a tissue with extremely low progerin expression that seems to be unaffected in HGPS. Mitochondrial function was restored in progeroid mouse fibroblasts treated with the isoprenylation inhibitors FTI-277 or pravastatin plus zoledronate, which are being tested in HGPS clinical trials. These results suggest that mitochondrial dysfunction contributes to premature organ decline and aging in HGPS. Prelamin A and progerin, aside from their effects on progeria, may also contribute to mitochondrial dysfunction and organ damage in normal aging, since these proteins are expressed in cells and tissues from non-HGPS individuals, most prominently of advanced ages [23].

Methylene-blue (MB) which is an antioxidant with positive effects on mitochondria (as in the HGPS there is a large proportion of abnormal mitochondria) enabling their survival, not only has apoptotic effects on cells removed from patients with HGPS but also restores the nucleus' shape [24]. Other analyses regarding MB suggested that it induces

release of progerin from the nuclear membrane, protects perinuclear hetero-chromatin and restores defective expression of genes isolated from cells of HGPS patients. These data as well suggested that the cellular energy deficit would be the factor leading to abnormalities of protein synthesis and processing, so abnormal proteins such as LMNA are produced. It is likely that in this case these above proteins interfere with mitochondrial function, as LMNA was found out also in the cellular mitochondrial fraction [25]. An interesting action of MB is the increase of chymotrypsin- and trypsin-like activities of the proteasome in the brain. [26]. This above MB effect supports the idea of defective protein processing as culprit of progeria and aging.
If the cellular energy deficit that occurs as a result of diminished signaling is also involved with progeria then some metabolic pathways such as IGF1/insulin are affected. Also, IGF1 delayed effects of progeria in mice [27] but these results are not valid in humans. Probably in humans the IGF1 levels are much lower than those in mice, so effects of this hormone would not be striking in comparison with small mammals. There is an inverse relationship between mammal's body weight and IGF1 levels [28], and in humans effects of IGF1 are probably more subtle.
In HGPS the sequence of phenomena could be the following: certain epigenetic changes favor mutations such as the LMNA. Cellular energy deficit manifests itself by affecting mitochondria and having as consequences apoptosis but especially fibrosis. As already mentioned children with HGPS have at birth thin skin and increased visibility vessels, these aforementioned characteristics showing deficient tissue development. Probably in these children transitions of energy metabolism from the embryonic period to the neonatal days, fail. Cells from these children-patients instead of proliferation differentiate largely and prematurely and hence, fibrosis occurs. A hypersecretion of collagen occurs as well in cultivated keratinocytes after a certain number of passages [29]. The above phenomena could explain the low incidence of cancer, in which there is on the contrary, an insulin/IGF1 hypersignaling.

CONCLUSIONS

By analyzing data with regard to syndromes of accelerated aging, which all are under the generic term of "progeria", we advance the following personal perspectives on the topic:
Patients with progeria develop degenerative pathologies ever since their childhood and hence in their case, the mature stem cell proliferations but also cellular differentiations are impaired. Moreover, in the Hutchinson Gilford syndrome, repair processes may not be much impaired and this fact might be an explanation for reduced frequencies of cancers and neurodegenerative processes.
Furthermore, taking into account the biochemical hypothesis on aging, we may draw the conclusion that both in the Werner syndrome and the Hutchinson-Gilford syndrome, cellular energy deficits and diminished cellular signaling occur but to different extents. While in the case of the Hutchinson – Gilford Progeria Syndrome, there is a key cellular energy deficit, in what regards the Werner syndrome a diminished cellular signaling might occur to a large extent.

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SIALIC ACID MODIFICATIONS IN AGING AND PATHOLOGY

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Abstract. Serum sialic acid (SA) is a marker that drew attention to its predictive role in various pathologies. Being an important component of glycoproteins and glycolipids, it intervenes in the antigenic characterization of cells, and by its content in platelets, erythrocytes and low density lipoproteins plays an important role in the atherogenesis process. The aim of this study was to determine the serum total sialic acid levels in elderly patients with various associated pathologies compared to a control group. Studies were carried out in 69 patients aged between 50-85 years, hospitalized at the "Ana Aslan" National Institute of Gerontology and Geriatrics. Depending on age and pathology, selected patients were divided into several study groups: a group of 32 presenescent patients (50-65 years), a group of 37 senescent patients (66-85 years), an ischemic heart disease (IHD) and hypertension group (n=24), a type 2 diabetes mellitus group (n=19), an obesity group (n=13) and a control group (n=13). Total serum sialic acid was determined by the Ehrlich's method using standard chemicals and reagents. The color reaction was quantitatively measured on the spectrophotometer at 565 nm. Increases in sialic acid serum levels were observed in all study groups, but statistically significant only in diabetic, obese and presenescent patients groups (92.11 ± 14.52, 87.82 ± 12.90, 88.24 ± 14.46, vs control group 79.41 ± 6.71mg/dL). These results are in agreement with other studies that attest the role of serum sialic acid as a predictive marker for cardiovascular complications.
Key words: sialic acid, elderly patients, ischemic heart disease, diabetes mellitus

MODIFICĂRILE ACIDULUI SIALIC ÎN ÎMBĂTRÂNIRE ŞI PATOLOGIE

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Rezumat. Acidul sialic seric (SA) este un marker care a atras atenţia asupra rolului său predictiv în diverse patologii. Fiind o componentă importantă a glicoproteinelor şi glicolipidelor, el intervine în caracterizarea antigenică a celulelor, iar prin continuţul său din trombocite, eritrocite şi lipoproteine de joasă densitate joacă un rol important în procesul de aterogeneză. Scopul acestui studiu a fost de a determina concentraţiile serice totale de acid sialic la pacienţii vârstnici cu diferite patologii asociate comparativ cu un grup de control. Studiile au fost efectuate pe 69 de pacienţi cu vârsta cuprinsă între 50-85 ani, internaţi la Institutul Naţional de Gerontologie şi Geriatrie "Ana Aslan". În funcţie de vârstă şi patologie, pacienţii selectaţi au fost împărţiţi în mai multe grupuri de studiu: un grup de 32 pacienţi presenescenţi (50-65 ani), un grup de 37 pacienţi senescenţi (66-85 ani), un grup cu boală cardiacă ischemică (BCI) şi hipertensiune arterială (n=24), un grup cu diabet zaharat de tip 2 (n=19), un grup cu obezitate (n=13) şi un grup de control (n=13). Acidul sialic total seric a fost determinat prin metoda Ehrlich, folosind substanţe chimice şi reactivi standard. Reacţia de culoare a fost măsurată cantitativ pe spectrofotometru la 565 nm. Creşteri ale concentraţiilor serice de acid sialic au fost observate la toate grupele de studiu, dar cu semnificaţie statistică numai la grupurile de pacienţi cu diabet zaharat, obezitate şi presenescenţi (92.11 ± 14.52, 87.82 ± 12.90, 88.24 ± 14.46, faţă de grupul de control 79.41 ± 6.71 mg/dL). Aceste rezultate sunt în concordanţă cu alte studii care atestă rolul acidului sialic seric ca marker predictiv pentru complicaţiile cardiovasculare.
Cuvinte cheie: acid sialic, pacienţi vârstnici, boală cardiacă ischemică, diabet zaharat

INTRODUCTION

In recent years, it has been observed that serum sialic acid (SA) is a marker which drew attention to its predictive role in various pathologies.
SA is an acute phase reactant, a terminal component of the non-reducing end of carbohydrate chains of glycoproteins and glycolipids [1]. Being an important component of glycoproteins and glycolipids, it intervenes in the antigenic characterization of cells. Also, being present in the composition of platelets, erythrocytes and low density lipoproteins plays an important role in the atherogenesis process [2].
Most of the researches described show total SA as being equally strong predictor with cholesterol level of cardiovascular mortality and its determination could prove useful in screening of populations at risk [3].
Low density lipoproteins (LDL-C) contain sialic acid in the carbohydrate chains in both the protein and the lipid part. Due to the fact that SA plays a role in glycoprotein metabolism, its LDL content could give new information about the atherogenicity of these lipoproteins. While SA affects cellular LDL catabolism, it can be assumed that this content is also associated with LDL metabolism *in vivo* [4].
Recent studies have been highlighted elevations of serum SA in patients with cardiovascular disease, or with diabetes, their levels correlating with carotid atherosclerosis independent of major cardiovascular risk factors [5]. In another study that took place over a period of 17 years, it has been shown that SA may be a long-term predictive marker of cardiovascular events in adults, especially in women [6].
The aim of present study was to determine the serum total SA levels in elderly patients with various associated pathologies compared to a control group.

MATERIALS AND METHODS

Studies were carried out in 69 patients (men and women) aged between 50-85 years, hospitalized at the "Ana Aslan" National Institute of Gerontology and Geriatrics in according to an inclusion / exclusion protocol in agreement with physicians. All the subjects gave informed consent to participate in the study.
Selected patients were divided in two study groups, from age point of view:
- a group of 32 presenescent patients aged between 50-65 years
- a group of 37 senescent patients aged between 66-85 years

From the pathological point of view, we divided patients into four study groups:
- a control group (n=13)
- an ischemic heart disease (IHD) and hypertension (n=24)
- a type 2 diabetes mellitus group (n=19)
- an obesity group (n=13)

Evaluation of biochemical parameters and lipid ratios

Venous blood samples were obtained from patients after 12 hours of fasting and serum was collected after 20 minutes of centrifugation at 1000 x g and stored at -70° C until use. Serum determinations of biochemical parameters (blood glucose, total cholesterol - TC, high density lipoproteins - HDL-C, low density lipoproteins, triglycerides - Tg) were performed using standardized methods with Konelab 301 SC autoanalyzer.
Lipid reports are required to assess the risk of cardiovascular disease beyond the usual lipid profile. These reports are:
- the atherogenic index (AI)
- the Castelli I risk index (CR I)
- the Castelli II risk index (CR II)
- atherogenic coefficient (AC)

The atherogenic index (AI) is based on the ratio of two major parameters, Tg and HDL-C; both being considered independent risk factors for cardiovascular disease.

The risk indexes Castelli I and Castelli II are based on three important parameters of the lipid profile (TC, HDL-C, LDL-C) and are calculated as a ratio between TC / HDL-C - the risk index Castelli I and LDL- C / HDL-C - Castelli II risk index.
The atherogenic coefficient (AC) calculated as {(TC-HDL-C) / HDL-C} is another report based on the importance of HDL-C in estimating the risk of cardiovascular disease [7].
AI values are associated with:
- low risk -0.3÷0.1
- medium risk 0.1÷0.24
- high risk >0.24

Total sialic acid determination
Total serum sialic acid was measured by standard Ehrlich's method, a colorimetric assay, using standard chemicals and reagents with an analytical grade purchased from Sigma Aldrich (Stockholm). In this method supernatant of serum containing SA will react with Ehrlich reagent producing a color reaction, wich is quantitatively measured on spectrophotometer at 565 nm.

Anthropometric measurements
Weight and height of the participants were measured when the participant had thin clothes on and was wearing no shoes by using the standard scales. Body mass index (BMI) was calculated for each individual by division of body weight (kg) by height (m^2).
Blood pressure was measured using a tensiometer, with the patient rested for 5 min in the seated position, arm supported and using an appropriate-sized cuff.

Statistical analysis
All values are represented as mean ± SD. Statistical analysis was done by using the Student "t" test (EXCEL 7.0 and SPSS 8.0 Microsoft Softwear). A p-value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

For all patients under study, were determined clinical parameters and lipid ratios (Tab. I and II).

Tab. I Biochemical and clinical characteristics of study groups

Biochemical and clinical parameters	**Control group n=13**	**Pathological group n=56**			**Age group n=69**	
		Diabetes patients n=19	**IHD and hypertension patients n=24**	**Obesity patients n=13**	**Presenescent patients n=32**	**Senescent patients n=37**
Glucose (mg/dL)	94.85±11.42	141±41.18	95.04±10.74	96.15±13.54	103.25±29.18	111.86±32.52
Total cholesterol (mg/dL)	195.84±31.67	215.16±60.07	211.95±45.26	263.31±25.55	214.62±57.25	223.67±41.80
HDL-cholesterol (mg/dL)	52.25±13.92	47.56±18.80	44.89±13.85	53.53±16.37	48.80±15.16	48.5±16.69
LDL-cholesterol (mg/dL)	120.05±24.63	145.54±57.14	143.79±42.10	166.12±23.99	138.33±48.72	148.91±38.03
Triglyceride (mg/dL)	115.15±34.24	145.10±88.70	158.79±78.97	135.84±46.45	144.28±78.74	140.91±64.42
BMI (kg/m^2)	24.87±3.21	29.88±3.69	22.99±2.77	33.53±5.40	27.58±6.35	26.92±4.68
Sistolic blood pressure (mmHg)	12.96±1.64	15.68±2.23	18.69±21.04	14.73±2.40	17.75±18.28	14.55±2.08
Diastolic blood pressure (mmHg)	7.69±0.66	8.5±1.2	8±1.02	8.92±1.19	8.36±1.22	8.19±1.04

Values are presented as mean ± D.S.

Tab. II Lipid ratios and total SA serum levels

	Control group n=13	Pathological group n=56			Age group n=69	
		Diabetes patients n=19	IHD and hypertension patients n=24	Obesity patients n=13	Presenescent patients n=32	Senescent patients n=37
Atherogenic coefficient	2.99±1.29	3.93±1.66	4.13±1.80	4.36±1.79	3.70±1.63	4.08±1.77
Atherogenic index	0.34±0.16	0.46±0.28	0.52±0.25	0.40±0.23	0.44±0.25	0.45±0.25
Castelli I risk index	3.99±1.29	4.93±1.66	5.13±1.80	5.36±1.79	4.70±1.63	5.08±1.77
Castelli II risk index	2.51±1.09	3.42±1.56	3.57±1.61	3.42±1.28	3.07±1.30	3.50±1.60
Total SA (mg/dL)	79.41±6.71	92.11±14.52*	83.11 ±12.44	87.82 ±12.90*	88.24 ± 14.46*	83.64 ±11.26

Values are presented as mean ± D.S.; *p<0.05 vs. control

There are differences between the all groups of study, regarding their serum levels of glucose, as well as their lipidic and lipoproteic parameters: TC, HDL-C, LDL-C and Tg (Tab. I).

For all groups studied, there are increases in lipid ratios, compared to the control group, and the atherogenic index has values higher than 0.24, indicating an increased risk of cardiovascular disease for all patients included in the study.

Also, increases in serum SA levels were observed in all study groups, but statistically significant only in diabetic, obese and presenescent patients groups (Tab. II).

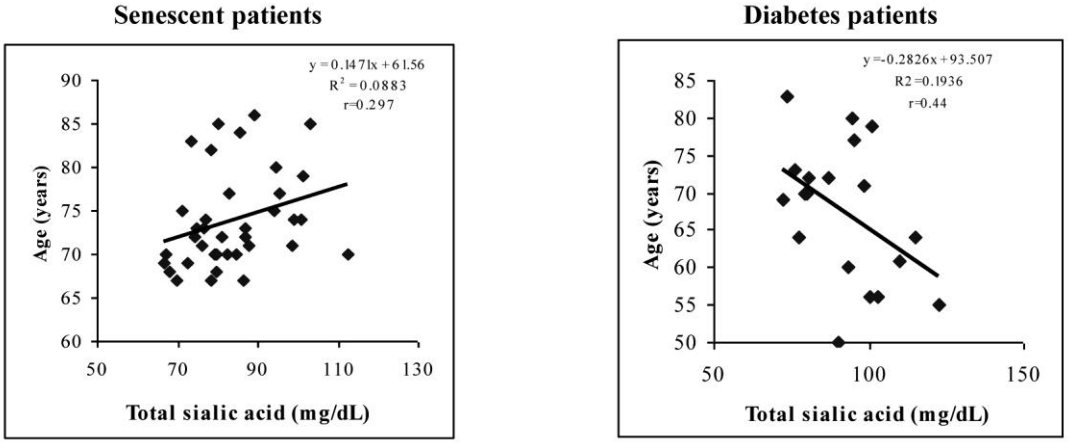

Fig. 1 The total SA variation with age in senescent and diabetes patients
Y=the linear regression equation; r=correlation coefficient

From linear regression equation, there was observed a positive correlation between total SA with age in senescent group, and a negative correlation, almost statistical significant (p=0.059), in diabetes mellitus group (Fig. 1). An explanation for the increase of the level of total SA with age would be a higher frequency of subclinically diseased individuals among the elderly, because are known that several diseases increase serum SA concentrations.

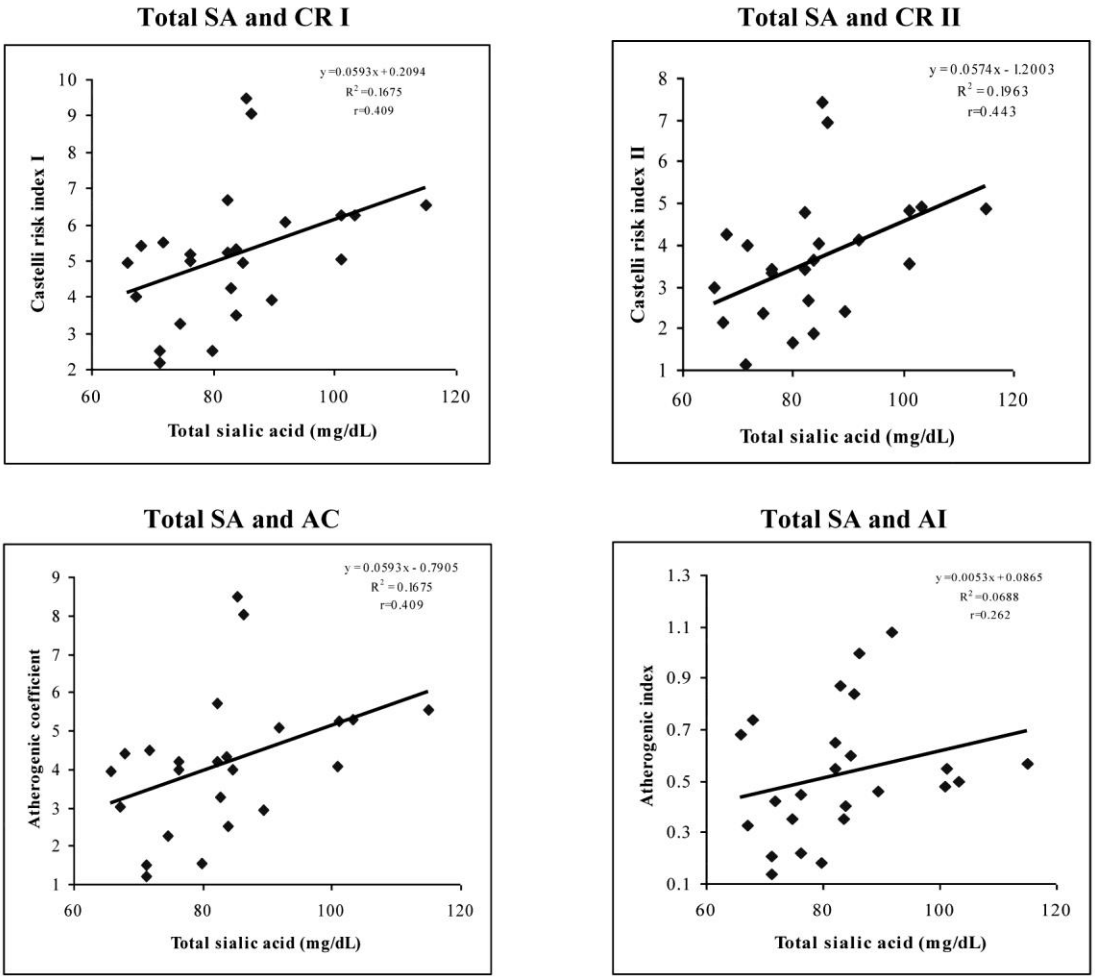

Fig. 2 Correlation between total SA and lipid ratios in IHD and hypertension patients
Y=the linear regression equation; r=correlation coefficient

The positive correlation was observed between the lipids ratios and total sialic acid in IHD and hypertension group (Fig. 2).

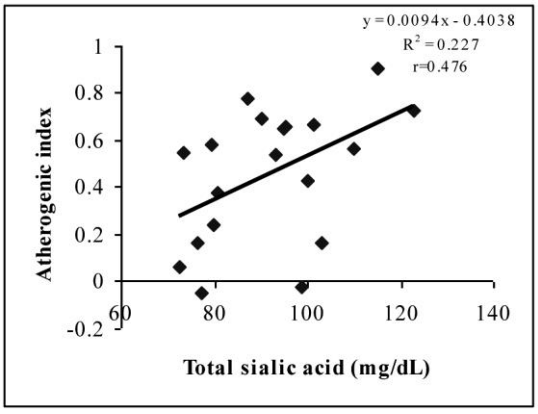

Fig. 3 Correlation between total SA and atherogenic index in diabetes group
Y=the linear regression equation; r=correlation coefficient

Other positive significant correlation between SA and atherogenic index (r = 0.477, p = 0.039) were also observed in diabetes patients group (Fig. 3).

From these results and from the results of other previous studies on elevated total SA concentrations in diabetic patients, patients with IHD and hypertension, and in obese patients it is suggested that it may reflect the degree of atherosclerotic progression involving inflammatory processes.

CONCLUSIONS

In this study we found that serum total SA concentrations were elevated in all study groups (diabetic, IHD and hypertension, obesity, presenescent and senescent).
Also, we established a significant correlation between total SA levels with lipid ratios in IHD and hypertension patients and with AI in diabetes patients.
These results are in agreement with other studies that attest the role of serum SA as a predictive marker for cardiovascular complications.

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RAGE PRODUCTION STIMULATION – POSSIBLE PROTECTIVE EFFECT IN DIABETES MELLITUS

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Abstract. The formation of advanced glycation end product (AGE) is markedly accelerated in diabetes because of the increased availability of glucose. The receptor for advanced glycation end products (RAGE) has been shown to be involved in the pathogenesis of diabetic complications. The study aims to follow the correlation between serum levels of RAGE with glycosylated hemoglobin (HbA1c) and blood sugar in elderly patients with diabetes mellitus. Serum levels of RAGE were determined by an ELISA method. The present study was carried on 77 elderly patients (mean 70 years) from INGG, divided into 3 groups: I-control (n = 16), II- prediabetes patients (n = 36) and III-diabetes patients (n = 25), classified by HbA1c values reference. We found a decrease in serum levels of RAGE in diabetic patients compared to controls (1085.29 vs 1321.7 ng/mL). Control group showed a significant negative correlation between RAGE and HbA1c ($r = -0.65$; $p<0.001$) and at diabetics a significant positive correlation ($r = 0.339$; $p<0.05$). Also, regression analysis pointed out a significant positive correlation of HbA1c with blood glucose at both prediabetes and diabetes patients ($r = 0.434$; $p<0.01$ respectively $r = 0.368$; $p<0.05$). Study reveals low levels of RAGE in diabetic patients, supporting the hypothesis that RAGE, by limiting interaction AGE - RAGE, can protect vessels against AGE toxicity. Thus, stimulation of RAGE production should be considered as a potential therapeutic target in diabetes mellitus and AGE- related vascular diseases.

Key words: receptor for advanced glycation end-product, glycosylated hemoglobin, diabetes mellitus

STIMULAREA PRODUCTIEI DE RAGE – POSIBIL EFECT PROTECTIV IN DIABETUL ZAHARAT

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Rezumat. Formarea produşilor finali de glicare avansată (AGE) este semnificativ accelerată în diabetul zaharat din cauza disponibilităţii crescute a glucozei. Receptorul produşilor finali de glicare avansată (RAGE) s-a dovedit a fi implicat în patogeneza complicaţiilor diabetice. Studiul îşi propune să urmărească corelaţia dintre nivelele serice de RAGE cu hemoglobina glicozilată (HbA1c) şi glicemia, la pacienţii vârstnici cu diabet zaharat. Nivelele serice de RAGE s-au determinat printr-o metodă imunoenzimatică ELISA. S-au luat în studiu 77 pacienţi vârstnici (media 70 ani) din INGG, împărţiţi în 3 loturi: I-control (n=16), II-prediabetici (n=36) şi III-diabetici (n=25), clasificaţi după valorile de referinţă ale HbA1c. Se observă o scădere a nivelelor serice RAGE la pacienţii diabetici faţă de control (1085.29 vs. 1321.7 ng/mL). La lotul control se constată o corelaţie negativă semnificativă între RAGE şi HbA1c ($r = -0.65$; $p<0.001$) iar la diabetici o corelaţie semnificativ pozitivă ($r = 0.339$; $p<0.05$). De asemenea, analiza de regresie arată o corelaţie semnificativ pozitivă a HbA1c cu glicemia atât la prediabetici cât şi diabetici ($r =0.434$; $p<0.01$ respectiv $r =0.368$; $p<0.05$). Studiul relevă nivele scăzute RAGE la pacienţii diabetici, susţinând ipoteza că RAGE, prin limitarea interacţiunii AGE - RAGE, ar proteja vasele sanguine de toxicitatea AGE. Astfel, stimularea producţiei RAGE ar putea fi considerată ca potenţială ţintă terapeutică la diabetici şi bolile vasculare legate de AGE.

Cuvinte cheie: receptorul produşilor de glicare avansată, hemoglobina glicozilată, diabet zaharat

INTRODUCTION

Forming compounds after a non-enzymatic reaction between sugars and proteins was originally described by Louis C. Maillard in 1912 and only in 1971 with the discovery of glycated hemoglobin (HbA1c) by Trivelli, it has been recognized that such reactions occur normally in the body.
Advanced glycation end products (AGE) make cross-links between proteins of the extracellular matrix, which is a physiological process, but in diabetic patients AGE is bound covalently to other AGE forming multiple cross-links between molecules such as laminin, elastin, collagen, a process that is accompanied by increase in stiffness of the extracellular matrix [1,2,3,4].
AGE formation profoundly alters the composition and interaction between various components, leading to alteration of adhesion, growth and secretory activity of various cell types.
AGE accumulation is involved in the onset of diabetic complications and typical medication that blocks the synthesis or loosens crosslinking reduces the incidence of these complications.
Although initially it was thought that AGE are toxic products, it proved that they interact with certain types of receptors, called receptor for AGE (RAGE). By binding AGE to these receptors are stimulated oxidative stress and inflammation and triggers reactions that ultimately give rise to endothelial and adipocytal dysfunction [5, 6].
Interaction between AGE and receptors and is now considered the key to induce pathological changes related to AGE. Trying to better understanding of the involvement of AGE in vascular dysfunction, the concept of a "two hit model" which involves initially interaction AGE-RAGE, causing cell activation and inflammation, followed by accumulation of lipoproteins, maintaining the inflammatory status and further acceleration of atherosclerosis [7,8,9].
In 2010, the American Diabetes Association (ADA) recommended inclusion of HbA1c in the diagnostic criteria for diabetes at a cut-off value of 6.5%. ADA also recommended that patients with HbA1c values in the range of 5.7-6.4% to be included in the category of high risk for diabetes, along with those who have impaired fasting glucose or impaired glucose tolerance [10].
Reference values according to ADA:
- Normal: 4.8-5.6%
- Increased risk of developing diabetes - prediabetes: 5.7-6.4%
- Diabetes :> = 6.5%
Therapeutic target in diabetic patients: <= 7%
The study aims to track the correlation between serum levels of RAGE with glycosylated hemoglobin and glycemia in elderly patients with diabetes mellitus.

MATERIALS AND METHODS

We have studied 77 elderly patients (mean 70 years), divided into 3 groups, classified by reference values of HbA1c:

- I-control (n = 16)
- II- prediabetes patients (n = 36)
- III-diabetes patients (n = 25)

Subjects were selected from I.N.G.G. "Ana Aslan", according to a protocol based on criteria of exclusion / inclusion. All persons included in the study were evaluated clinical and laboratory. All patients signed an informed consensus. Anthropometric measurements were performed under standardized conditions. Blood pressure measurement was carried out during the clinical examination after 5 minutes of rest.
Serum levels of RAGE were determined by ELISA method with spectrophotometric detection at 450nm, and the concentration was expressed as pg / mL.

Statistical analysis

All values are presented as mean ± standard deviation. The results were statistically analyzed by using Student's "t" test, by Pearson's correlation coefficient and $p<0.05$ is considered to be statistically significant. The relationship between RAGE, HbA1c and glycemia was assessed using a linear regression model.

RESULTS AND DISCUSSIONS

Initially AGE and RAGE were reported to be involved in microvascular and macrovascular complications of diabetes mellitus, renal failure and peritoneal injury of long-term peritoneal dialysis patients. They are also implicated in various other pathologic situations such as aging, neurological disorders and inflammatory states.

Study results shows a decrease in serum levels of RAGE in diabetes patients compared to controls (Tab.I).

Tab.I Main parameters at prediabetes and diabetes patients vs. control

	Age (years)	**Glycemia (mg/dL)**	**HbA1c (%)**	**RAGE (pg/mL)**
Group I-Control	67.4±11.6	98.56±12.54	5.30±0.28*	1321.17±639.75
Group II-Prediabetes patients	70.1±8.4	101.56±12.5^{t}	6.01±0.22**	1191.95±578.7
Group III- Diabetes patients	69.8±7.18	141.28±42.69^{tt}	7.54±1.23***	1085.29±595.0

Results are presented as means±D.S., $^{*}p<0.0001$ vs. II, $^{**}p<0.0001$ vs. III, $^{***}p<0.0001$ vs. I, $^{t}p<0.0001$ vs. III, $^{tt}p<0.0001$ vs. I

In the control group reveals a significant negative correlation between RAGE and HbA1c ($r = -0.65$; $p<0.001$) and at group III-diabetes patients a significant ($r = 0.339$; $p<0.05$) positive correlation (Fig.1, Fig.2).

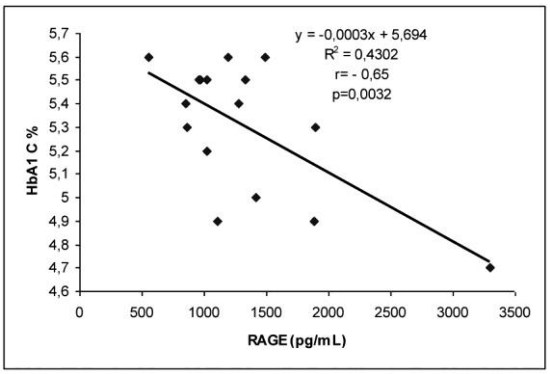

Fig.1 Correlation between RAGE and HbA1c at control group
Curve fitting was by linear regression; r = correlation coefficient

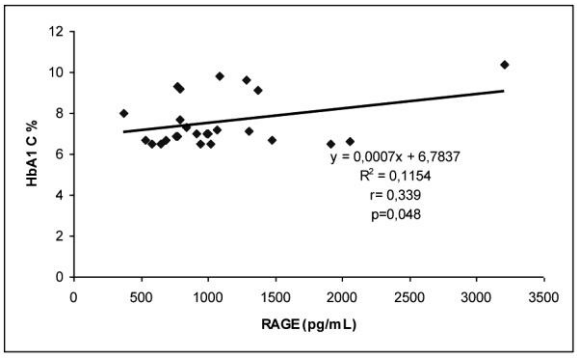

Fig.2 Correlation between RAGE and HbA1c at diabetes patients
Curve fitting was by linear regression; r = correlation coefficient

The regression analysis (Fig.3, Fig.4) showed a significant positive correlation between blood glucose and HbA1c at both prediabetes and diabetes patients (r = 0.434; $p<0.01$ respectively r = 0.368; $p<0.05$).

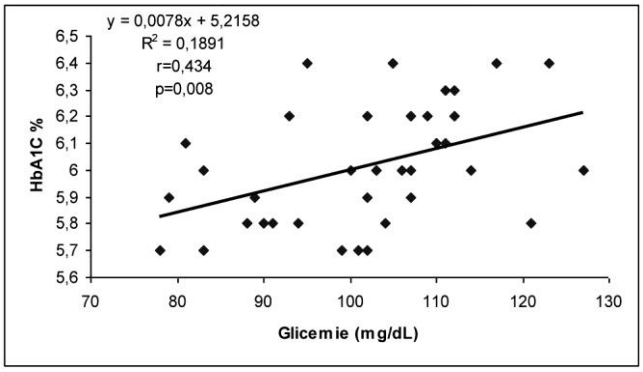

Fig.3 Correlation between glycemia and HbA1c at prediabetes patients
Curve fitting was by linear regression; r = correlation coefficient

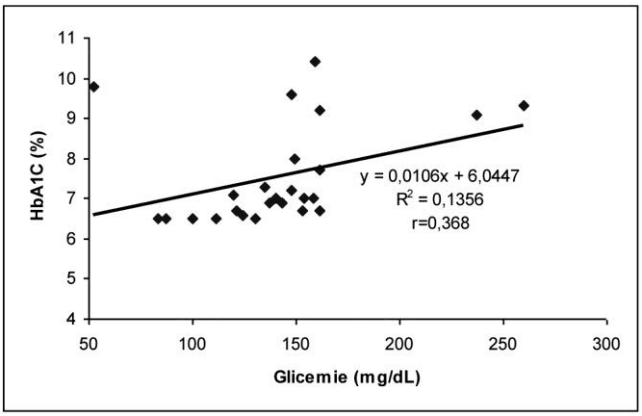

Fig.4 Correlation between glycemia and HbA1c at diabetes patients
Curve fitting was by linear regression; r = correlation coefficient

It has been demonstrated that numerous pathophysiological situations enhance the expression of RAGE and its ligands, including diabetes mellitus, renal failure, aging and smoking. Inflammation increases the production and accumulation of AGE, HMGB1, S100 proteins and amyloid fibrils deposits and subsequently leads to the increased activation of RAGE and further enhances the inflammatory

state. The relationship between the up-regulation of RAGE/RAGE ligands and the level of 'protective' RAGE levels is of obvious clinical interest. The use of RAGE as a therapeutic agent may prevent some inflammatory/autoimmune diseases in animal models, and therefore highlights the administration of RAGE or the V-domain ligand binding as potential therapeutic targets for inflammatory disease.

RAGE mechanisms by which serum levels are lower in diabetic patients are not fully understood [1, 11, 12].

One hypothesis would be that hyperglycemia inhibits the production of RAGE directly or by increasing the levels of cytokines and / or hyperglycemia-induced AGE.

Another explanation is that low levels RAGE may be due to an increase in ligand complexes AGE / RAGE clearance.

Therefore RAGE reduction in diabetes may be a consequence of diabetes or a causal factor. Prospective clinical and fundamental studies are needed to clarify the relationship cause-effect between AGE, RAGE, high glycemia and HbA1c.

CONCLUSIONS

- Study reveals low levels of RAGE in diabetic patients, supporting the hypothesis that RAGE, by limiting interaction AGE - RAGE, protect blood vessels toxicity AGE
- Stimulation of RAGE production could be considered as a potential therapeutic target in diabetes and AGE- related vascular diseases

ACKNOWLEDGMENT

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PARTICULARITIES OF AGING PROCESS IN THE STRUCTURE OF HUMAN GASTRIC MUCOSA

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Abstract. The present study is a post-mortem one in which we followed the histological features of gastric mucosa in old healthy human subjects, means with no gastric diseases diagnosed in vivo. We prelevated gastric tissue samples from antrum and body part of the stomach by post-mortem procedures, tissue so obtained being further processed by histopathological methods, consisting mainly in parrafin embedding, sectioning and then staining with hematoxilin-eosin and Van Gieson techniques. In the analyzed cases we found mostly the same tissular parameters consisting in a lymphocytic inflammatory infiltrate, pseudopyloric metaplasia of body gastric mucosa with increasing number of the mucus secretory cells, patchy atrophy with some area of lamina propria expansion dissociating glands. With age, there is a broadly functional decline which is based on progressive changes of the morphological background. At gastric level constant findings in our examined cases were chronic inflammatory infiltrate and zonal atrophy, together with pseudopyloric metaplasia with proximal advancing of antrum-body junction.
Key words: aging, gastric mucosa, histological features, pseudopyloric metaplasia

PARTICULARITĂŢILE PROCESULUI DE ÎMBĂTRÂNIRE LA NIVELUL STRUCTURII MUCOASEI GASTRICE UMANE

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Rezumat. Studiul prezent este un studiu post-mortem în care am urmărit caracteristicile histologice ale mucoasei gastrice la subiecții umani în vârstă sănătoși, înțelegând prin aceasta că nu au o patologie gastrică diagnosticată *in vivo*. S-au prelevat probe de țesut gastric din antrum și partea corpului stomacului prin proceduri post-mortem, țesutul astfel obținut fiind prelucrat în continuare prin metode histopatologice, constând în principal în includerea la parafină, secționarea și apoi colorarea cu tehnici de hematoxilină-eozină și Van Gieson. În cazurile analizate am constatat în principal aceiași parametri tisulari constând într-un infiltrat inflamator limfoplasmocitic, metaplazia pseudoplorică a mucoasei gastrice a corpului cu creșterea numărului de celule secretoare ale mucusului, atrofia neuniformă cu o anumită zonă de dilatare a glandelor de dilatare lamina propria. Cu vârsta, există un declin general funcțional care se bazează pe schimbări progresive ale fundalului morfologic. La nivelul gastric, constatările constante în cazurile examinate au fost infiltrarea inflamatorie cronică și atrofia zonală, împreună cu metaplazia pseudoplorică însoțită de avansarea proximală a joncțiunii antrum-corp.
Cuvinte cheie: îmbătrânirea, mucoasa gastrică, trăsături histologice, metaplazie pseudopilorică

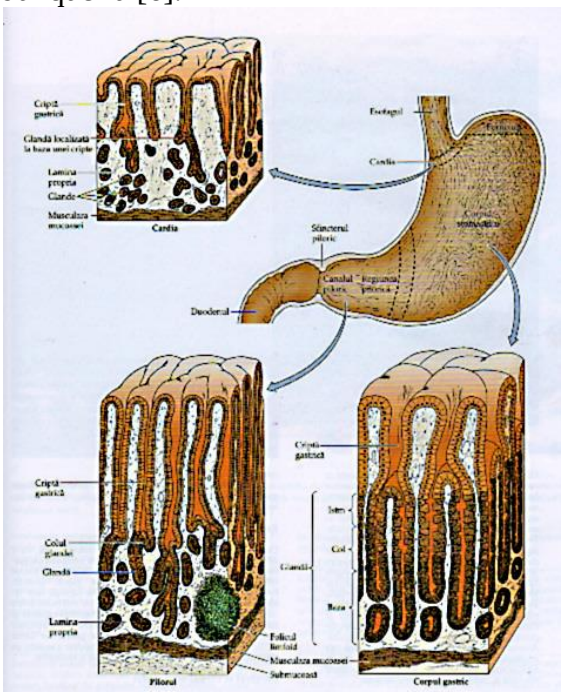
INTRODUCTION

Through the last years there is a marked tendency of population all over the world to live longer: this phenomenon increases naturally the interest for a better understanding of the normal aging process, including at morphological level, as well as for the advanced age prevailing diseases. Since the structural and functional integrity of the gastrointestinal tract are maintained by a constant renewal of lining cells, a

detailed knowledge of the events that initiate and modulate the proliferative processes of mucosa is essential for a better understanding of the natural aging process and the associated dysfunctions [1]. Among these, an important one is the malignancy which represents an impairment of tissue growth process. Studies on Fisher rats evidentiated an increase of proliferative activity of gastrointestinal mucosa in old subjects at the same time with decrease or even cessation of functional activity [2]. So, the decreasing secretion of acid and pepsin goes together with the decrease of gastrin production. In contrast to this, the level of antral gastrin increases in this time and so does the histidin decarboxylase activity too. According to these the diminishing of gastrin secretion could be explained by increase of cellular ratio between D cells somatostatin secretive and G cells gastrin secretive in antral mucosa. The increased proliferative activity in advancing age couldn't be due to the trophic action of gastrin or bombesin because these don't induce significant changes in rats.

The renewal of gastric epithelium is a relative rapid process, regeneration being different according to the zone. The surface epithelium is changed in normal conditions at 3-4 days and the parietal and chief cells in one to three years. The renewal is based on the mitotic activity of stem cells from the istm and neck of the glands. The gastric mucosa has an extremely high regenerative capacity when a superficial damage occurs as in aspirin, alcohol and toxics intake. Repair is realized by migration of viable cells from the profound part of the foveolae. If there is only a little damage this is made in thirty minutes.

The initial form of the migrating cells is cuboidal, and then they begin to grow and start mucus secretion [3]. Apoptosis is frequently observed in juxtaluminal portion of the epithelium and has a significant role in sustaining the normal turn-over. Repair can create confusion with dysplastic lesions because there are a big number of mitoses at the neck level of gland, together with anisocitosis and anisocaria which can occur frequently [4]. Still, major changes in architecture or in nucleo-cytoplasmic ratio are in this case not present. In elderly appears a cvasiconstant decrease of acid production that is reflected by changes in the histological structure of the stomach [5]. The gastric histology is illustrated in the following schematic drawing, after Junqueira [6].



The hypoclorhidria of old patient is most often a result of chronic gastritis [7]. The surface of fundic mucosa decreases and the pyloric one expands. So, the pyloro-fundic junction becomes more proximal and this change is defined as pseudopyloric metaplasia [8]. By this transformation, the mass of secretory cells of fundic glands are replaced by mucus secreting cells.

Concerning the histopathological features analyzed in this postmortem study, these can be synthetised as it follows:

-inflammation, defined as presence of lympho-plasmocytic infiltrates

-activity of gastric mucosa, defined as presence of neutrophils in superficial or profound layers

-atrophy of gastric mucosa, defined as a loss of glandular tissue

-pseudopyloric metaplasia defined as replacement of the fundic type of glandular cells mass with pyloric type of glandular cells mass
All these changes can be graded in: absent, mild, moderate and severe. Regarding the inflammation, interpretation can be subjective because the lympho-plasmocytic infiltrate is a normal feature in antral region

MATERIALS AND METHODS

The study group was made of twenty cases of patients who died in our clinic with age between 78 and 88 years old and the gender distribution was twelve women and eight males.
Gastric samples were prelevated from antrum and gastric body by post-mortem procedures, fixed in buffered formaline 10 % and processed by histopathological methods. The paraffin embedded tissues were serially sectioned transversely at 5 µm thickness and stained with routine hematoxylin - eosin technique and Van Gieson technique, the later one used to point out the connective tissue. The tissue samples so obtained were examined in light microscopy.
The technique used for electronic microscopy consists in using as fixator a glutaraldehide solution 4% and osmic tetraoxide for after fixation. Samples were embedded in EPON 812, and the contrast was obtained by uranyl acetate and Reynold's solution. Sections were made at 60 nm thickness.

RESULTS AND DISCUSSIONS

Following the analysis in optic microscopy, we noticed in all twenty cases similar tissue parameters, represented in different proportions from mild, to moderate and abundant. The most common histological feature was the inflammatory lympho-plasmocytic mostly moderate to mild (Fig 4, 5). This chronic infiltrate was encountered especially at antral level where it tends to form lymphoid pseudofollicles (Fig.1) and in a less significant measure at the body level (Fig.3). At the bottom of the foveolae, some typical mitoses could be observed locally as a sign of normal turn-over. We also presented an ultrastructural benchmark for gastric epithelium and inflammatory cells. (Fig.6, 7)
In patches, the expansion of lamina propria tendency to dissociate glands was conspicuous, change that is a sign of atrophy of gastric mucosa by diminishing glandular tissue (Fig.1).
In mucosa fragments taken from the body of the stomach it could be observed in some areas the phenomenon of pyloric metaplasia (Fig.2) consisting in increasing number of mucus secreting cells, phenomenon that appears in a progressive manner to old subjects and expressed by a front line moving upper from antrum-body junction to cardia. This can explain the morphological background of the reduced acid production that appears in advanced age.
By examining all twenty cases, we encountered mostly the same structural features consisting first in an inflammatory lympho-plasmocytic infiltrate, then in the pseudopyloric metaplasia of gastric mucosa of the body with increasing number of the mucus secretory cells, variable areas of atrophy with expansion of lamina propria dissociating glands and an overall decreasing of glandular mass.

CONCLUSIONS

The constant histopathological changes observed in all twenty cases of our study were:
- presence of variable lympho-plamocytic infiltrate graded in mild, moderate and abundant
- presence of pseudopyloric metaplasia of the gastric body mucosa
- presence of atrophic zonal changes expressed by flattening of gastric mucosa and diminishing of glandular mass

All of these are included in a predictable evolution of aging process reflected in gastric morphology that occurs at the level of microstructure of the gastric mucosa and represents the morphological background of the gastric functional decline that appears in advanced age.

Acknowledgements: Special thanks to the Victor Babes Institute's electronic microscopy department, for the electronomicroscopic images of gastric mucosa and to Natalia Oniga, principal technician, laboratory of pathology, for histopathological processing of gastric tissues.

LEGEND OF THE FIGURES

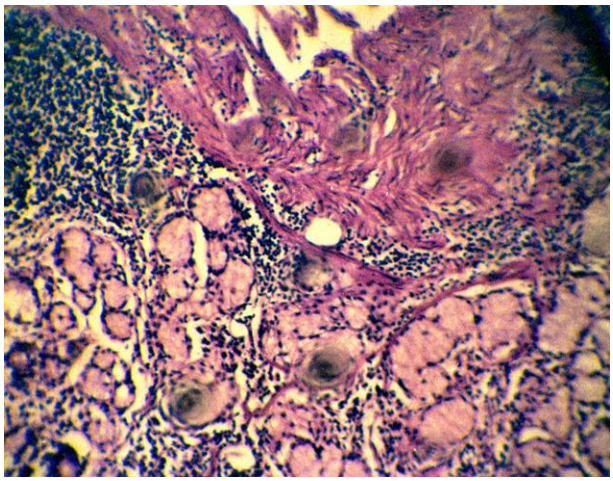


Fig.1 Inflammatory infiltrate tending to form lymphoid pseudofollicles and expanding lamina propria that dissociates glands, antral zone. H-E stain, magnified x200

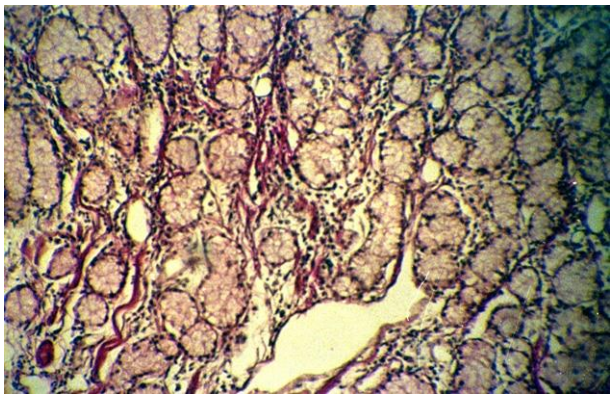


Fig.2 Mucous glands with moderate lymphocytic inflammatory infiltrate in the lower body part: pseudopyloric metaplasia .V.G. stain, magnified x200

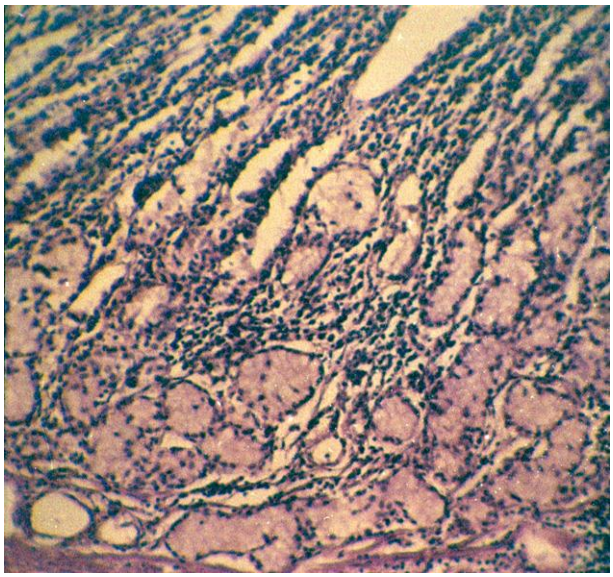


Fig.3 The foveolar part (the upper half) and spaced mucous glands with moderate inflammation of the stroma.H-E stain, magnified x200

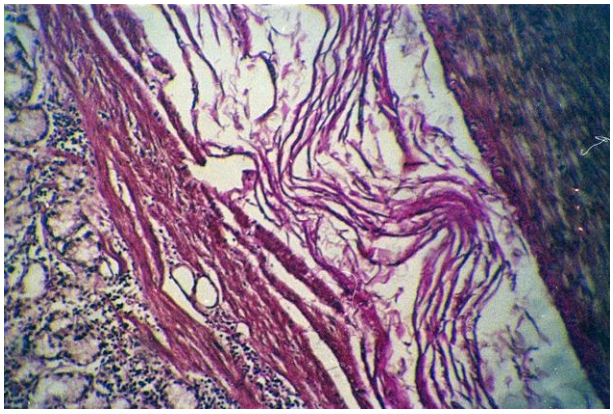


Fig.4 Gastric epithelium with moderate inflammatory infiltrate (left) and all the compounding layers in depth from left (mucosa) to the right (muscularis) H-E stain, magnified x200

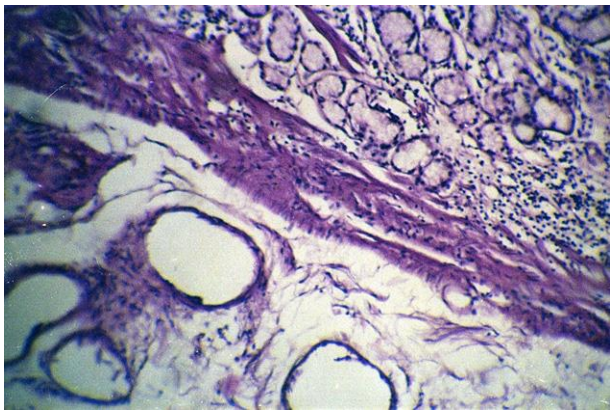


Fig.5 Gastric epithelium showing the compounding layers from mucosa with mild inflammation (upper right), muscularis mucosae (crossing center) and submucosa (bottom left) with well defined vascular lumens H-E stain, magnified x200

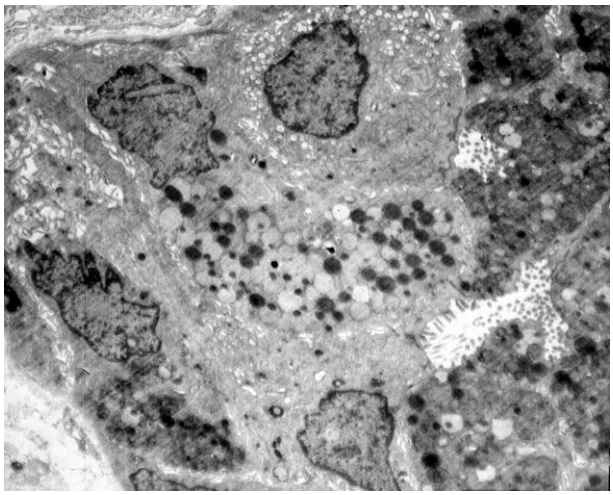


Fig.6 Gastric epithelium with basal membrane (bottom left) and microvilli (upper right) with secretory granules EM x4500

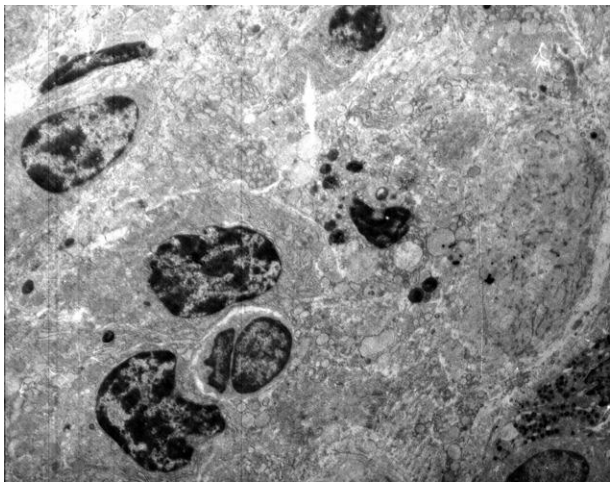


Fig.7 Interstitial inflammation EM X 3400

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MYELOPEROXIDASE IMPLICATIONS IN PATHOLOGY

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Abstract. Myeloperoxidase (MPO) is a pro-inflammatory enzyme, which is involved in oxidative stress, in inflammation, and plays a pathophysiological role in atherogenesis and degradation of fibrous plaque. Modified serum levels of MPO have been linked to the pathology of different diseases, including atherosclerosis, myocardial infarction, atrial fibrillation, diabetes mellitus, multiple sclerosis, Alzheimer's disease, lung cancer and transplant rejection. Therefore, MPO it is considered a significant biomarker with diagnostic properties for a number of cardiovascular diseases, and also with a prognostic value in regards to future untoward cardiovascular events. MPO is a covalently bound tetrameric complex, an heavily glycosylated peptidic enzyme, a member of the heme peroxidase superfamily. MPO is stored in the azurophilic granules of polymorphonuclear neutrophils, monocytes and macrophages from where it is secreted like response to local inflammation. MPO activity can be measured in the blood and tissue by enzyme-linked immunosorbent assays (ELISA), colorimetric assays using substrates and by gas chromatography followed by mass spectrometry.
Key words: myeloperoxidase, cardiovascular disease, diabetes mellitus, obesity

IMPLICAŢIILE MIELOPEROXIDAZEI ÎN PATOLOGIE

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Rezumat. Mieloperoxidaza (MPO) este o enzimă proinflamatorie care e implicată în stresul oxidativ, în inflamaţie şi joacă un rol fiziopatologic în aterogeneza şi degradarea plăcii fibroase. Modificarea nivelelor serice ale MPO au fost legate de patologia diferitelor boli incluzând ateroscleroza, infarctul miocardic, fibrilaţia atrială, diabetul zaharat, scleroza multiplă, boala Alzheimer, cancerul pulmonar şi respingerea transplantului. De aceea, MPO este considerată ca un biomarker important, cu valoare diagnostică pentru o serie de boli cardiovasculare, şi de asemenea, cu valoare prognostică în ceea ce priveşte viitoarele evenimente cardiovasculare nedorite. MPO este un complex tetrameric legat covalent, o enzimă peptidică puternic glicozilată, membră a superfamiliei hem-peroxidaze. MPO este stocată în granulele azurofile ale neutrofilelor polimorfonucleare, monocite şi macrofage, de unde este secretată ca raspuns la inflamaţia locală. Activitatea MPO poate fi măsurată în sânge şi ţesuturi prin metoda imunoenzimatică ELISA, teste colorimetrice cu substraturi şi prin gaz cromatografie urmată de spectrometria de masă.
Cuvinte cheie: mieloperoxidaza, boli cardiovasculare, diabet zaharat, obezitate

INTRODUCTION

Myeloperoxidase (MPO) is a covalently bound tetrameric complex, an heavily glycosylated peptidic enzyme, a member of the heme peroxidase superfamily which also includes eosinophil peroxidase and lactoperoxidase.

The mature MPO has a molecular weight of 146-150 kD and is formed after linkage of two identical dimmers, composed of 2 light chains and 2 heavy chains, which is identical and functionally independent. MPO is stored in the azurophilic granules of polymorphonuclear neutrophils, monocytes and macrophages from where it

is secreted like response to local inflammation. In macrophages, MPO is taken up by endocytosis of whole neutrophils, because the ability of MPO synthesis is lost and monocytes gradually lose their MPO**.** MPO represents more than 5% of the total cellular protein content in neutrophils and 1% in monocytes [1, 2].
For a long time, MPO was considered to be a bactericidal enzyme whose main function is to generate reactive oxygen species that contribute to the destruction and killing of bacteria and other pathogens. Recent evidence showed that MPO is involved in cellular homeostasis and is an important factor in the initiation and progression of various inflammatory diseases, most prominently cardiovascular diseases (CVD) [3].
Additionally, MPO has been detected in a variety of cells that are not related to the infectious process such as Kupffer cells in the liver, microglia, granule-containing neurons, and pyramidal neurons of the hippocampus [4].
Factors that influence serum MPO levels include age, gender, smoking in men and use of contraceptive pills in women. Neutrophilic MPO activity is higher in women, while serum MPO levels are increased with age in both genders [5].

MYELOPEROXIDASE DETECTION METHODS

Determination of MPO levels in human tissues and biological fluids is of great importance in experimental and clinical studies because the most important characteristics of a biomarker are the feasibility, reproducibility and reliability of the measurement method.
The MPO activity can be determined in the blood and tissues by spectrophotometric assay using hydrogen peroxide and racemic o-dianisidine as substrate [6], while the content of MPO can be measured in the neutrophils as an index of degranulation by a counter and flow cytometry and circulating MPO by enzyme-linked immunosorbent assays (ELISA).
For clinical reasons, the most widely measurement method used for MPO detection and quantification is the ELISA method which uses MPO-specific monoclonal or polyclonal antibodies. This is a very sensitive and accurate method that detects MPO levels from the nanogram to picogram range showing a high specificity for MPO without interfering with other peroxidases or enzymatic antagonists.
In research fields, a very common method for detection of MPO is colorimetric assay using substrates such as 3,3',5,5'-tetramethyl-benzidine (TMB) and guaiacol which was widely used for detection of neutrophil and monocyte recruitment in areas of inflammation [7].
An additional technique, is gas chromatography followed by mass spectrometry (MS), procedure which uses advanced methods such as product or precursor ion scanning and neutral loss analysis to detect hypochlorous acid (HOCl)-induced oxidative effects, like the formation of 3-chlorotyrosine, which is a valid marker of MPO tissue activity in inflammatory conditions [8].
The latest techniques for non-invasive detection of MPO activity include imaging technologies utilizing magnetic resonance imaging, single photon emission computed tomography, fluorescence and bioluminescence imaging [9].

MYELOPEROXIDASE MECHANISMS OF ACTION IN PATHOLOGY

MPO is a pro-inflammatory enzyme, which is involved in oxidative stress, in inflammation, and plays a pathophysiological role in atherogenesis and degradation of fibrouse plaque. It has been shown to be involved in the pathology of different diseases, including atherosclerosis, myocardial infarction, atrial fibrillation, diabetes mellitus, multiple

sclerosis, Alzheimer's disease, lung cancer, and transplant rejection [10-12].

MPO plays an important role in the initiation, progression and the complications of atherosclerotic CVD.

Thus, have been described several mechanisms by which MPO promotes atherosclerosis:

a) The induction of endothelial dysfunction, by consumption derived nitric oxide, which can lead to plaque formation.

b) Conversion of low density lipoprotein to an atherogenic form that is subsequently taken up by macrophages, resulting in the formation of lipid-laden foam cells

c) Physiologically functional modification of high density lipoprotein into "dysfunctional" form that loses its antiatherogenic effects.

The MPO physiologically substrate is hydrogen peroxide (H_2O_2) which is used to oxidize chloride, resulting the formation of proinflammatory oxidants, hypochlorous acid (HOCl), which reacts with different amino acids and proteins to produce chloramines (chlorothyrosine) and the modified protein (HOCl – modified low density lipoprotein), which are detected in human atherosclerotic lesions [13, 14]. Fu et al. [15] reported that HOCl production by MPO could represent a physiological mechanism that link degradation of matrix proteins by metalloproteinases.

In addition, MPO may participate in the ischemic complications of atherosclerosis by serum proteases of the cascade activation and promotion of endothelial cell apoptosis, which leads to a breakdown of the fibrous cap of atherosclerotic plaque.

Clinical studies have shown that high levels of MPO in blood, was closely linked to a higher risk of developing coronary artery disease, being associated with post-infact ventricular remodeling and congestive heart failure progression [16].

Increased plasma levels of MPO (unlike those of troponin T, creatine kinase-MB and C-reactive protein) are also useful for risk stratification of patients with chest pain, so it could serve as a marker for the incidence of cardiovascular events [17].

Even in healthy people, high levels of MPO were considered a risk factor for cardiovascular disease and may predict future cardiovascular events. In contrast, individuals with low serum values of MPO in hereditarily were found to be cardio-protected [18].

Some studies speculate that the relationship between MPO and cardiovascular disease is stronger on a background of hyperglycemia, being associated with risk for developing macrovascular diabetic complications [19].

Hyperglycaemia stimulates the production of H_2O_2 used by MPO like physiological substrate to form hypochlorous acid, resulting in an increase in MPO activity [20].

Glycosylated hemoglobin (HbA1c) is a marker of long term glycemic control (2-3 months before) and has a lower biological variability than glucose, therefore, it is recommended to estimate retrospective glycemic status and the risk of diabetes [21]. Zang Y. et al. [22] showed that each 1% reduction in mean HbA (1c) was associated with a 14% reduction in risk for myocardial infarction, and a 1% increase in HbA1c levels among patients with type 2 diabetes was associated with an increase of 15% for coronary artery disease.

In one of our studies (in manuscript), it is not founded a statistically significant correlation between the MPO serum and HbA1c, but the association between MPO and risk of cardiovascular disease was intensified by the increased level of HbA1c, indicating that a high concentration of glucose can interact with MPO and subsequently increase the risk of cardiovascular disease. We also found an increase in the MPO levels, this being due to both the process of atherosclerosis and age.

Also, literature studies showed (by multiple logistic regression analysis) that MPO, blood pressure and HbA1c were associated with the occurrence of coronary

artery disease among patients with diabetes, suggesting that interactions between blood pressure, blood glucose and levels of MPO accelerates the progress of atherosclerosis.
Other conditions of patients, such as obesity, have also an influence on MPO elevated levels. In this regard, Ghanbari et al. [23] have shown that MPO levels are elevated in obese people compared to non obese. In a similar study, Gandley et al. [24] have reported that people with BMI>30 had elevated MPO circulant levels.
In another study, Nicholls et al. [13] showed that besides elevated levels of MPO and changes in lipid and glucose metabolism, were found an increased number of total leukocytes, neutrophils and monocytes in the group with obesity and systemic inflammation. The explanation is that in inflammatory conditions, activated monocytes and neutrophils release MPO, which plays an important role in the evolution of cardiovascular diseases.
Andrade et al. [25] observed an increase of approximately 29 % in MPO levels in obese women as compared to lean women, demonstrating that the activation of neutrophils was probably caused by an inflammatory condition linked to obesity.

CONCLUSIONS

MPO is a pro-oxidant enzyme that is released from granules of activated leukocytes, (neutrophils, monocytes and macrophages) at inflammatory sites.
It generates reactive oxygen species that contribute to the destruction of pathogens ingested, but this antimicrobial activity can lead to oxidative damage of the endothelium and vessel wall, by the fact that both MPO and its oxidants are found in atheroma and atherosclerotic lesions.
To be secreted, MPO does not require cellular necrosis (such as troponins) or mechanical activation (such as brain natriuretic peptide, BNPs) which is probably the reason why this protein can be detected so early in the disease process.
Therefore, MPO could be considered as a significant biomarker with diagnostic properties for a number of cardiovascular diseases, and also with a prognostic value in regards to future untoward cardiovascular events.

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METABOLIC PROFILES REFLECT RISK OF DISEASE PROGRESSION IN OCTOGENARIANS

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Abstract. Non-atherogenic lipid panels in old patients are easy to highlight because of normal serum lipid values found out. However, in a group of octogenarian patients, low values of triglycerides and VLDL-C respectively, called our attention. This work aimed to point out low VLDL-C and any other combination of clinical biochemistry parameters, which indicate risk of disease progression in these octogenarians. Materials and methods: For 20 male inpatients mean age 86+2 years, and 50 female inpatients ages 86+3 years, admitted at NIGG Ana Aslan from Feb 2014 to July 2015, data of biological parameters were collected from their medical records. None of these octogenarian patients had malignant disease. 10 (20%) octogenarian female patients had type 2 diabetes and none of these male patients had diabetes type 2. Results: Data of biochemical and hematological evaluations were within the normal ranges; glycemia 93+9 in male patients and 112+39 in female patients, respectively. Only one male patient had elevated total cholesterol whereas 18 (36%) female patients had cholesterol above 200 mg/dl. 25% of these octogenarians male patients and 5 female patients had VLDL-C between 9 and 13 mg/dl. We found out significant differences between female octogenarian inpatients and male octogenarian inpatients regarding glycemias (P=0.0354) and HDL-C levels (P=0.0228). AST/ALT ratio 1.6+0.4 in these female patients and 1.5+0.5 in octogenerian male patients. The ratio AST/ALT was higher than 2 in 9 female patients (18%) and in 4 male patients (20%). Conclusion: A tendency is noticeable for metabolic profiles of male octogenarian patients to be significantly different as regards some parameters than those of female octogenarian patients. Determinations of apoB could have confirmed LDL-C levels.
Key words: octogenarian inpatients, VLDL-C, metabolic profiles

PROFILURILE METABOLICE REFLECTĂ RISCUL PROGRESIEI BOLILOR LA OCTOGENARI

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Rezumat. Panelurile lipidice non-aterogene la pacienţii cu vârste avansate sunt uşor de evidenţiat prin valorile normale ale lipidelor şi lipoproteinelor. Totuşi, ne-au atras atenţia valorile scăzute ale trigliceridelor şi VLDL-C la un grup de octogenari. Scopul lucrării este evidenţierea unor niveluri scăzute de VLDL-C şi a unor combinaţii de parametri de biochimie clinică care pot indica riscul progresiei unor boli asociate vârstei înaintate. Materiale şi metode: Pentru 20 bărbaţi vârsta medie 86+2 ani şi 50 femei vârsta medie 86+3 ani, internaţi la INGG "Ana Aslan", între feb 2014- iulie 2015, datele parametrilor biologici s-au colectat din buletinele de epicriză. Niciunul dintre pacienţi sau paciente nu avea boli maligne. 10 paciente (20%) aveau diabet de tip 2 controlat, dar niciun pacient octogenar nu avea diabet tip 2. Rezultate: Datele evaluării biochimice şi hematologice au fost în limitele normale; glicemia 93+9 mg/dl la pacienţii octogenari şi 112+39 mg/dl în cazul acestor paciente. Numai un

pacient a avut colesterol total crescut, iar 18 (36%) paciente octogenare au avut colesterolul total mai mare decât 200 mg/dl. 25% dintre pacienţii octogenari şi 5 paciente au avut VLDL-C între 9 şi 13 mg/dl. Am constatat diferenţe semnificative între pacientele octogenare şi pacienţii octogenari privind glicemia (P=0.0354) şi nivelurile de HDL-C (P=0.0228). AST/ALT 1.6+ 0.4 în cazul pacientelor şi 1.5+ 0.5 la pacienţii octogenari. Raportul AST/ALT a fost mai mare decât 2 în cazul a 4 octogenari şi 9 (18%) paciente. Concluzii: Se observă tendinţa profilurilor metabolice ale pacienţilor octogenari de a fi semnificativ diferite privind unii parametri, faţă de profilurile metabolice ale pacientelor octogenare. Determinările de apoB ar fi putut confirma nivelurile de LDL-C.
Cuvinte cheie: pacienţi octogenari, VLDL-C, profiluri metabolice

INTRODUCTION

It appears that elderly who reach advanced ages have a less significant load of co morbidities. However, study-groups of "very elderly"(Sheedfar), for which we collected data on metabolic profiles have been distinct due to increased frequencies of glycemias specific of prediabetes. For adults recent researches have pointed to genetic factors encoding for insulin resistance and described phenotypes according to body mass indexes and metabolic profiles [1,2].

Phenotypes described were of lean and healthy subjects, lean and unhealthy (thin outside and fat inside), obese and healthy (obese but insulin-sensitive) and obese and unhealthy adults. The aforementioned metabolically obese patients who are metabolically healthy do not have dyslipidemia, symptoms of hypertension and are characterized by a high sensitivity to insulin [1]. As well, Mc Ardle described in the North-American population the metabolically unhealthy obese subjects as having serum elevated cholesterol, increased triacylglycerol and free fatty acid levels [2].

Another factor contributing to insulin resistance in old patients might be steatosis, the early stage of nonalcoholic fatty-liver disease NAFLD, which has wrongly been considered a less important condition and undiagnosed in our population samples [3]. Most recent studies have highlighted that patients with NAFLD had more than double the risk of all-cause mortality compared with those with type 2 diabetes but no record of NAFLD [4]. Accumulation of fat in the liver causes hepatic insulin resistance, peripheral insulin resistance and glucose intolerance. Conventional risk factors are very similar in type 2 diabetes mellitus and NAFLD, so lifestyle changes such as physical exercise, weight loss and restriction on alcohol intake are being recommended in both conditions [4]. Specialized literature with regard to steatosis has provided as well a phenotype of individuals having fatty liver, metabolically unhealthy and who do not respond to exercise and therefore need pharmacological treatments [5]. Studies have mentioned that progression of steatosis and iron overload associated with a zinc deficit lead to insulin resistance [6]. Estimation on iron overload remains nevertheless inconvenient as it should be conducted by means of high-performance magnetic resonance imaging in order to have accurate data in this regard.

Aim: Although low values of serum VLDL-C are not of much concern except those in patients with hepatitis C-virus, decreased values of serum triglycerides and VLDL-C called our attention. This work aimed to point out low VLDL-C and any other combination of parameters of clinical biochemistry, which indicate risk of progression of diseases in octogenarians.

MATERIALS AND METHODS

This work is part of an ongoing collection of data regarding metabolic profiles and co morbidities in old subjects, ages over 80 and 90 years. Data were collected for this descriptive work out of 87 medical records of inpatients, mean age 86+3 years who were admitted at Ana Aslan NIGG between Feb 2014-July 2015. Subjects gave their written informed consent for their data to be included in the study.

Collection of results was conducted only for 50 women and 20 men as 17 medical records were incomplete. Some data on glycated hemoglobin, BMI and MCV were missing. MCV would have been useful to calculate the index for alcohol consumption according to the formula " -58.5+0.637xMCV+3.91(AST/ALT)-0.406xBMI+6.35 " for male patients. Almost all patients had hypertension, namely 47 female patients out of 50 (94%) and 19 male patients out of 20 (95%) and mainly cardiovascular disease (ischemic heart disease, atrial fibrillation) and arthrosis. We showed results as mean + standard deviation for female and male patients. To compare means of the two groups (male and female octogenerian patients, we used the t test calculator, GraphPad Software, 2017 [7].

RESULTS

Non-atherogenic characteristics of lipid panels in these old patients were evident as shown in the table below. However, 18 (36%) female patients and only one male patient had elevated total cholesterol (above 200 mg/dl). Low HDL-C was found out only in the case of diabetic subjects. In this study group 10 female patients (20%) had controlled type 2 diabetes but no elder male patient had it. 25% of these male patients and 5 female patients had VLDL-C between 9 and 13 mg/dl.

Tab. I Clinical biochemistry results in study-group female patients (n=50)

Total cholesterol mg/dl	LDL-C calculated mg/dl	HDL-C mg/dl	Triglycerides mg/dl	VLDL-C calculated mg/dl	Glycemia mg/dl	AST IU	ALT IU	Ratio AST /ALT	Hb g/L
191±49	105±39	62±17	120±51	24±11	112±39	22±10	14±5	1.6±0.4	12.4±1.3

Results are presented as means ± D.S

Tab. II Clinical biochemistry results in study-group male patients (n=20)

Total cholesterol mg/dl	LDL-C calculated mg/dl	HDL-C mg/dl	Triglycerides mg/dl	VLDL-C calculated mg/dl	Glycemia mg/dl	AST IU	ALT IU	Ratio AST/ALT	Hb g/L
171±22	100±22	52±14	115±49	22±11	93±9	23±7	16±7	1.5±0.5	12.2±1.6

Results are presented as means±D.S

We found out significant differences between female octogenarian inpatients and male octogenarian inpatients regarding glycemias (P=0.0354) and HDL-C levels (P=0.0228). The ratio AST/ALT was higher than 2 in 9 female patients (18 %) and 4 octogenarian male patients.

Limitations: Study-sample too small to analyze levels of triglycerides in a subgroup to which lipid lowering drugs were (probably) administered. Also, a lack of any indication on nutritional status of these elderly, except for a small number of patients for whom total proteins were measured.

DISCUSSION

Results for this small study-sample of patients showed that there were significant differences between male and female octogenarian patients concerning their glycemia and HDL-C values. Although in these octogenarian patients clinical biochemistry results were within the normal ranges, we noticed low serum triglycerides, VLDL-C and ratios AST/ALT higher than 2.

In case of intrahepatic lipid accumulation resulting in steatosis (hypothetical in our case), Sheedfar pointed out that despite evidence for normal transaminases'

activities, 5% of the hepatocytes are nevertheless affected. Also, advanced liver fibrosis "is often accompanied by a reduction in steatosis to the point of complete fat loss", which is reflected by the low prevalence of non-alcoholic fatty liver (21.1%) in the very elderly [8]. Other data showed that when the liver is affected by fibrosis, the reduced functioning tissue synthesizes transaminases but in lower amounts and so, transaminases (activities) appear normal instead of increased [9]. Mancone pointed out that low VLDL (hypobetalipoproteinemia) are specific of hepatitis C-virus and along with steatosis contribute to hepatitis progression [10].

VLDL assembly is dependent on phosphatidylcholine, which is an important part of the the phospholipid component of VLDL. Synthesis of the aforementioned is dependent on choline and methionine and also microsomal triglyceride transfer protein (MTTP). According to Mancone "VLDL synthesis occurs in two different stages: assembly and intracellular maturation". In the first stage, microsomal triglyceride transfer protein, an endoplasmic reticulum-localized enzyme, "promotes the addition of small amounts of triglycerides to apoB-100, while it translocates across the endoplasmic reticulum. In the second stage, VLDL precursors are fused with triglycerides-rich droplets to form mature and secretion-competent VLDL particles"[10]. Studies conducted in patients with liver disease pointed out disturbances in the two stages of VLDL synthesis. Martinez-Una found out in patients with NAFLD that increased S-adenosylmethionine (synthesis of phosphatidyl-choline) SAMe "enhanced VLDL clearance from the blood stream" and improved patients'serum lipid profiles. Nevertheless, the increased lipoprotein-lipid supply to tissues as associated with the aforementioned VLDL clearance might cause lipid storage in muscle, heart or adipose tissue leading to extrahepatic complications of NAFLD [11]. Studies also showed that phosphatidyl ethanolamine –methyl transferase PEMT and methionine adenosyltransferase MAT activities were low in subjects with cirrhosis [12]. In adult patients with different genotypes of the hepatitis C-virus a statistically significant inverse correlation between MTTP RNA levels and the degree of steatosis was pointed out, regardless the hepatitis C- virus genotype [13]. Also, reduced apoB levels were inversely related with those of plasma pro-inflammatory cytokines [14,15].

Another presumable cause for the low VLDL-C values may be diets deficient in organic nutrients. Experimental studies showed ways in which aforementioned diets, especially choline and methionine-deficient diets affect first the synthesis of phosphatidylcholine and secondly, the VLDL secretion, the consequence being an intrahepatic accumulation of triglycerides. In laboratory animals (mice) Raubenheimer compared effects of choline-deficient and also choline and methionine deficient diets, on pathogenic mechanisms of the diet-induced steatohepatitis [16]. This study found out that phosphorylation of the hepatic insulin receptor substrate was impaired because of inflammatory signals relating to steatohepatitis and confounding sensitivity to insulin. Like the choline and methionine deficient diet, the diet deficient only in choline induced fatty liver but without body weight loss, less significant steatohepatitis and as well it improved both insulin sensitivity and glucose tolerance [16].

CONCLUSION

A tendency is noticeable for metabolic profiles of male octogenarian patients to be significantly different as regards some parameters than those of female octogenarian patients. Determinations of apoB could have confirmed LDL-C levels.

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THE CORRELATION BETWEEN ANXIETY DISORDERS AND COGNITIVE DISTORTIONS

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Abstract. Anxiety is the pathological state characterized by a feeling of "fear without object" accompanied by somatic signs that indicate the hyperactivity of the autonomic nervous system. People suffering from anxiety disorders do not just complain that they are often very restless, but need support to face certain impairment thoughts that occur repeatedly and that they cannot judge in a realistic manner or control them, thus becoming upsetting for them. The purpose of the present study is to identify the correlation between the variables anxiety disorder and affected operations at the cognitive triad level. We have operated the cognitive impairment through the existence of abnormalities at the specific thinking level: errors of judgment, difficulties in the discrimination of the logical elements and sensing the absurd. The general hypothesis of the study it was that anxiety disorder trigger the occurrence of the distortions at the level of the cognitive triad. The results of the study were obtained by applying quantitative assessment scales: DASS – Depression, Anxiety and Stress scale, Montreal Cognitive Assessment Test (MoCA), Wechsler Test - verbal comprehension scale and the Projective Test - Inkblot Test, over a lot of 100 subjects aged 35-80 years. Correlation of test results validates the initial hypothesis: the presence of an anxiety disorder is associated with the presence of disorders at the triad of abstract thinking, more specific at the level of stimulus mediation compartment.
Key words: anxiety disorder, cognitive disorders, assessment scales

CORELAŢIA DINTRE TULBURĂRILE ANXIOASE ŞI DISTORSIUNILE COGNITIVE

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Rezumat. Anxietatea este starea patologică ce se caracterizează printr-un sentiment de „teamă fără obiect" însoţită de simptome somatice care indică hiperactivitatea sistemului nervos autonom. Persoanele care suferă de tulburări anxioase nu se plâng doar de faptul că sunt frecvent foarte neliniştite, ci au nevoie de sprijin pentru confruntarea cu anumite gânduri eronate care apar în mod repetat şi pe care nu le mai pot judeca realist şi nici controla, devenind astfel supărătoare. Tulburarea de anxietate este întreţinută de multiple distorsiuni cognitive, de aici apare caracterul iraţional şi necritic al gândurilor negative disfuncţionale ce intregesc tabloul clinic. Studiul de faţă îşi propune identificarea corelaţiei dintre prezenţa tulburării anxioase şi apariţia unei afectări la nivelul triadei cognitive. Am operationalizat afectarea cognitivă prin existenţa unor erori de judecată şi a unor dificultăţi în discriminarea elementelor logice şi sesizarea absurdului. Ipoteza generală a studiului a fost aceea că tulburarea de anxietate antrenează apariţia unor distorsiuni cognitive. Rezultatele au fost obţinute prin aplicarea unor scale cantitative de evaluare: DASS - scala de depresie, anxietate şi stres, testul de evaluare cognitivă Montreal (MoCA), testul Wechsler - scala verbală, şi testul proiectiv - Testul Petelor de Cerneală Rorschach, pe un lot de 100 de subiecţi, cu vârsta cuprinsă între 35-80 de ani. Corelarea rezultatelor testelor validează ipoteza iniţială: prezenţa unei tulburări anxioase este asociată cu afectarea la nivelul calităţii gândirii, mai specific la nivelul capacităţii de mediere a stimulilor.
Cuvinte cheie: tulburare anxioasă, distorsiuni cognitive, scale de evaluare

INTRODUCTION

In everyday life, each of us goes through difficult moments, complicated, tense or loaded or certain intensity. Unrest and worries about everyday problems and situations are, to a point, naturally. Often these concerns, this prudence can help increase efficiency in certain situations; also, in certain special circumstances a high level of concern and care can be considered normal. When these concerns become overwhelming and are associated with an invalidation of everyday life then we can talk about the possibility of an anxiety disorder.

Anxiety was defined as a fear without object, manifested by psychomotor restlessness, autonomic and behavioral changes [1]. Pathological anxiety is different from the usual restlessness or fear felt by every person in front of new situations or situations with a high degree of difficulty, states which have a positive impact on activities (concentration, mobilizing resources). In fact, the literature distinguishes between **facilitating anxiety** which stimulates and accesses resources and **blocking anxiety** that paralyzes and hijacks one's self control and the ability to think logical and rational [2].

Anxiety has a trait of potentiality, deforming living presence in relation to the future foreboded as hostile and predetermined as such. People who suffer from anxiety disorders complain not only about being frequently restless, but need support to face certain fears that occur repeatedly and that they can not judge critically, thus becoming troublesome. Anxiety has the following characteristics:

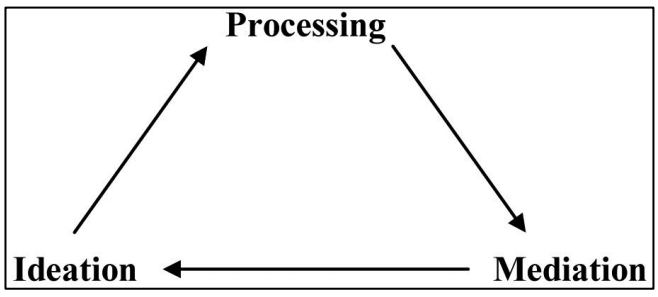
- is unjustified
- refers to an indeterminate and imminent danger, to which an attitude of hypervigilance is manifested
- is accompanied by the belief of helplessness and disorganization in the face of danger
- associating a vegetative sympto-matology generating somatic
- creates a vicious circle that enables anxiety to sustain itself

Anxiety's effects can **not be easily controlled** and the foundation on which they are developed is represented by disorders is the quality level of thinking. Anxiety disorder is sustained by multiple cognitive distortions that maintain the irrational and uncritical nature of dysfunctional negative thoughts.

Data regarding the cognitive activities of a person are usually known as the Cognitive Triad. These three clusters consist of:

- Information processing, which involves the mental procedures entailed in the input of information.
- Cognitive mediation, involving the mental operations which occur when information that has been input is translated or identified.
- Ideation, which refers to the thinking process that occurs after inputs have been identified and which leads to some form of mental conceptualization of the infor-mation that has been translated.

Research findings from experimental psychology suggest that the results of the operation in one element can have a very direct influence on the operations in either of the other two elements [3]. Therefore, it is not unrealistic to assume that, as a collective of functions, they reflect a continuous process that forms the basis for essentially all deliberate and/or meaningful behaviors. The process can be illustrated simply as circle because in many instances pre-established conceptual sets influence the tactics applied in processing information. Likewise, the manner in which an input is mediates can easily impact on the conceptualization of an input



MATERIALS AND METHODS

Subjects

- Demographic variables: urban and rural environments
- Social variables: aged 35-80, lot 100 people; sex distribution 83,33% F; 16,67%M

Metodology

The theoretical objective of the present study is to identify the presence of a correlation between the variables anxiety disorder and affected operations at the cognitive triad level. The premise we started from was that there is a relationship of determinism between anxiety disorder and cognitive impairment. We have operationalized the cognitive impairment through the existence of abnormalities at the specific thinking level: errors of judgment, difficulties in the discrimination of the logical element and sensing the absurd.

The specific objective is to identify the affected compartment and how these dysfunctions train changes at the level of the other operations and the manner in which they together or separately generate the anxiety disorder.

The general hypothesis is that anxiety disorder is causing impairments of qualitative thinking.

The working hypothesis is that the level at which the impairments occur is especially to be the mediation and ideation.

Assessment scales

- DASS depression, anxiety and stress scale

The DASS is a set of three self-report scales designed to measure the negative emotional states of depression, anxiety and stress [4]. The DASS was constructed not merely as another set of scales to measure conventionally defined emotional states, but to further the process of defining, understanding, and measuring the ubiquitous and clinically significant emotional states usually described as depression, anxiety and stress.

Each of the three DASS scales contains 14 items, divided into subscales of 2-5 items with similar content.

The Depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest/involvement, anhedonia, and inertia.

The Anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect.

The Stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset/agitated, irritable/over-reactive and impatient.

Subjects are asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each state over the past week. Scores for Depression, Anxiety and Stress are calculated by summing the scores for the relevant items.

In addition to the basic 42-item questionnaire, a short version, the DASS-21, is available with 7 items per scale.

As the scales of the DASS have been shown to have high internal consistency and to yield meaningful discriminations in a variety of settings, the scales should meet the needs of both researchers and clinicians who wish to measure current state or change in state over time (e.g., in the course of treatment) on the three dimensions of depression, anxiety and stress.

- Montreal Cognitive Assessment Test (MoCA)

The MoCA was designed as a rapid Mini-Mental State Examination (MMSE) in distinguishing clients with mild cognitive impairment from normal elderly clients [5]. Thus, the MoCA is intended for clients with memory complaints who score within the normal range on the MMSE. The MoCA assesses the following cognitive domains: attention and concentration, executive functions, memory, language,

visuoconstructional skills, conceptual thinking, calculations, and orientation.

- Inkblot Test Rorschach

The Rorschach Inkblot Test is a projective psychological test consisting of 10 inkblots printed on cards (five in black and white, five in color) created in 1921 with the publication of *Psychodiagnostik* by Hermann Rorschach (6). During the 1940s and 1950s, the test was synonymous with clinical psychology. Throughout much of the 20th century, the Rorschach inkblot test was a commonly used and interpreted psychological test.

In 1969, John E. Exner, Jr. decided to undertake the creation of a new, comprehensive Rorschach scoring system that would take into account the best components of these five existing systems, combined with extensive empirical research on each component. A foundation was established in 1968 and the significant research began into creating a new scoring system for the Rorschach. The result was that in 1973, Exner published the first edition of *The Rorschach: A Comprehensive System*. In it, he laid out the new scoring system that would become the new gold standard (and the only scoring system now taught).

The Rorschach Inkblot test was not originally intended to be a projective measure of personality. Instead, it was meant to produce a profile of people with schizophrenia (or other mental disorders) based upon score frequencies. Rorschach himself was skeptical of his test being used as a projective measure.

The Rorschach is, at its most basic level, a problem-solving task that provides a picture of the psychology of the person taking it, and some level of understanding the person's past and future behavior. Imagination is involved most often in the embellishment of a response, but the basic process of the task has little to do with imagination or creativity.

- Wechsler Test – verbal comprehension scale

The Wechsler intelligence scales were developed by Dr. David Wechsler, a clinical psychologist with Bellevue Hospital. His initial test, the Wechsler-Bellevue Intelligence Scale, was published in 1939 and was designed to measure intellectual performance by adults. Wechsler constructed the WBIS based on his observation that, at the time, existing intelligence tests for adults were merely adaptations of tests for children and had little face validity for older age groups.

In recognition of emerging demographic and clinical trends, the WAIS-IV was developed to provide the most advanced measure of cognitive ability and results that can be trust when addressing the changing clinical landscape [7].

The Verbal Comprehension subtests include: Similarities, Vocabulary, Information and Comprehension.

RESULTS AND DISCUSSIONS

The data obtained after the clinical investigation were statistically analyzed using the Pearson correlation coefficient. A correlation between the presence of an anxiety disorder and disorder at the specific thought functions level resulted after the analysis (Fig. 1). This means that lack the necessary ingredients required to test reality correlates with the presence of the anxiety disorder. Thus, we can explain and understand differently the pathology specific to the anxiety disorder: irrationality related to deep and unwarranted fear is due to the presence of a disorder at the logical thought operations level and errors in judgment..

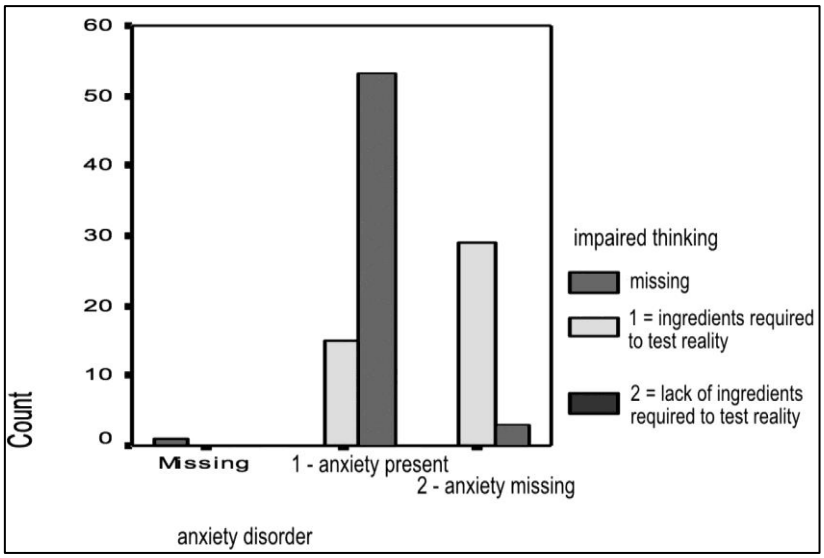


Fig.1 Correlation between the anxiety disorder and disorder at the specific thought functions level

The cognitive triad and its related compartments represented the next point of focus for the study. The question that was raised was: Which of the three affected departments correlate with anxiety disorders?

The disturbances occur in the first compartment (the stimuli scanning strategies one, in which the subject perceives the stimulus situation in an erroneous manner), the second compartment (in which the collected data are averaged with the existing ones, process that results in a more or less distorted reasoning) or at the third compartment level (of concepts and principles already formulated that mediates data obtained from scanning the stimulus situation).

Judgment can be defined as the capacity to assess situations and draw sound conclusions after careful consideration of the relevant circumstances. From a neuropsychological perspective, judgment falls under the domain of executive functioning [8] and includes both a cognitive appraisal process (determining what to do in a situation) and the behavioral follow-through (engaging in the adaptive/safe behavior). Numerous processes are involved in the execution of good judgment including generating appropriate strategies to approach a problem, identifying suitable goals, shifting from one idea to another, evaluating the potential consequences of different courses of action, inhibiting inappropriate responses, initiating and carrying out purposeful behavior, and monitoring the progress and effectiveness of a chosen solution.

Cognitive distortions is a concept from Cognitive-Behavioral Therapy and is referring to biased ways of thinking about oneself and the world around us. The model essentially states that there are specific ways people distort their thinking. These irrational thoughts and beliefs (distortions) can lead to problematic emotional states and behavior, like anxiety, low self-esteem, depression and relationship conflicts [9]. More rational thinking tends to lead to more positive emotional and behavioral experiences. All human beings use these cognitive distortions to greater and lesser degrees.

Errors of judgment and cognitive distortions induce qualitative changes at the thought operations level. At the first cluster level, the cognitive distortions that may arise are related to the strategies that the individual uses in scanning the stimulus situation. Overgeneralization, denial, rationalization etc., if used consistently lead to an erroneous perception of the situation and the

formation of a distorted image of reality and hence engage changes in the other clusters. Incorrect information on a perceived situation reach the next cluster where it's combined with existing data (that may also be distorted), and, after this mediation, behaviors are adopted and, subsequently concepts are formulated. Being distorted, they further engage judgment errors, erroneous beliefs and maladaptive and inefficient behaviors.

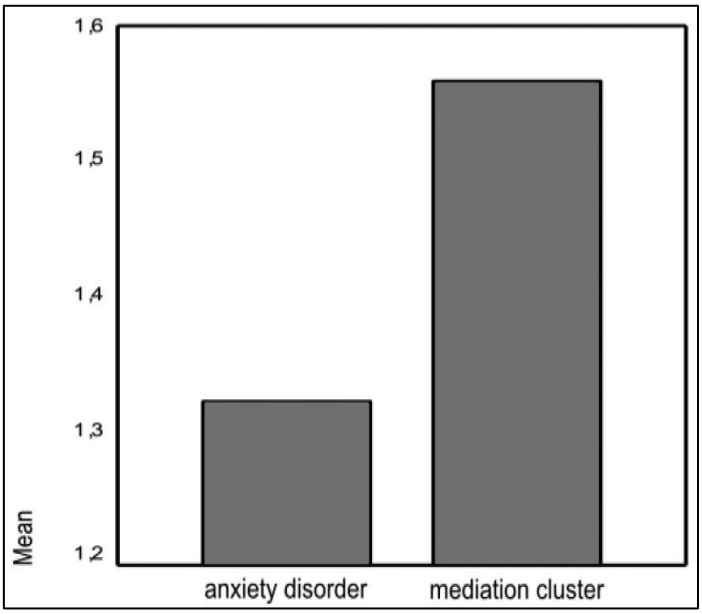


Fig. 2 Correlation between disorder variable and the mediation ability

Data processing showed the existence of a negative correlation between the panic disorder variable and the second compartment of the cognitive triad: the mediation ability, aspect explained psychologically by the presence of the ingredients necessary for testing reality at this level (Fig. 2). The non-presence of anxiety disorder correlates with a proper and efficient mediation capacity.

The data from this section indicate the manner in which images are identified and translated. The variables in this section cover a wide range of elements and perhaps most important aspect is the level of ordinary, unusual or inappropriate that you touch. They also consider the circumstances in which inappropriate mediation occurs.

We notice the idea that the presence of an anxiety disorder is a particular context in which errors of judgment and wrong reasoning can happen. There is a mutual influence between the two variables in the sense that a permanent and persistent working model is established.

The statistically processed data did not show significant correlations for the other two compartments which underlines the idea that the mediation compartment is the most vulnerable and at its level we see the erroneous processing that further engage inadequate and irrational behavior as they appear in the clinical picture of anxiety. The judgment may also be expanded and we can state that mediation and wrong testing of reality leads to the formulation of distorted beliefs and principles are encoded into long-term memory and which will subsequently undergo a new process of mediation.

CONCLUSIONS

The presence of an anxiety disorder is associated with the presence of disorders at the cognitive triad level: stimuli processing, the capacity for mediation and the ideation.

The disorders influencing and maintaining the clinical image of anxiety occur within the mediation and cognitive triad compartment. The ingredients required for testing reality exist and are thus affected at this level.
The disorders occurring at the data processing and ideation compartment level do not influence or sustain at a significant level the anxiety disorder.
Going deeper into the study by exploiting the brain imaging towards identifying the way in which the thought disorders occur at this level and the way in which they generate and maintain the anxiety disorder.

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