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1H NMR STUDIES OF ERYTHROCYTE MEMBRANE PERMEABILITY IN RATS WITH EXPERIMENTAL ATHEROSCLEROSIS. THE IMPACT OF PROCAINE OR ASLAVITAL TREATMENT

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Abstract. The aim of this study was related with investigation of erythrocyte membrane permeability of rats fed on reach cholesterol diet by means of 1HNMR technique. 16 rats aged 32 months old divided into 4 groups of 4 rats each have received a reach cholesterol diet(animal lard) combined with Aslavital or Procaine for 6 weeks (4mg/kg body weight). Group (A) Control. The animals fed on cholesterol reach diet were divided into 3 groups: group (B) –hypercholesterolemic diet, group (C) also with Aslavital (i.m. 4mg/Kg body weight); group (D) – also with Procaine treatment (4 mg/Kg body weight).to test its properties as an antiatherosclerotic drug. Our data have pointed out a decrease of proton half time within erythrocyte which accounts for an accelerated proton exchange in all groups of rats with high level of serum cholesterol. In controls the exchange of water through red blood cell membrane is accelerated in parallel with increase in local temperature. Red blood cell membrane permeability towards water can be accounted as an index of cardiovascular system recovery, important in maintaining a dynamic equilibrium with vascular destruction phenomenon due to the high blood pressure associated to experimental atherosclerosis.
Key words: experimental atherosclerosis, Aslavital, Procaine, erythrocyte membrane permeability, cholesterol, 1H Nuclear Magnetic Resonance (1H NMR)

STUDII 1H RMN ALE PERMEABILITATII MEMBRANEI ERITROCITARE LA SOBOLANI CU ATEROSCLEROSA EXPERIMENTALA. IMPACTUL TRATAMENTULUI CU PROCAINA SAU ASLAVITAL

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Rezumat. Scopul acestui studiu a fost investigarea permeabilitatii membranei eritrocitare la sobolanii hraniti cu dieta bogata in colesterol, timp de 6 saptamani, cu ajutorul tehnicii de 1H NMR. 16 sobolani in varsta de 32 de luni impartiti in 4 grupe de cate patru sobolani fiecare au primit dieta bogata in colesterol combinata cu Aslavital sau Procaina timp de 6 saptamani (4mg/kg corp). Grupul (A) Control. Animalele hranite cu colesterol au fost impartite in 3 groupuri: grupul (B) – cu dieta hipercolesterolemianta, grupul (C)-dieta hipercolesterolemianta plus Aslavital i.m. 4mg/Kg corp); grupul (D) – dieta hipercolesterolemianta plus tratament cu Procaina (4 mg/Kg corp) pentru a testa proprietatile antiaterosclerotice ale acestor medicamente. Datele noastre au evidentiat o scadere in timpul de viata al protonilor eritrocitari care pledeaza pentru un schimb accelerat de protoni in toate grupele de sobolani cu niveluri crescute ale colesterolului seric. La grupul de control schimbul de protoni ai apei

prin membrana eritrocitara este accelerat in paralel cu o crestere in temperatura locala. Permeabilitatea membranara a eritrocitelor fata de apa poate fi considerata drept index al recuperarii sistemului cardiovascular, important in mentinerea unui echilibru dinamic cu fenomenul de distrugere vasculara datorita presiunii crescute a fluxului sanguin asociat cu ateroscleroza experimentala.
Cuvinte cheie: ateroscleroza experimentala, Aslavital, Procaina, permeabilitatea membranei eritrocitare, colesterol, 1H Rezonanta Nucleara Magnetica (1H RMN)

INTRODUCTION

High blood pressure and its major complications is one of the major problems of medical research, the attention being concentrated towards elucidation of physiopathological mechanisms which interfere during evolutionary stages of the disease.

The aim of this paper was related with the investigation of some biophysical aspects which seem to be altered in aging animals fed on cholesterol reach diet and which received Aslavital tretment which contains in composition Procaine chlorhydrate, glutamic acid (as activator factor) and benzoic acid (as an antiaterogenic factor). This drug has an regenerative eutrophic, antiaterogenic (lipotrop) action and of regulation of fat metabolism and cholesterol which is used for prophylactic and curative treatment of cerebral and cardiovascular aging.

MATERIAL AND METHOD

Our study on experimental hyperthensive animal models using biophysical methods was designed to investigate the proton transverse relaxing times of intracellular water and of membrane permeability to water.

1. Biological Material

Our study has been done on: 32 months old White Wistar rats group fed for six weeks on reach cholesterol diet (animal fat) and on Control Group (A). The group of animals fed on reach cholesterol diet has been divided into: group (B) – which has received Procaine treatment along with high reach cholesterol diet. Group (C) – which also received Aslavital treatment (Intra peritoneal injections with Aslavital 4mg/Kg body weight.); Group (D) –which received treatment with Procaine (I.P injections with Procaine solution 4 mg/Kg body weight). Procaine treatment has been done in order to establish if this drug formula is efficient in preventing atherosclerotic effect of high reach cholesterol diet.

2. Determinations of 1H Nuclear Magnetic resonance (1H NMR)

The biological material used in our study was venous blood harvested on heparin and an adequate volume of $MnCl_2$ in such a way to obtain in extracellular compartment a concentration of 20 mM $MnCl_2$.

1H NMR method has been used for evaluating proton transverse relaxing times of intra erythrocyte water and also of extra erythrocyte water, as well as for evaluation of times for exchange of water and calculus for water permeability.

The method's principle: Consists of characterising of a system composed of two compartments - A and B – of the two relaxing times - T_{2a} and T_{2b} – of the same type of nuclei originating from the same compartment.

1H NMR determinations have been done using an AREMI`78 Spectrometer in impulses at a frequency of 25 MHz, using the standard sequence CARR-PURCELL-MEIBOOM-GILL with an interval of 1 ms between impulses. Have been measured transverse proton relaxing times in intracellular compartment in the presence of water exchange between intracellular and extracellular compartment fed with Mn2+ obtaining in such a way the apparent relaxing time $T_{2a'}$

RESULTS AND DISCUSSIONS

Modifications of membrane permeability to water at the level of blood erythrocyte are similar with those at the cardiovascular level and are produced in the same way. Also the water permeability can be modified also by changing the proportion and distribution of lipid membranes.

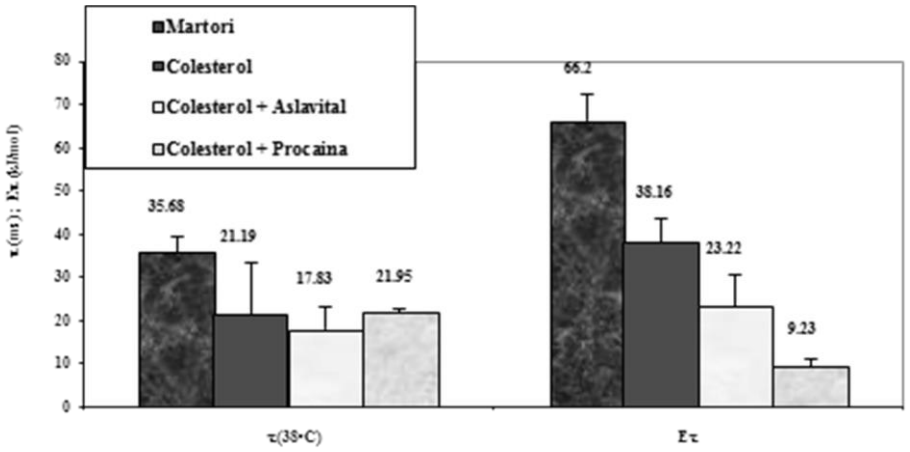

Fig.1. The exchange time of water at the level of erythrocyte membrane (τ) and activation energy of water exchange at the level of erythrocyte membrane E_τ, in Control or cholesterol, Aslavital and Procaine treated animals in different combinations

Figure 1 presents the aspects related to the dynamics of protons along the erythrocyte membrane and of energetic modifications due to exchange of water protons. There is a decrease of half life time of proton in the erythrocyte (**τ**), which suggests an accelerated proton exchange which appears in all groups where Cholesterol exceedes the normal range, even the animals have received/or not drug therapy.

The activation enery of water exchange along erythrocyte membrane (E_τ) becames lower and lower in groups fed only on cholesterol reach diet, then, in combination with Aslavital, and the smallest values have been recorded for Procaine treated group.

This accounts for the fact that high cholesterol level makes the water exchange to get faster despite the drug administration, and the transmembrane exchange process is partialy deconected under the influence of thermic processes with heat liberation, being positively affected following the drug administration.

When the specific energetic processes of an exchange between two implicated compartments, that means that one of the compartments is responsable for a deviation in energetic field, either the two compartments under grow this process (1).

By studying the state of plasma-erythrocyte biocompartimental system from the point of view of activation energy processes of proton relaxation (Fig. 2), we can observe significant increase of energy activation in plasma (E_{plasma}) in all treated groups, the highest value being obtained in Procaine's presence (7 times higher than in Controls).

Activation energies inside the erythrocyte are modified in a smaller proportion and have different aspects: they decrease comparatively to Controls in cholesterol reach diet animals and they increase a little in comparison with those last ones, following the treatment with Aslavital and Procaine, remaining still under the Control values.

The erythrocyte membrane permeability to water (PMEA) is a parameter which characterizes both the water exchange along the erythrocyte membrane as well as those which are taking place at the vascular walls (2).

This correlation is allowed by the presence of the same type of aquaporine -AQP1- both in the erythrocyte membrane as well as in the vascular endothelial membranes at all levels.

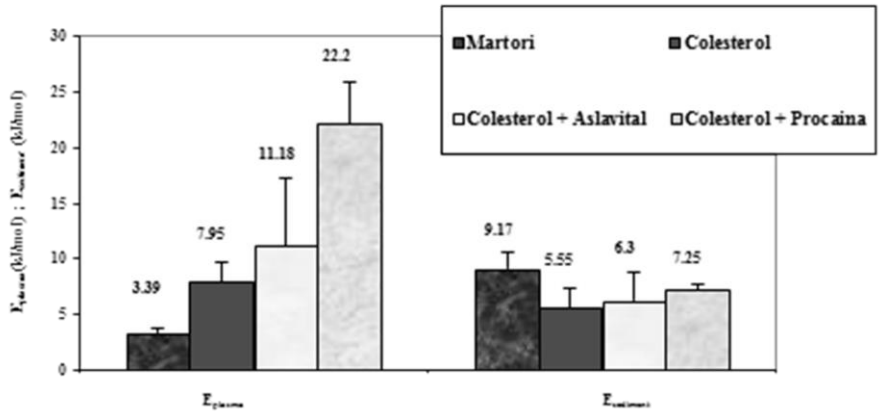

Fig.2 The water exchange time along erythrocyte membrane (τ) and activation energy of water exchange along erythrocyte membrane E_τ in Controls or cholesterol treated, Aslavital and Procaine in different combinations

In Figure 3 it can be observed that permeability to water increases very much in high reach diet animals (from 2.19 cm-s*10^{-3} in Controls to 5.8 cm-s*10^{-3} high reach diet animals. Aslavital and procaine induce a decrease of this parameter, but the values are still above the Controls values.

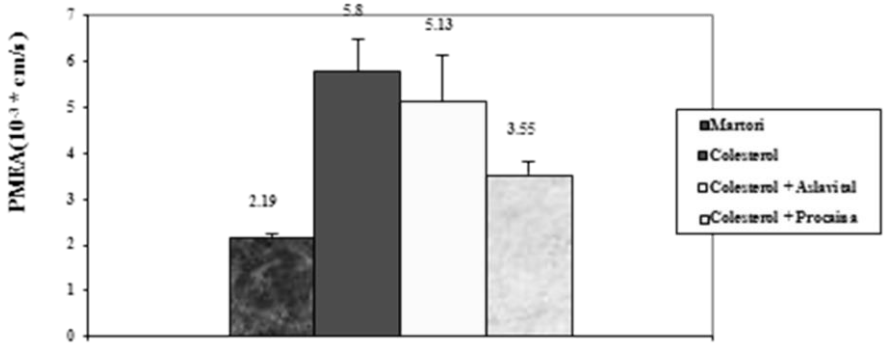

Fig.3 Erythrocyte membrane permeability for water (PMEA) in Control or treated animals with cholesterol, Aslavital and Procaine in different combinations

From our previous studies (1) we have seen that permeability to water is increased in the onset stages of the disease and is a defense reaction against the high blood pressure, and after maintaining it in an increased plateau there is a sudden decrease this fact is correlated with an increased risk of stroke (3).

Therefore, not always the readjustment of an increased physiological parameter to the normal values in a pathological context of a disease is wellcomed because the organism adaptively has created a new state of equilibrium; if the primary course of deregulation of normal equilibrium is not corrected, it can lead to a more serious pathological event (4).

It is mentioned the fact that singular administration of drugs which decrease water permeability of membranes in hypertensive patients is risky and it is recommended that these to be associated with drugs with an opposite effect upon membrane permeability (5).

In this study it is not the case because it has been demonstrated that Aslavital and Procaine have a very weak effect upon membrane permeability. High EPMW and

low values of intracellular T2 suggest a hydration phenomenon of cells from high reach cholesterol fed animals.

CONCLUSIONS

From the above presented results we can drawn the following conclusions:

• There is a decrease in proton half life in erythrocytes which suggests an accelerated proton exchange present in all studied groups of animals where the cholesterol levels exceedes the normal range.

• In young animals (Controls) the water exchange along the erythrocyte is accelerated in parallel with the increase in local or global temperature (generated by the activation of metabolical reactions) with heat release in the intracellular environment), drug administration conducting to the stabilization of water exchanges in the context of high values of temperature.

• Membrane permeability to water (MPW) may be accounted for as an index of recovery of cardiovascular system, important în mentaining a dynamic equilibrium with phenomenons of vascular destruction due to the high blood pressure.

• Aslavital or Procaine treatment exhibits upon the studied parameters effects which emerge in the same direction, the Procaine effect is more pronounced. In general, Aslavital is behaving as a buffered Procaine with a more adequate action.

REFERENCES

[1] Gatina R, Balta N, Moisin C, Burtea C., Botea S. *Research on red cell membrane permeability in arterial hypertension* - Roum J Physiol, 1998,35(3-4), 285-302.

[2] Revnic C.R,.Nica A.S.,Ginghina, C.,Revnic F.,Botea S. *Biophysical studies of erythrocyte membrane permeability in patients with cardiovascular pathology New Horizons in Coronary artery disease*. Proceedings of the 7th International Congress on Coronary Artery Disease,Venice Italy. Ed.Medimond International Proceedings ISBN 978-88-7587, 2007,pp 37-41.

[3] Badimon L1, Vilahur. *LDL-cholesterol versus HDL-cholesterol in the atherosclerotic plaque: inflammatory resolution versus thrombotic chaos*. Ann N Y Acad Sci., 2012,Apr;1254:18-32.

[4] Jesper Brahm. *The permeability of red blood cells to chloride, urea and water*. Journal of Experimental Biology, 2013,216: 2238-2246.

[5] Shanahan CM, Connolly DL, Tyson KL, et. al. *Aqouaporin-1 is expressed by vascular smooth muscle cells and mediates rapid water transport across vascular cell membranes* - J Vasc Res, 2000,36(5): 353-62.

CORRELATION BETWEEN ATHEROGENIC INDEX AND HEARTSCORE IN ELDERLY PATIENTS

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Abstract. Atherogenic index (AI) has been shown to be weighty predictor for future cardiovascular events. The aim of the study was to assess the prevalence of risk factors for cardiovascular diseases-AI and to calculate 10-year risk of cardiovascular mortality based on the HeartScore, in elderly patients. We analyzed clinical data from 196 patients with polypathology: a senescent group-over 65 years (n=136), compared to the adult group (n=60). We calculated AI and HeartScore and based on their values, we estimated the cardiovascular risk in 3 categories: low, medium and high. AI has no variation between senescent and adult patients; HeartScore values are raised with 61.08% (4.24 vs. 1.65, $p<0.0001$) in senescent patients compared with adults. For both groups, AI has a value above 0.24 indicating a high risk for all patients included in the study. Linear regression equation revealed a positive significant correlation between HeartScore and AI at both senescent ($r=0.348$, $p<0.0001$) and adult patients ($r=0.336$, $p<0.01$).There is no correlation between AI and age, but HeartScore is positively statistically significant correlated with age ($r=0.434$, $p<0.0001$). Thus, changes in lipid profile begin early in adult life, and level of risk remains high as we age. On the contrary, risk of developing cardiovascular diseases is at low-medium level for adults and rose at high-very high level at elderly. Correlating obtained data with recent studies it can be concluded that aging and risk for cardiovascular diseases are in a strong and close relationship and together, AI and HeartScore can be a useful tool for diagnosis, treatment and prevention of chronic cardiovascular disease and major cardiac events.
Key words: atherogenic index, HeartScore, aging

CORELAȚIA DINTRE INDEXUL ATEROGENIC ȘI HEARTSCORE LA PACIENȚII VÂRSTNICI

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Rezumat. S-a aratat ca indexul aterogenic (AI) este un predictor puternic al viitoarelor evenimente cardiovasculare. Scopul studiului a fost de a evalua prevalenta factorilor de risc cardiovasculari-AI si de a calcula riscul pe 10 ani de mortalitate cardiovasculara, pe baza HeartScore, la pacientii varstnici. Am analizat datele clinice de la 196 pacienti cu polipatologie: un grup senescent-peste 65 ani (n=136), comparativ cu un grup de adulti (n=60). Am calculat AI si HeartScore si pe baza valorilor obtinute, am putut estima riscul cardiovascular in 3 categorii: scazut, mediu si ridicat. AI nu prezinta nici o variatie intre pacientii senescenti si adulti; valorile HeartScore sunt crescute cu 61.08% (4.24 vs. 1.65, $p<0.0001$) la senescenti comparativ cu adulti. Pentru ambele grupe, AI are o valoare peste 0.24, indicand un risc ridicat pentru toti pacientii luati in studiu. Ecuatia de regresie lineara releva o corelatie pozitiva semnificativa intre HeartScore si AI atat la senescenti ($r=0.348$, $p<0.0001$) cat si la adulti ($r=0.336$, $p<0.01$). AI nu se coreleaza cu varsta, insa HeartScore prezinta o corelatie pozitiva semnificativ statistica cu varsta ($r=0.434$, $p<0.0001$). Astfel, din timpul vietii adulte au loc schimbari in profilul lipidic, la nivel de risc ridicat, si ramane asa pe masura ce imbatranim. Contrar, riscul de a dezvolta o boala cardiovasculara este la un nivel scazut-mediu la adulti si creste la nivel ridicat-foarte ridicat odata cu imbatranirea. Corelarea datelor obtinute, cu cele din literatura, duce la concluzia ca riscul cardiovascular si imbatranirea sunt intr-o relatie stransa si puternica, iar AI si HeartScore impreuna pot fi o unealta utila pentru diagnosticul, tratamentul si preventia bolilor cardiovasculare cronice si evenimentelor cardiace majore.
Cuvinte cheie: indicele aterogenic, HeartScore, imbatranire

INTRODUCTION

Lipid profile and atherogenic index (AI) have been shown to be weighty predictors for metabolic disturbances including dyslipidemia, atherosclerosis, hypertension and cardiovascular diseases (1). Any changes in the levels of lipids make the individuals more inclined to develop atherosclerotic cardiovascular diseases as well as endothelial dysfunction (1,2,3,4)

Ratios of LDL and total cholesterol with HDL, according to Grover et al (5), are good predictors for future cardiovascular events, but after Gotto et al (6) who conducted a study of 8 years in a predominantly male population, the serum levels of triglycerides (TG) may represent alone an important risk factor for future cardiovascular events (7).

Recent studies have indicates that TG/HDL ratio transformed logarithmically can estimate the atherogenic risk better than all others. TG and HDL perfectly reflects the balance between atherogenic lipoproteins and protective lipoproteins. Clinical studies revealed that AI can estimate the cardiovascular risk, this index is also sensitive to pharmacological treatment being a barometer of therapeutic success (8,9,10).

In the prevention of cardiovascular diseases, the European Society of Cardiology recommends the SCORE scale. The SCORE scale, available in the form of tables, allows estimation of the 10-year risk of the first fatal incident due to an atherosclerotic cause, including myocardial infarction, stroke, aortic aneurysm, or other incident. Most other systems can only estimate the risk of coronary artery disease. The ESC guidelines indicated the advantages of using the SCORE scale. These are, among others: it is an intuitive, easy to use method that takes into account the multifactorial nature of cardiovascular diseases, and providing flexibility of treatment – if a perfect result for a particular risk factor is not achieved, the global risk can still be reduced by obtaining larger changes in other risk factors. HeartScore is an electronic, interactive version of the SCORE scale. It allows for quick calculation of risk and data archiving (11,12,13).

To calculate the 10-year risk of cardiovascular death, the HeartScore calculator was used, which included age, systolic blood pressure, plasma total cholesterol level, and smoking habits. The relative risk of death was compared with the risks acceptable for the age of each person and the difference was calculated. The HeartScore risk estimation is based on the following risk factors: sex, age, smoking, systolic blood pressure, and total cholesterol. The calculated value of the score refers to relative risk, not percentage risk. The threshold for high risk based on fatal cardiovascular events is defined as "higher than 5%" (14,15).

The aim of the study was to assess the prevalence of risk factors for cardiovascular diseases -atherogenic index and to calculate 10-year risk of cardiovascular mortality based on the HeartScore, in elderly patients.

MATERIALS AND METHODS

Subjects

We analyzed clinical data from 196 patients (men and women) with polypathology: a senescent group-over 65 years (n=136), compared to the adult group (n=60). We calculated AI and HeartScore, and based on their values, we can estimate the cardiovascular risk in 3 categories: low, medium and high.

AI values are associated with:

- low risk -0.3 ÷ 0.1
- medium risk 0.1 ÷ 0.24
- high risk above 0.24

The HeartScore risk was divided into three subclasses according to the various algorithms:

- low risk (HeartScore<2%),
- intermediate risk (HeartScore2% but<5%) and
- high risk(HeartScore>5%)

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 HeartScore, AI and age was assessed using a linear regression model.

RESULTS AND DISCUSSIONS

Our study revealed increased values of lipid fractions; AI has no variation (but high values) between senescent and adult patients; HeartScore values are raised with 61.08% ($p<0.0001$) in senescent patients compared with adults.

For both groups (Table 1), AI has a value above 0.24 indicating a high risk for all patients included in the study. This clearly suggests relevant lipid reports against individual lipid parameters, especially in situations where medication management may be affected.

Table 1. Atherogenic index and HeartScore risk at senescent patients compared to adults

	Senescent patients (73.86 ± 6.41 years)	**Adult patients** (57.11± 5.02 years)
Atherogenic index (AI)	0.399 ± 0.26	0.391 ± 0.25
HeartScore	4.24 ± 2.54*	1.65 ± 1.4

Results are presented as means±D.S.; p*<0.0001 vs. adults

SCORE scale shown low risk at 56.66% adults respectively 0% at senescent patients and high risk 0% at adults respectively 18.38% for senescent patients (Fig.1, Fig.3).

From the point of view of AI, there is very little variation between % risk categories in senescent compared to adult patients (Fig.2, Fig.4).

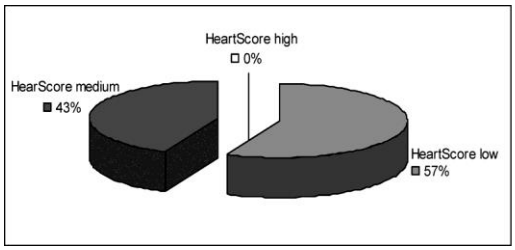

Fig. 1 HeartScore risk categories at adult patients

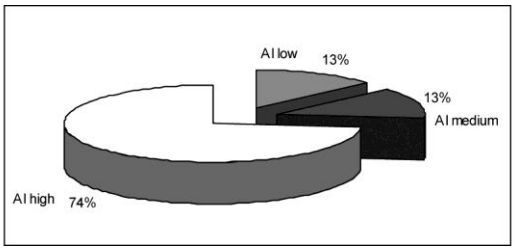

Fig.2 AI risk categories at adult patients

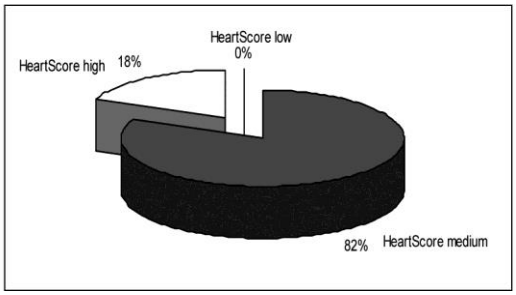

Fig.3 HeartScore risk categories at senescent patients

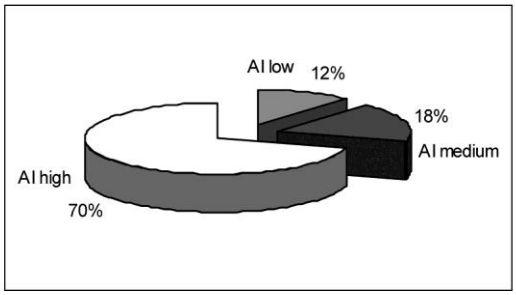

Fig. 4 AI categories at senescent patients

Furthermore, linear regression equation revealed a positive significant correlation between HeartScore and AI at both senescent-Fig.5 (r=0.348, p<0.0001) and adult patients-Fig.6 (r=0.336, p<0.01).

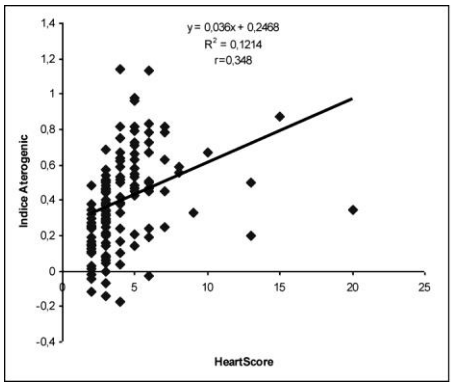

Fig.5 Correlation HeartScore and AI at senescent patients. Curve fitting was by linear regression; r = correlation coefficient

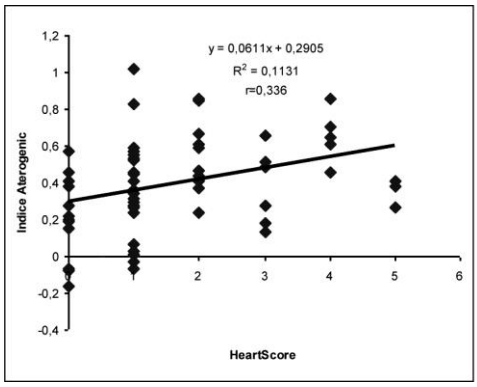

Fig.6 Correlation HeartScore and AI at adult patients. Curve fitting was by linear regression; r = correlation coefficient

There is no correlation between AI and age, but HeartScore is positively statistically significant correlated with age- Fig.7 (r=0.434, p<0.0001). It seems that lipid reports are not influenced by age, only by other risk factors like diet, smoke, blood pressure and cardiovascular disease.

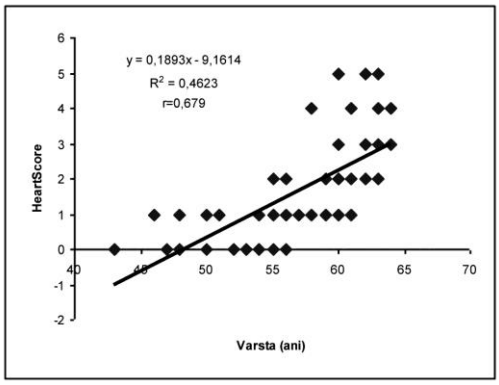

Fig.7 Correlation HeartScore and age at adult patients
Curve fitting was by linear regression; r = correlation coefficient

Thus, changes in lipid profile, e.g. atherogenic indices, begin early in adult life, and level of risk remains high as we age. On the contrary, risk of developing cardiovascular diseases is at low-medium level for adults and rose at high-very high level at elderly. Correlating obtained data with recent studies it can be concluded that aging and risk for cardiovascular diseases are in a strong and close relationship.

CONCLUSIONS

AI and HeartScore are much higher in terms of statistics and diagnosis of cardiovascular events. HeartScore proved to be a better tool for predicting cardiovascular risk and seems to be a useful method to eval¬uate the risk of cardiovascular mortality. Comparison with the value of risk acceptable for the age and

sex may oblige the physician to take action to reduce it.

Together, AI and HeartScore can be a useful tool for diagnosis, treatment and prevention of chronic cardiovascular disease and major cardiac events (especially myocardial infarction and stroke).

REFERENCES

[1] Parinita K. *Study of serum lipid profile in individuals residing in and around Nalgonda*. Int J pharm Bio Sci., 2012,2:110-116.

[2] Pallavi S. Kanthe , Bheemshetty S. et al. *Atherogenic index as a predictor of cardiovascular risk among women with different grades of obesity*. International Journal of Collaborative Research on Internal Medicine & Public Health, 2012,10, 1767- 1774.

[3] Nwagha UI. *Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria*.African Health Sciences, 2010,10(3): 248-252.

[4] Khaki-Khatibi F, Yaghoubi A.R, Rahbani N.M. *Study of antioxidant enzymes, lipid peroxidation, lipid profile and immunologic factor in coronary artery disease in East Azarbijan.* Int J Med Biomed Res, 2012,1(2):147-152.

[5] Grover S, Dorais M, Coupal L. *Improving the prediction of cardiovascular risk: interaction between LDL and HDL cholesterol.* Epidemiology; 2003,14(3):315-20.

[6] Gotto AM. *Triglyceride; the forgotten risk factor*. Circulation,1998;97:1027-8.

[7] Shilpa Bhardwaj et al. *Atherogenic index of plasma, Castelli risk index and atherogenic coefficient-new parameters in assessing cardiovascular risk*. Int J Pharm Bio Sci, 2013,3, 359-364.

[8] Santos AP, Vieira MS, Deus DF et al.*Atherogenic indices and HDL particle size as laboratory parameters to evaluate cardiovascular risk in the presence of dyslipidemia*. J Biophy Chem, 2014,5, 24-32.

[9] Ikewuchi CJ. *Alteration of Plasma Lipid Profile and Atherogenic Indices of Cholesterol Loaded Rats by Tridax Procumbens Linn: Implications for the Management of Obesity and Cardiovascular Diseases*.Biokemistry.; 2009,21(2):95199.

[10] Njajou O, Kanaya AM, Holvoet P, Connelly S, Harris T. *Association between oxidized LDL-C, obesity and type 2 diabetes.* Diabetes Metab Res; 2009,25(8):733-9.

[11] Alpert JS, Thygesen KE. For the joint European Society of Cardiology/American College of Cardiology Committee. *Myocardial infarction redefined – a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction.* Eur Heart J, 2000,21,1502–13.

[12] Pearson TA, George A. Mensah, R. Wayne Alexander, et al *Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association*. Circulation, 2003,107:499-511.

[13] *European Guidelines on cardiovascular disease prevention in clinical practice (version 2012)* The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. Eur Heart J, 2012; 33: 1635–701.

[14] Krzysztof J, Jolanta G, Dorota L. *Prevalence of the coronary artery disease risk factors and 10-year risk of cardiovascular mortality based on HeartScore in people qualified for kidney donation.* Ann Transplant, 2013,18: 393-398.

[15] Diederichsen AC, Mahabadi AA, Gerke O, et al. *Increased discordance between HeartScore and coronary artery calcification score after introduction of the new ESC prevention guidelines.* Atherosclerosis, 2015,239(1):143-9.

THE ROLE OF VITAMINE B12 IN ELDERLY

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Abstract. Vitamin B12, or cobalamin (cyanocobalamin), is the largest and most complex of all vitamins chemical structure, having an important role for the human body. This has a beneficial role on the nervous system, acts essentially in the production of DNA and red blood cells in the bones, increases mental and physical performance, prevents accumulation of fat in the liver and prevents atherosclerosis and other cardiovascular diseases. In general, vitamin B12 level declines with age, studies showing that the prevalence of cobalamin deficiency among the elderly can range between 5 – 40%, depending on the diagnostic criteria used. Many studies use serum vitamin B12 level, with or without additional testing its metabolites, such as homocysteine and methylmalonic acid, to estimate the prevalence of vitamin in the patients. As we know, elderly people are particularly at risk of vitamin B12 deficiency. The main etiologies can be divided under two main categories: inadequate dietary intake and impaired absorption of vitamin B12. Therefore it is important to determine whether, indeed, deficiency of cobalamin is a significant problem in elderly people, especially because it is easily treatable and its effects are reversible if detected and treated in time.
Key words: cobalamin, elderly, vitamin B12 deficiency

ROLUL VITAMINEI B12 LA VÂRSTNIC

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Rezumat. Vitamina B12, sau cobalamina (cianocobalamina), are cea mai mare si complexa structura chimica dintre toate vitaminele, avand un rol important pentru organismul uman. Aceasta are un rol benefic asupra sistemului nervos, actioneaza in mod esential in productia de ADN si in formarea globulelor rosii la nivelul oaselor, creste performantele fizice si psihice, impiedica acumularea de grasimi in ficat si previne ateroscleroza si alte afectiuni cardiovasculare. In general, nivelul de B12 scade cu varsta, studiile aratand ca prevalenta deficitului de vitamina B12, printre varstnici, poate varia intre 5 – 40 %, in functie de criteriile de diagnostic utilizate. Multe studii folosesc nivelul seric de vitamina B12, cu sau fara testarea suplimentara a metabolitilor sai, cum ar fi homocisteina si acidul metilmalonic, pentru a estima prevalenta vitaminei la pacienti. Dupa cum stim, persoanele in varsta sunt in mod deosebit expuse riscului de deficit de vitamina B12. Principalele etiologii pot fi impartite in doua categorii principale: aportul alimentar inadecvat si diminuarea absorbtiei de vitamina B12. Prin urmare, este important sa se determine daca intr-adevar deficitul de cobalamina este o problema semnificativa la persoanele in varsta, mai ales pentru ca este usor tratabil si efectele sale sunt reversibile daca este detectat si tratat la timp.
Cuvinte cheie: cobalamina, varstnici, deficienta vitaminei B12

INTRODUCTION

Vitamin B12 or cobalamin (cyanocobalamin) is a water soluble vitamin that plays a fundamental role in DNA synthesis, optimal haemopoesis and neurological function (1). Cobalamin is a atypical vitamin, formed from a tetrapyrole plane with a central cobalt atom, a group of nucleotide (5,6-dimethylbenzimidazole) below the plane and prosthetic part above the plane (Figure 1). The molecular formula for vitamin B12 is $C_{63}H_{88}CoN_{14}O_{14}P$.

Depending on the prosthetic group are identified multiple forms of cobalamin:
- methylcobalamin: predominates in the plasma and in the cytoplasm
- adenosylcobalamin: prevails intramitochondrial
- cyanocobalamin: a stable pharmacological preparation to be converted into other forms to be metabolically active

The active forms of vitamin B12 in the body are methylcobalamin and deoxyadenosine cobalamin.

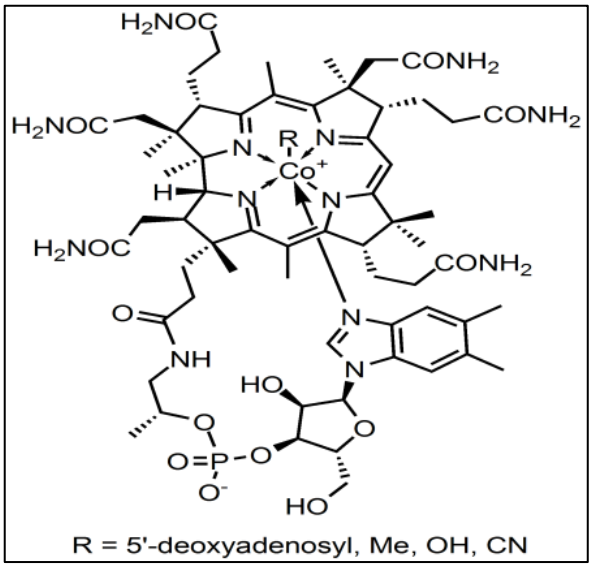


Fig. 1. Chemical structure of vitamin B12

METABOLISM AND FUNCTION OF VITAMIN B12

Absorption depends mainly on intrinsic factor, which is secreted by the gastric mucosa. Intrinsic factor binds cobalamin forming a complex that is absorbed by the terminal ileum. This mechanism is responsible for the absorption of at least 60% of oral cobalamin (2).

The transport of vitamin B12 in the blood as well as its tissue and hepatic uptake require the presence of transcobalamins (TCBs). Normally, there are three vitamin B12 transport proteins present in the plasma, which are known as transcobalamines (TCB I - TCB II - TCB III)

TCB types I (TCB I) and III (TCB III) ensure the binding of approximate 80% of circulating vitamin B12; however, TCB type II (TCB II) plays the predominant role in the key processes of tissue and hepatic uptake of vitamin B12. The physiologically active is TCB II which complex in a 1:1 ratio with vitamin B12. The complex is then bound to specific surface receptors on developing blood cells in the bone marrow. Vitamin B12 is then released by hydrolysis. The TCB II is not reutilized. The congenital absence of TCB II causes megaloblastic anaemia within weeks of birth (3).

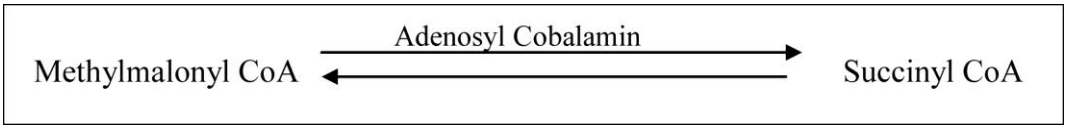
Transcobalamines I and III are α-globulins synthesized by granulocytes and known as R-binders that are found in a wide range of body fluids. TCB I and III do not readily release vitamin B12 to the developing tissues. The congenital absence of them causes no physiological impairment (4).

Cobalamin metabolism is complex and requires many processes, anyone of which, if not present, may lead to cobalamin deficiency (5).

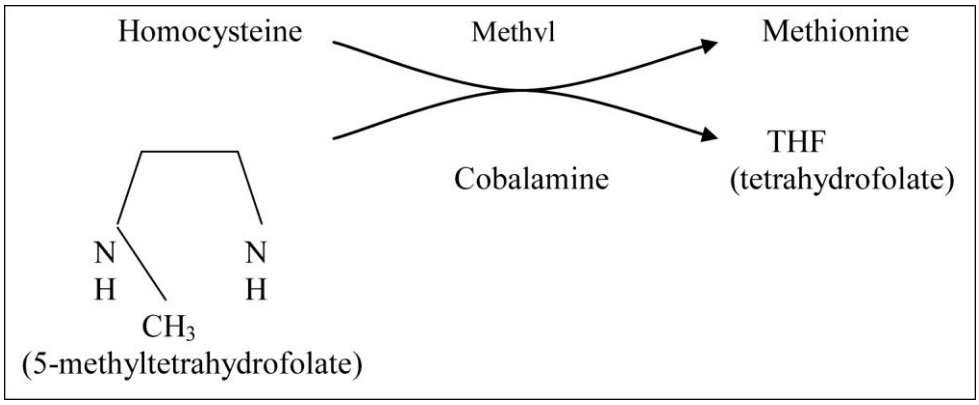
Once metabolized, cobalamin is a cofactor and coenzyme in many biochemical reactions (6).

Vitamin B12 is required as coenzyme for two metabolic reactions:

1. Isomerization of L-methylmalonyl CoA to succinyl CoA. This is important substrate in Hb synthesis



2. Methylation of homocysteine to methionine. This step is important in intracellular synthesis of folate coenzyme



Role of vitamin B12

• it helps the development and proper functioning of the nervous system
• acting essentially in the production of DNA and red blood cells in the bone marrow
• increase physical and mental performance
• prevents fat accumulation in the liver
• prevents atherosclerosis and other cardiovascular diseases

DEFICIENCY OF VITAMIN B12

Pathophysiological changes, multiple co-morbidities, coupled with multiple drug intake, and increasing dependency associated with ageing can lead to malnutrition due to inadequate intake and malabsorption of vitamin B12, resulting in deficiency (Table I).

Table I. Causes of vitamin B12 deficiency

Cause	Particulars
Food deficiency	Insufficient intake, poor diet or vegetarian total nonsupplemented
Impaired absorption	It can be caused by lack of intrinsic factor, abuse of antacids, Helicobacter pylori (H. pylori) infection, surgeries and overgrowth of microbial flora
Interactions with certain drugs	Long-term administration of metformin or lithium
Increased requirement of vitamin B12	In pregnancy, hyperthyroidism
Increased excretion and destruction of vitamin B12	Alcoholism Administration the high doses of vitamin C

Deficiency of vitamin B12 in elderly

Vitamin B12 is essential for the normal metabolism and functioning of all cells in the body.

Commonly, vitamin B12 level declines with age and therefore prevalence of vitamin B12 deficiency increases with age.

Elderly people are particularly at risk of vitamin B12 deficiency because of the high prevalence of atrophic gastritis–associated food-cobalamin (vitamin B12) malab-sorption, and the increasing prevalence of pernicious anaemia with advancing age (7).

Vitamin B12 deficiency can cause significant adverse effects to organ and systems with high cell turnover and metabolism like the bone marrow, gastro-intestinal tract, and brain.

Symptoms of B12 deficiency

Vitamin B12 deficiencies are slowly progressing; they may take several years to start affecting people. Initial signs and symptoms may be subtle, but in time, they become more noticeable. Vitamin B12 deficiency (8) is associated with hematologycal, neurological, and psychiatric manifestations (Table II).

Table II. Symptoms of B12 deficiency

General	Neurological	Hemathologycal
General weakness	Paresthesia (numbness, tingling)	Anemia
Tiredness	Balance disorders	Shortness of breath
Painful sensitivity of the tongue and mouth	Leg weakness	Macrocytosis
Loss of appetite	Muscle pain	Hyper segmented neutrophils
Weight loss	Memory loss	Isolated thrombocytopenia and neutropenia
Hair loss	Psychiatric changes, Dementia	Hemolytic anemia
	Combined sclerosis of the spinal cord	

In a recent study, Andres E. and colab. reported the principal causes of cobalamin deficiency in 172 elderly patients (aged 70 years) hospitalized in the University Hospital of Strasbourg, France (5). The main causes included food-cobalamin malabsorption (53%), pernicious anemia (33%), insufficient nutritional vitamin B12 intake (2%), postsurgical malabsorption (1%) and as much as 11% of the patients suffered from cobalamin deficiency of undetermined etiology. In elderly patients, cobalamin deficiency is classically caused by pernicious anemia, or Biermer's disease, which is an autoimmune disease characterized by the destruction of the gastric mucosa, especially fundal mucosa, by a primarily cell-mediated process (9).

Some authors have speculated about the reality and significance of cobalamin deficiency related to food-cobalamin malabsorption, because many patients displayed mild clinical or haematological features.

Over 40% of patients older than 80 years have gastric atrophy that might or not be related to H. pylori infection (10). Other factors that contribute to food-cobalamin malabsorption in elderly people include chronic carriage of H. pylori and intestinal microbial proliferation, situations in which cobalamin deficiency can be corrected by antibiotic treatment, long-term ingestion of antiacids such as H2-receptor antagonists and proton-pump inhibitors, particularly among patients with Zollinger–Ellison syndrome, and metformin (11, 12).

EPIDEMIOLOGY

As we know, elderly people are particularly at risk of vitamin B12 deficiency. Studies have shown that prevalence of vitamin B12 deficiency among elderly can range between 5% and 40% depending on the definition of vitamin B12 deficiency used (13).

The Framingham study demonstrated a prevalence of 12% among elderly people living in the community. Furthermore, reports have indicated that institutionalised elderly with multiple co-morbidities and with increasing dependency are more prone to vitamin B12 deficiency than non-institutionalised (free-living) elderly (14).

EVALUATION AND DIAGNOSIS OF VITAMIN B12 DEFICIENCY IN THE ELDERLY

I. Serum cobalamin level < 150 pmol/L (on two separate occasions)

II. Serum cobalamin level < 150 pmol/L and

- total serum homocysteine level > 13 μmol/L
- methylmalonic acid > 0,4 μmol/L (in the absence of renal failure and folate and vitamin B6 deficiency)

Many studies have used serum vitamin B12 level with or without additional tests for its metabolites like homocysteine and methylmalonic acid (MMA) to estimate

the prevalence of vitamin B12 in the population. When there is a clinical suspicion of vitamin B12 deficiency, the initial laboratory assessment includes serum vitamin B12 levels, complete blood count, and blood film examination (15).

The falsely low vitamin B12 level can be related to the disturbance in vitamin B12 metabolism but may not be associated with any tissue vitamin B12 deficiency.

On the other hand, falsely normal serum vitamin B12 level may occur in the presence of liver disease, myeloproliferative disorder, congenital transcobalamin II deficiency, and intestinal bacterial overgrowth (16).

When serum vitamin B12 results are normal but still the clinical suspicion of deficiency exists, additional "confirmatory testing" may help to identify vitamin B12 deficiency.

There is compensatory elevation of homocysteine and MMA levels preceding the drop in serum vitamin B12 level and these are regarded as more sensitive indicators of vitamin B12 deficiency than just low serum vitamin B12 level (17). Elevated serum homocysteine and MMA level has a sensitivity of 95.9% and 98.4%, respectively to diagnose vitamin B12 deficiency. However, the reference intervals for serum MMA and homocysteine are variable among different laboratories.

THERAPEUTIC MANAGEMENT OF COBALAMIN DEFICIENCY

Classical treatment of cobalamin deficiency, when the cause is not deficiency food, it is vitamin parenteral administration (18) usually by intramuscular injection (as cyanocobalamin and rarely hydroxocobalamin).

In France, the recommended practice to build up the tissue stores of the vitamin quickly and correct serum cobalamin hypovitaminosis, particularly in the case of pernicious anemia, involves administration of 1000 μg of cobalamin per day for 1 week, followed by 1000 μg per week for 1 month and then by 1 injection of the same dose once per month, normally for the rest of the patient's life (19).

In cases other than those caused by nutritional deficiency, alternative routes of administration have recently been proposed: oral or nasal (20).

CONCLUSION

• The cobalamin deficiency occurs frequently in the elderly population, but due to subtle clinical manifestations, it is difficult to diagnose.

• The falsely low vitamin B12 level are not associated with disruption but with its metabolism.

• Elevated serum levels of metabolites of vitamin B12 - homocysteine and methylmalonic acid - are more sensitive indicators of the deficiency.

Therefore it is important to determine whether indeed deficiency of cobalamin is a significant problem in elderly people, especially because it is easily treatable and its effects are reversible if detected in time.

REFERENCES

[1] Wolters M., Strohle A., Hahn A. *Cobalamin: a critical vitamin in the elderly*. Prev. Med. 2004; 39: 1256–66.

[2] Andres E., Affenberger S., Vinzio S., et al. *Food-cobalamin malabsorption in elderly patients: clinical manifestations and treatment.* Am. J. Med. 2005; 118:1154–9.

[3] Ermens A.A., Vlasveld L.T., Lindemans J. *Significance of elevated cobalamin (vitamin B12) levels in blood.* Clin. Biochem. 2003; 36:585–90.

[4] Chiche .L, Jean R., Romain F., et al. *Implications cliniques de la decouverte d'une hypervitaminemie B12 en medecine interne.* Rev. Med. Interne. 2008; 29:187–94.

[5] Andres E., Loukili N.H., Noel E., et al. *Vitamin B12 (cobalamin) deficiency in elderly patients.* CMAJ,2004; 171:251–60.

[6] Klee G.G. *Cobalamin and Folate evaluation: measurement methylmalonic acid and homocysteine vs vitamin B12 and Folate*. Clin. Chem. 2000; 46:8(B), 1277-1283.
[7] Chui C.H., Lau F.Y., Wong R., et al. *Vitamin B12 deficiency—need for a new guideline*. Nutrition,2001; 17:917–20.
[8] Serraj K., Mecili M., Andres E. *Signes et symptomes de la carence en vitamine B12: revue critique de la litterature.* Med. Ther. 2010; 16:13–20.
[9] Carmel R. *Current concepts in cobalamin deficiency*. Annu. Rev. Med. 2000; 51:357–75.
[10] Carmel R., Aurangzeb I., Qian D. *Associations of foodcobalamin malabsorption with ethnic origin, age, Helicobacter pylori infection, and serum markers of gastritis*. Am. J. Gastroenterol. 2001; 96:63–70.
[11] Jensen R.T. *Consequences of long-term proton pump blockade: insights from studies of patients with gastrinomas*. Basic. Clin. Pharmacol. Toxicol. 2006; 98:4–19.
[12] Liu K. W., Dai L. K., Jean W. *Metformin-related vitamin B12 deficiency.* Age Ageing,2006; 35:200–1.
[13] Loikas S., Koskinen Irjala K., et al. *Vitamin B12 deficiency in the aged: a population-based study.* Age Ageing,2007; 36:177–83.
[14] Clarke R., Grimley Evans J., Schneede J., et al.*Vitamin B12 and folate deficiency in later life.* Age Ageing,2004; 33:34–41.
[15] Clarke R., Refsum H., Birks J., et al. *Screening for vitamin B-12 and folate deficiency in older persons.* Am. J. Clin. Nutr. 2003; 77:1241–7.
[16] Oosterhuis W.P., Niessen R.W., Bossuyt P.M., et al. *Diagnostic value of the mean corpuscular volume in the detection of vitamin B12 deficiency.* Scand. J. Clin. Lab. Invest. 2000; 60:9–18.
[17] Wolters M., Strohle A., Hahn A. *Cobalamin: a critical vitamin in the elderly*. Prev. Med. 2004; 39:1256-66.
[18] Kaltenbach G., Noblet-Dick M., Barnier-Figue G., et al. *Early normalization of low vitamin B12 levels by oral cobalamin therapy in three older patients with pernicious anemia.* J. Am. Geriatr. Soc. 2002; 50:1914–5.
[19] Lane L.A., Rojas-Fernandez C. *Treatment of vitamin B12 deficiency anemia: oral versus parenteral therapy.* Ann. Pharmacother,2002; 36:1268–72.
[20] Andrès E., Kaltenbach G., Noel E., et al. *Efficacy of short-term oral cobalamin therapy for the treatment of cobalamin deficiencies related to food-cobalamin malabsorption. A study of 30 patients*. Clin. Lab. Haematol. 2003; 25:161-6.

DISPHONY AS A MANIFESTATION OF MYASTHENIA GRAVIS

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Abstract. Myasthenia gravis it is not a common disease. According to statistics of the National Association of Myasthenia Gravis in Romania, this,, invisible disease "appears in the population at a frequency of 1: 10,000 persons, regardless of age. It is a condition that affects quality of life, and can significantly develop serious life-threatening risk. The disease occurs due to a defect in the transsynaptical transmission of nerve impulses from nerve to muscle fiber. It is considered to be an autoimmune disease due to antibodies anti Achetylcoline and muscle specific tyrosinkinasis. The exact cause is not specified, and a genetic determination cannot be ruled out. Patients are seeking medical care for nonspecific symptoms (eye muscle weakness, diplopia, hoarseness, trouble breathing, difficulty in controlling facial expression, generalized muscle weakness) most often framed in the clinical picture of other diseases. Consider though the variability of symptoms that are less pronounced in the morning when the patient is rested and accentuated vesperally when fatigue occurs. Electrophysiological studies or laryngeal electromyography show that insertional activity is normal. There is no abnormal spontaneous activity but at a minimal muscle contraction the motor unit potentials undergo changes in amplitude, meaning that there is a disorder of neuromuscular transmission. Repetitive nerve stimulation, single fiber electromyography, the edrophonium test, dosing acetylcholine receptors antibodies and tyrosine kinase antireceptor (anti Musk) are the important ancillary tools we use in the diagnosis of myasthenia gravis. Other imaging tests are important too, as radiographs / mediastinal CT, brain CT, thyroid gland ultrasound, fiberscopy and stroboscopic laryngeal examination to exclude other causes of hoarseness with normal vocal cord macroscopic. The authors will present the case of a patient who presented to an ENT consult with a 16 years old dysphonia at which time it was diagnosed and treated as chronic laryngitis delaying the start of proper treatment.
Key words: myasthenia, dysphonia, electromyography

DISFONIA CA MANIFESTARE A MIASTENIEI GRAVIS

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Rezumat. Miastenia gravis nu este o boala frecventa. Potrivit statisticii Asociatiei Nationale de Miastenia gravis din Romania aceasta ,,boala invizibila" apare in populatie cu o frecventa de 1:10000 de persoane, indiferent de varsta. Este insa o boala care afecteaza calitatea vietii, si care poate evolua grav cu risc vital semnificativ. Apare ca urmare a unui defect in transmiterea transsinaptica a impulsului nervos de la nerv la fibra musculara. Este considerata a fi o boala autoimuna din cauza producerii anticorpilor antireceptor pentru acetilcolina si tirozinkinaza specific musculara. Cauza exacta nu este precizata, nu este exclusa nici determinarea genetica. Pacientii se adreseaza medicului pentru simptome nespecifice (slabiciunea musculaturii oculare, diplopie, raguseala, tulburari respiratorii, dificultati in controlul expresiei faciale, slabiciune musculara generalizata) de cele mai multe ori fiind incadrate in tabloul clinic al altor afectiuni. De notat insa variabilitatea simptomatologiei care este mai redusa dimineata cand pacientul este odihnit, si se accentueaza vesperal cand apare oboseala.

Studiile electrofiziologice, respectiv electromiografia laringiana arata ca activitatea insertionala este normala. Nu exista activitate spontana anormala dar la o minima contractie musculara potentialele de unitati motorii sufera modificari in amplitudine si durata insemnand ca exista dereglari ale conducerii nervoase prin jonctiunea neuromusculara. Stimularea repetitiva, electromiografia de fibra unica, testul la edrophonium, dozarile anticorpilor antireceptor de acetilcolina si antireceptor pentru tirozinkinaza (anti Musk) precizeaza diagnosticul de miastenia gravis.Sunt importante si alte investigatii imagistice: radiografia/CT pulmonara, CT cerebral, ecografia de glanda tiroida, fibroscopia si stroboscopia laringiana pentru excluderea altor cauze de disfonie cu corzi vocale normale macroscopic. Autorii vor prezenta cazul unei paciente care s-a prezentat la un consult ORL pentru o disfonie trenanta de mai bine de16 ani timp in care a fost diagnosticata si tratata ca laringita cronica intarziindu-se inceperea tratamentului corespunzator.
Cuvinte cheie: miastenie, disfonie, electromiografie

INTRODUCTION

Myasthenia gravis is a rare disease with an incidence of 1: 10,000 persons. It might occur at any age, most commonly between 20-30 years. The evolution could be severe and life-threatening

Why does it appear?

It is known as an autoimmune disease; don't exclude the genetic hypothesis (1).

It is characterized by a defect in the transsynaptic transmission of nerve impulse to the muscle fiber and the presence of anti-Racha (anti-Acetylcholine) and anti-MuSK (anti- muscle fiber).

The laryngeal event may be the first and only systemic sign of myasthenia gravis. It can occur in systemic disease or a focal disorder similar to ocular myasthenia. Myasthenia gravis can be the cause of a dysphony with fluctuating and intermittent character and fonastheny. The examination of the larynx can reveal changes in vocal cord movements suggesting as cause, nervous fatigue. Neuromuscular junction disorders such as myasthenia may affect the intrinsic muscles of the larynx and limit their mobility (2) Neurolaringological disorders should not be neglected, their diagnosis and treatment is done in mixed team ORL-neurophysiologist.

How does it act?

The symptoms are nonspecific and fluctuate throughout the day. They can occur isolated as a unique manifestation or together (2,3):

- muscle eye weakness
- generalized muscle weakness
- diplopia
- disphonia
- respiratory disfunctions
- difficulty in controlling facial expression

Each symptom requires different diagnosis.

Bilateral and symmetric ophthalmoplegia will be distinguished by (4):

- Kearns-Sayre syndrome (chronic progressive external ophthalmoplegia)
- Botulism
- Myasthenia Gravis
- Sarcoidosis
- Thyroid Pathology

Dysphonia may have a laryngeal origin (2):

- Paresis / paralysis of the vocal cord
- Myopathy
- Laringeal tumor/infections
- Cricoarytenoid joint ankylosis

Generalized muscle weakness and respiratory distress may have multiple etiologies:

- lung pathology
- cardiac pathology
- metabolic pathology(diabetes mellitus)
- neurological pathology

Diagnosis of MG is not easy. The diagnosis protocol includes more investigations that will exclude step by step the causes considered in the differential diagnosis:

- general clinical exam
- interdisciplinary consults: Neurology, Ophthalmology, ENT, Endocrinology, Psychological
- pulmonary radiography, barital transit, EKG
- spirometry
- thyroid gland and orbit ecography
- CT / MRI brain orbit
- EMG-Repetitive stimulation and single muscle fiber (2,3)
- Laryngeal muscle –EMG (2,3)

Laboratory studies with electromyographic recordings show the balance toward myasthenia gravis diagnosis.

- Mg low, low parathyroid hormone,high lactic acid, pyruvic acid, CPK high, high aldolase proteins in CSF Kearns-Sayre syndrome high
- thyroid hormone (Basedow Graves)
- positive test for anti-RAch myasthenia gravis
- positive test for anti-MuSK in Myasthenia Gravis
- prostigmine-test to significant improvements in symptoms of myasthenia gravis (1,2,5)

Myasthenia gravis can be sorted by muscle distribution achieved (6):
- myastenia of the eye
- myastenia bulbar palsy
- miyastenia generalized

Classification of Myasthenia gravis forms depending on the presence of certain antibodies (5,6):
- myastenia with anti-RAch
- myastenia with anti-MuSK
- double seronegative -myastenia
- myastenia with anti-LRP4

CASE REPORT

The patient is 80 years old, lives in urban area, former nurse, nonsmoker, no drinking, consuming coffee occasionally hospitalized in our clinic: fatigue, 4 years dysphonia, coxalgia, cervicodorsolombar pain.

Medical history: esential hypertension in treatment, faringolaringitis, appendectomy (at 50 years), HBsAg +, alterations of the intervertebral discs. When she had 63 years old a doctor has raised suspicion of myasthenia gravis but didn't recommended investigations to confirm the disease.

General exam:
- Kyphotic chest, left hip disease in walking, cervical lumbar pain exacerbated by movement
- palpebral ptosis partial bilateral
- cold extremities
- TA 170/100 mm Hg

Paraclinical investigations:

Chest radiograph show normal transparency of the lung fields, apparently normal thyroid ultrasound.

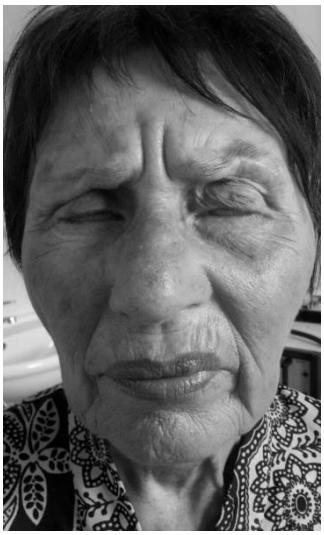
CT brain without significant changes

Spirometry- suggestive graphics for restrictive syndrome

Laboratory tests: ESR 23mm Hg

There were calls for more interdisciplinary consult:

Ophthalmologic:



Diagnostic: catharactis, palpebral ptosis with insertion modification of the mm levator at left eye, without diplopia

Neurologic consult:
- narrowing fissure slots, asymmetrical ptosis, eyelids down incomplete when eyes are closed tendon reflexis diminished on the left, bitonal voice; EMG recommend

ENT consults:

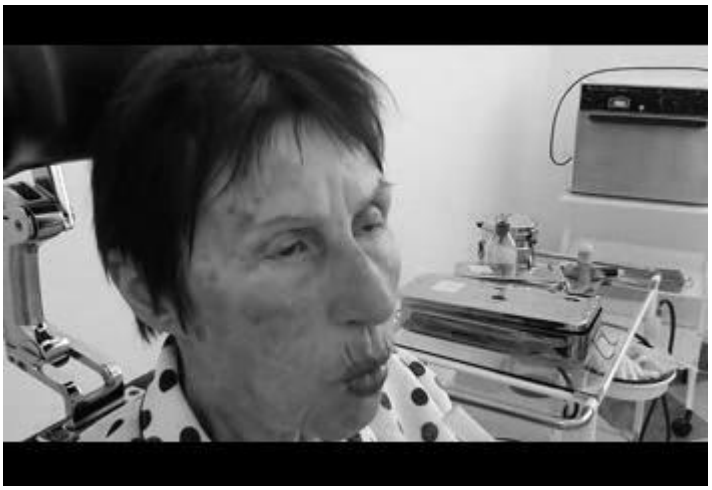
Voice perceptual analysis
- simultaneously emision on different frequencies in the vocal patterns-falsetto and bass

- fundamental altered frequency with severe vocal timbre modification with impaired quality of life
- VRQOL (Voice relation with quality of life) of average quality
- VHI (The voice handicap index American Speech-Language-Hearing Association) = 49

Indirect laryngoscopy exam:

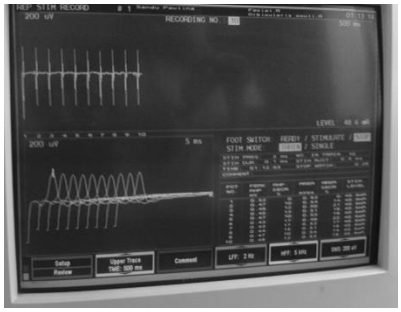
- vocal cords with normal macroscopic aspect, symmetrical movements, little glottal insufficiency
- voice not held on the same frequency during reading

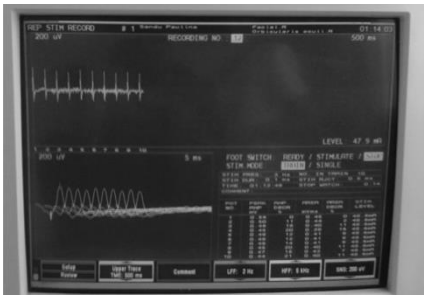
Seven sessions were conducted exercises ortophonic using the VOX getting LAX fundamental frequency for 10 seconds.



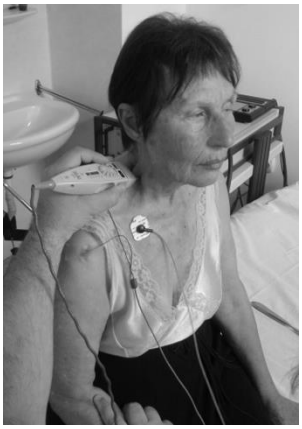
EMG exams were made for more muscles.

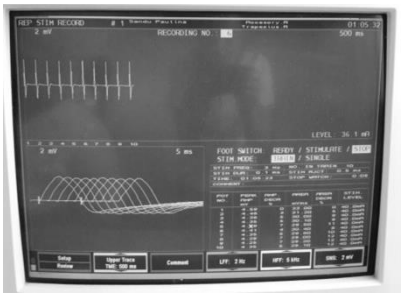
EMG for mm orbicularis oculis right by repetitive stimulation (3)of the temporo-zigomatic facial nerve



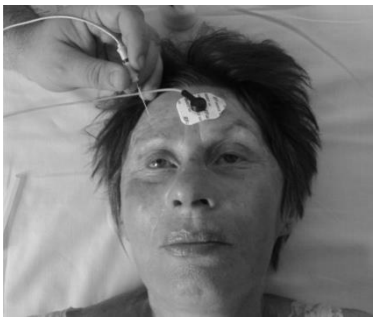


EMG for the trapez muscle with repetitive stimulation (3) for accesor nerve with decrement suggestive for myastenia

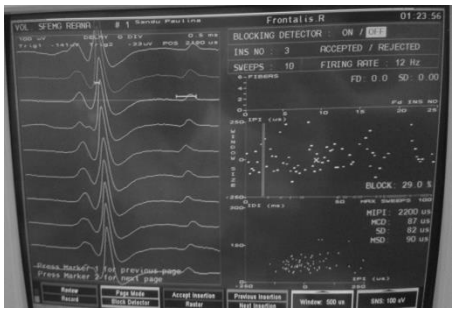




EMG single fiber- jitter determination (3) with electrod-needle for right frontalis muscle



It used an concentric electrod-needle 25 mm



Jitter extended is suggestive for block neuromuscular transmission

Laryngeal EMG for right tiroaritenoidian muscle with monopolar electrod introduced in the right vocal fold by cricotiro-idian membrane (1)

There is no abnormal spontaneous activity but at a minimal muscle contraction the motor unit potentials undergo changes in amplitude, meaning that there is a disorder of neuromuscular transmission.They were recorded and analyzed characters of the waves at the insertion, phonation the vowel "e" and, "i" with vocal fold adduction, abduction inhale sharply and repeatedly with vocal cords, swallowing with additional adduction of the vocal cords.

There were no recorded PUM (potential insertion motor units) maneuvers adduction / abduction PUM recruits were normal in amplitude and duration.

Recommended immunological tests indicated the absence of anti wicker Ac Ac anti MuSK. X3 prostigmine tested at 30 mg / day resulted in rapid improvement in fatigue with obvious influence of ptosis and voice.

The final diagnosis was: Myasthenia gravis double seronegative

After 3 months of treatment with Mestinon 60 mg / cps, (½ cpx3)/ day patient was reevaluated neurological and ENT.

CONCLUSIONS

Myasthenia gravis require a multidis-ciplinary approach to be diagnosed.

Imaging cerebrovascular is used for differential diagnostic.

Electromyographic and electrophysiological explorations confirm the diagnosis in cases with double seronegative antibodies.

Administered properly and on time treatment significantly influences the course of disease and significantly increasing the quality of life.

REFERENCES

[1] Romeo V. *Myotonic Dystrophy Type 1 or Steinert's disease*. Adv Exp Med Biol. 2012, 724:239-57.

[2] Robert T. Sataloff, Steven Mandel, Yolanda Heman-Ackah.*Laryngeal electromyography.* Second edition, Plural Publishing INC. San Diego, Oxford, Brisbane, 2006, pg.38,67,73,105.

[3] Constantin I. Bogdan. *Foniatrie Clinica*, Vol 1.Vocea, Editura Viata medicala Romaneasca, 2001, pg.174-178; 281-282.

[4] Souilem S, Chebel S, Mancuso M, et al. *A novel mitochondrial tRNA(Ile) point mutation associated with chronic progressive external ophthalmoplegia and hyperCKemia.* J Neurol Sci. ,2011,Jan 15, 300(1-2):187-90.

[5] Leite MI, Waters P, Vincent A. *Diagnostic use of autoantibodies in myasthenia gravis.* 2010,Aug, 43(5-6):371-9.

[6] *Adams and Victor's Principles of Neurology*. Tenth edition, International Edition, 2014, pg1305-1306, 1472-1487.

PERSPECTIVES REGARDING AGING AND STEM CELLS TREATMENTS FOR DEGENERATIVE DISEASES

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Abstract. Stem cells for long having been known in plants, opened up ways for novel research directions in humans and animals. This work attempts to review some experimental results in animals for future possible applications in humans, with a view especially on neurodegenerative diseases but not in the least therapy of aging. As utilization and studies regarding embryonic totipotent stem cells, which can differentiate in any type of tissue, encountered ethical problems, there is search for modalities of restoring stem cells' capacity to differentiate more widely. In this sense, factors involved in maintaining undifferentiation are being investigated as well as those of restoring undifferentiation of stem cells which were already differentiated. But numerous results have shown that stem cells can differentiate in various types of cells under the influence of physical not only chemical factors and recent data have evidenced that environment might determine transdifferentiation without transition stage of stem cells. Results of last years have shown numerous difficulties in using treatments with stem cells. These difficulties will be reviewed along with other results from the standpoint of the biochemical hypothesis of aging we have advanced, mentioning also some predictions of this hypothesis, which could lead to solving problems regarding stem cells. Most hopes concerning cell therapy are related to aging and degenerative diseases. To cope with eventual problems raised by the unknown functions of transplanted stem cells, an ideal solution would be to find out modalities to stimulate intrinsic cells in view of repair and regeneration of the envisaged tissue but cautiously.
Key words: stem cells, aging, degenerative diseases

PERSPECTIVE PRIVIND ÎMBĂTRÂNIREA ȘI TRATAMENTELE CU CELULE STEM PENTRU BOLILE DEGENERATIVE

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Rezumat. Celulele stem, cunoscute de mult la plante, au deschis calea unor directii de cercetare inedite la animale si om. Acesta lucrare incearca trecerea in revista a unor rezutate obtinute experimental pe animale, cu posibile aplicatii in viitor si la om, cu referire in special la maladiile neurodegenerative, dar nu in ultimul rand la terapia imbatranirii. Deoarece utilizarea si chiar studiile legate de celulele stem embrionare, tutipotente, care se pot diferentia in orice tip de tesut, se lovesc de probleme etice, se cauta modalitati de restaurare a capacitatii de diferentiere cat mai larga a celulelor stem adulte. In acest sens, se urmaresc factorii implicati in mentinerea nediferentierii, eventual restaurarea nediferentierii celulelor stem deja diferentiate. Dar numeroase rezultate arata ca celulele stem se pot diferentia in diverse tipuri de celule sub influenta unor factori fizici, nu numai chimici, iar date recente arata ca mediul ar putea determina transdiferentiere fara tranzitia prin celule stem. Rezultatele din

ultimii ani arata numeroase dificultati ale tratamentului cu celule stem. Vom discuta aceste dificultati, precum si alte rezultate, prin prisma propriei ipoteze biochimice a imbatranirii, mentionand si cateva predictii ale acesteia, care ar putea conduce la rezolvarea unor probleme legate de celulele stem. Cele mai multe sperante ale terapiei celulare se leaga de imbatranire si maladiile degenerative. Pentru a surmonta eventualele probleme ridicate de necunoscutele comportamentului celulelor stem transplantate, solutia ideala ar fi sa se gaseasca modalitati de a se stimula celulele intrinseci in vederea repararii si regenerarii tesutului vizat, dar si acestea cu prudenta.
Cuvinte cheie : celule stem,imbatranire,boli degenerative

INTRODUCTION

Stem cells almost like no other discovery in biology and medicine have raised great interest, controversy and concern about ethics in the scientific and non-scientific world.

What are stem cells?

The stem cell is defined as an undifferentiated cell, which through mitotic division results in two cells having the capacity to either remain in the stage of stem cells (thus preserving the undifferentiated character) or to differentiate following successive divisions. In this way, the cell can divide symmetrically, in which case two new cells or two differentiated cells result, or it can divide asymmetrically and a stem cell and a differentiated cell result. The capacity of the stem cell to produce another identical cell has been termed self-renewal, whereas the capacity to result in two different types of cells is termed differentiation. Sperm cells and ovocytes are stem cells.

Researches on stem cells developed extensively in the last years because there has been great hope for treatment of some degenerative diseases using stem cells, replacement of tissues damaged in the course of diseases or by factors causing damage and not in the least, for treatments in aging (1).

Research data have suggested that stem cells when introduced in an adequate cell medium, under the influence of growth factors can transform themselves in a desired type of tissue. When stem cells are introduced in the organism they can reach to the injured areas where they transform themselves in cells typical of that tissue because of the tissue generated microenvironment, thus improving the function of the tissue. For instance, by use of this procedure, there is hope that Parkinson's, Alzheimer's diseases, diabetes, cancer, heart failure will be (better) treated, to the extent that knowledge in the field advances (2). There were identified cellular signals that control proliferation, differentiation and survival of stem cells (growth factors, cytokines, adhesion molecules and intracellular mechanisms regulating stem cells (cell signal transduction secondary messengers, transcription factors, telomerase) (3).

New techniques of genetics pointed out a series of genes whose expression has been related to the particular stem cells' state. Genes expressed by different types of stem, embryonic, hematopoiesis stem cells are diverse and their overlapping is very little and hence, a great complexity of these genes' functions.

Research endeavors have aimed at discovering and understanding genes' functioning that maintain stem cells undifferentiated, as undifferentiating is considered the key to the stem cells' function of repair.

The more a stem cell is undifferentiated, the more it produces many types of cells through transdifferentiation. From this point of view embryonic totipotent stem cells are distinguished from adult stem cells of various organs and tissues (almost all, bone marrow, adipose tissue, muscle, skin), which are multi-potent. The aforementioned preserve their distinctiveness as stem cells (due to the modality of division), but have passed through a few stages of differentiation. Which are the factors involved in maintenance of stem cells' toti-potency? The Nanog gene is noteworthy as it appears to be responsible for preserving an undifferentiated state of embryonic stem cells. This gene codes a transcription factor the overexpression of which is involved in preservation of the multi-potency state of

human embryonic stem cells, whereas this gene's absence leads to differentiation. Other such transcription factors are oct-4 si SOX2, the expression of which together with that of Nanog mutually potentiate thus blocking differentiation pathways of the embryonic stem cells (4).

Certainly, embryonic stem cells are preferred to adult stem cells from various organs. But due to ethical considerations, in some countries studies on embryonic stem cells have been forbidden partially or totally. Under these circumstances, there are attempts at developing treatment methods based on adult stem cells. In vitro studies seem promising, but nevertheless some problems still have be solved.

Problems raised by the treatment with stem cells

Firstly, there is little knowledge about stem cells functioning in vivo. One study generated many controversies because it presumed that in fact transdifferentiation of stem cells which are introduced in the organism would take place in target organs through fusion of undifferentiated and differentiated cells of that organ tissue. The result of these processes would be polyploid nuclei along with all risks of genetic instability, which can lead to cancer. Under these circumstances, the problem of safety of an eventual treatment with stem cells has been raised. Other studies declined these results and reported that transdifferentiation without cellular fusion was obtained (5). Anyway, it is not clear how transdifferentiation takes place and moreover, the in vivo correspondent of embryonic stem cells is not known yet. The aforementioned cells obtained in vitro, derived from blastocyst, which permanently preserves its state of pluripotency.

A major problem regarding transplantation of embryonic or pluripotent stem cells, including those with an induced pluri-potency, is the occurrence of benign tumors, teratoma, which can turn into malignant tumors, teratocarcinoma (6). In theory, at present, data converge towards the idea that from every human cell, any other type of cell can be produced. The aforementioned seems to be a normal fact considering that life relates to chemistry and with appropriate reagents, if one has the knowledge about how to add these reagents to a medium and control the later mentioned then he can obtain the desired reaction products. The aspiration (dream) to produce multipotent stem cell lines already became a fact. Two research teams, Japanese and American, produced multipotent stem cell lines from differentiated epithelial cells using transcription factors Oct3/4, Sox2, c-Myc, and Klf4 (7) and Oct4, Sox2, Nanog and Lin28, respectively (8).

The problem of stem cell transplantation regards differentiation. It is difficult to produce the desired type of differentiated cell because cells' differentiation is not uniform. Sometimes cells appear to differentiate to totally undesired directions (9).

Malignization itself regards differentiation. Transcription factors involved in maintaining undifferentiation, pluri-potency of embryonic stem cells have been encountered in tumors oct-4 (10), Nanog (11.12), Sox-2 (13), thus being useful in defining such tumors.

Why does this phenomenon occur? The biochemical hypothesis we advanced (14) could explain it in that aging would result from diminishment of certain cellular signals that are related to particular functioning of an organism also having evolutionary and ecological implications. (In the course of aging), signals involved in cell differentiation and repair, which are stimulated under certain conditions of cellular functioning, diminish at a rate that differs among various species Maintenance of a young phenotype would result from stimulating these reactions through organism's functioning or by slowly diminishing them.

Data regarding maintenance of pluripotent stem cell lines are in accord with the above mentioned hypothesis. There are two

theories concerning maintenance and loss of totipotent stem cell undifferentiation. According to one of them, the type of division plays the main role in maintaining and loss of undifferentiation. Stem cells divide symmetrically and same stem cells result but they also divide asymmetrically and a stem cell as well as a progenitor cell result which after several divisions differentiates into a mature cell. In this sense, the polarizing cell, plasma membrane and cytoplasm polarizations would play roles in distributing membrane proteins (receptors) (15) and gradient of cytoplasmatic substances. The role of polarizing in (cell) regeneration is well-known and as related to this, induction of polarization could lead to restoring stem cells' capacity to differentiate.

According to biochemical hypothesis of aging, it is polarization, among other factors, which is lost once a number of divisions take place and this fact would explain progenitor cells' but also stem cells'diminished capacities to differentiate with age.

Another theory is related to biochemical niche of stem cells in the organism. When these cells are removed from that niche, they lose their capacity to differentiate. Experiments using Drosophila have been a good illustration of this view (16, 17), which is also according with the advanced biochemical hypothesis on aging. During the stage of aging, actually any cellular microenvironment, including that of stem cells is changing biochemical through diminishment of signals. Loss of capacity to differentiate occurs with age, probably due to lack of environmental stimuli and division which is less asymmetrical. Stem cells differentiate and are depleted because environmental factors change. Division itself abates signals and this fact is the reason for asymmetry (actually cell polarization) being important. Asymmetry is reduced through division, if it is no longer stimulated.

Prediction would be that adequate signals could restore stem cells capacity to differentiate. In this sense, a study of embryology would be very useful. It is not enough stem cells be very undifferentiated, pluri or totipotent as embryonic stem cells are, what counts is how broad their differentiative capacity is in terms of cell polarization and capacity to specifically synthesize (substances).

Also, taking into account similarities of stem cells to malignant cells, in presence of adequate factors not only cancer could be treated, but malignant cells with their indefinite capacity to divide, after proliferation, could turn into stem cells and this fact provides a huge capacity to regenerate.

It is important that in adult organisms, stem cells are being found permanently. In the brain of old people there are neuronal stem cells. Therefore, adult organisms use stem cells for all time in order to repair. Then the ideal solving would be ad hoc producing stem cells at localizations where stem cells are needed or at least stimulation of existing stem cells' function. The question remains whether these cells have to be much undifferentiated. It may be possible that stock of stem cells from certain organs be depleted, meaning the number of stem cells decreases until their differentiation becomes impossible. Probably this phenomenon takes place in some degenerative diseases and menopause (18) and for instance, apoptosis under the influence of auto-immune factors would be causing it. Even in this case, somatic stem cells undergo biochemical changes through deficit of signals, which makes them vulnerable to apoptosis, their differentiation becomes impaired and even these cells differentiate, their function is impaired.

In other words, therapy with stem cells could aim at restoring stem cells potential to differentiate by use of biochemical treatments with transcription factors and eventually substances that stimulate synthesis of transcription factors, which finally lead to expression of genes responsible for restoring the initial functionality of stem cells. When the

number of stem cells is too reduced, local proliferation of stem cells without their differentiation, could be stimulated. Under these conditions, restoring stem cell functioning could be the simple and efficient solution.

This idea, which actually is not circulated, copes with inconveniences of treatments with stem cells by introducing these cells in the organism.

Apart from risks, treatments with stem cells implying the aforementioned and other unknown (risks), the very high cost of such a highly personalized treatment has to be taken into consideration.

Perspectives regarding cell therapies in neurodegenerative diseases

Even at present levels of knowledge, stem cells are being used to treat successfully some diseases.

Umbilical stem cells removed at birth are utilized in treating some forms of leukemia and in some countries, including Romania, there are such stem cell biobanks. Cells of this type are relatively cheap as they are present at birth, and there is no need for sophisticated procedures to remove them, nor do these aforementioned raise ethical problems.

Cell therapies utilizing stem cells for neurodegenerative diseases are at an early stage (19).

The major challenge in using them is that of identification of molecular determinants of stem cell proliferation and control exertion on these stem cells and their untoward genetic alterations.

For neurons from stem cells it is necessary to induce their functional integration in existing afferent and efferent synaptic networks.

Brain's potential for self-regeneration is unexplored. New tehnologies of genetically labeling of stem cells are needed for precisely establishing where neurogenesis takes place and what types of cells are involved.

Genomic and proteomic techniques will aim at identification of molecular markers which regulate different stages of neurogenesis through a concerted action. It is also necessary to develop models utilizing experimental animals and mimicking human diseases and only based on this knowledge, strategies for therapies to use in humans could be elaborated. Not in the least evaluation of the ratio risk/benefit is needed before utilizing the stem cell therapy in humans (20).

In rodents but also in humans some data suggested that stem cells could be stimulated to produce new neurons, a fact which is contrary to the canon of neurons that do not regenerate (1). Dr. Kunlin Jin has shown that neuronal stem cells can be handled by use of growth factors such as FGF-2, EGF, VEGF and SCF even through nasal administration. Also, Dr. Jin and colaborators have demonstrated that cerebral ischemia (focal cerebral ischemia) was reversed in old mice by adminstration of plasma from young mice (1). These results were as well according to the biochemical hypothesis of aging, whereas parabiosis (old animals were given blood from young animals) was very important in designing the hypothesis of aging (14).

If we were to summarize, the major succes of stem cell treatment would be the treatment of multiple sclerosis by transplant of bone marrow stem cells (21). The aforementioned regards autologous grafts of bone marrow stem cells handled using a procedure which was proposed twenty years ago, but in the meantime developed through an increasingly efficient isolation of stem cells. After removing bone marrow stem cells, a patient's immune system is inactivated by the chemotherapy, similarly to what happens during tratment of leukemia and subsequently stem cells are transplanted. Conditions of many patients who were selected by tight criteria, greatly improved when this transplant treatment was performed under the frame of a randomized multicenter study.

This treatment is certainly very invasive and not recommended for patients aged over fifty years because it affects other

organs. A great disadvantage of treatments with stem cells is the requirement for immunosupression in order to remove the patient's cells or so that his immune system does not attack the transplanted stem cells. The patient's own stem cells are a solution for this second case.

Stem cells and aging

If aging were a degenerative condition as aging itself is origin for degenerative diseases (aging related pathology), then expectations regarding stem cells in treatments of degenerative conditions should advance also towards treatment of aging as such. Under these conditions we had to answer the very difficult question „what is aging?" and how important alterations of stem cells' functions in aging are. The above biochemical hypothesis of aging has responses in the evolutionary context to this question (14). Anyway, in mammals aging appears not to result from stem cells' replicative exhaustion which should take place after completion of the maximum lifespan in the case of humans, at the least. However, there seems to a functional impairment of this type of cells (22). For instance, in the case of skin epithelial cells there were some modifications, number of stem cells decreased but as well their differentiation capacity, as shown by loss of melanocytes involved in skin and hair pigmentation. From physiological changes associated with aging we can assume that there are involvements of some proliferation and differentiation deficits of several types of stem cells of skin, muscle, organs and brain. In young animals, stem cells divide asymmetrically, thus producing other stem cells and cell lines which are differentiated during tissue's homeostasis and regeneration. Some stem cells lose their specificity with age and produce impaired descendants, a fact which determines loss of tissue's integrity and physiological decline, even the number of stem cells is unaffected. Some stem cells lose their renewal capacity by undergoing symmetrical divisions that result in two differentiated cells and a gradual loss of the stem cell stock. Senescence of stem cells can also contribute to loss of functional stem cells. Increase in malignant phenomena with age, especially epithelia of a high turn-over has been assumed as originating in the stem cells' compartment or stem cells' early descendants (22).

Depending on tissues' capacities to proliferate and regenerate, there are three types of tissues: tissues with proliferative (turn-over) and high regeneration capacities (epidermis, blood, intestine), which have well represented stem cell compartment; tissues with a more reduced turn-over, but high regenerative capacity (muscle, liver) for which there are different strategies of regeneration and repair such as need for stem cells in the case of muscles and proliferation of differentiated hepatocytes in the case of liver; tissues with reduced turn-over and regenerative capacity (brain, heart) for which repair is limited and takes place through recruiting stem cells (23). Taking into account the modalities in which various tissues are torn over time, we can detect mechanisms determining aging, levels at which capacities of regeneration and renewal of systems are affected meaning the level of stem cells and cells' impaired differentiation.

Which is the determinant of aging? Is it that cells lose their capacity as stem cells, their capacity to differentiate or do they more poorly differentiate and the resulting cells are less functional?

As regards stem cell functioning most of the studies were conducted on hematopoietic stem cells (HSC), which can be used for bone marrow repopulation in cases of severe irradiations. Bone marrow transplant gained its utility in clinics at the end of sixties, in cases of acute leukemia and hereditary immune deficiencies. Then it was found out that actually hematopoietic stem cells from the bone marrow migrate to the blood (peripheral HSC) and can restore the hematopoietic and immune functions in patients through stimulation with cytokines

or in the recovery stage after myelo-suppressive therapy (24).

However, apart from hematopoietic stem cells' utility for the clinics, much is unknown regarding these cells and most knowledge is due to studies conducted on mice. Even though the model of immune suppression in mice is not similar to that of bone marrow repopulation through transplant after lethal doses of radiations, it provides information about the HSC repopulation potential.

From existing data, the age of the donor appears to be determinant for a successful transplant. In aging it seems that the ratio adipose cells to hematopoietic cells of the bone marrow (24) changes and more so, the number of CD34+ cells (having a specific antigen that has immune function) decreases. A modality to solve the problem of a low number of hematopoietic cells in the case of these grafts is cells' multiplication in cultures and re-implantation. Nonetheless, it appears that the multiplication potential of CD34+ cells is limited and data are controversial. Umbilical stem cells CD34+ can be multiplied only for a limited time, a fact suggesting their "aging"

Generally, HCS could provide interesting information about adult stem cells' dynamics. Also, in the case of HSC (being nevertheless reserved due to controversial results and manipulation techniques), changes in multiplication and differentiation capacities are found out. Which would be the factors involved in these changes?

There are often discussions about the influence of microenvironment on stem cells. Recent data have shown that the environment could determine transdifferentiation without transition involving stem cells. Neurons were produced from skin cells (fibroblasts) subsequently to manipulations (25). Under these conditions we can hope that through control of the environment, transdif-ferentiations can be obtained more easily, more cheaply and by avoidance of ethical aspects which stem cells raise. In the case of these cells, apart from the success mass media distributed, there were as well many failures familiar to those working in the field. At the same time new perspectives open up in cell therapy, similar to the heart reconstituted from the fibrous skeleton (which resulted from chemical removal of cells) by use of repopulation with cardiomyocytes from new born rats (26). The rat heart thus formed started to beat eight days after growth under laboratory conditions and stimulation by electrodes. It is interesting how these various tissues formed (blood vessels) through these cells' differentiation as if the fibrous skeleton transmitted certain signals. The physical and chemical environment appears to be decisive in the case of any cell, regardless its advanced differentiation. Cells are open systems. Complex influences the environment exerts on cells are nevertheless least known.

Another aspect would be that of the environment of differentiated tissues, not only that of stem cells. Cells from differentiated tissues (or host tissues in the case of grafts with stem cells) could change biochemical with age, and this fact might lead to limiting differentiation of stem cells reaching those tissues or of stem cells responsible for regeneration of aforemen-tioned tissues. For instance, HCS can lead to regeneration of tissues outside of the hematopoietic bone marrow. The phenomenon of differentiation of stem cells transplanted in other types of cells than the desired type was already mentioned. According to predictions of the biochemical hypothesis of aging, the above phenomenon might be treated through transcription factors eventually having local action and promoting stem cell differentiation. With a view to restoring the initial cellular biochemical features of stem cells from different tissues, epigenetic prints of tissues from young ages could play an orienting role. Future researches could have answers in this sense, thus contributing to deciphering the aging process. Despite all difficulties

stem cells' research encountered, it has shown promising ways for cell therapy emerging as important part of regenerative medicine.

REFERENCES

[1] Ilia Stamler. *Stop aging and disease*, ICAD 2014, conference report, Aging and Diseases, 2015,Vol. 6, 2:76-94.

[2] Banerjee S.,Williamson D., Habib N., et al. *Human stem cell therapy in ischaemic stroke: a review.* Age and Ageing, 2011,40: 7–13.

[3] Yamazaki S., Iwama A., Takayanagi S., et al. *Cytokine signals modulated via lipid rafts mimic niche signals and induce hibernation in hematopoietic stem cells*. EMBO J., 2006,vol. 25: 3515–3523.

[4] Boyer L.A., Lee T.I., Cole M.F., et al. *Core transcriptional regulatory circuitry in human embryonic stem cells.* Cell, 2005,vol. 122, 6: 947–56.

[5] Almeida-Porada G., Porada C.D. *Formation of human hepatocytes by human hematopoietic stem cells in sheep*.Blood, 2004,104: 2582-2590.

[6] Abad M. A., Mosteiro L., Pantoja C., et al. *Reprogramming in vivo produces teratomas and iPS cells with totipotency features*. Nature, 2013,vol. 502, 747: 340–5.

[7] Takahashi K. , Tanabe K., Ohnuki M., et al. *Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors.* Cell, 2007,Vol. 131, 5: 861–872.

[8] Yu J., Vodyanik M. A., Smuga-Otto K. et al. (2007). Induced Pluripotent Stem Cell Lines Derived from Human Somatic Cells. Science, vol. 318, 5858: 1917-1920.

[9] Moore,K.L.,Persaud T.V.N. and Torchia M.G. *Before we are born:Essentials of Embriology and Birdh Defects*, 2013,Philadelphia,PA:Saunders,Elsevier Print.

[10] Hochedlinger K.,Yamada Y., Beard C., Jaenisch R.*Ectopic expression of Oct-4 bloks progenitor-cell differentiation and causes dysplasia in epithelial tissues*.Cell, 2005,vol 121,3: 465-77.

[11] Ibrahim E.E.,Babaei-JadidiR.,Saadeddin A.,et al.*Embrionic Nanog activity defines colorectal cancer stem cells and modulates through AP1-and TCF-dependent mechanisms.* Stem cells , 2012,vol.30,10, 2076-87.

[12] Santagata S.,Hornich J.L.,Ligon K.L. *Comparative analysisof germ cell transcription factors in CNS germinoma reveals diagnostic utility of NANOG*.. The American Journal of Surgical Pathology, 2006,vol.30,12, 1613-1618.

[13] Kregel S.,Kiriluk K.J.,Rosen A.M.,et al. *Sox2 is an androgen receptor-repressed gene that promotes castration-resistant prostate cancer.* PloS ONE8(1):e53701,2013 doi:10.1371/ journal.pone. 00537010. PMC3543364.PMID 23326489.

[14] Diaconeasa A.G.,Rachita M.,Stefan-van Staden R-I. *A new hypothesis of aging*. Med Hypothesis, 2015,84,252-257.

[15] Beckman J.,Scheitza S.,Wernet P. et al.*Asymetric cell division within the human hematopoietic stem and progenitor cell compartment:identification of asymmetricaly segregating proteins.*Blood, 2007,vol.109,12, 5494-501.

[16] Xie T.,Spradling A.C.*Decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary*. Cell, 1998,94(2), 251-60.

[17] Song X.,Zhu C.H.,Doan C.,Xie T. *Germline stem cells anchored by adherens junctions in the Drosophila ovary niches.* Science, 2002,Vol 296, 5574,1855-7.

[18] Diaconeasa A.G., Rachita M., Dumitrescu I.*A new hypothesis of the origin of menopause*. Romanian J.of Gerontology and Geriatrics , 2013,no.1, 19-24.

[19] Lindvall O.,Kokaia Z.,Martinez-Serranoa.*Stem cell therapy for human neurodegenerative disorders-how to make it work.* Nature publishing Group, 2004, http://www.nature.com/naturemedicine.

[20] Mimeault M.,Batra S.K.*Recent progress on tissue-resident adult stem cell biology and their therapeutic implications.* Stem Cell Rev , 2009,4 (1), 27-49.

[21] Bakhuraysah, M. M., Siatskas, C., & Petratos, S. *Hematopoietic stem cell transplantation for multiple sclerosis: is it a clinical reality?* Stem Cell Research & Therapy, 2016,7, 12. http://doi.org/ 10.1186/s13287-015-0272-1

[22] Liu L., Rando T. *Manifestatios and mechanisms of stem cell aging*. J.Cell.Biol, 2011,vol.193, 2, 257-266.

[23] Rando T.A. *Stem cells, ageing and the quest for immortality*. Nature, 2006,441, 1080-1086.

[24] Ho A.D.,Punzel M. *Hematopoietic stem cells; can old cells learn new trics?* J.Leukoc. Biol, 2003,73, 547-555.

[25] Pang Z.P., Yang N., Vierbuchen T.et al. *Induction of human neuronal cells by defined transcription factors.* Nature, 2011,476, 7359, 220-3.

[26] Otti H., Matthiesen T., Goh S. et al. *Perfusion-decellularized matrix: Using nature's platform to engineer a bioartificial heart.* Nature Medicine. 2008;doi:10.1038/nm1684

PRELIMINARY DATA REGARDING HEALTH CONDITION AND SOCIAL RELATIONS IN ELDERLY

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Abstract. Active longevity should not be limited to or synonym for a trendy concept which is widely circulated in programs of public policies. Active longevity is above all a state of mind that implies to enjoy living, to have the feeling of being useful socially (family relations, relations among relatives and friends) and not lastly a state of wellbeing (from the psychosocial point of view but also health/pathology). Fulfilling these preconditions must be analyzed from a double perspective: external and internal/individual. In other words, safety from the economical viewpoint (decent living) and ensuring an optimal social climate are external important factors, but together with the individual's affective and pathological states cumulatively ensure major conditions for aging/active longevity as a stage in life to be lived altogether.This study had a solid theoretical and methodological grounds consisting in a rigurous literature search regarding aging/active longevity as assumed by political policies for vulnerable people (third age included) and as well methods and tools of investigation on elderly respondents' perceptions (two groups of subjects). The latter consists of questions regarding quality of life and social relationships, ways to adopt preventive behaviors in relation to health condition, namely a self-evaluation of the psychological „status". We used in this study both standardized Depression Scale in elderly subjects and the Scale for satifaction with life. Finally, a set of conclusions and recommendations has been elaborated and also there have been described several profiles of persons prepared/vulnerable in assuming aging/active longevities.
Key words: longevity/active longevity, health condition, depression scales, quality of life.

DATE PRELIMINARII REFERITOARE LA CONDIȚIILE DE SĂNĂTATE ȘI RELAȚIILE SOCIALE LA VÂRSTNICI

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Rezumat. Longevitatea/imbatranirea activa nu trebuie nicidecum redusa sau echivalata cu un concept la moda, larg vehiculat in programele si politicile publice. Dincolo de orice definitie "de manual", longevitatea/ imbatranirea activa este inainte de toate o stare de spirit ce presupune bucuria de a trai, sentimentul de utilitate sociala (relatia cu familia, rudele, pritenii) si, nu in ultimul rand, o stare de bine (din punct de vedere psihosocial, dar si somatic/patologic). Indeplinerea acestor preconditii trebuie privita dintr-o dubla perspectiva: externa si interna/individuala. Cu alte cuvinte, securitatea economica (traiul decent) si asigurarea unui climat social optim sunt factori externi care, alaturi de starea individuala afectiva si psihologica, asigura (cumulativ), conditiile esentiale in care imbatranirea, ca etapa a ciclului vietii, se manifesta in mod plenar. Cercetarea pe care o propunem se bazeaza pe un fundament teoretico-metodologic solid: pe de o parte pe o documentare riguroasa in ceea ce priveste imbatranirea activa, asumata prin politici publice dedicate categoriilor vulnerabile (inclusiv varsta a III-a), iar pe de alta parte - pe utilizarea unor metode si instrumente de investigare a perceptiilor repondentilor de varsta a III-a (2 loturi de subiecti). Chestionarul aplicat cuprinde intrebari despre calitatea vietii si a relatiilor sociale, despre modalitatile de adoptare a unor comportamente preventive in raport cu starea de sanatate, respectiv o autoevaluare a "tonusului" psihic. Am utilizat aici 2 teste standardizate: Scala depresiei la

presoanele varstnice si Scala privind satisfactia de viată. In final a fost elaborat un set de concluzii si recomandari, inclusv ca rezultat al testarii unor ipoteze statistice, fiind totodata elaborate profile de persoane pregatite/vulnerabile in a-si asuma o imbatranire/longevitate activa.
Cuvinte cheie: longevitate/longevitate activa, stare de sanatate, scala de depresie, calitatea vietii

INTRODUCTION

Active longevity should not be limited to or synonym for a trendy concept which is widely circulated in programs of public policies. Beyond any definition of the manual, active longevity is above all a state of mind that implies to enjoy living, to have the feeling of being useful socially (family relations, relations among relatives and friends and not lastly a state of wellbeing (from the psychosocial point of view but also health/pathology). Meeting these prerequisites should be regarded from a double perspective: an external one and an individual one. In other words, safety from the economical viewpoint (decent living) and ensuring an optimal social climate are external important factors, but together with the individual's affective and pathological states cumulatively ensure major conditions for aging/active longevity as a stage in life to be lived altogether.

The research we propose has a solid theoretical and methodological ground consisting in on the one hand, a rigurous literature search regarding aging/active longevity as assumed by political policies for vulnerable people (third age included) and on the other side, the research is based on using methods and tools of investigation on elderly respondents' perceptions (two groups of subjects (n=129; patients from the NIGG and subjects randomly selected who did not benefit healthcare services of the NIGG until the questionnnaire was given). The latter consists of questions regarding quality of life and social relationships, ways to adopt preventive behaviors in relation to health condition, namely a self-evaluation of the psychological „status". We used in this study two standardized tests: the Depression Scale in elderly subjects and the Scale for satifaction with life.

Finally, the aim was to support, based on scientific ground, the importance of adopting values and desirable lifestyles for in elderly an active longevity be ensured.

MATERIALS AND METHODS

In view of the aim, at first the key-concepts of the specialized literature (1) were defined and secondly, factors influencing the elderly health conditions were pointed out.

Currently operating definitions: Given the predominantly applicative type of this work, under the frame of this section, we limited ourselves to elaboration of definitions with operating distinctions as regards nomenclature in view of easing under-standing and further going in-depth with results, conclusions and recommendations.

Lifestyles are in brief distinct models of social or personal behavior of a person or group. The concept of lifestyle may be considered an alternative to that of „social class", lifestyles being patterns concerning values, tastes, behaviors which the person does not have to adopt, because there are multiple options to choose from (those above enumerated). It folllows that when we say lifestyles specific of the third age, we should refer to values, tastes (habits) and behaviors of that social class (the latter as a notion in its sociological sense).

Risk: in an operational sense, it represents the probability higher or lower for a person to be injured or suffer from a danger. The term "vulnerability" is associated to risk and it depicts characteristics of a person related to his more or less exposure to dangers. In the case of elderly but also other people categories (young, women, minorities) we may say about a "social vulnerability", the latter type being determined by factors such as: poor health condition, poverty, limited access to resources, social injustice, marginalization, dependence on resources, difficult access to infrastructure, and inadequate quality of housing.

Old age, third age: in the specialized literature, the period of time of the old age is defined according to three criteria: chronological, functional and cyclic. In a broader sense, aging is considered a multidimensional phenomenon which includes physical changes of the human body after adulthood, psychological changes, but also social changes in ways a person is perceived with regard to what the person itself expects and expectations of others related to him. Consequently, the age at which a person starts to be called old depends on historical period and culture (2). Culture has an important role in approaches regarding aging, as it has influence on perceptions on the third age, attitudes towards roles, rights and responsabilities of elderly and as well, support and care systems for the old persons. Old age image, which a certain society circulates is extremely important for way in which people perceive elderly, as well as old persons' self-esteem.

According to WHO, active and as well healthy aging is a process of optimising opportunities related to health, social participation and safety aiming at improving quality of life of elderly. Longevity refers to chronological age and the fact that we wish an old person to stay active does not mean at all that he is free of diseases. Healthy aging is an ambitious aspiration, which is very difficult or impossibly to attain under present circumstances. This is why Prof. Ana Aslan expressed the above aspiration in a sintagm, which is „ adding life to years not years to life".

Strategies and public policies: Strategy is a record of public policies on long and medium term, which defines the govern's policy with regard to a certain field of public policies for which decisions should be taken with respect to a wide range of issues. The strategy is elaborated in view of designing a new public policy or in case the former areas of public policy need a significant improvement (3). It follows that these policies represent all activities carried out by specialized public administration in view of solving problems identified and ensuring development of public policies necessary for a certain field.

Methodological references and statistical tools: Detailed statistical processing (information collected through the questionnaire given to two groups of subjects depending on their demand for healthcare NIGG provides) was doubled by a secondary analysis (studies, reports) and a documenting one (legislation, public policies).

The study addresses a new paradigm (mix of methods (1), in that it addresses both qualitative methods (documenting analysis) and methods as well as quantitative tools (survey, questionnaire).

The documenting analysis allowed study from the point of view of active longevity and quality of lifestyles, of some reports relevant for third age related issues (programs, strategies, various other reports of public policy.

The questionnaire was structured on the following items:

• socio-demographic data;

• level of satisfaction regarding health, life and social relations (with specifications on accessing NIGG services);

• frequency of access to healthcare specialists (family physician, geriatrician, pharmacist);

• lifestyles, evaluation of depressive condition and satisfaction with life.

Socio-demographic data were analysed using specific statistical tests regarding the following variables as relevant for our analysis: the group to which belong respondents (NIGG patients or outpatients), sex, education level and occupation, place of origin (urban/rural), level of income, potential social and family support (number of children, number of grandchildren, housing), marital status.

We showed next, in brief, the two standardized scales used in the last part of the questionnaire.

The Scale of Depression in Elderly – GDS (4) comprises a set of thirty items by use of

which respondents expressed their agreement or disagreement.
Finally, the following interpretation resulted:
a. normal=0-9 points;
b. slightly depressive =10-19 points;
c. severe depression=20-30 points.
The Satisfaction With Life Scale - scales regarding satisfaction with life (SWLS). SWLS consisted in five items in relation to which agreement or disagreement was requested (5). A scale from 1 to 7 was used;
Scores were by interpreting:
a. 5-9 extremely unsatisfied
b. 10-14 unsatisfied
c. 15-19 slightly unsatified;
d. 20-neutral;
e.; 21-25- slightly satisfied;
f.; 26-30 satisfied;
g.. 35-31 very satisfied;
Data were collected in Microsoft Office, Excel and analysed using SPSS (Statistical Package for the Social Sciences), in order to point out relevent differences between medians, ranks, depending on what case and significant correlations.
Testing hypotheses of work was conducted by using correlations' tests (Pearson, Spearman, depending on distribution and category of variables), *t* test and Independent Samples Mann U Test respectively. The later was used for data that were not utilized in correlations.
• The null hypothesis (H0): absence of relationship/absence of a significant difference;
• The alternative hypothesis (H1): presence of a relationship/presence of a significant difference (actually the negative for the null hypothesis);
Taking into account the descriptive type for this study, the modality to present arguments in support of these two hypotheses imply a synthetic approach (questions addressing perceptions of respondents, small interviewed population sample and the short section as dedicated to methodology.

RESULTS AND DISCUSSION

This section has two apparently distinct parts of analysis but found out as depending on one another. The first part is dedicated to interpretation of results of the quantitative study and the other part is an analysis of context under programs, projects, public policies that mainly focuses on active longevity. Dependence on one another was evident through qualitative interpretation as a transfer from the documenting analysis to quantitative data (for certain, not by tresspassing rules specific of comparative analysis).

Tabel I. Levels of satisfaction expressed by respondents

Please indicate levels of satifaction in relation to the following aspects: (scores from 1 to 10, for which 1 – very low level 10 – very high level) – school like scoring	*mean*	*stdev*
	7,07	1,94
Social relations	6,48	2,69
Dervices provided by the NIGG*	6,42	3,65
Health condition at present	6,57	1,80
Subject's health condition in perspective	6,36	1,89

*Services provided by the NIGG - only for respondents who use these services (group2)

Since it is a quantitative study we preferred an interpretation the type of „question by question". But for reasons of managing adequately the size of this paper, we showed only tools and tables that were relevant to the study.

Regarding the first set of items under evaluation, levels of satisfaction expressed by respondents were shown in the above table (Table I).
Satisfaction with regard to quality of life NIGG correlated negatively with age (in

younger persons satisfaction is higher), but correlated positively and strongly with education and professional levels (the higher the levels, the greater the satisfaction with quality of life).
This type of satisfaction correlated negatively with age (older persons have a lower level of satisfaction and inversely was also found out, the correlation being a bidirectional one) but positive in relation to education and professional levels (the higher the education level, the more visible, the profession).
The satisfaction with regard to quality of NIGG services was higher among persons who used these services more frequently (group 1 of respondents; median for group 1 8,20, median for group 2 4,37, t = 6,7, p = 0,01).
Satisfaction regarding perspectives on subjects' health conditions correlated negatively with age (in younger persons satisfaction is higher). This type of satisfaction was higher in persons under 69 years old (compared with that of persons aged 70 years old and over).
Respondents of the group 2 (those who did not require services of the NIGG) called the family physician more often, while pacients of the NIGG called the geriatrician specialist (Table II).

Table II. Most frequently calls, for respondents of the group 2

Used to call: (scores from 1 to 10, in which 1 –very rarely, 10 –very often)	*mean*	*st.dev.*
Family physician	7,74	2,13
Geriatrician specialist	5,95	3,58
For pharmacist's advices	5,38	3,01

Application of specific tests pointed out that:
1. Health condition (present) - a better one in the case of subjects that did not require healthcare services from the NIGG correlates negatively with age (in younger persons satisfaction is higher with regard to own health condition); satisfaction regarding own health condition being higher in persons under 69 years old (compared with that of persons aged 70 years old and over). It is also relevant that satisfaction with own health condition correlates with education and professional levels (the higher the levels, the greater the satisfaction). Finally, those with higher incomes meaning those living in urban areas also have a higher level (degree) of satisfaction (in comparison with those on low incomes who live in rural areas);
2. Quality of life –is higher in subjects, especially men who do not require healthcare services of the NIGG (interpretations are not cumulative). The level of satisfaction is higher in persons from urban areas who are in a marital relation (marriage/domestic partnership/ cohabitation) and have higher incomes. In other words, a dark perspective on life is that of respondents living alone on low incomes in rural areas (significant differences being individualized on of the aforementioned sociodemographic variable);
3. Satisfaction regarding social relations is higher for persons on higher incomes, namely those living in urban areas. From the point of view of social relations its correspondence is in a higher level of satisfaction with the milieu of social relationships;
4. NIGG services – a result predictable in our case is that satisfaction with regard to quality of NIGG services is higher among persons who use these services more frequently. There were no correlations or significant differences as related to other variables (sex, age, education level, marital status, area of residence).
5. Future of subjects' health conditions: Satisfaction regarding future of health correlates positively with education and profession level (in our study those with higher education levels and most valued professions [6]. Finally, those on high incomes but also living in urban areas are more optimistic about their health condition in the future (compared with subjects on low

incomes and those living in rural areas, respectively).

Study limitations: The small size of the population sample under study, 129 subjects of a relatively heterogeneous sample from the point of view of age groups and absence of validation data at national level, led to the need for meeting requirements of normalcy of distribution on sets of sociodemographic variables that are relevant (sex, age, education level, category of income). The aforementioned was one reason for addressing in parallel parametric and non-parametric statistics.

CONCLUSIONS

• Respondents have been satisfied with the quality of life and their social relations;

• Slight unsatisfaction has been associated only with the state of wellbeing (present state and state in perspective);

• Values for standard deviation in the case of satisfaction with own social relations suggest a polarization of options (satisfaction vs. unsatisfaction).

When they have a health problem, persons who were given the questionnaire, most frequently call the family physician and rarely the geriatrician, and the aforementioned happens when the pharmacist advises.

Additionally, women tend to look for pharmacist's advices more than men do, as they pay much attention to their looks more so since cosmetics penetrated the market of drugstores.

Some habits are in view of a healthy lifestyle (food, rest), other habits envisage being healthy physically, and while aspects related to social relations managing and attainment of a desirable emotional balance are being evaluated.

Beyond a classification of evaluated aspects, we noticed that "negative" statements (which identify an unhealthy/inactive lifestyle) received the lowest appreciations. Exception to the aforementioned is the statement of "Occasionally, I have various activities in order to gain some extra money". For this statement the evaluation should be related to relatively low post-retirement possibilities for carrying out profitable activities and receiving payment for these latter. In this case we witness a list of causes that point to not only to a poor health condition (people cannot work after a certain age) but also to lack of mechanisms of social insertion. The latter is specific for persons of the third age (needed jobs needed as to be dedicated to elderly, employers more willing to recruit from old age group, tolerance towards the elderly. The above mentioned represents excessively protective mentalities of adults/ children/grandchildren and is pointed out by cultural aspects residing in prejudices such as "it is shameful to let your elderly parent work" and not agreeing to this sort of prejudice, is publicly criticized.

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REFERENCES

[1] Chelcea, S. *Metodologia cercetării sociologice: metode cantitative şi calitative*. Bucureşti, ed. a III-a, 2007, Editura Economică, 74-83.

[2] Sorescu, Maria E. *Asistenţa socială a persoanelor vârstnice*. Craiova, Editura Universităţii din Craiova, 2005,6-10.

[3] *Strategia naţională pentru Promovarea Îmbătrânirii Active şi Protecţia Persoanelor Vârstnice 2014–2020*, Sources: http://www.mmuncii.ro/j33/images/Documente/Transparenta/Dezbateri_publice/2015-07-15_Anexa1_ProiectHG_SIA.pdf

[4] *Scala depresiei geriatice*, http://web.stanford.edu/~yesavage/Rumanian.html . Developed by: Jerry Yesavage, MD Source: http://web.stanford.edu/~yesavage/GDS.english.long.html .

[5] *Satisfaction With Life Scale* Developed by Ed Diener and coll., 1985 (Diener, Emmons, Larsen & Griffin, 1985). Sources: http://www.tbims.org/combi/swls/

[6] *"Fii activ la orice vârstă", proiect finanţat prin Programul Operaţional Sectorial pentru Dezvoltarea Resurselor Umane 2007 – 2013*, Axa prioritară 4 „Modernizarea Serviciului Public de Ocupare", Domeniul major de intervenţie 4.1 „Întărirea capacităţii SPO pentru furnizarea serviciilor de ocupare", numărul de identificare al contractului: POSDRU/123/4.1/S/131974, beneficiar: Agenţia Naţională pentru Ocuparea Forţei de Muncă - (2014), Sources: http://www.anofm.ro/files/In%20primele%206%20luni....pdf

THE IMPACT OF TYPE 2 DIABETES MELLITUS ON GASTROINTESTINAL FUNCTION IN OLDER AGE

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Abstract. Although very common, the gastrointestinal complications of Type 2 Diabetes Mellitus (T2DM) in older people are often undiagnosed in clinical practice and are associated with significant morbidity. This study aims to evaluate the prevalence of gastrointestinal symptoms in older diabetic patients and to assess the relationship between the duration of diabetes, metabolic control, and frequency of digestive manifestations. The study sample included 60 patients 50-64 years of and 60 patients 75 years of age and older diagnosed with T2DM. There were significantly fewer asymptomatic older adults as compared to younger subjects (21,6% versus 46,6%, p=0,01). The most frequent gastrointestinal symptoms in both groups were abdominal pain (30,83%), pyrosis (23,33%), constipation (21,66%) and anorexia (20%). A moderate positive correlation was identified between symptoms frequency and duration of DM in years which was statistically significant ($\tau = 0,46$, $p = 0,004$). A poor metabolic control was associated with a significantly higher frequency of gastrointestinal symptoms in older adults but not in younger participants (p=0,005). Older age, a long duration of T2DM, female genre and the presence of diabetic peripheral neuropathy are associated with a higher prevalence of gastrointestinal symptoms in diabetic patients.
Key words: diabetes, older age, gastrointestinal symptoms

IMPACTUL DIABETULUI ZAHARAT TIP II ASUPRA FUNCȚIILOR GASTROINTESTINALE LA VÂRSTNICI

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Rezumat. Desi foarte frecvente si asociate cu o morbiditate importanta, complicatiile gastrointestinale ale Diabetului Zaharat de Tip 2 (DZT2) raman adesea nediagnosticate in practica clinica. Acest studiu isi propune sa estimeze prevalenta simptomelor gastrointestinale la varstnicii diabetici si sa evalueze relatia dintre durata DZT2, controlul metabolic si frecventa acuzelor cu punct de pornire in tractul gastrointestinal. In studiu au fost inclusi 60 de pacienti cu varste intre 50-64 de ani si 60 de pacienti cu varste ≥ 75 de ani, diagnosticati cu DZT2. In grupul adultilor vartsnici au fost semnificativ mai putini pacienti asimptomatici comparativ cu cei din grupul cu varste mai tinere (21,6% versus 46,6%, p=0,01). Cele mai frecvente simptome gastrointestinale in ambele grupuri de varsta au fost: durere abdominala (30,83%), pirozis (23,33%), constipatie (21,66%) si anorexie (20%). O corelatie pozitiva, moderata semnificativ statistic, a fost identificata intre frecventa simptomelor digestive si durata in ani a DZT2 ($\tau = 0,46$, $p = 0,004$). Controlul metabolic insuficient a fost semnificativ asociat cu o frecventa mai mare a simptomelor gastrointestinale la pacientii varstnici (p=0,005). Varsta inaintata, durata lunga a DZT2, sexul feminin si prezenta neuropatiei diabetice periferice sunt asociate cu o prevalenta mai mare a simptomelor cu punct de plecare in tractul gastrointestinal la pacientii diabetici.
Cuvinte cheie: diabet, vârstnic, simptome gastrointestinale

INTRODUCTION

Diabetes Mellitus (DM) is affecting more and more people globally and it is one of the main causes of morbidity and mortality especially in older people [1]. Increasing longevity is translated into increasing need of health care services due to existing comorbidities, atypical course of disease and multiple complications. The older population is very heterogeneous in terms of coexistence of multiple comorbidities and geriatric syndromes thus, diabetes therapeutic management requires a multidisciplinary approach and individualized dietary and life style recommendations [2].

The negative impact of type 2 DM on digestive function is present at all anatomical segments from the oral cavity to the external anal sphincter [3,4]. Frequent gastrointestinal diabetic complications are esophageal dysmotility, gastro-esophageal reflux disease, gastroparesis, enteropathy and non-alcoholic fatty liver disease [5-8]. Autonomic neuropathy, microangiopathy, prolonged hyperglycemia and hepatic insulin resistance are considered to be key players in the pathological pathways of digestive dysfunctionalities associated with diabetes [6-10].

The age related changes in gastrointestinal function include presbyphagia, decreased gastric elasticity and evacuation rate, increased risk of peptic ulcer disease and atrophic gastritis, decreased lactase levels, changes in gut microbiota composition, decreased metabolic liver ability, diminished hepatobiliary function, increased prevalence of constipation and increased risk of colonic diverticular disease [11-13]. Diminished functional reserve capacities in older age augment the consequences of diabetes complications [14]. Although very common, the gastrointestinal complications of DM in older people are often undiagnosed in clinical practice and are associated with significant morbidity. Early diagnosis that would enable timely intervention is essential for prognosis and quality of life improvement [15].

This study investigates the relationship between type 2 DM and gastrointestinal function in older people. Identifying the prevalence of gastrointestinal function impairment in older diabetic patients, evaluating the distribution of gastrointestinal symptoms in different age groups and genres and assessing the correlation between the presence of gastrointestinal symptoms and the duration of DM and metabolic control are the main objectives of the study.

METHODS

Subjects

All study participants were inpatients admitted to the National Institute of Gerontology and Geriatrics "Ana Aslan", Bucharest, on referral from their GP or other specialists for various subacute or chronic conditions during January – June 2015. After being checked against the exclusion criteria, two groups of diabetics patients of equal numbers of men and women were selected using the age criteria as follows: patients 50-64 years of age and patients 75 years of age and older. The exclusion criteria were: type 1 DM, any neoplasia in the last 5 years, excessive consumption of alcohol, hemochromatosis, hiatal hernia and current treatment with anti-inflammatory drugs, bisphosphonates, antibiotics or iron supplementation.

Data collection

According to our set objectives, several parameters were recorded: presence and type of gastrointestinal symptoms, duration of DM in years, glycated hemoglobin (HbA1c) and body mass index (BMI). Macrovascular and microvascular diabetic complications were evaluated by clinical and neurological examination, peripheral circulation assessment (either Duplex Ultrasonography or ankle-brachial index test), electrocardiogram, retinal

examination, reagent test strip specific for albumin and albumin-to-creatinine ratio in a morning urine sample. The presence of the following gastroenterological conditions was identified based on anamnesis and medical records: gastroesophageal reflux disease, gastritis in the presence of Helicobacter Pylori, biliary lithiasis and steatosis hepatic. All data were identified from patients' medical records.

Statistical analysis

This is a case control study where type 2 DM was considered the risk factor to which the patients were previously exposed and the dependent variables were the presence of: anorexia, dysphagia, pyrosis, postprandial nausea and/or emesis, early satiety, meteorism, diarrhea, constipation, faecal incontinence and abdominal pain unrelated to a specific cause. The frequency distribution was used to identify the prevalence of gastrointestinal symptoms in diabetic patients. To compare the frequency of gastrointestinal symptoms in different age and genre groups we used the *Independent Samples T–test* equations. The distribution of gastrointestinal symptoms in patients with good versus poor glycemic control (defined with a glycated hemoglobin cutoff of 7%)[16] was evaluated with the *Independent Samples T–test*. To evaluate the associations between the frequency of gastrointestinal symptoms and the duration of DM in years we used the *Kendall rank correlation* analysis. Statistical analysis was performed with DATAPLOT programme.

RESULTS

After being checked against inclusion and exclusion criteria, a total number of 120 diabetic patients were selected. Similar numbers of women and men were included in each study group (39 women and 21 men). Both groups have comparable socio-cultural and demographic characteristics as presented in Tab. I. The majority of the participants have a high school or university degree and lives in urban areas (Tab. I).

Tabel I. Socio-cultural study groups' characteristics

		Adults (50-64 years) N=60	Older adults (≥ 75 years) N=60
		%/Mean (SD)	%/Mean (SD)
Age		59,83 (2.46)	79,13 (3.02)
Education	Primary school	13,33	18,33
	High school	46,66	33,33
	University	40	48,33
Settlement	Urban	76,6	78,3
	Rural	22,3	21,6

The average duration of DM was 8,86 years in the adults group and, as to be expected, significantly longer in the older adults group (15,95 years). Diabetes mellitus metabolic control, reflected by the glycated haemoglobin levels, was significantly poorer in the older age (68,3% respectively 33,3% had HbA1c ≥ 7%, $p<0,05$) compared to younger patients. A larger number of older adults as compared to younger participants were either underweight or suffered from obesity, and fewer had BMI values within the normal range (Table II). All recorded diabetic complications were significantly more frequent in the older adults group (Table II).

Table II. DM characteristics in adults and older adults groups

		Adults (50-64 years) N=60	Older adults (≥ 75 years) N=60
		%/Mean (SD)	%/Mean (SD)
Number of years DM		8,86 (1.37)*	15,95 (2.08)*
Glycated haemoglobin	≥ 7%	33,3*	68,3*
BMI	Underweight	3.3*	11,6*
	Normal weight	22,3*	13,3*
	Overweight	26,6	20
	Obesity	65*	55*
DM complications	Chronic Ischaemic Heart Disease	56,6*	78,3*
	Stroke	8,3*	26,6*
	Retinopathy	8,3*	16,6*
	Nephropathy	5*	15*
	Peripheral neuropathy	58,3*	80*

* = T- Test p < 0,05 (CI 95%)

The most frequent gastrointestinal symptoms in both groups were abdominal pain (30,83%), pyrosis (23,33%), constipation (21,66%) and anorexia (20%). In the case of anorexia, constipation, nausea, pyrosis, faecal incontinence and dysphagia the age related frequency distribution difference was statistically significant (Fig. 1). All gastrointestinal symptoms were more frequent in the older adults group as compared to younger participants (Fig. 2).The gastrointestinal symptoms were more frequent in women in both age groups (65,62% respectively 68,08%).

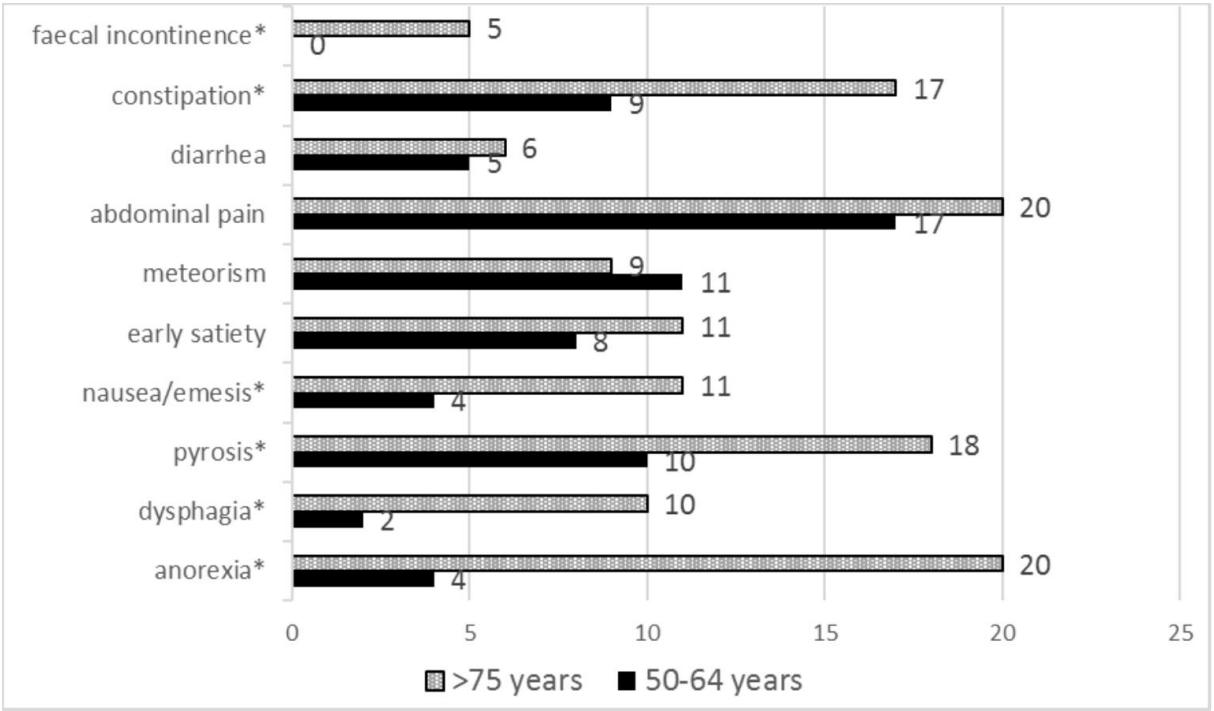
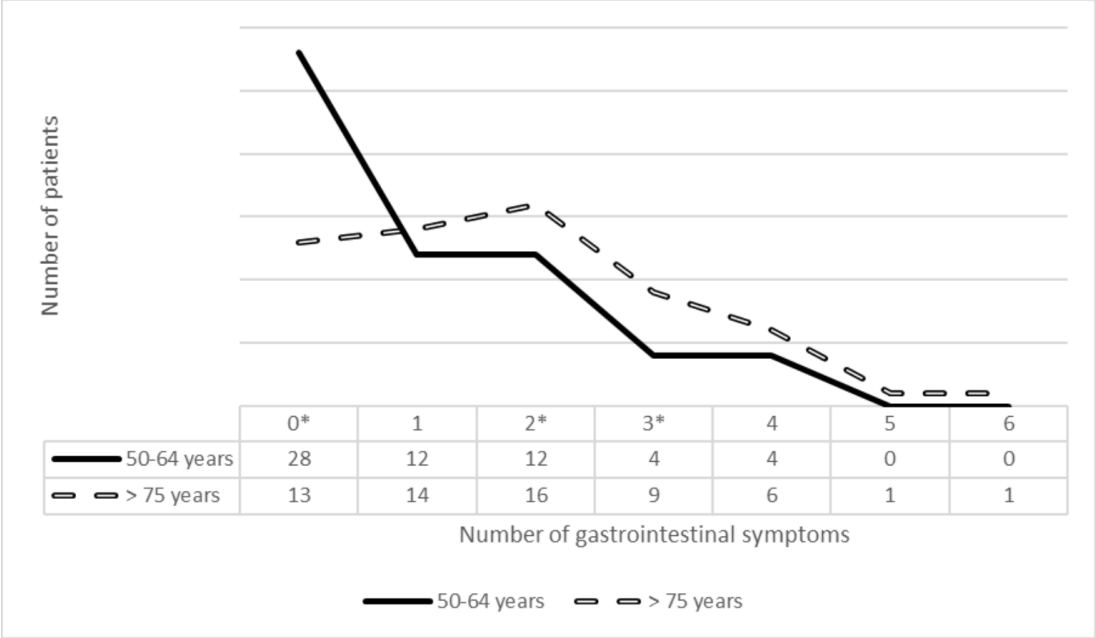


Fig. 1. Distribution of gastrointestinal symptoms in adults and older adults groups;
* = T- Test p < 0,05 (CI 95%).

There were significantly fewer asymptomatic older adults as compared to younger subjects (21,6% versus 46,6%, p=0,01)(Fig. 2). The number of patients

with concomitant gastrointestinal symptoms was higher in the older adults group, most reporting one or two manifestations (Fig. 2). A poor metabolic control was associated with a significantly higher frequency of gastrointestinal symptoms in older adults but not in younger participants (p=0,005)(Fig. 3). A moderate positive correlation was identified between symptoms frequency and duration of DM in years which was statistically significant (τ = 0,46, p = 0,004).

Peripheral neuropathy was present in 60% of the 50-64 years of age participants and in 80% of the 75 years of age and older subjects. The coexistence of peripheral neuropathy and gastrointestinal symptoms was present in 66,6% of the adults and in 81,2% of the older patients.



	0*	1	2*	3*	4	5	6
50-64 years	28	12	12	4	4	0	0
> 75 years	13	14	16	9	6	1	1

Fig. 2. Participants distribution considering the number of gastrointestinal symptoms;
* = T- Test p < 0,05 (CI 95%).

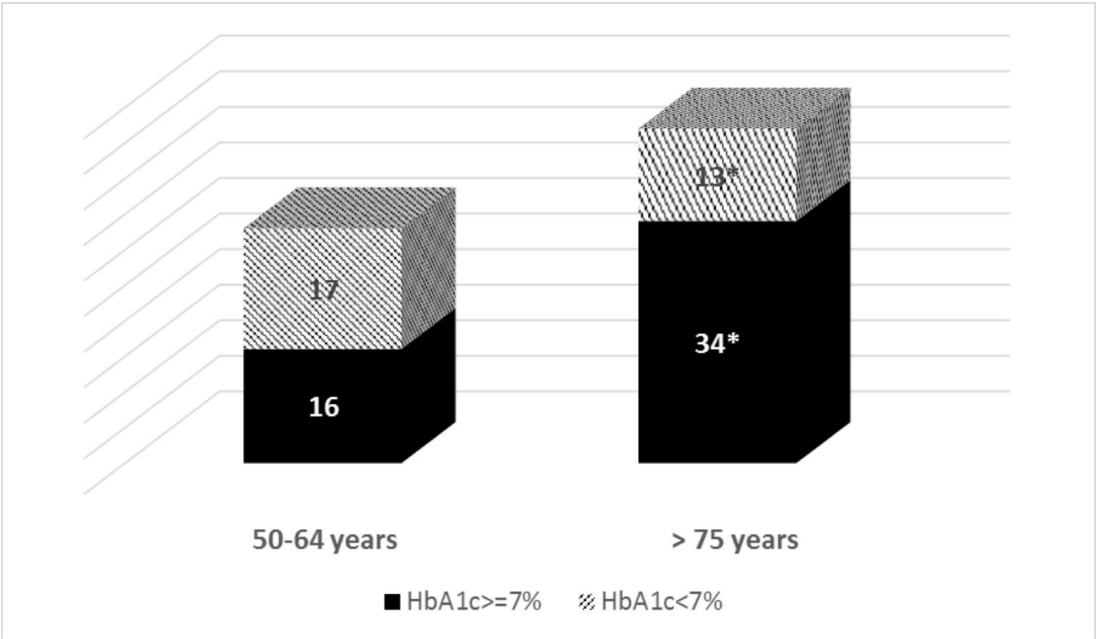


Fig. 3. Number of patients with at least one gastrointestinal symptom considering DM metabolic control;
* = T- Test p < 0,05 (CI 95%).

The prevalence of gastrointestinal diseases in the study sample was 21,6% for steatosis hepatis, 19,1% for biliary lithiasis, 12,5% for Helicobacter Pylori gastritis and 10,8% for gastroesophageal reflux disease. The prevalence of all gastrointestinal disorders was higher in older adults compared to younger patients. Steatosis hepatis was significantly more frequent in older men compared to women and younger men ($p = 0,004$ respectively $p = 0,003$). The prevalence of biliary lithiasis was significantly higher in women compared to men in any age group ($p=0,003$ respectively $p=0,005$).

DISCUSSIONS

The results of this study highlight a poorer metabolic control in older diabetic patients compared to younger ages. Diabetic complications were also more prevalent in older age. Gastrointestinal symptoms were highly prevalent in our study sample and significantly more frequent in older age. One in two diabetic patients 50-64 years of age had at least one gastrointestinal symptom, while one in two participants 75 years of age and older reported two or more concomitant manifestations. The results confirm the study hypothesis and demonstrate that the prevalence of gastrointestinal symptoms in diabetic patients is age related. The distribution of different types of digestive manifestations varies with age, in older adults the most frequent symptoms were anorexia, constipation, nausea, pyrosis, faecal incontinence and dysphagia. With regard to genre differences, in our study, the susceptibility to develop gastrointestinal symptoms was higher in women compared to men at any age. The longer duration of DM and the presence of peripheral neuropathy were moderately correlated with a higher prevalence of digestive tract manifestations. A poor metabolic control was associated with a higher frequency of gastrointestinal symptoms in older adults.

The gastroesophageal reflux disease is known to be associated with obesity, smoking and DM [17, 18]. In our study, more than half of the cases were identified in older adults. The relationship between DM and Helicobacter pylori gastritis is not clear. Some studies reported a higher prevalence of Helicobacter pylori infection in diabetic patients and an augmented risk of developing DM in patients diagnosed with Helicobacter infection [19-21]. In this study sample, the prevalence of Helicobacter pylori gastritis was 10% in adults and 15% in older adults. It has been argued that steatosis hepatis exists as a spectrum of disorders and the most common form is associated with obesity and type 2 DM [22, 23]. In this study, steatosis hepatis was identified predominantly in older age men. Among the risk factors for cholelithiasis formation are advancing age, female gender, obesity, lipid abnormalities and DM [24,25]. The prevalence of biliary lithiasis was 19% in our sample and significantly higher in women compared to men.

Because of the small sample, it was not possible to analyse gastrointestinal symptoms in different groups of interest, such as diabetic therapeutic management or other drugs. Another limitation of this study is the recruitment of participants from patients admitted to a single centre.

CONCLUSIONS

Older age, a long duration of type 2 DM, female genre and the presence of diabetic peripheral neuropathy are associated with a higher prevalence of gastrointestinal symptoms in diabetic patients. The most frequent digestive manifestations in patients over 50 years of age diagnosed with type 2 DM are abdominal pain, pyrosis, constipation and anorexia. Diabetic patients' susceptibility to steatosis hepatis, Helicobacter pylori gastritis, gastroesophageal reflux and biliary lithiasis is higher in older age. The

prevalence of steatosis hepatis is augmented in older diabetic men and the prevalence of biliary lithiasis is higher in diabetic women regardless of age.

REFERENCES

[1] Shaw JE, Sicree RA, Zimmet PZ, *Global estimates of the prevalence of diabetes for 2010 and 2030*. Diab Res Clin Prac, 2010/7:4-14.

[2] International Diabetes Federation, IDF Diabetes, 7 ed., 2015, Brussels, Belgium: International Diabetes Federation. http://www.diabetesatlas.org.

[3] Horowitz M, and Samsom M, eds., *Gastrointestinal function in diabetes mellitus*. 2004, John Wiley & Sons.

[4] Shaw KM and Cummings MH, eds., *Diabetes chronic complications*. 2012, John Wiley & Sons.

[5] Forgacs I, and Raja O, *Diabetes and the gastrointestinal tract*. Medicine, 2015, 43(6):347-51.

[6] Tolman KG., et al. *Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease*. Diabetes Care, 2007,30(3): 734-43.

[7] Hâncu N, Vereşiu IA et al. *Diabetul zaharat, nutritia, bolile metabolice - manual clinic pentru medicii de familie.* 1999, Editura National.

[8] Ionescu-Tîrgoviste C et al. *Tratat de diabet Paulescu*.Editura Academiei Române, 2004, Bucuresti.

[9] Lupu LD. *Aspecte ale bolii hepatice alcoolice si ale steatohepatitei nealcoolice*. Editura Universitară ”Carol Davila”, 2003, Bucuresti.

[10] Feldman M, Friedman L and Brandt L. *Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology, diagnosis, management*. 2010, Elsevier Health Sciences.

[11] Cecil RL, Goldman L and Schafer AI. *Goldman's Cecil Medicine*. 24th ed. Philadelphia: 2012, Elsevier/Saunders.

[12] Bhutto A, Morley JE. *The clinical significance of gastrointestinal changes with aging*. Curr Opin Clin Nutr Metab Care, 2008,11(5):651-60.

[13] Brown NA, Levine JL and Zenilman ME. *Age-Related Changes in the Gastrointestinal Tract*. Rosenthal RA, Zenilman ME and Katlic MR (eds) Principles and Practice of Geriatric Surgery, 2011, Springer New York, 711-27.

[14] Sinclair AJ ed. *Diabetes in old age*. 2009, John Wiley & Sons.

[15] Krishnan B et al. *Gastrointestinal complications of diabetes mellitus*. World J Diabetes, 2013,4(3): 51-63.

[16] American Diabetes Association. *Standards of Medical Care in Diabetes-2016.* Diabetes Care, 2016,39 Suppl 1:81-6.

[17] Bashashati M, Sarosiek I, McCallum RW. *Epidemiology and mechanisms of gastroesophageal reflux disease in the elderly: a perspective*. Ann N Y Acad Sci., 2016, Aug 15. doi: 10.1111/nyas.13196. [Epub ahead of print]

[18] Kulig M et al. *Risk factors of gastroesophageal reflux disease: methodology and first epidemiological results of the ProGERD study*. J Clin Epidemiol, 2004, 57(6):580-9.

[19] He C et al. *Helicobacter pylori infection and diabetes: Is it a myth or fact?* World J Gastroenterol, 2014, 20(16): 4607–17.

[20] Kayar Y et al. *Relationship between Helicobacter pylori Infections in Diabetic Patients and Inflammations, Metabolic Syndrome, and Complications*. International Journal of Chronic Diseases, 2015.

[21] Buzás GM. *Metabolic consequences of Helicobacter pylori infection and eradication*. World J Gastroenterol, 2014, 20(18):5226-34.

[22] Firneisz G. *Non-alcoholic fatty liver disease and type 2 diabetes mellitus: The liver disease of our age?* World J Gastroenterol, 2014, 20(27): 9072–89.

[23] Portillo-Sanchez P et al. *High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels*. J Clin Endocrinol Metab, 2015,100(6):2231-8.

[24] Stinton LM and Shaffe EA. *Epidemiology of Gallbladder Disease: Cholelithiasis and Cancer Gut Liver*, 2012, 6(2): 172–87.

[25] Amigo L et al. *Risk factors and pathogenesis of cholesterol gallstones: state of the Art.* Eur Rev Med Pharmacol Sci , 1999, 3:241-6.

TRAUMA IN THE ELDERLY

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Abstract. Methodology: The objective of this study has been to identify the symptoms of PTSD according to DSM-5, in people over 65 years who had the following traumatic events: trauma of loss, impaired physical and mental health in the last five years. Subjects*:* The research was conducted in a total of 40 subjects aged between 65 and 87 years. The total study-group included two subgroups with the same number of elderly subjects (n=20) who had no symptoms of PTSD at the time of evaluation and elderly who had PTSD symptoms at the time of evaluation. Tools*:* Interviews, Montreal Cognitive Assessment (MoCA), Short Mood Scale and a Screening based on PTSD Criteria from DSM-5, were used. Results*:* Not all of the elderly, who had experienced a traumatic event in their lifetime, had PTSD symptoms. Depression was stronger in older people with PTSD symptoms, whereas anxiety was stronger in elderly who had no PTSD symptoms.
Key words: trauma, elderly, depression, anxiety

TRAUMA LA VÂRSTNICI

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Rezumat. Metodologie**:** Obiectivul acestui studiu este identificarea simptomelor tulburarii de stres posttraumatic conform DSM-5, la persoanele de peste 65 de ani, care in ultimii 5 ani au trecut prin urmatoarele evenimente traumatice: trauma de pierdere, afectarea sanatăţii fizice si psihice. Subiecti*:* Cercetarea s-a desfasurat pe un numar de 40 de subiecţi cu varste cuprinse intre 65 si 87 de ani. Lotul total a fost format din doua loturi egale ca numar, persoane varstnice care prezentau si persoane varstnice care nu prezentau simptome ale tulburarii de stres posttraumatic la momentul evaluarii. Instrumente*:* Am utilizat interviul, Evaluarea Cognitiva Montreal (MoCA), Scurta Scala de Dispozitie, Screening pe baza criteriilor pentru PTSD din DSM-5. Rezultate*:* In urma studiului efectuat, s-a observat ca nu toate persoanele varstnice care au avut parte de un eveniment traumatic pe parcursul vietii lor, prezinta si la momentul actual simptome ale tulburarii de stres posttraumatic. Am observant de asemenea ca depresia este mai puternica la varstnicii care prezinta simptome ale tulburarii de stress posttraumatic, iar anxietatea este mai puternica la varstnicii care nu prezinta simptome ale tulburarii de stress posttraumatic.
Cuvinte cheie: trauma, varstnici, depresie, anxietate

INTRODUCTION

The period of old age, the one that makes the subject of this work occurs after 65 years. During this period, the personal integrity crisis, the opposite of despair, appears as various retrospectives such as lifetime achievements, and how his life was lived.Currently, a person who has a sense of acceptance of his life is considered fully integrated in this age. [1]

The most traumatic situations which appear in elderly lives are related to professional and marital status. Degenerative diseases make of this fragile period an estrangement of social uselessness and abandon, as at the time of this personal crisis, children become

adults and leave parental home together with their own families.

Other important traumatic situations would be the aforementioned children leave from home in order to have their own new family, elderly leaving the old home to live together with their children, family conflict relations, adapting to solitude after death of one partner, lower incomes and constraints to accept institutional placement. [2]

Many seniors are in these traumatic situations without anyone's assistance, often because of interpersonal relationships which they no longer are able to keep, or seniors' refusals to maintain them.

As a result of psychological trauma, the elderly often escape grievance but fall into by depression or anxiety as the most common affective mental illnesses.

From psychological point of view, at sensorial level compensation phenomena occur despite changes which already took place and this compensatory ability is even greater as exercised profession has been linked to the sensorial level above mentioned [3].

Cognitive changes occur because there is a decreased capacity to acquire new information, but there is a remarkable ability to use existing knowledge. Intellectual abilities remain relatively active between 65 and 75 years, though they are not as fluent as before and there are moments of intellectual vacuum [4].

In terms of affection, the elderly's emotions become primitive; depression becomes common and affects both internal balance and relationships with others. This depression is most often accompanied by the idea of death. [5]

In few elderly, depression is regret for happy periods of personal life but the majority has depression in its most accentuated form when life partner dies.

With the onset of depression, hopelessness idea occurs which leads to a tragic experience of the phenomenon of death.

In the second stage of aging, most people are concerned about them and become easily irritable with no reason. We may say that there is a self hypertrophy, "dilation" of lived events and personal rights. It has been confirmed that suicide is more common in men aged over 65 years in spite of the fact that true personality structure disruption occurs after the age of 70-75 years.

A psychotraumatic moment in elderly lives is the withdrawal from professional activity. Trauma left by retirement is so great that it has been called "social death" or "retired shock".

Most of the elderly perceive the phenomenon of "retirement" as a major loss, an immense change in a person's life. Many of them do not know how to accept it and try to make sense of it before it happens. For this, in the UK, was conducted an education program prior to retirement was conducted and dedicated to people who were on the brink of retirement.

Other important traumatic situations would be the departure of children from the parental home in order to build their own family, abandonment of their home to live with children, conflicting relations in the family, adapting to solitude in the death of one partner, lower revenues and obligation to accept institutional placement.

Many elderly people live in these psychotraumatic situations without receiving help from someone, often because of interpersonal relationships that often are not able to maintain them, or refuse to maintain them.

Many social, demographic, psychosocial and biological factors contribute to a certain mental state. Seniors are more likely to have suffered heavy losses or physical disabilities that affect their emotional wellbeing and mental harm. Also they may be mistreated at home or in institutions where they are cared for, with long-term psychological consequences, such as depression or anxiety. [6]

Social support and interaction with family can enhance the dignity of older people and can be protective on their mental health. Solidarity between generations is shrinking, especially in high-income countries, while those with medium or low income,

grandparents are living with grandchildren more often.

Demographic changes bring not only challenges but also opportunities: in many countries, older people are healthier than in the past and can make important contributions - beyond the usual role of wise counselor - as family members, volunteers or even employees due to increasing retirement age. [7]

A trauma is a vital experience of discrepancy between threatening situational factors and the individual's coping capacities, that is associated with feelings of helplessness and lack of any protection, thus creating a permanent instability of the self concept and perception of the world.[8]

The essence of human existence includes entry attachment in relationships. Attachments are established primarily through the experiences and the emotional experience ensures durability and permanence of the relationship. Only when between two people or more there is an emotional bond they stay together and care for each other. [9]

Some traumatic losses occur suddenly; others are announced slowly, for example, the death of a partner or a child from an incurable disease. But you can prepare for announced losses ahead of time by contrast to a sudden death. And yet these losses can cause long lasting trauma.

Posttraumatic Stress Disorder
Diagnostic Criteria 309.81 (F43.10)

Posttraumatic Stress Disorder

A. Exposure to actual or threatened death, serious injury, or sexual violence in one (or more) of the following ways:

1. Directly experiencing the traumatic event(s).

2. Witnessing, in person, the event(s) as it occurred to others.

3. Learning that the traumatic event(s) occurred to a close family member or close friend. In cases of actual or threatened death of a family member or friend, the event(s) must have been violent or accidental.

4. Experiencing repeated or extreme exposure to aversive details of the traumatic event(s) (e.g., first responders collecting human remains: police officers repeatedly exposed to details of child abuse).

Note: Criterion A4 does not apply to exposure through electronic media, television, movies, or pictures, unless this exposure is work related.

B. Presence of one (or more) of the following intrusion symptoms associated with the traumatic event(s), beginning after the traumatic event(s) occurred:

1. Recurrent, involuntary, and intrusive distressing memories of the traumatic event(s).

2. Recurrent distressing dreams in which the content and/or affect of the dream are related to the traumatic event(s).

3. Dissociative reactions (e.g., flashbacks) in which the individual feels or acts as if the traumatic event(s) were recurring. (Such reactions may occur on a continuum, with the most extreme expression being a complete loss of awareness of present surroundings.)

4. Intense or prolonged psychological distress at exposure to internal or external cues that symbolize or resemble an aspect of the traumatic event(s).

5. Marked physiological reactions to internal or external cues that symbolize or resemble an aspect of the traumatic event(s).

C. Persistent avoidance of stimuli associated with the traumatic event(s), beginning after the traumatic event(s) occurred, as evidenced by one or both of the following:

1. Avoidance of or efforts to avoid distressing memories, thoughts, or feelings about or closely associated with the traumatic event(s).

2. Avoidance of or efforts to avoid external reminders (people, places, conversations, activities, objects, situations) that arouse distressing memories, thoughts, or feelings

about or closely associated with the traumatic event(s).

D. Negative alterations in cognitions and mood associated with the traumatic event(s), beginning or worsening after the traumatic event(s) occurred, as evidenced by two (or more) of the following:

1. Inability to remember an important aspect of the traumatic event(s) (typically due to dissociative amnesia and not to other factors such as head injury, alcohol, or drugs).

2. Persistent and exaggerated negative beliefs or expectations about oneself, others, or the world (e.g., "I am bad," "No one can be trusted," 'The world is completely dangerous," "My whole nervous system is permanently ruined").

3. Persistent, distorted cognitions about the cause or consequences of the traumatic event(s) that lead the individual to blame himself/herself or others.

4. Persistent negative emotional state (e.g., fear, horror, anger, guilt, or shame).

5. Markedly diminished interest or participation in significant activities.

6. Feelings of detachment or estrangement from others.

7. Persistent inability to experience positive emotions (e.g., inability to experience happiness, satisfaction, or loving feelings).

E. Marked alterations in arousal and reactivity associated with the traumatic event(s), beginning or worsening after the traumatic event(s) occurred, as evidenced by two (or more) of the following:

1. Irritable behavior and angry outbursts (with little or no provocation) typically expressed as verbal or physical aggression toward people or objects.

2. Reckless or self-destructive behavior.

3. Hypervigilance.

4. Exaggerated startle response.

5. Problems with concentration.

6. Sleep disturbance (e.g., difficulty falling or staying asleep or restless sleep).

F. Duration of the disturbance (Criteria B, C, D, and E) is more than 1 month.

G. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

H. The disturbance is not attributable to the physiological effects of a substance (e.g., medication, alcohol) or another medical condition. [10]

Specify whether:

With dissociative symptoms: The individual's symptoms meet the criteria for posttraumatic stress disorder, and in addition, in response to the stressor, the individual experiences persistent or recurrent symptoms of either of the following:

1. Depersonalization: Persistent or recurrent experiences of feeling detached from, and as if one were an outside observer of, one's mental processes or body (e.g., feeling as though one were in a dream; feeling a sense of unreality of self or body or of time moving slowly).

2. Derealization: Persistent or recurrent experiences of unreality of surroundings (e.g., the world around the individual is experienced as unreal, dreamlike, distant, or distorted).

Note: To use this subtype, the dissociative symptoms must not be attributable to the physiological effects of a substance (e.g., blackouts, behavior during alcohol intoxication) or another medical condition (e.g., complex partial seizures).

Specify if:

With delayed expression: If the full diagnostic criteria are not met until at least 6 months

after the event (although the onset and expression of some symptoms may be immediate).

The objective

The objective of this study is to identify PTSD symptoms according to DSM-5, in people over 65 years that have been through the following traumatic events: trauma of

loss, impaired physical and mental health in the last five years.

Hypotheses

- Elderly people who have gone through a traumatic event show symptoms of PTSD at the time of evaluation.
- Elderly people showing symptoms of PTSD present a higher score of depression.
- Elderly people showing symptoms of PTSD present a higher score of anxiety.

Presenting the lot of subjects

The research was conducted in a total of 40 subjects aged between 65 and 87 years. The total group included two equal size groups, elderly with symptoms of PTSD and older people without symptoms of PTSD at the time of evaluation. Both groups of elderly, were assessed and interviewed at the Institute of Gerontology and Geriatrics "Ana Aslan" Bucharest Headquarters - Clinics I and II. The two study- groups of subjects were independent heterogeneous batches, each consisting of about 20 people aged between 65 and 87 years.

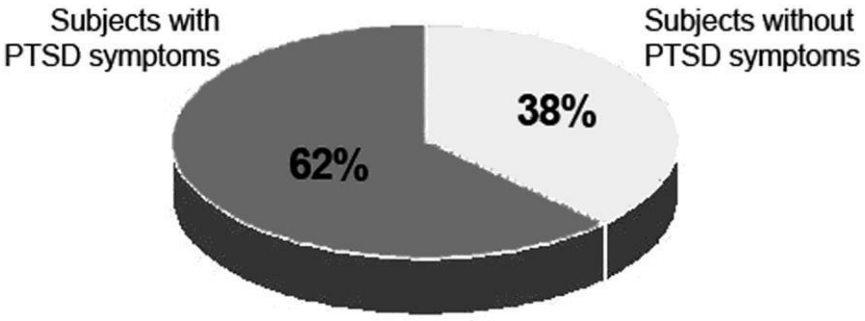

Figure 1. Presentation of the total group of subjects

Issues encountered in subjects: bereavement, ill health, depression and anxiety. The instruments used were the interview, cognitive tests and affective tests (Montreal Cognitive Assessment (MoCA), Short Mood Scale, screening for PTSD based on the criteria of DSM-5 [10]

Operational criteria for selecting subjects:

- The presence of a traumatic event in the past 5 years;
- Visual acuity and hearing or adequate correction;
- Compliance and cooperation for assessment;
- Apt in clinically;
- Without psychiatric medical history;

Operational criteria for excluding subjects:

- Age less than 65 years;
- Sensorial deficiences or severe language impairments;
- Compliance and non-cooperation;
- Clinically unfit;
- With psychiatric history;

The first working hypothesis was that older people who have experienced a traumatic event in the past 5 years had symptoms of PTSD at the time of evaluation. Null hypothesis showed that there were two distinct groups, some with PTSD, and others without PTSD.

Null hypothesis was confirmed in that not all people who have been through a traumatic event had symptoms of PTSD.

T1 score for 19 degrees of freedom, was 35, 29 for the group of elderly showing PTSD symptoms at the time of assessment and the T2 score for 19 degrees of freedom, was 9.6 for the second group, elderly people who had no symptoms PTSD at the time of evaluation. For both scores, statistical significance was $p < 0.01$, so the differences between averages were statistically significant.

							Evaluation's results				
Nr. Crt.	Name	Gendre	Age	Marital status	Studies	MoCA	Short Scale of Disposition		Criterias for PTSD	Traumatic events	Time passed
							Anxiety	Depression			
1	P.M.	F	65	D	medium	27	7	2	6	bereavement	1 year
2	S.L.	F	81	D	medium	20	5	9	7	bereavement	1 year
3	C.A.	F	72	C	7 classes	20	7	6	6	bereavement	2 years
4	A.M.	F	67	D	medium	28	4	6	6	ill health	5 years
5	H.C.	F	87	V	7 classes	20	3	3	5	ill health	3 months
6	L.M.	F	74	C	medium	21	3	6	6	bereavement	3 years
7	R.T.	F	71	V	4 classes	20	3	7	7	bereavement	2 years
8	D.I.	F	77	V	7 classes	21	1	1	6	bereavement	1 year
9	S.E.	F	65	C	8 classes	25	9	6	5	despression	1 year
10	T.N.	M	74	C	6 classes	20	2	2	6	ill health	5 years
11	M.S.	M	72	C	medium	21	4	5	5	ill health	4 years
12	S.M.	F	78	C	8 classes	23	9	8	5	ill health	3 years
13	S.E.	F	82	C	4 classes	20	7	9	5	ill health	1 year
14	A.I.	F	73	V	7 classes	28	5	3	5	anxiety	2 years
15	N.G.	M	68	V	7 classes	25	9	5	6	ill health	4 years
16	P.L.	F	70	V	7 classes	21	3	9	7	bereavement	4 years
17	B.F.	F	66	D	superior	28	5	4	7	depression	2 years
18	B.E.	F	79	V	6 classes	23	5	4	7	bereavement	3 years
19	N.M.	F	65	C	7 classes	25	3	6	6	depression	1 year
20	M.M.	F	80	C	8 classes	24	2	3	7	bereavement	1 year

Table I. Elderly with symptoms of PTSD when assessing

							Evaluation's results				
Nr. Crt.	Name	Gendre	Age	Marital status	Studies	MoCA	Short Scale of Disposition		Criterias for PTSD	Traumatic events	Time passed
							Anxiety	Depression			
1	D.M.	F	69	V	7 classes	30	4	2	4	bereavement	5
2	M.B.	F	84	V	superior	25	6	3	3	bereavement	4
3	T.C.	F	77	C	medium	20	6	4	3	anxiety	5
4	P.E.	F	82	V	medium	24	2	4	4	ill health	5
5	V.P.	M	83	C	superior	25	1	2	4	bereavement	3
6	H.A.	F	65	C	medium	21	3	7	2	ill health	2
7	M.V.	F	74	C	medium	22	6	6	1	ill health	1
8	N.F.	F	80	V	4 classes	11	2	2	3	bereavement	3
9	C.G.	F	66	V	medium	25	9	5	2	ill health	4
10	P.I.	M	73	C	7 classes	25	8	8	1	depression	2
11	T.M.	F	65	C	medium	24	9	5	0	anxiety	1
12	P.S.	F	78	D	6 classes	21	5	6	3	bereavement	1
13	A.S.	M	68	C	superior	24	4	4	4	bereavement	2
14	P.M.	F	87	V	7 classes	16	7	1	2	anxiety	3
15	B.A.	F	65	C	7 classes	28	9	6	1	depression	1
16	R.N.	F	69	C	medium	28	3	2	4	bereavement	4
17	P.E.	M	86	C	medium	12	3	2	2	depression	2
18	P.A.	F	81	V	medium	25	3	7	4	bereavement	2
19	C.M.	M	73	C	medium	26	4	6	3	ill health	3
20	T.I.	M	82	V	superior	19	6	7	2	bereavement	1

Table II. Elderly showing no symptoms of PTSD when assessing

The second working hypothesis was that older people showing PTSD symptoms at assessment had a higher score of depression. The variation coefficient was 5.95 in the first batch, meaning the elderly who had symptoms of PTSD, and 4.57 in the second batch, meaning the elderly who had no symptoms of stress disorder posttraumatic.

The coefficient of variation less than 15% of the subscales values in the two groups (the elderly group with symptoms of PTSD v = 5.95 and elderly group without symptoms of PTSD v = 4.57) confirmed groups'homogeneity, the dispersion was small, so averages were representative.

A third working hypothesis was that older people showing symptoms of PTSD at evaluation have a higher score of anxiety.

The variation coefficient was 6.06 in the first batch, ie the elderly showing symptoms of PTSD, and 6.21 in the second batch, ie the elderly who had no symptoms of stress disorder posttraumatic.

The coefficient of variation less than 15% of the value scales in the two groups (the elderly group with symptoms of PTSD v = 6.06 and elderly group without symptoms of PTSD v = 6.21) confirmed once again groups'homogeneity, the dispersion was small, so averages were representative.

CONCLUSIONS

The study conducted noticed that depression is more pronounced than anxiety in the elderly with symptoms of PTSD at the time of the assessment, a fact that was explained by Franz Ruppert (2012). After a traumatic experience no man is the one before. In the trauma of loss, processing spiritual wound is rather incomplete and the personality of affected human is cleaved. A part of personality takes over the memory feeling the loss and retires (dissociates), and another part continues to live denying feelings reminiscent of the trauma.

The part of personality carrying the memory of the trauma of loss lives in a state that could be defined as a chronic sadness or "depression". Symptoms of this condition are well known in psychiatry and psychotherapy. Losses that are experienced as tension and stress lead to depressive reactions, which fade after about six months at most, and after a few years it is as if these reactions never even existed.

Trauma of loss are due to depression, which become gradually more severe and chronic then.

I also noticed that not all of the people aged over 65 who have experienced a traumatic event, are showing symptoms of PTSD at the time of evaluation, although they went through this process at some point and I could put this issue on behalf of an increased resilience of the elderlys, than others.

Because lots that have made this research were small, I leave room for a more detailed research on this subject.

REFERENCES

[1] Balaceanu-Stolnici C. *Geriatrie*. Ed. Medicala, 1997, Bucuresti, 5-27

[2] Draghici R. *Rolul familiei in asistarea persoanei varstnice cu probleme*, in Rada C.&Faludi C. (coord.), Functii si disfunctii ale familiei contemporane. O abordare socio-psiho-medicala, Editura Universitara, 2015, Bucuresti, 71-83.

[3] Fontaine R. *Psihologia imbatranirii.* 2008, Iasi: Polirom, 11-34.

[4] Gusic V.I. *Biologia varstelor si lupta impotriva batranetii*. Ed. Stiintifica si Enciclopedica, 1984, Bucuresti.

[5] Draghici R. *Depresia geriatrica – importanţa evaluarii depresiei la persoanele varstnice*, in Popa C. & Ciobanu A.M. (coord), Tulburarea depresiva, Vol. II, Elemente de psihodiagnostic, terapii moderne si studii de caz, Editura Academiei Romane, 2014, Bucuresti, 45-54.

[6] Vrasti R. *Masurarea Sanatatii Mentale* (carte in format electronic), 01/2009.

[7] Institutul Naţional de Statistica. *Populaţia Romaniei la 1 iulie 2008*. Principalele *caracteristici demografice*. Anuarul I.N.S., 2008.

[8] Fischer G. und Riedesser P. *Tratat de psihotraumatologie*. Editura TREI, 1999, Bucuresti, p. 79 .

[9] Vasile D.-L. *Trauma familiala si resursele compensatorii*. Editura SPER, 2012, Bucuresti, 19-22.
[10] American Psychiatric Association. *DSM-5*. Ed. Medicala Callisto, 2013, Bucuresti, 271-272.