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THE PHYSIOLOGY OF ENGLISH AS A *LINGUA FRANCA* IN MEDICINE

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ABSTRACT

Based on the theoretical research on the concepts of cross-linguistic influence and interference, the study outlines the effects of cross-linguistic influence of English on Romanian, especially at the lexico-semantic level, in the scientific field of medicine. The analysis is pursued first diachronically, with focus on the advent of English as a lingua franca in medicine, and finally synchronically, providing a short list of neological medical Anglicisms, and some samples of texts in which lexical English borrowings occur. Notions such as language contact, bilingualism, positive and negative transfer are passed into review, and the review closes on the necessity of a more thorough analysis of the present-day Romanian medical terminology.
Keywords: lingua franca, medical language, language contact, cross-linguistic influence, bilingualism

ENGLISH AS A LINGUA FRANCA IN MEDICINE – DIACHRONICAL ACCOUNT

In outline, physiology, as defined by one of the "giants in the fields of physiology and medicine", Dr. Arthur Guyton, means "the science of function in living organisms" (9). My treatment of what I call "physiology of English" relies upon analogy. It is a non-standard, own view of the topic, in which the functioning of a language may be seen as resembling the functioning of the human body, or of any organ of the human body.

A few words about the metalanguage would be of use now. The term lingua franca refers to the earliest Romance-based pidgin and has gained the meaning of a widely used auxiliary language to enable communication between people of different mother tongues (13).

In medicine, English replaced, one by one, other lingua francas of communication. Latin was the lingua franca of Western medical writing for several centuries. The roots of Western medicine lie in Greek. Medical learning was transmitted in Latin translations of Greek and Arabic texts, mostly by translators whose first language was not a European vernacular, but Arabic or Greek. Galen's texts became available in the XIIIth century in Latin commentaries, with several layers of additions. Medical texts began to be translated into vernacular languages such as French, English, German, Portuguese, and Catalan in the XIVth and XVth centuries, almost simultaneously in different parts of Europe. However, at that time, Latin retained its strong position as a pan-European language of science. The situation started to change in France, at the end of the XVIth century, and in England, at the end of the XVIIth century (14), when several authors began to publish in both vernacular languages and Latin. But Latin still retained its position longer in other parts of Europe, for example in German-speaking countries. Several scholars think that publication in medicine from the XVIIth century onward played a part in nationalizing medical communication (13). In 1551, one of the first French medical dictionaries, Traicté familier des noms grecs, latin, arabiques, ou vulgaires, aveques les définitions de toutes les maladies qui surviennent superficiellement au corps humain, was published (11).

In the early XXth century, there were rival languages for the lingua franca position in science: French, German, and English. German was a very strong candidate before the Second World War. German had served as a lingua franca in large parts of Europe for centuries, for example, in the Baltic area since the Middle Ages, and the names of several scholarly journals and series in many fields are still in German.

French medical literature, dictionaries and treatises were also at their peak till the 1950s and 1960s, when the situation started to change in favour of English with the increasing impact of Anglo-American culture. After the World War II, all the countries in Western Europe were affected to a greater or lesser degree by the dominant role of the United States of America, which was related to several well-known factors, such as their military, political, economic, scientific, and technological leadership, as well as the creation of the Atlantic Alliance and the diffusion of the culture, lifestyles, and behaviours of the English-speaking world.

The contemporary status of English as a global language is primarily the result of two factors: the former expansion of British colonial power, which peaked towards the end of the XIXth century, and the emergence of the United States of America as the leading power of the XXth century. It is the latter factor that continues to explain the position of the English today, much to the discomfiture of some in Britain who find the loss of linguistic preeminence unpalatable (7).

On the other hand, it is sometimes thought that English has achieved the worldwide status because of its intrinsic linguistic features. People have claimed that it is inherently a more logical or more beautiful language than others, easier to pronounce, simpler in grammatical structure, or larger in vocabulary. This kind of reasoning is naive, inadequate, there are no objective standards of logic or beauty to compare languages, and questions of phonetic, grammatical, or lexical complexity

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are never capable of simple answers. For example, English may not have many inflectional endings, but it has a highly complex syntax.

In the recent years, English has become a prime vehicle for the transmission of information, which explains its nearly absolute dominance in most scientific fields, because not only the world's most widely cited medical journals but also most of the best contributions in science and medicine are published in English in international European or American journals. To this overall presence of English in traditional written communication systems, we must add the World Wide Web and the computer networking Internet, whose predominantly English voice has been rapidly exported from and imported into many languages. "Therefore, non-English-speaking scientists, researchers, and practicing doctors have no other option but to learn English if they want to be informed of the latest developments in their fields" (1).

"As English has turned into the primary medium of international specialized publication, many non-English-speaking scientists, being aware of the relevance of medical literature in English to their work and wanting to obtain responses to it, find it more effective to publish in English than in their native language" (1). In this respect, it is interesting to note that many nations, Romanian academic life included, measure the productivity of their top international scientists and scholars by the number of times their works are quoted in English-language publications with an impact factor by the Science Citation Index.

Apart from being the primary medium of scientific publication, English has likewise emerged as the main language of international meetings of specialists and of international scientific exchanges. In fact, the high level of technical and scientific knowledge, the necessity of collaboration among several specialists in order to establish a common base for work, and the complexity of the organization of production and of services in today's society are all factors that foster the use of the same technical terms contemporaneously.

"This trend to increasingly use one lingua franca, and in relatively few journals for each science, favours a smoother communication between scientists and, consequently, a rapid progress in science" (1).

Thus, the continually increasing contact between non-English-speaking scientists and the English-speaking scientific world, mainly through reading and, to a lesser extent, through writing and attending conferences, reaches even national meetings, everyday informal conversations between colleagues, and national journals, such as the following Romanian medical journals: Physiology, The Journal of Cardiovascular Surgery, Timisoara Medical Journal etc., in which articles are published directly in English.

However, despite the obvious advantages of the present-day status of English as a lingua franca, its achievement nevertheless runs parallel to a series of interrelated pitfalls, of which we focus on the great number of loan words from English.

LANGUAGE CONTACT AND BILINGUALISM

One of the drawbacks entailed by the supremacy of English in the world of science would be that the language contact between English and other vernacular languages brings about massive linguistic transfer, which could end up in altering the cultural integrity of a language (8). In all cases of language contact, speakers of one language may, deliberately or unconsciously, introduce into their language features of another language to which they have been exposed. Language contact is a process that determines the use of two or more languages, therefore, it is a source of individual or group bilingualism (3), or of multilingualism. Contact between languages only happens in the minds of bilingual (or multilingual) speakers.

Nowadays, most of the world's population is thought to be bilingual or multilingual. Taking Europe as reference, many millions of Europeans are at least bilingual, speaking both their mother tongue and the national language of the country they live in, and many of them can additionally speak a global language or world language, like English, which is a good reason to believe that bilingualism or multilingualism has been the norm for most human beings at least for the last few millennia.

On the one hand, language contact results in cross-linguistic influence or language transfer, but on the other, language transfer is not always positive, on the contrary, it may be negative, ranging from limited lexical borrowing with casual contact and limited bilingualism to heavy structural influence from very intensive contact and bilingualism.

ANGLICISMS IN THE ROMANIAN MEDICAL LANGUAGE

Romanian medical terminology, which, as different from other non-English European medical languages, seems to have been unduly overlooked in the recent years, rests on a fundamentally Latin nomenclature and on neologisms built up with roots, prefixes, and suffixes drawn from Greek and Latin, especially in the fields of anatomy and physiology. From the latter half of the XVIIIth century, when Romanian medical writing appeared, to the recent past, Latin and French influenced the Romanian medical language. Traces from other European languages, such as Italian, and German may be found as well.

As English has turned into the most powerful medium of medical and scientific communication in Europe, it is not surprising to find many English words in Romanian medical language, which have also entered Romanian medical dictionaries (11), a proof of their acceptance within Romanian medical communities. We provide a short list of neological Romanian medical Anglicisms, open to completion: banding, borderline, bridge to recovery, floppy, bridge to transplant, bypass, clubbing, pacemaker, end-stage, flail, flapping tremor, flutter, follow-up, graft, guideline, patch, marker, rash, scallop, prick, pattern, screening, scratch, thrill, turnover, slice, stripping, trigger, shunt, stem cell, target, feedback.

The lexemes of the above list are examples of univerbal lexical Anglicisms with geminated vowels and consonants (eg. flutter, patch, pattern, scratch), multiverbal lexical Anglicisms with consonant groupings (eg. feedback), compounds that contain a noun and a particle, generally considered difficult to translate adequately (eg. follow-up, bypass, turnover), phrases with nouns related by a preposition, also considered difficult to translate (eg. bridge to transplant, bridge to recovery), and –ing simple and compound terms, extremely popular with specialist writers (eg. banding, screening, stripping) (1).

The influence exerted by English on the Romanian medical language has affected all levels of linguistic systems, ranging from lexis and semantics to syntax and pragmatics, with the borrowing of vocabulary items being nevertheless by far the most common.

As previously stated, language transfer may be positive, when the loan-words occur in response to a demand for the expression of a new concept originating in another country, and the word/phrase adopted fits the phonetico-phonological and lexico-semantic Romanian environment, but also negative. Without a disregard for the former type of cross-linguistic influence, a thorough survey of the latter is almost compulsory for the medical language, where any terminological ambiguity or error, affecting the oral code, as well as the written discourse, may have serious consequences in real life. Instance the following different cases of negative transfer: false friends (Engl. dramatically - Rom. dramatic; Engl. murmur - Rom. murmur; Engl. insult - Rom. insultă etc.), polysemantic words (switch, cleft, marker, management), inadequate calques, either lexical or grammatical (Engl. in the population - Rom. în populația), and English doublets (synonymous variants) for

already existing words in Romanian (Engl. rash / Rom. erupție; Engl. pacemaker / Rom. stimulator cardiac).

And finally, as lexical items only function in texts, the following samples of texts, taken from a national medical journal, the Romanian Journal of Cardiovascular Surgery (4), in which the majority of the articles are written in English, with a few exceptions, reflect the present state of the Romanian written discourse. The main terms are introduced in italic and bold letters:

1. Ecocardiografia a devenit metoda de neînlocuit în evaluarea valvulopatiilor: toate guideline-urile pentru valvulopatii folosesc numai criterii ecocardiografice în algoritmul de decizie (5).

2. Valva trebuie considerată, metaforic vorbind, ca o structură dinamică, formată din şase cuspide dispuse în perechi de câte două scallop-uri, scallop-ul A1 vs P1, scallop-ul A2 vs scallop-ul posterior P2 şi perechea A3-P3 (5).

3. Sunt posibile şi alte soluții, deşi sunt mult mai rar folosite pentru acest tip de leziune – folosirea de corzi artificiale de goretex pentru restabilirea coaptării valvei la nivelul scallop-ului sau a tehnicii "edge to edge" [. . .] (5).

4. Sunt deja clare astăzi atât ecocardiografiştilor cât şi chirurgilor conceptele de valvă mitrală floppy, de valvă mitrală flail, ca şi de prolaps valvular mitral (5).

5. Corecția chirurgicală anatomică (switch arterial) a transpoziției de vase mari a devenit în ultima decadă intervenția de elecție în țările dezvoltate [. . .] (6).

6. Am analizat datele pre-, peri- şi postoperatorii a 1461 pacienți care au fost supuşi unor operații primare de bypass aortocoronarian (on şi off pump) [. . .] (2).

A WORD IN CLOSING

The present article, as incomplete and open to further discussions and individual viewpoints, claims, and suggestions it may be, points out that the linguistic material the present-day Romanian medical literature provides us with is at least interesting, if not fascinating to survey, and a linguistic study would be extremely helpful, not to say absolutely necessary for a proper assimilation of the numerous Anglicisms to the Romanian linguistic rules. English medical terms borrowed should be at first well understood, in their phonological, semiotic and morphosyntactic behaviour, and then adapted to our language, with a maximum of precision, clarity, and accuracy.

Without a close survey from linguists, Romanian medical terminology is on the way of becoming more and more heterogeneous, a mixture of Hellenisms, and English neologisms, and medical discourse, written and oral, in a constant effort to keep up with the English medical language, is adopting mechanically/ad litteram English words, phrases or grammatical structures, thus giving rise to dangerous ambiguities and confusions.

The study of language, as Ferdinand de Saussure clearly defined it, in his celebrated langue—parole dichotomy, is the study of life in so far it is the study of society (12). That is why I imagined a parallel between a linguistic aspect, the functioning of language, and the functioning of the living organisms. Therefore, to conclude this article in the same spirit it started, I will quote from Dr. Guyton's address to the American Physiological Society in 1975, appropriately entitled Physiology, a Beauty and a Philosophy: "What other person, whether he be a theologian, a jurist, a doctor of medicine, a physicist, or whatever, knows more than you, a physiologist, about life? For physiology is indeed an explanation of life. What other subject matter is more fascinating, more exciting, more beautiful than the subject of life?" (10).

In its turn, medical terminology, with all the linguistic and extra-linguistic aspects entailed, offers both terminologists and medical practitioners a large and extremely beautiful field of research. However, as a Danish physician says, "there is no recognized discipline called medical linguistics, but perhaps there ought to be one", as the study of the language of medicine may be a challenge for linguists and doctors, offering to the latter a new dimension to their professional language (15).

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FIZIOLOGIA ENGLEZEI CA *LINGUA FRANCA* ÎN MEDICINĂ

REZUMAT

Având la bază studiile teoretice asupra conceptelor de influență şi interferență lingvistică, articolul urmăreşte influența limbii engleze asupra limbii române din domeniul ştiințific medical, în special la nivel lexico-semantic. Analiza, la început diacronică, cu accent pe impunerea limbii engleze ca lingua franca în medicină, este urmată de o abordare sincronică, oferind o scurtă listă de anglicisme medicale neologice şi câteva fragmente de text în care apar împrumuturi lexicale din limba engleză. Sunt, de asemenea, trecute în revistă conceptele de contact lingvistic, bilingvism, transfer pozitiv şi negativ, iar trecerea în revistă se încheie cu afirmarea necesității unei analize mai profunde a terminologiei medicale româneşti actuale.

Cuvinte cheie: lingua franca, limbaj medical, contact lingvistic, influență lingvistică, bilingvism

MOLECULAR REGULATION OF SKELETAL MUSCLE ATROPHY

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ABSTRACT

This review focuses on the most recent findings related to molecular regulation of muscle atrophy. Prolonged periods of skeletal muscle inactivity due to bed rest, denervation, hindlimb unloading, immobilization, or microgravity can result in significant muscle atrophy. Skeletal muscle health involves maintenance of an intricate balance between protein synthesis and degradation. Atrophy results from a perturbation in this intricate modulation of the various synthetic and proteolytic pathways and very little is known about mechanisms involved in initiation of the imbalance Muscle atrophy in a range of conditions is thought to be due to an increased expression of the ubiquitin-proteasome proteolytic pathway. Catabolic agents such as cytokines, proteolysis-inducing factor, and reactive oxygen species are causing an increased gene expression of proteasome subunits. Glucocorticoids cause activation of transcription factors possibly through an increase in expression of myostatin.

Key words: muscle atrophy, ubiquitin-proteasome system, glucocorticoids, myostatin.

INTRODUCTION

Muscle is a highly plastic tissue able to adapt to changing functional demands. Increased load on muscle results in an increase in its mass or hypertrophy, whereas unloading or disuse leads to a decrease in mass or atrophy. Exercise is a key regulator of muscle mass, as is nutrition (18). Muscular atrophy regularly occurs as a consequence of immobilization or disuse after sports injuries. Immobilization is a frequently used treatment for musculoskeletal injuries despite well-documented resulting muscle cell atrophy, intramuscular fibrosis, and loss of muscle extensibility, strength, and endurance (12). Prolonged periods of skeletal muscle inactivity due to bed rest, denervation, hindlimb unloading, immobilization, or microgravity can result in significant muscle atrophy. Several experimental models deal with muscle atrophy and are suitable for investigations of the underlying mechanisms of muscle atrophy. Strength loss is the most evident response to atrophy. Muscle strength decreases most dramatically during the first week of immobilization; little further weakening occurs later on. This is reflected in changes in the EMG of disused muscles and can also be observed in muscle weight and size of muscle fibers (1). Slow muscles with predominantly oxidative metabolism are most susceptible to atrophy as indicated by various findings: slow muscle fibers show greater atrophy than fast fibers; their relative and probably absolute number is decreased in atrophic muscles; in addition, the oxidative enzyme content is most severely affected by disuse. Autophagic activities probably play an important role in early stages of muscular atrophy. The oxygen supply to disused muscle may be impaired, although myoglobin content is increased in atrophic muscle. The complete loss of mitochondrial function during the first days of disuse may be of etiological importance. The amount of connective tissue is increased in atrophic muscle and surrounding periarticular tissue which may lead into a vicious circle of musculoskeletal degeneration. An almost complete recovery from atrophy is possible, yet often the recovery phase is much longer than the total immobilization period (1). The muscle atrophy is characterized as decreased muscle fiber cross-sectional area and protein content, fiber diameter, reduced force, fatigue resistance, increased insulin resistance as well as a slow to fast fiber type transition. Skeletal muscle atrophy attributable to muscular inactivity has significant adverse functional consequences (10). While the initiating physiological event leading to atrophy seems to be the loss of muscle tension and a good deal of the physiology of muscle atrophy has been characterized, little is known about the triggers or the molecular signaling events underlying this process. Decreases in protein synthesis and increases in protein degradation both have been shown to contribute to muscle protein loss due to disuse, and recent work has delineated elements of both synthetic and proteolytic processes underlying muscle atrophy. It is also becoming evident that interactions among known proteolytic pathways (ubiquitin-proteasome, lysosomal, and calpain) are involved in muscle proteolysis during atrophy. Factors such as TNF, glucocorticoids, myostatin, and reactive oxygen species can induce muscle protein loss under specified conditions. Also, it is now apparent that the transcription factor NF-B is a key intracellular signal transducer in disuse atrophy. Transcriptional profiles of atrophying muscle show both up- and downregulation of various genes over time, thus providing further evidence that there are multiple concurrent processes involved in muscle atrophy. The decreases in protein synthesis and increases in protein degradation rates account for the majority of the rapid loss of muscle protein due to disuse (10, 22). Because different events initiate atrophy in different conditions, it seems that the regulation of protein loss may be unique in each case. In fact differences exist between the regulation of the various atrophy conditions, especially sarcopenia, as evidenced in part by comparisons of transcriptional profiles as well as by the unique triggering molecules found in each case. By contrast, recent studies have shown that many of the intracellular signaling molecules and target genes are similar, particularly

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among the atrophies related to inactivity and cachexia (11).

SKELETAL MUSCLE ADAPTATIONS TO INACTIVITY

Because skeletal muscle is the most abundant tissue of the human body, the deceases in its mass possibly will profoundly impact the whole body metabolism and ultimately lead to the development of lifestyle-related diseases (2). Marked decreases in absolute and relative protein contents of skeletal muscles in early stages of disuse is one of the most significant adaptations of this tissue to reduced tension, resulting in fiber shrinking and weakening. Disuse-induced skeletal muscle atrophy is accompanied by a whole-body negative nitrogen balance in humans during spaceflight or bed rest (2, 17). In addition, hindlimb suspension or immobilization for 7 days significantly decreased the total RNA content and the α-actin and cytochrome c mRNA expression in the muscle in rats (2). Moreover, other studies also revealed that the RNA-to-DNA ratio in atrophic muscle decreases considerably in rats and human, indicating reduced capacity for protein synthesis (9). Thus, it can be deduced that changes in protein turnover (concomitatnt upregulation of protein degradation and downregulation of protein synthesis) in myofibers is one of the key mechanisms that orchestrate adaptation of skeletal muscle to unweighting. The balance between protein synthesis and degradation is a critical determinant of muscle cross-sectional area (18). Net protein synthesis results in greater myofibrillar content which is accommodated in a larger myofibres. Significant myofibre hypertrophy also requires an increase in the number of myonuclei so that a constant myonuclear domain (volume of cytoplasm supported by a single nucleus) is maintained. In a muscle, the ratio of DNA/protein is fairly constant (16). Myofibres are post-mitotic cells, and their nuclei do not proliferate. New myonuclei are provided by a population called satellite cells (18). These cells lie just under the basal lamina of myofibres, and are normally found in a quiescent state. Once activated by exercise or muscle damage, satellite cells proliferate and fuse with existing muscle fibres, thus providing new nuclei for hypertrophy and repair. The absence of a satellite cell proliferative response following γ-irradiation of the muscle limits hypertrophic gains. Protein synthesis depends on the energy status in the muscle as it is an ATP-dependent process, and therefore is also regulated by the AMP-dependent kinase AMPK (4, 18). Treatment of rats with an AMPK-activating drug leads to a reduction in protein synthesis accompanied by a decrease in activation of mTOR, p70S6K and 4E-BP1. Protein degradation resulting from disease or disuse can be inhibited by AKT activation. This occurs because AKT phosphorylates and thereby prevents nuclear translocation of the FOXO family of transcription factors. FOXO1 and FOXO3 regulate the expression of two ubiquitin protein ligases in muscle. Ubiquitin ligases link ubiquitin to proteins thereby targeting them for degradation by the ubiquitin–proteasome, an ATP-dependent proteolysis complex. Another pathway of protein degradation in skeletal muscle is autophagy, the bulk degradation of proteins and organelles by lysosomal enzymes. The mechanisms responsible for the induction and regulation of the autophagy programme are poorly understood but appear to involve FOXO transcription factors as well, in particular FOXO3. Autophagy can be inhibited by AKT, but not rapamycin. Thus, FOXO3 controls the two major systems of protein breakdown in skeletal muscle, the ubiquitin–proteasomal and autophagic/lysosomal pathways (15, 18). However, the hormonal and molecular regulators that act in response to weightlessness or unloading, and ultimately bring about skeletal muscle wasting, are not fully understood (2).

In an article of Haddad F (9), muscular atrophy (model of spinal cord isolation) was associated with a reduced transcriptional activity (via pre-mRNA analyses) of myosin heavy chain (MHC) and actin. In addition, there was an increased gene expression of enzyme systems impacting protein degradation (calpain-1; plus enzymes associated with polyubquitination processes) that could further contribute to the protein deficits in the spinal cord isolation muscles via degradative pathways. IGF-I receptor and binding protein-5 mRNA expression was induced throughout the 15-day period of spinal cord isolation, whereas IGF-I mRNA was induced at 8 and 15 days. These responses occurred in the absence of an upregulation of translational regulatory proteins (p70 S6 kinase; eukaryotic 4E binding protein to compensate for the decreased protein translational capacity. These data demonstrate that: the molecular changes accompanying spinal cord isolation - induced muscle atrophy are not necessarily the reverse of those occurring during muscle hypertrophy, and the rapid and marked atrophy that defines this model of muscle inactivity is likely the result of multifactorial processes affecting transcription, translation, and protein degradation. In conclusion, the atrophy process in response to spinal cord isolation is heavily impacted by a reduction in the transcriptional activity of genes encoding key sarcomeric proteins (actin and myosin) of the muscle. This response lowers the mRNA substrate available for translation and is not offset by significant increases in the functional activity of enzymes regulating protein translation processes. Furthermore, the protein deficits appear to be exacerbated by increased gene expression of enzymes postulated to be associated with disassembly of the cytoskeletal and myofibril framework, and ubiquitination of proteins targeting them for degradation by the proteasomal machinery. These data suggest that the rapid and marked atrophy associated with the spinal cord isolation model is likely the result of multifactorial processes affecting gene transcription and of protein translation and degradation.

The work of Wang X (19) demonstrates that Merg1a channel function participates in initiation of skeletal muscle atrophy in response to muscle disuse or cachexia by signaling an increase in ubiquitin proteasome pathway proteolysis. Merg1 channel function is an initiating factor acting upstream of atrophy: Merg1 proteins are detected (day 4 of suspension) before the onset of significant atrophy (day 7 of suspension) genetic and pharmacologic attenuation of Merg1 channel function prevents atrophy in hindlimb-suspended mice. These studies also demonstrate that the Merg1a splice variant is expressed in skeletal muscle, while Merg1b is not detected, which strongly suggests that the Merg1 channel in this tissue is composed of Merg1a α subunits only. Also, expression of Merg1a, and not Merg1b, results in decreased muscle fiber size and increased ubiquitin proteasome pathway activity in wt-bearing mice. Perhaps the more extensive Merg1a NH_2 terminus is necessary for up-regulation of the ubiquitin proteasome pathway. Further, our data strongly suggest that Merg1a channel function is necessary to the atrophic process because both expression of the dysfunctional Merg1a mutant and astemizole treatment (pharmacologic channel block) inhibit the decrease in fiber size induced by suspension. Interestingly, although ubiquitin proteasome pathway activity is known to function during atrophic remodeling of the heart, physiologically relevant levels of Merg1 current is necessary for normal cardiac and are not likely to induce atrophy. Perhaps expression of the Merg1b splice variant in heart is involved in this regulation. Therefore, the functional consequence of Merg1 channel current conduction may be determined by Merg1 channel α subunit composition. In summary, Merg1a channel function is an initiator of disuse- and cachexia-stimulated atrophy, acting upstream of ubiquitin proteasome pathway proteolysis.

Skeletal muscle size is regulated by anabolic (hypertrophic) and catabolic (atrophic) processes (13). Hypertrophy in adult skeletal muscle is accompanied by the increased expression of insulin-like growth factor-1 (IGF-1). IGF-I increases the size of human myotubes whether treatment begins while myoblasts are still proliferating or after proliferation has ceased (4, 18). IGF-I appears to regulate human myotube size by activating protein synthesis, inhibiting protein degradation and inducing fusion of reserve cells. During differentiation in culture, the majority of cells exit the cell cycle and fuse, but there is always a small number of so-called reserve cells that remain mononucleated. Fusion of a greater proportion of reserve

cells increases the number of nuclei found within myotubes (fusion index) and this will result in larger myotubes. The effect of IGF-I on reserve cell recruitment appears to be indirect and to result from increased production of the cytokine interleukin-13 by treated myotubes. It remains to be demonstrated whether induction of satellite cells fusion is induced by interleukin-13 in vivo and whether expression on this cytokine in muscle is regulated by IGF-I. It is also unclear whether fusion of nuclei is a cause or consequence of activation of protein synthesis and cell size increase. When IGF-1 was overexpressed in the skeletal muscle of transgenic mice an increase in muscle size resulted. Furthermore, addition of IGF-1 in vitro to differentiated muscle cells promotes myotube hypertrophy, supporting the idea that IGF-1 is sufficient to induce hypertrophy (13). Skeletal muscle atrophy, denoted by a decrease in muscle mass and fiber size, can be driven by such disparate stimuli as denervation, immobilization, sepsis, cachexia, or glucocorticoid treatment. Atrophy is characterized by increases in protein degradation processes, particularly the ATP-dependent proteolytic ubiquitin-proteasome pathway. During atrophy, there is an increase in ubiquitin-protein conjugates and increased transcription of components of the ubiquitin degradation pathway. A screen for genetic markers of atrophy identified two genes that are up-regulated rapidly in multiple models of muscle atrophy in vivo, including dexamethasone-induced wasting, which also show highly muscle-specific expression. By studying both atrophy and hypertrophy conditions simultaneously, the study establishes a new set of regulated genes, those transcripts that are not only perturbed by an atrophy stimulus but that are inversely regulated during hypertrophy. This inversely regulated subset of genes would presumably constitute an even more reliable set of genetic markers than genes simply regulated by either atrophy or hypertrophy individually, since the ability to be regulated by the opposing conditions makes the mRNA profile of this group of genes a barometer of the growth state of the muscle. From a clinical perspective, the finding that IGF-1 can dominantly and inversely modulate key atrophy-induced helps to further validate this pathway as a target for activation by anti-atrophy therapeutics (13).

THE ROLE OF HSP IN MUSCLE ATROPHY

In the paper of Broome CS (19), it is show that skeletal muscle aging is characterized by atrophy, a deficit in specific force generation, increased susceptibility to injury, and incomplete recovery after severe injury. The ability of muscles of old mice to produce heat shock proteins (HSPs) in response to stress is severely diminished. Studies using HSP70 overexpressor mice demonstrated that lifelong overexpression of HSP70 in skeletal muscle provided protection against damage and facilitated successful recovery after damage in muscles of old mice. The mechanisms by which HSP70 provides this protection are unclear. Aging is associated with the accumulation of oxidation products, and it has been proposed that this may play a major role in age-related muscle dysfunction. The cellular mechanisms underlying this age-related decline are unclear, although considerable support exists for a role of reactive oxygen species (ROS) in modulating the aging process (3, 19, 21). Changes in markers of ROS production in skeletal muscle during aging have received some attention , although the functional effect of these changes has not been clearly examined. Skeletal muscle contractions result in an increased ROS generation (14, 19), which can be potentially damaging. However, muscle cells have defense systems that provide protection against an increase in the production of ROS. The two major endogenous defense systems involved in this adaptation in muscle are the antioxidant defense enzymes (including superoxide dismutase - SOD, catalase, and glutathione peroxidase) and heat shock proteins. Up-regulation of these systems occurs in muscle in response to increased ROS production via activation of redox-responsive transcription factors. Nuclear factor-κB (NF-κB) and activator protein-1 (AP1) transcription factors are involved in the up-regulation of antioxidant enzymes such as SOD and catalase in response to oxidative stress (19), whereas HSP expression in response to acute stress in eukaryotic cells is primarily regulated by the transcription factor heat shock factor 1 (HSF1). In summary, the inability of muscles of old mice to produce HSPs after stress results in the accumulation of cellular oxidation products and that overexpression of HSP70 protects against the age-associated increase in ROS-mediated damage to cellular components and preserves the ability of muscle cells to activate redox-responsive transcription after stress, which results in protection against the development of age-related functional deficits.

In June 2009, Dodd SL (7), show that heat shock protein 25/27 (Hsp25/27) is a cytoprotective protein that is ubiquitously expressed in most cells, and is up-regulated in response to cellular stress. In nonmuscle cells, Hsp27 inhibits TNF-alpha-induced NF-kappaB activation. During skeletal muscle disuse, Hsp25/27 levels are decreased and NF-kappaB activity increased, and this increase in NF-kappaB activity is required for disuse muscle atrophy. Therefore, the purpose of his study was to determine whether electrotransfer of Hsp27 into the soleus muscle of rats, prior to skeletal muscle disuse, is sufficient to inhibit skeletal muscle disuse atrophy and NF-kappaB activation. The 35% disuse muscle-fiber atrophy observed in nontransfected fibers was attenuated by 50% in fibers transfected with Hsp27. Hsp27 also inhibited the disuse-induced increase in MuRF1 and atrogin-1 transcription by 82 and 40%, respectively. Furthermore, disuse- and IKKbeta-induced NF-kappaB transactivation were abolished by Hsp27. In contrast, Hsp27 had no effect on Foxo transactivation. The conclusion of this study was that Hsp27 is a negative regulator of NF-kappaB in skeletal muscle, in vivo, and is sufficient to inhibit MuRF1 and atrogin-1 and attenuate skeletal muscle disuse atrophy.

Carmeli E (2009) show that certain proteins such as matrix metalloproteinase -2(MMP-2) and heat shock protein 70(HSP-70) play a role during the degradation process (6). They hypothesized that tetracycline can be used to reduce tissue degradation in skeletal muscles exposed to immobilization. The right knee of old rats (20-months-old) was immobilized by a rigid external fixator (EF) device for 1, 2, 3 and 4 weeks. Aqueous Tetracycline solution was administrated 3 times a week, following 2 days after the EF was constructed. Control group I was immobilized for 3 weeks, did not receive tetracycline but did received saline injection, and control group II only received tetracycline for 3 weeks. MMP-2 and HSP-70 protein and mRNA levels in the gastrocnemius and soleus muscles were analyzed at the molecular level by RT-PCR and the protein level using SDS-PAGE gels and western blots. We have shown that rats treated by Tetracycline reduce the MMP-2 expression and HSP-70. Theses changes mainly occurred in type IIb and type IIa muscle fibers. Tetracycline administration has beneficial effect on expression of enzymes involved in protein degradation. This may suggest a protective effect on protein degradation during immobilization. The mechanisms by which lifelong overexpression of HSP70 may preserve muscle function in old mice are unclear (5). It is suggest that dysfunction occurs in muscle and other cells as a consequence of the accumulation of oxidative damage to cellular components. HSPs are known to provide protection against acute reactive oxygen species (ROS) mediated cell damage, although the effects of increased HSP expression on changes in markers of ROS production have not been examined. The inability of muscles of old mice to produce HSPs after stress results in the accumulation of cellular oxidation products and that overexpression of HSP70 protects against the age-associated increase in ROS-mediated damage to cellular components and preserves the ability of muscle cells to activate redox-responsive transcription after stress, which results in protection against the development of age-related functional deficits (5).

SIGNALING MOLECULES INVOLVED IN MUSCLE ATROPHY

Various signaling proteins have been studied for roles in regulating disuse

atrophy. The identification and molecular characterization of distinct pathways implicated in the pathogenesis of muscle atrophy have revealed potential targets for therapeutic interventions. However, an effective application of these therapies requires a better understanding of the relative contribution of these pathways to the development of muscle atrophy in distinct pathological conditions.

UBIQUITIN-PROTEASOME SYSTEM IN DISUSE ATROPHY

There is evidence that loss of lean body mass is usually caused by activation of the ubiquitin-proteasome proteolytic pathway in muscle, but the pathophysiological triggers that accelerate protein degradation are controversial (20). Inflammation is often suggested as a trigger because many illnesses causing loss of lean body mass are associated with increases in circulating cytokines. However, inflammation can be linked to insulin resistance because high levels of circulating TNFα and possibly other cytokines can cause insulin resistance. Another potential proteolytic trigger of muscle protein breakdown is a decrease in the responses to insulin or IGF-I. For example, there is evidence that insulin deficiency causes muscle protein breakdown by activating the ubiquitin-proteasome proteolytic pathway in processes that include transcription of genes encoding subunits of this system. This is relevant because catabolic conditions that stimulate muscle protein degradation by the ubiquitin-proteasome proteolytic pathway such as aging, acidosis, chronic kidney disease, or acidosis are often associated with insulin resistance (20). The presence of these complicating factors raises the question of whether insulin resistance by itself will stimulate protein metabolism and, if so, by what mechanisms. An activation of the ubiquitin–proteasome pathway has been reported in disuse muscle atrophy, possibly by a passive–active mechanism. The process of substrate ubiquitination involves the cooperative interaction of at least three classes of proteins termed E1 (ubiquitin activating), E2 (ubiquitin conjugating), and E3 (ubiquitin ligating) enzymes. The activation of the noncanonical NFkB pathway, which involves p50 and Bcl-3, and is not induced by inflammatory cytokines, has also been reported in experimental models of disuse atrophy. It is possible that reactive oxygen species (ROS) activate NFkB directly. ROS can also stimulate FOXO activity. This evidence link the oxidative stress produced in the muscles during unloading and immobilization with the activation of the ubiquitin–proteasome pathway. Inhibition of the proteasome with agents available since 1994 has shown significant interference of muscle proteolysis in disuse muscle. In addition, there are significant increases in the expression of components of both the process of ubiquitination and of the many proteasome subunits with disuse.

Myostatin is a member of the TGFβ family of signal transduction proteins that negatively regulates muscle mass in the adults by inhibiting muscle regeneration and is a strong negative regulator of muscle growth. The increase in muscle mass observed in myostatin-null animals predominantly results from an increase in the number of muscle fibers (hyperplasia). Myostatin has been isolated as the gene mutated in cattle characterized by abnormal hypertrophy of the skeletal muscle (8). Similarly, myostatin-null mice display an increase in muscle mass relative to control animals, and gene mutation that precludes myostatin expression has been identified in humans and dogs showing a hyper-muscular phenotype. Knockout or mutation of this protein produces animals with markedly enlarged muscles as a result of hypertrophy and hyperplasia (10). Consistently, systemic overexpression of myostatin in mice causes significant loss in muscle mass, and the effect is reversed by follistatin administration (8). Thus, factors that interfere with myostatin activity can be considered anabolic signals. Systemic administration of this negative growth regulator leads to muscle wasting in mice, and treatment of cultured muscle cells with recombinant myostatin has resulted in the loss of protein and reduced protein synthesis rates (10). Moreover, myostatin expression is increased in some types of muscle atrophy). Human immunodefiency virus (HIV)-infected men have shown higher levels of serum myostatin, indicating that myostatin may contribute to cachexia-type atrophy.

GLUCOCORTICOIDS

In skeletal muscle, glucocorticoids decrease the rate of protein synthesis and increase the rate of protein degradation (10). Disuse atrophy is associate with increases in circulating glucocorticoid levels. Moreover, the binding capacity of corticosteroids also was increased markedly with disuse atrophy, and so it seemed plausible that glucocorticoids could be an important trigger. However, when adrenalectomized animals underwent unloading, with or without cortisol treatment, atrophy still occurred (10). Importantly, treatment of unloaded rats with an inhibitor of glucocorticoids, RU-38486, also did not inhibit disuse atrophy. Thus glucocorticoids do not appear to be required for disuse atrophy. In the case of cachexia, glucocorticoids seem to be a contributing factor to muscle wasting, in part because rats treated with RU-38486 plus TNF showed reduced proteolysis, but protein loss was not completely attenuated (10). Glucocorticoids have been shown to cause atrophy of fast-twitch or type II muscle fibers (particularly IIx and IIb) with less or no impact observed in type I fibers. Therefore, fast-twitch glycolytic muscles (i.e., tibialis anterior) are more susceptible than oxidative muscles (i.e., soleus) to glucocorticoid-induced muscle atrophy. The mechanism of such fiber specificity is not known. In skeletal muscle, glucocorticoids decrease the rate of protein synthesis and increase the rate of protein breakdown contributing to atrophy. The severity and the mechanism for the catabolic effect of glucocorticoids may differ with age. For example, glucocorticoids cause more severe atrophy in older rats compared with younger rats. Furthermore, glucocorticoid-induced muscle atrophy results mainly from increased protein breakdown in adult rats but mostly from depressed protein synthesis in the aged animals (1, 9, 10).

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REGLAREA MOLECULARA A ATROFIEI MUSCULATURII SCHELETICE

REZUMAT

Acest articol se refera la cele mai recente descoperiri legate de reglarea moleculara a atrofiei musculare. Periodele prelungite de inactivitate ale musculaturii scheletice datorate repausului la pat, denervarii, imobilizarii sau imponderabilitatii, pot duce la atrofie musculara semnificativa. Pastrarea sanatatii musculaturii scheletice presupune mentinerea echilibrului intre sinteza si degradarea proteinelor. Atrofia apare ca urmare a perturbarii acestui echilibru si din pacate se cunosc inca destul de putine date despre mecanismele care contribuie la initierea acestui dezechilibru. In cursul atrofiei musculare s-a constatat cresterea expresiei sistemului proteolitic ubiquitin-proteozom. Agenti catabolici cum ar fi: citokinele, factorul care induce proteoliza, speciile reactive ale oxigenului determina cresterea expresiei genelor subunitatilor de proteozomi. Glucocorticoizii determina activarea transcriptiei unor factori care probabil cresc expresia miostatinei.

Cuvinte cheie: atrofie musculara, sistemul ubiquitin-proteozom, glucocorticoizi, miostatina.

TENDENCIES IN SMOKING STATUS AND SMOKING HABITS AMONG MEDICAL STUDENTS DURING THE FIRST THREE YEARS OF MEDICAL STUDIES

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ABSTRACT

The purpose of our longitudinal study was to evaluate whether smoking status and smoking habits changed during the first three years of medical studies. A total number of 350 medical students (126 male and 224 female) were surveyed every year, between 2003 – 2006, using a questionnaire including demographic details, data of smoking status and smoking habits evaluated by the Fagerström test for nicotine dependence (FTND). The results of our study revealed that percentage of male subjects current smokers was significantly higher than the same parameter evaluated in female subjects only in the first ($p = 0.03$) and the second year of study ($p = 0.04$). The percentage increase of new smokers related with the year of study was significant in female subjects ($p = 0.01$). Severity of nicotine dependence, estimated using the FTND score, significantly increased every year of study, both in male subjects ($p = 0.002$), as well as in female subjects ($p < 0.001$). Upward trend in tobacco use, particularly in women, is a reason for concern. The relatively less encouraging smoking data among our medical students suggest the need to promote tobacco education and intervention efforts in this population segment.

Key words: cigarette smoking, medical students, Fagerström questionnaire

INTRODUCTION

The most important determinant of human health is the increase in tobacco related mortality and disability. Tobacco related deaths have been projected to increase from 3.0 million in 1990 to 8.4 million in 2020, which made tobacco the largest single health problem at this time (1).

There are 80 - 90% of deaths from chronic obstructive lung disease attributed to tobacco, and smokers have 6 times the risk of contracting this disease compared with non-smokers. Similarly, 80 - 85% of lung cancer deaths are attributed to tobacco use, with smokers having 10 times the risk compared with non-smokers (2).

Physicians who take their professional role seriously have the opportunity and responsibility to act on various levels to combat smoking, acting as models, educators, therapists, and antismoking advisers. It has been observed that doctors who smoke tend to be more permissive, and are less inclined to advise their patients against tobacco use, and adopt a passive attitude towards smoking (3).

A comprehensive education for physicians on the subject of smoking dependence is imperative, and the best possible time for the training is when they are students.

The smoking habits of medical students have only rarely been the object of studies and the interventions in Romania. A review of the results obtained in other countries reveals certain trends in the evolution of the smoking habits of this population.

The purpose of our longitudinal study was to evaluate whether smoking status and smoking habits changed during the first three years of medical school studies.

MATERIAL AND METHODS

A total of about 350 medical students, 224 female (age 21 ± 1.9 years) and 126 males (age 22 ± 2.20 years), were surveyed every year, between 2003 – 2006, using a questionnaire included: demographic details (name, age, gender), data of smoking status (current, new, former, never) and data of smoking habits evaluated by Fagerström test for nicotine dependence (FTND).

Subjects who smoked daily were asked to rate themselves as current smokers, those who had smoked for the first time in the previous 12 months as new smokers, those who had not smoked in the previous 12 months or longer as former smokers, while those who had smoked < 100 cigarettes in their lifetime were asked to consider themselves non or never smokers.

The FTND is a widely used and validated 6-item questionnaire to asses severity of nicotine dependence using a score range from 0 to 10 (Table I) (4, 5). FTND also provides a scale for nicotine dependence severity as low, medium and high dependence, and allows analysis of other characteristics related to smoking behavior, which define severity of nicotine dependence. A score ≤ 4 suggests a low level of nicotine dependence, and a score ≥ 6 usually indicates a high level

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of nicotine dependence.

Table I. The Fagerström Test for Nicotine Dependence

Questions	Score
1. How soon after you wake up do you smoke your first cigarette? ▪ within 5 min ▪ 6 to 30 min ▪ 31 to 60 min ▪ after 60 min	 3 2 1 0
2. Do you find it difficult to refrain from smoking in places where is forbidden? ▪ Yes ▪ No	 1 0
3. Which cigarette would you hate most to give up? ▪ The first in the morning ▪ Any other	 1 0
4. How many cigarettes per day do you smoke? ▪ 10 or less ▪ 11 – 20 ▪ 21 – 30 ▪ 31 or more	 0 1 2 3
5. Do you smoke more frequently during the first hours after waking than during the rest of the day? ▪ Yes ▪ No	 1 0
6. Do you smoke if you are so ill that you are in bed most of the day? ▪ Yes ▪ No	 1 0

Statistic analysis was performed using Excell Microsoft Office 2003 and EpiInfo 6 software. The central tendencies of the variables were expressed as a mean (M), and the dispersion ones as standard deviation (sd). In order to perform the statistic comparisons the „t"-Student test, as well as the variance analysis (ANOVA), were used for continuous variables, and the Chi – square (X^2) test for categorical variables. The values achieved were considered significant for $p < 0.05$.

RESULTS

Smoking Status

Distribution of smoking status' variables was determined on each year of our study, both in male gender subjects, and in female gender subjects.

We can observe the general tendency of increasing the percentage of current smokers in the diagrams presented above, which follows each year of study, both in male subjects ($p = 0.22$), and in female subjects ($p < 0.001$), respectively. Significant differences between the percentage of male and female subjects current smokers were revealed in the first ($X^2 = 4.26$, $p = 0.03$) and the second year of study ($X^2 = 3.97$, $p = 0.04$), respectively.

In the third year of medical education, this difference became insignificant ($X^2 = 0.13$, $p = 0.72$), thus suggesting the progressive increase of female smokers percentage along with each year of study.

Graphs showed in Fig 1 and Fig 2 also reveal the increasing percentage of new smokers along with each year of study, both in male subjects ($p = 0.36$), and in female subjects ($p = 0.01$), respectively. The highest rate of new smokers percentage increase was showed in male subjects in the third year of study ($X^2 = 4.28$, $p = 0.03$), and in female subjects in the second ($X^2 = 9.46$, $p = 0.002$) and in the third year of study ($X^2 = 3.70$, $p = 0.05$), respectively.

Considering the status of former smokers, we did not found significant differences related to the year of study ($p = 0.30$) or subjects' gender ($X^2 = 1.91$, $p = 0.16$ for the first year; $X^2 = 0.69$, $p = 0.40$ for the second year; $X^2 = 3.70$, $p = 0.05$ for the third year).

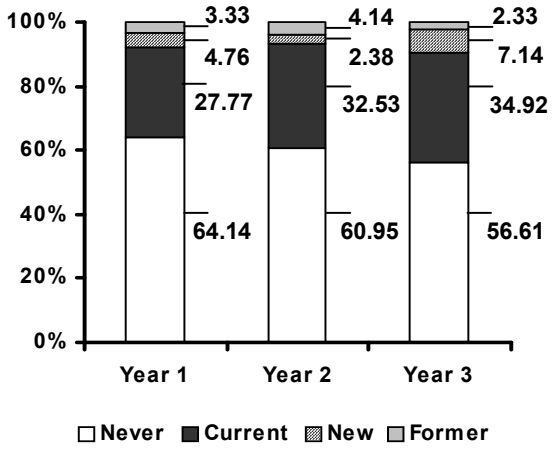

Fig. 1. The pattern of smoking status on *male* group

Severity of nicotine dependence

Severity of nicotine dependence was estimated using Fagerström score, which

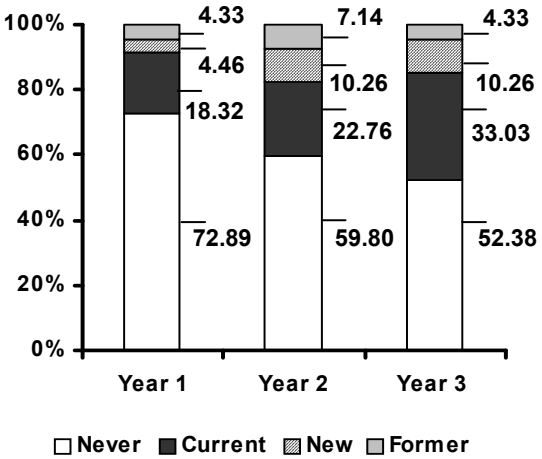

Fig. 2. The pattern of smoking status on *female* group

proved to have a higher significance when analyzing the male subjects, compared

with female subjects, during all years of study (Tab.II).

Significant increase of Fagerström score was also revealed together with the next year of study, both in male subjects ($p = 0.002$), as well as in female subjects ($p < 0.001$).

Table II. FTND - Nicotine dependence score

Year	Male	Female	p
Year 1	5.19 ± 1.85	4.11 ± 1.59	0.003
Year 2	6.35 ± 1.95	5.25 ± 2.17	0.006
Year 2	6.51 ± 2.05	5.39 ± 2.09	0.001

Due to an increase of Fagerström score in the year of study, we examined the evolution of subjects' percentage distribution, divided in categories of nicotine dependence severity, both in male persons, as well as in female subjects.

In Fig. 3 it is shown that there is a pattern in male subjects, characterized by a higher percentage of high nicotine dependence subjects, which remains unchanged along with the year of study ($p = 0.86$). This tendency was also revealed in case of medium nicotine dependence ($p = 0.42$), and low nicotine dependence ($p = 0.74$) percentage subjects, respectively. But, in case of female subjects (Fig.4), the pattern was modified along the year of study, in the way of a significant increase of high nicotine dependence ($p < 0.001$), decrease of medium nicotine dependence ($p = 0.007$) percentage subjects, without any change of low nicotine dependence ($p = 0.31$) percentage subjects.

These results are also sustained by comparative analysis using severity categories of nicotine dependence in each gender, along with the year of study. Thus, in the first year of medical studies, male subjects were placed in the high nicotine dependence category ($X^2 = 28.71$, $p < 0.001$), while female subjects in low nicotine dependence ($X^2 = 5.16$, $p < 0.05$) and medium nicotine dependence ($X^2 = 4.93$, $p < 0.05$) category. In the second year, the male subjects remained in high nicotine dependence category ($X^2 = 4.01$, $p < 0.05$), while in the third year there were no significant differences related to gender, for any of the severity categories ($X^2 = 3.50$, $p = 0.06$ for low nicotine dependence ; $X^2 = 0.02$, $p = 0.96$ for medium nicotine dependence ; $X^2 = 2.07$, $p = 0.15$ for high nicotine dependence).

Smoking habits of nicotine dependence

Different aspects of smoking behavior associated with nicotine dependence were analyzed using FTND. Statistic analysis was based on comparing intermediate scores obtained at each of the 6 items (Q) of FTND, both in male subjects (Tab. III), as well in female subjects (Tab.IV). Data presented in Tab.III and Tab.IV show significant change of smoking behavior along with each year of study only in case of female subjects.

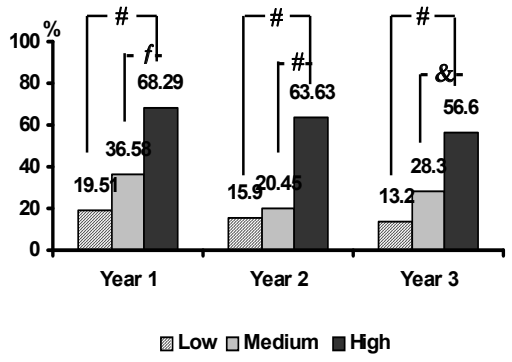

Fig. 3. The pattern of nicotine dependence severity on *male* group ($^{\#}p<0.001$, $^{f}p=0.009$, $^{\&}p=0.002$).

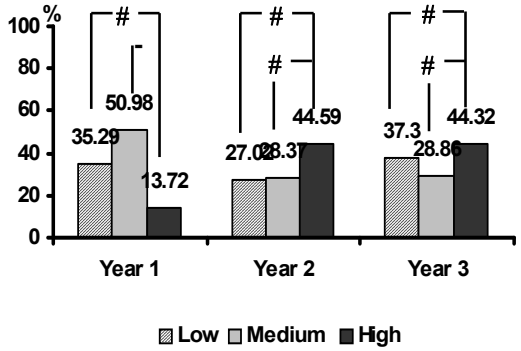

Fig. 4. The pattern of nicotine dependence severity on *female* group ($^{\#}p<0.05$, $^{f}p<0.001$).

Table III. FTND – intermediary points for male group

Q	Year 1 (n = 41)	Year 2 (n = 44)	Year 3 (n = 53)	p
1.	2.42 ± 0.96	2.40 ± 0.93	2.44 ± 0.79	0.96
2.	0.50 ± 0.50	0.35 ± 0.48	0.29 ± 0.46	0.11
3.	0.69 ± 0.46	0.63 ± 0.49	0.62 ± 0.48	0.82
4.	1.14 ± 0.52	1.04 ± 0.67	1.16 ± 0.72	0.62
5.	0.80 ± 0.40	0.82 ± 0.38	0.81 ± 0.39	0.98
6.	0.30 ± 0.46	0.33 ± 0.47	0.35 ± 0.48	0.91

The results are expressed as M ± sd

Q = question of Fagerström test of nicotine dependence (FTND)

Table IV. FTND - intermediary points for female group

Q	Year 1 (n = 51)	Year 2 (n = 74)	Year 3 (n = 97)	p
1.	1.36 ± 0.97	1.38 ± 0.92	1.94 ± 1.17	<0.001
2.	0.40 ± 0.49	0.55 ± 0.49	0.58 ± 0.49	0.01
3.	0.17 ± 0.38	0.14 ± 0.35	0.46 ± 0.50	<0.001
4.	0.28 ± 0.53	0.53 ± 0.72	0.75 ± 0.70	<0.001
5.	0.36 ± 0.48	0.40 ± 0.49	0.69 ± 0.46	<0.001
6.	0.03 ± 0.19	0.05 ± 0.19	0.19 ± 0.39	0.002

The results are expressed as M ± sd

Q = question of Fagerström test of nicotine dependence (FTND)

DISCUSSIONS

Results of population studies performed in Romania starting with the '90 revealed a concerning evolution of smoking prevalence, both within general population, as well as within population groups, such as female gender persons and

adolescents, but also professional groups, such as doctors and pharmacists.

Dynamics of tobacco use in Romania between 1995 and 2000 indicates the increase of adult smokers' prevalence, from 28.5% in 1995 to 36.1% in 2000. This increase is due mainly to the high prevalence of smoking in adult women, from 15.2% in 1995 to 25.0% in 2000, while the increase for the male subjects was from 42.7% in 1995 to 48.0% in 2000 (6).

Results of our study showed that smoking prevalence between the students in the first three years of medical education was 34.28%, and 35.50% in male subjects, and 33.03% in female subjects ($p = 0.25$). Our research also highlighted the existence of an increasing tendency in new smoker's percentage, but also in nicotine dependence severity, along with year of study, both in male, as well as in female subjects. However, prevalence of male smokers situated under the level estimated for the entire population, while the female smokers' prevalence was higher to the general population.

Compared with data from literature concerning smoking prevalence in students from medicine faculties in Europe, smoking prevalence in our subjects was higher than the same parameter evaluated in other countries such as Great Britain -11% (7), Czech Republic - 18% (8), Nederland - 18% (9), Hungary - 20.9% (10), or Germany - 23.7% (11), but was comparable with prevalence in students from Turkey - 23.9% – 45.9% (12). A survey investigating the 4th year students from Medical Faculty in Bucharest showed 32.7% smokers (30% females and 36.5% males) and 7.8% former smokers (6).

Our study also revealed the elevated number of female medical students smoking compared with the same population segment in the western countries (16% – 23%) (13), but which was very close to the same parameter in the eastern countries, such as the former Yugoslavia (24% - 35.1%) (14) or Russia (30% – 35%) (15).

Our results suggest that male subjects became smokers previous to commitment to University and remained high degree of severity smokers at least in the first three years of studies. On the other hand, female subjects became smokers only after starting the medical education, and progressively acquired a smoking behavior similar to male subjects. Following the same rationale, studies performed in Spain (16), Turkey (17) or Poland (18) showed that medical students are more likely to become smokers after coming to University, rather than quit smoking, and seem to increase the number of smoked cigarettes, rather than decrease it.

High percentage of smokers revealed even from the first year of medical education can be also analyzed from the perspective of smoking permanent increasing tendency among students in medium or high schools. Thus, numerous studies conducted in Bucharest high schools showed that tobacco use generally starts at the age of 14 (2.8%), significantly increase up to the age of 16 (12.3%), reaching a peak at the age of 18 (28.5%) (6). These studies also revealed the tendency of permanent increase in girls smoking during high school.

Although our study was limited to the first three years of medical education and there were some inconveniencies related to the large number of female gender subjects, considerably larger compared with the male subjects, the percentage increase of new smokers, and also the reduced tendency of quitting smoking present in equal proportion in both genders suggest the progressive development of smoking habit even in the superior years of study. Evidence can consolidate our conclusion based on results of enquiries performed between 1997 and 1999 among doctors in Romania, which showed 43.2% prevalence of smoking doctors, with a pick of prevalence in doctors of 30 – 49 years old (44.3%). A high rate of smokers was also found in family doctors (40% in males, and 19% in females), as well in specialist doctors (62.6%), pneumologists (60.5% in males, and 40% in females) and surgeons (45.8%) (6).

Although some data from smoking habits among medical students study are relatively old, the findings revealed that medical students generally have poor knowledge of smoking related diseases as coronary artery disease, lung cancer, pulmonary emphysema and peripheral vascular disease, bladder cancer, and neonatal mortality (19-21).The deficiencies in knowledge among medical schools reflect a general failure of medical schools globally to include teaching about tobacco in the curriculum.

CONCLUSIONS

The results of our study are far from being complete understanding of the subject. Our finding can only suggest that the high rate and severity of smoking among medical students may have an impact on public health, since medical students of today will by called upon to lead by either their non-smoking example or their clinical advice and practice the way to the reduction of tobacco use and smoke-cessation.

The relatively less encouraging smoking data among our medical students suggest the need to promote tobacco education and intervention efforts within this population segment.

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TENDINTE IN STATUSUL SI OBICEIURILOR FUMATORILOR IN CADRUL GRUPULUI DE STUDENTI LA MEDICINA DIN ANUL TREI

REZUMAT

Scopul acestui studiu longitudinal a fost sa evalueze modificarea statusului si obiceiurilor fumatorilor in timpul primilor trei ani de studii medicale. In fiecare an, intre 2003-2006, au fost supravegheati 350 studenti la Medicina (126 baieti si 224 fete), folosind un chestionar care includea detalii demografice, date legate de statusul de fumator si obiceiurile legate de fumat, care au fost evaluate prin testul Fagerström de dependenta la nicotina (FTND). Rezultatele studiului nostru au aratat ca procentul subiectilor de sex masculin care erau fumatori curenti a fost semnificativ mai mare decat acelasi parametru evaluat la subiectii de sex feminin doar in primul ($p = 0,03$) si al doilea an de studiu ($p = 0,04$). La subiectii de sex feminin, a fost semnificativa cresterea procentului de noi fumatori relationata cu anul de studiu ($p = 0,01$). Severitatea dependentei de nicotina, estimata cu ajutorul scorului FTND, a crescut semnificativ in fiecare an de studiu, atat la subiectii de sex masculin ($p = 0,002$), cat si la cei de sex feminin ($p < 0,001$). Este ingrijoratoare tendinta de consum a tutunului in continua crestere mai ales intre subiectii de sex feminin. Datele relativ descurajatoare legate de fumat, in randul sudentilor la Medicina, sugereaza nevoia promovarii unei educatii anti-tabac si a unor campanii de interventie in randul acestui segment populational.

Cuvinte cheie: fumat, studenti medical students, chestionar Fagerström

ESTABLISHMENT, PROPAGATION AND MAINTENANCE OF PRIMARY PULMONARY FIBROBLASTS

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ABSTRACT

Primary fibroblasts represent a heterogeneous population of cells that can be separated into subsets based on cell markers. Obtaining fibroblasts culture initially presume collecting tissue explants from tissues such as the lung, mechanically disrupted it and enzyme digested, in order to obtain an uni-cellular suspension. Once established the primary culture of fibroblasts, it is necessary to characterize the new strain of cells to ensure their purity and fibroblastic phenotype. The methods described in this paper outline the procedure used to initiate and establish primary fibroblast from pulmonary tissue and fibroblast characterization using morphological features and fibroblast markers, like vimentin, alpha-smooth muscle actin (α-SMA) and myosin.

Key words: fibroblasts, cell culture, morphologic features, immunohistochemistry

INTRODUCTION

Asthma is a chronic inflammatory disease of the airway characterized by airflow limitation and airway hyperresponsiveness (AHR) to nonspecific irritants. The AHR is defined by exaggerated airway narrowing, which can usually be reversed by bronchodilators that relax airway smooth muscle (ASM). The observations that the basic features of asthma are associated with airway inflammation and changes in airway structure have led to the predominant view in the past decade that ASM was primarily an effecter, whereas airway inflammation was thought to be the causal pathophysiological mechanism underlying AHR (1). This concept has been studied, showing dissociations between AHR and airway inflammation. The chronic inflammatory response in the airway in asthma is characterized by the presence of increased numbers of Th2 lymphocytes, eosinophils, and activated mast cells (2). Besides the presence of inflammatory cells in the airway, there are different levels of structural changes termed airway remodeling (3). Characteristic structural changes of airway remodeling include epithelial cell mucus metaplasia, smooth muscle hypertrophy/hyperplasia, subepithelial fibrosis, and increased angiogenesis. Increased myocyte smooth muscle mass, is thought to be due to an increase in myofibroblast proliferation (4) and a more specific role in this has the relation between fibroblasts and myofibroblasts (5). Fibroblasts are a population of heterogeneous cells which play a vital role in maintaining the structural integrity of organs such as lungs, skin, heart, sclera, and the orbit, in both physiological and pathological circumstances, like healing and reparative processes, and in the pathogenesis of scarring (fibrosis) disorders. Fibrosis is the result of fibroblast hyperplasia, as well as abnormal and accelerated production of extracellular matrix. These alterations are succeeded by fibroblast activation (6). Even if the fibroblasts don't normally have contractile elements, they are able to develop a minimal contraction, due to cytoskeleton rearrangement, enabling them to migrate to affected areas and to secrete matrix proteins, this meaning in fact the fibroblasts-myofibroblasts transformation (7). That myofibroblasts represent an intermediate state between fibroblasts and smooth muscle cells is best demonstrated in the prostate (8), in the pericytes surrounding the fetal vessels of the placental stem villi (9) and in stromal myofibroblasts of the breast (10). In both cell culture in vitro and native tissues in situ, myofibroblasts possess several distinguishing morphological characteristics. They display prominent cytoplasmic actin microfilaments (stress fibers), and they are connected to each other by adherence and gap junctions (11). Immunohistochemical characterization of myofibroblasts is based on antibody reactions for two of the three filament systems of eukaryotic cells (12). These three systems are composed of actin, a component of the microfilaments, vimentin, desmin or lamin, members of the intermediate filament system and the tubulins of the microtubules. Myofibroblasts have not been characterized with regard to tubulins. Beta and gamma actins are expressed by all cells, including myofibroblasts, which may also express α-SMA. Myofibroblasts stain negatively for alpha-cardiac and alpha-skeletal actin (13). Myofibroblasts are not well characterized regarding myosin isoforms (12). Vimentin, desmin, and α-SMA are the three filaments most often used to classify myofibroblasts (14). Expression of these proteins may vary with the tissue studied, within species and whether the cells are studied in situ or in culture and, even in a given tissue, if the cells are activated by hormonal or cytokine treatment or by disease (15). Based on immunohistochemical staining of these filaments, a classification system has been proposed (9). Myofibroblasts that express only vimentin are termed V-type myofibroblasts, those that express vimentin and desmin are called VD-type, those that express vimentin, α-SMA, and desmin are called VAD-type, those that express vimentin and α-SMA are called VA-type, and those that express vimentin and myosin are called VM-type.

MATERIAL AND METHODS

- **Pulmonary explants collection**

The samples were collected during pulmonary lobectomy, from patients with lung cancer. The samples were obtained from 5 patients (4 male, 1 female), aged 54.6±16.8, from areas distal the tumor, which were histological free of cancer. All

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samples were collected after the patients had signed the informed consent. The samples were collected in 50 ml Falcon tubes, in PBS supplemented with Penicillin 200 UI/ml, Streptomycin 150 µg/ml and Gentamicin 50 µg/ml. The samples were stored at 4°C until processing.

- **Isolation and cultivation of primary fibroblasts**

The samples were processed in 100 mm^2 Petri dishes. The bronchial and vascular structures were removed from the sample, and afterwards the lung parenchyma was cut in <0.5 cm pieces with a sterile scalpel. The samples were then washed twice with PBS at room temperature, to remove as many erythrocytes as possible. The tissue was then processed in three different ways in order to obtain cells from lung parenchyma, using aseptic techniques, for minimal contamination of the primary culture.

a. Lung explants: small pieces (less than 0.5 cm across) are placed in 25 mm^2 Petri dishes which were previously held for 30 minutes at 37°C, in order to increase surface adherence (Fig. 1). Then the lid is placed on top of the plate, for 10 minutes, at room temperature, in order for the tissue to adhere to the plate. Afterwards, the tissue is resuspended in DMEM High Glucose culture medium supplemented with FCS 20%, L-Glutamine 2 mM, Penicillin 150 UI/ml, Streptomycin 100 µg/ml. The plates are then stored for 4 days without changing the medium. After the 4 days, the medium is changed twice a week, until a confluence of 60-70% is reached.

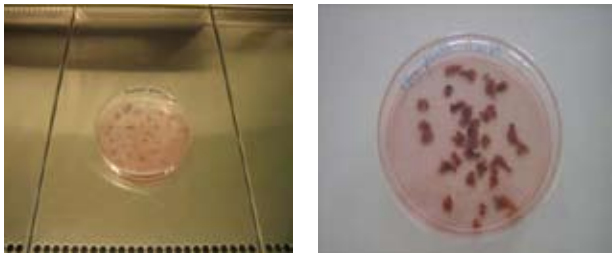

Fig. 1. Pulmonary explants

b. Collagenase digestion: the lung parenchyma fragments obtained after removal of connective bronchial and vascular tissue are placed in a 15 ml Falcon tube with 5 ml collagenase 200 UI/ml, for 30 minutes at 37°C. In order to obtain a uni-cellular suspension, the tissue is filtered through a 70 µm pores cell strainer, washing continuously with PBS. The cell suspension thus obtained, together with the collagenase solution in which the tissue was kept, are centrifuged at 1500 rpm for 8 minutes, at room temperature. The supernatant is removed with a Pasteur transfer pipette, and the cell pellet is resuspended in 7.5 ml DMEM High Glucose culture medium supplemented with FCS 20%, L-Glutamine 2 mM, Penicillin 150 UI/ml, Streptomycin 100 µg/ml, and placed in a T25 culture flask with filtered cap. The flask is incubated at 37°C, a relative humidity of 95%, and the level of CO_2 is maintained at 5%. The medium is changed after 4 days.

c. Trypsin digestion: the lung parenchyma from the biopsies is placed in a 15 ml Falcon tube, in 5 ml trypsin 0.25%, for 30 minutes at 37°C. For obtaining the uni-cellular suspension, the tissue is filtered through 70 µm pores cell strainer, washing continuously with PBS. The cell suspension plus the trypsin solution, in which the tissue was kept, are centrifuged at 1500 rpm for 8 minutes, at room temperature. The supernatant is removed with a Pasteur transfer pipette, and the cell pellet is resuspended in 7.5 ml DMEM High Glucose culture medium supplemented with FCS 20%, L-Glutamine 2 mM, Penicillin 150 UI/ml, Streptomycin 100 µg/ml, and placed in a T25 culture flask with filtered cap. The flask is incubated in same conditions as previously described. The medium is changed after 4 days.

During the first two weeks, the cells which are not fibroblasts do not adhere to the plate surface and are weeded out, while the fibroblasts proliferate, becoming the dominant cells in the culture. Once the fibroblasts from the explants have grown, or the ones from the cell suspension have adhered, forming a cell monolayer with a confluence of at least 80%, the first cell passage is possible.

Cell passaging is made by trypsinization, following the next steps:

- The cells are washed once with PBS;
- They are detached from the plate surface by adding 5 ml of 0.25% trypsin EDTA solution, at room temperature (the trypsin-EDTA solution should not be left on the cells for longer then 5 min.);
- Once the cells have separated from the plate, they are resuspended in an equal volume of 4°C complete cell culture media for neutralizing the trypsin action;
- The obtained cell suspension is placed in a 15 ml Falcon tube and centrifuged at 1500 rpm for 5 minutes, at room temperature;
- The cell pellet is resuspended in 1 ml culture medium;
- The cells are then counted with a hemacytometer, according to standard cell culture methods;
- The cells are then seeded in T25 or T75 flasks containing cell culture media (depending on the number of cells), for a density of $1x10^4$ cells/cm^2.
- The growth medium is changed twice a week until the next passage;
- The primary fibroblasts can be used between 3^{rd} and 10^{th} passage, and they can be stored indefinitely in liquid nitrogen.

Freezing the cells:

- The cells are rinsed once with PBS;
- They are detached from the plate surface by adding 5 ml of 0.25% trypsin EDTA solution, at room temperature;
- The trypsin action is attenuated by adding 5 ml of culture medium supplemented with FCS 20%, Penicillin 1%, Streptomycin 1%;
- The cell suspension is placed in a 15 ml Falcon tube and centrifuged at 1500 rpm for 5 minutes, at room temperature;
- The cell pellet is resuspended in 1 ml FCS;
- The cells are then counted with the hemocytometer;
- The freezing solution is prepared, using FCS + 20% dimethylsulfoxide (DMSO);
- The cells are placed in 2 ml cryotubes, $1.5x10^6$ cells/cryotube, using in 1:1 cell suspension and freezing solution.

- **Confirmation of fibroblasts culture by immunohistochemistry**

The cells obtained from lung biopsies were analyzed immunohistochemically. The cell characteristics involve surface or intracellular markers, as well as the cell fenotype. The fibroblasts have a typical morphology. They are elongated cells and have an oval nucleus. They can be star-like, with irregular cytoplasmic prolongations. Lung fibroblasts express Vimentin and Collagen, while CD45, cytokeratin – epithelial cell marker, and smooth muscle actin – expressed by myofibroblasts and smooth muscle cells are consistently absent (6).

For the immunohistochemical analysis, the cells were placed in 4 well plates, at 4000 cells/cm^2 cellulr density, and 7000 cells/well. After 4 days of incubation at 37°C, when the cells have reached a confluence of 60-70%, the medium was removed, the cells washed twice with PBS, and immunohistochemistry was performed for Vimentin, actin and myosin, following the next steps:

1. The cells are fixed by washing with 0.02 % formaline, for 5 minutes.

2. Rinse gently with PBS at room temperature, for 5 minutes.

3. To stain the cells for Vimentin, actine, or myosine incubate cultures with the primary antibody anti-vimentin (Dako, N1521), anti- α-SMA clone 1A4 (Dako, N1584), or anti- havy chain myosine (Dako, M3558), diluted in PBS, at room temperature, for 10 minutes. These reagents are supplied ready-to-use, so blocking is unnecessary.

4. The cells are washed twice with PBS, for 2 minutes, at room temperature.

5. The secondary biotinylated antibody is added, for 10 minutes, at room temperature.

6. The cells are washed twice with PBS, for 2 minutes, at room temperature.

7. The Streptavidin-HRP is a applied enough to cover specimen, for 10 minutes, at room temperature.

8. The cells are washed twice with PBS, for 2 minutes, at room temperature.

9. The substrate (AEC) is added, 2 drops/well, for 10 minutes, until the color turns red.

10. The cells are washed with distilled water, for 5 minutes.

11. Hematoxylin is added, for 30 seconds.

12. The cells are washed with distilled water, for 10 minutes.

13. The mounting solution and the cover slip are applied.

• In vitro fibroblasts differentiation through myofibroblasts

The following experiment will be designed to assay the effect of pro-inflammatory factors on the phenotypic change of fibroblasts. The cells will be plated in 6 well plates (4000 cells/cm^2), referring as day 2. Twenty-four hours later (day 1) the FCS concentration will be reduced to 4% to decrease the proliferating rate and maintained for the rest of the experiment. Twenty-four hours later the medium will be replaced by medium containing IL-4 (R&D, 204-IL-050, 25 ng/ml), IL-13 (R&D, 213-IL-025, 25 ng/ml), TGF- β_1 (Sigma Aldrich, T7039, 1.5 ng/ml), TGF- β_2 (Sigma Aldrich, T2815, 1.5 ng/ml) and one medium which will contain a mixture of all factors in same concentrations as above. One well will be maintained with diluents only (0 ng/ml factor). This will be referred as day 0. Each experiment will be proceeded in duplicate – one sample for reverse transcription polymerase chain reaction (RT-PCR) and the other for immunohistochemistry. The cells pulsed for 14 days, will be assessed for Vimentin, alpha-smooth muscle actin (α-SMA), myosine havy chain and desmine.

RESULTS

• Isolation and cultivation of primary fibroblasts

The human primary lung fibroblasts were isolated by using all of the three methods presented above (lung explants, collagenase digestion and trypsin digestion), but the establishment of cell cultures and the cell passaging were obtained only by using the digestion methods. The cells obtained from the lung explants resembled fibroblasts morphologically (Fig. 2), but after two weeks of cultivation they did not reach a confluence of at least 70%, and detached themselves from the plate surface.

Lung fibroblast cultures were obtained by using both digestion methods, but with collagenase, were obtained more uniform cultures (Fig. 3).

• Confirmation of fibroblasts culture by immunohistochemistry

The cells obtained from pulmonary biopsies presented the fibroblasts morphology, and concerning the markers, they respected the fibroblasts phenotype. All cells stained positive for Vimentin and negative for α-SMA and myosine (Fig. 4).

Fig. 2. Pulmonary fibroblasts isolated from tissue explants - 14 days culture

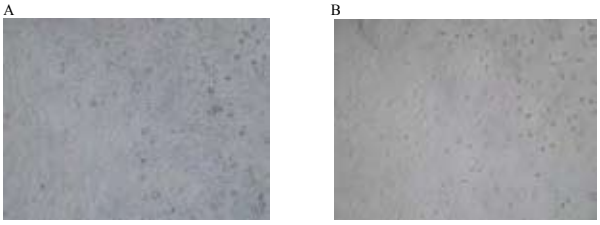

Fig. 3. Pulmonary fibroblasts isolated by (A) colagenase digestion and (B) trypsin digestion

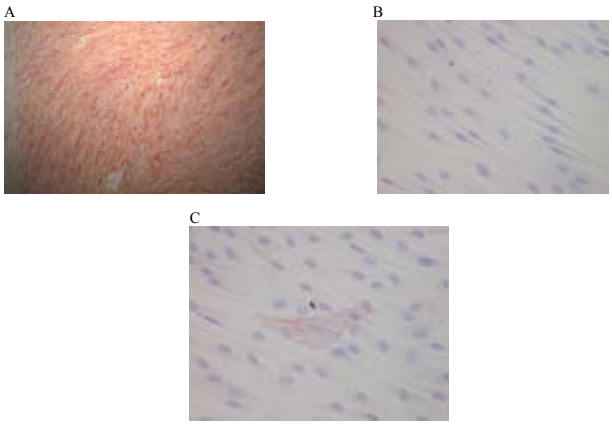

Fig. 4. Representative immunohistochemical staining of positive (A - vimentin) and negative (B - α-SMA and C - myosine) fibroblasts markers

CONCLUSIONS AND DISCUSSIONS

1. Primary fibroblast cultures may be initiated from lung tissue, but not limited to it and could be extended at various organs.

2. Mechanically disrupting tissue can be used for isolate fibroblasts, but alternative methods including enzymatic dissociation with collagenase are preferable in order to obtain primary fibroblasts strains.

3. Once established the fibroblasts cultures, media should be replaced twice a week and the cultured cells used between passages 3 and 10, because primary fibroblast strains have a finite life span.

4. Fibroblasts at an early passage may be frozen and stored indefinitely in liquid nitrogen. For limited period of time cells can be kept frozen in isopropanol freezing containers at –80°C. Before freezing, cells should be counted and resuspended in freezing media (FCS supplemented with 10% DMSO).

5. Cultures derived from lung tissue usually consist of fibroblasts only, because of the selecting media culture, but it is important to document the purity of each strain. The usual strategy involves assessing the presence of cell markers and phenotypic proprieties of fibroblasts.

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STABILIREA, PROPAGAREA SI MENTINEREA CULTURII PRIMARE FIBROBLASTICE

REZUMAT

Fibroblastii primari reprezinta o populatie celulara heterogena, ce poate fi impartita in subtipuri celulare, pe baza marker-ilor caracteristici. Obtinerea unei culturi celulare presupune initial prelevarea tesutului pulmonar, segmentarea sa si digestia enzimatica pentru a forma o suspensie uni-celulara. Dupa stabilirea culturii de fibroblasti este necesara caracterizarea celulelor derivate din prima generatie de celule, pentru a stabili omogenitatea poulatiei. Metodele descrise in continuare releveaza procedura de obtinere si perpetuare a unei culturi de fibroblasti din tesut pulmonar, precum si caracterizarea celulelor folosind caracteristicile morfologice si markeri imunohistochimici, precum vimentina, alfa-actina de muschi neted si miozina.
Cuvinte cheie: fibroblasti, culturi celulare, caracteristici morfologice, imunohistochimie

DENDRITIC CELLS AND T CELLS ACTIVATION

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ABSTRACT

Dendritic cells are the most important antigen presenting cells that possess the unique ability to stimulate naïve T cells. DCs are derived from bone marrow progenitors and circulate in the blood as immature precursors prior to migration into peripheral tissues. They are enables to have direct contact with incoming antigens, take up, process and present them on the cell surface linked to major histocompatibility molecules. After antigen uptake, DCs undergo further maturation and migrate to draining lymph nodes, where they present antigens to T cells and induce an immune response toward a Th1 or a Th2 profile.

Key words: dendritic cells, costimulatory molecules, T cells, Th1/Th2 response, cytokine

ANTIGEN-PRESENTING CELLS – GENERAL CONSIDERATIONS

Immune response initiation and establishment are based on a close interactions between antigen-presenting cells (APCs) and T lymphocytes. It is known that T lymphocytes cannot recognize antigens in their native form. They require antigen-presenting cells for processing antigens into short fragments and presenting them on major histocompatibility complex molecules (MHC). APCs include macrophages, B lymphocytes and dendritic cells (DCs). These specialized cells are distinguished by two important properties: class II MHC molecules expression on their membranes and the ability to deliver a co-stimulatory signal necessary for T cell activation. DCs are the most effective antigen-presenting cells, expressing high levels of class I and class II MHC molecules, as well as high levels of B7-1 and B7-2 glycoproteins, the principal co-stimulatory molecules. For this reason, DCs are very potent activators of naive, memory and effector T cells. In contrast, activated macrophages are common activators of memory and effector T cells, but are not able to activate naive T cells. This type of APCs up-regulate their expression of class II MHC molecules and co-stimulatory B7 molecules. Resting B cells express class II MHC molecules, but fail to express co-stimulatory B7 molecules and cannot activate naive T cells. Activated B cells up-regulate their class II MHC molecules expression and begin expressing B7 molecules, being able to activate now naive as well as the memory and effector T cells (1).

DENDRITIC CELLS

DCs were described in all lymphoid organs, blood, bone marrow, and in several other organs, including the lung, liver, heart, kidney and urogenital tract (2). The isolation of these cells from tissue samples is difficult because the conventional procedures for cell isolation damage their long extensions, but are developed isolation techniques including enzymatic organ digestion, gradient centrifugations and gentler dispersion (3). In contrast to other leukocytes, DCs cannot be immunophenotypically identified using one single antigenit marker. They are usually identified by the very intense expression of class II MHC molecules (HLA-DR) and the absence of T, B, NK cell, monocytic and granulocytic lineage markers.

The major histocompatibility complex function as antigen-recognition molecules, but they do not possess the fine specificity for antigen characteristic of antibodies and T cell receptors. The MHC loci encode two major classes of membrane-bound glycoproteins: class I and class II MHC molecules. Generally, T helper cells (Th) recognize antigen combined with class II MHC molecules, whereas cytotoxic T cells (Tc) recognize antigen combined with class I MHC molecules (4).

Although most mature dendritic cells have the same function, the presentation of antigen to T cells, there are known four types of dendritic cells: myeloid dendritic cells (mDCs), limphoid dendritic cells (pDCs), Langerhans cells and interstitial dendritic cells. They descend from hematopoietic stem cells through both the myeloid and lymphoid lineages. The most relevant distinction between mDCs and pDCs is functional because these cells are activated by a different set of pathogenic stimuls (5).

DCs are strategically mobilized to anatomic sites with high antigen exposure, thanks in part to their sensitivity to a whole array of chemoattracting inflammatory signals.

Because of theirs location, immediately above the basement membrane of the epithelium, DCs are enables to have direct contact with incoming antigens (6). Mucosal DCs, named immature DCs, have a high endocytic and processing

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Table I. Characteristics of immature dendritic cells

Very intense expression of CD1a and CD11c
Low expression of HLA-DR
High endocytic and processing the antigens activity
Weak capability to activate T cells
Maturation inhibited by IL-10

Table II. Characteristics of mature dendritic cells

Very intense expression of HLA_DR, CD80 and CD86
Intense expression of CD83 and CD1a
Low expression of CD14
Weak capacity to take up antigens
Ability to stimulate naive T cells
Intense IL-10 production

the antigens activity and a weak capability to activate T cells. After antigen uptake, they lose their capacity to take up antigens, become mature DCs, and carry processed antigen from nonlymphoid tissues to the draining lymph node, where they become able to stimulate naive T cells and induce the polarization of the immune response toward a Th1 or a Th2 profile (7,8).Table I. Characteristics of immature dendritic cells

There is another type of dendritic cells, named follicular dendritic cells, which do not function as APCs. These cells do not express class II MHC molecules, but express high levels of antibody membrane receptors. They are located in lymph node follicles, which are rich in B cells, playing an important role in B cells response (9).

Antigens are not generally recognized in their entirety by lymphocytes. Exogenous antigens, produced outside of the cells, are internalized by APCs, either by phagocytosis or by endocytosis, degraded into peptide fragments and then displayed on their surface bound to a class II MHC molecule. Exogenous peptide presentation is restricted to APCs, because class II MHC molecules expression is limited to these cells. CD4+ T cells recognize antigens combined with this molecule class. Endogenous antigens, produced within the host cells itself, are degraded into peptide fragments that bind to class I MHC molecules within the endoplasmic reticulum, from where are then transported to the cell membrane. Class I MHC molecules are present on all nucleated cells and antigens combined with these molecules are recognized by CD8+ T cells (10).

T CELLS SUBSETS

Expression of CD4 or CD8 defines two major types of T lymphocytes. CD4+ T cells generally function as T helper lymphocytes because they help phagocytes to destroy ingested microbes and stimulate B lymphocytes to produce antibodies. This type of T lymphocytes is class II MHC molecules restricted. CD8+ T cells are called cytotoxic T lymphocytes because they kill other cells which contain intracellular microbes, and are class I MHC molecules restricted. There are another class of lymphocytes, called natural killer (NK), because they function as mediators of innate immunity (11).

IMMUNE RESPONSE ORIENTATION

The first exposure to antigen generate an immune response, called primary immune response, mediated by naive lymphocytes, which not had previously recognized and responsed to an antigen. Th lymphocytes are activated by recognition of antigen-class II MHC complex on APCs. During the primary immune response are generated memory lymphocytes. Subsequent encounters with the same antigen generate clone of memory cells and activates previosly generated cells, leading to a better immune response, called secondary immune response. These lymphocytes secrete diferent cytokines, which play an essential role in the activation of other cells participating to the immune response. Pattern nature of these cytokines influence the orientation of immune response toward a Th1 or Th2 profile. Th1 cells are the inflammatory reaction support promoting cellular immune response through T lymphocytes and macrophages activation. In contrast, Th2 cells are involved in the humoral response and allergic reaction.

Differentiation into Th1 or Th2 response depends on many factors like: the nature and the dose of the antigen, the route of exposure, the genetic background of the individual, type of costimulatory molecules expressed on the surface of the DCs and the polarizing cytokines microenvironment during antigen presentation.

It is known that the type of costimulatory molecules expressed on DCs is essential for determining Th differentiation. Mature DC is characterized by a very intense expression of HLA-DR, CD80 and CD86 (HLA-DR^{+++}/CD80-CD86^{+++}), an intense expression of CD83 and CD1a (CD83^{++}/CD1a^{++}) and a low expression of CD14 (CD14^{-}). The most important DCs costimulatory molecules involved in Th differentiation are CD80 and CD86. The role of CD86 molecule seems to be more important than CD80 in the induction of a Th2 response. In contrast, CD80 is preferentially associated with Th1 type T-cell responses (12). It was observed that B cells from patients with atopic dermatitis express CD86, and this expression is correlated with the total serum IgE level (13).

The orientation toward a Th1 or a Th2 profile is also dependent on the polarizing cytokines present during the interaction of DCs with T cells, IL-10 and IL-12, both produced by DCs. It is postulated that IL-10 inhibits type 1 response, thus promoting a Th2 response. In contrast, IL-12 favours Th1 responses enhancing IFN-γ production by T cells (14). This cytokine stimulates, also, TNF-α production and reduces IFN-γ suppression IL-4 mediated (15). On the other hand, IFN-γ and even IL-4 enhance IL-12 production induced by appropriate stimuli, while prostaglandin E2 and IL-10 have an inhibitory effect (16). Levels of IL-12 in DCs vary genetically and with age, low levels promoting a Th2 response (17). IFN-γ secretion by Th1 cells activates macrophages, increasing their microbicidal activity and induces IgG antibody-class that supports phagocytosis and complement fixation. In addition, IFN-γ and IL-2, produced by the same cells, promote the Tc cells differentiation from CD8+ precursors and inhibits the Th2 cells expansion. Th2 cells secrete IL-4 and IL-5, which induce humoral immune responses caracterized by tendency to produce excessive IgE such as in allergic immune reaction. There is a close association between high IL-4 production and atopy. IL-4 seems to play a role in prevention of autoimmunity by down-regulation of Th1 cells. The tissue affected by organ-specific autoimmune diseases present low levels of IL-4 production (18). It seems that the capacity to induce Th2 immune response is a property of DCs which do not produce Th1 polarizing cytokines, because they have losed their IL-12 or IFN-γ producing capacity under some conditions[16]. Some authors report that the dynamics of DC migration to the draining lymph nodes may result in rapid changes in DC composition and cytokine content in T cell areas. Because of that, during the early phases of the immune response, Th1 cells are preferential primed, and this may be followed by priming of Th2 cells, in the later phases. Explanations of this could be that, in the early phases of immune response, stimulated DCs enter in T cell areas in large numbers and in the later phases, the influx of DCs decreases and the surviving DCs lose their capacity to produce IL-12 (19).

CONCLUSIONS AND FUTURE ADVANCES

All these significant observations suggest that dendritic cells and other antigen-presenting cells have main importance in immunity. They play two essential roles in immune response mediation : naive Th cells activation and activated Th cells polarization toward Th1 or Th2 effector cells. Th cells differentiation may depend on both the type of APCs and microenvironment signals. Because of their fundamental role in initiating T cell responses, they could have the same relevance in many aspects of immune regulation, including autoimmunity and cancer, due to their role in anti-cancer host responses and potential use as biological adjuvants in tumour vaccines. In this case it becomes more clearly that the new strategies for different diseases therapies should be focused them because they are a powerful tool for manipulating the immune system.

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CELULELE DENDRITICE SI ACTIVAREA LIMFOCITELOR T

REZUMAT

Celulele dendritice sunt cele mai importante celule prezentatoare de antigen caracterizate prin capacitatea unica de a stimula limfocite T naïve. Aceste celule provin de la nivelul maduvei hematogene si circula in sange sub forma de celule dendritice imature. In tesuturile periferice ele vin in contact cu antigenele, pe care le capteaza, le proceseaza si le prezinta la suprafata celulei pe complexului major de histocompatibilitate. Odata cu preluarea antigenului ele devin celule dendritice mature si migreaza in ganglionii limfatici, unde stimuleaza limfocitului T inducand diferentierea raspunsului imun spre unul de tip Th1 sau Th2.

Cuvinte cheie: celule dendritice, molecule co-stimularoare, cellule T, raspuns Th1/Th2, citokine

CORRELATION OF ANTHROPOMETRIC PARAMETERS WITH AGE AND SEX IN POST PUBERTY

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ABSTRACT

Postpuberty, with limits between 15-19 years old, is dominated by the reduction in growth velocity of height and weight, with the installation of puberty. It was studied a representative population of 2908 adolescents from Timis county of 15-19 years old. Anthropometry was the work method. The results showed a continuous increase of height and weight in both genders; higher mean values in boys than in girls for both somatometric indicators; higher mean values for height and weight in both genders as compared to national and regional mean values. Periodical surveillance of somatometric indicators represents a monitoring method for health and nutrition status during adolescence.
Keywords: postpuberty, physical development, health state

INTRODUCTION

Adolescence is an important period in human growth and maturation. Changes now occurr with unique significance, now the adult earns many traits. Situated around biological maturity and adult age, adolescence may offer the last opportunities for medical and social surveillance, for preventing major health problems of adults (1, 4).

Peripubertal stages (prepubertal period, 9-11 years old; early adolescence, 11-14 years old; postpubertal period, 15-19 years old) are runned trought under the influence of biological and growth changes, but also of temperament and personality, social influences and mature expectations.

Postpuberty (15-19 years old) is defined by features described briefly below.

Physical development

- Most young people have entered or completed phase of puberty.
- Less variation in levels of physical growth and sexual maturation.
- Many young people have achieved adult height and other thresholds of adult physical development.

Cognitive development

- Vast majority of thinking skills: can think abstractly and hypothetically; can discern the basic principles of phenomena and may apply them in new situations, can think about the future, taking into account more possibilities and results of hypothetical events.
- A greater ability in perspective may result in empathy and consideration for others, new interest in topics about society.

Moral development

- Less self-centredness with aging. Increased empathy for abstract values and moral principles.
- For some, increased skills in taking the perspective of others, can see society more widely and appreciate the principles of laws, the "principal" morality.
- More proportions of cognitive and emotional development; the adolescent often follows a value that he violates in the same time.

The concept of self

- The identity formation proces is intense. Experimentation of different roles: appearance, sexuality, values, friends, ethnicity, occupations in particular.
- Some girls can live obesessive disorders in diet food, especially those overweighted or those that have major family conflict relations and are chronic depressed.
- Young people can explore various patterns of identity formation: strong ethnic identity, bicultural identity ; assimilation into the majority culture or alienation from the majority culture.

Psychological and emotional traits

- For some, increased ability to agree with others. Vulnerability to concernes, depression, and care toward others, especially among girls.
- Many shows an increase in responsible behaviors.

Relations with parents and other adults

- Conflicts with parents are more rare with age: enhanced ability to watch their parents and taking their views into account, most maintain good relationships with parents.
- Increased interest in assuming the responsibilities of „adult humans": own management accounts, washing their laundry, buying clothes, preparing meals, the execution of repairs.
- Usually take most decisions themselves, preparing for their own family.
- They need to balance between time spent with adults and time spent with their fellows.
- Continue to benefit from some parental limits and monitoring, often

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objecting them.

- Usual conflicts because of money, imposed rules, domesticities, appearance and activities with fellows.

Relations with fellows

- Fellows help the youth to explore and develop their own identity.
- Friendships between the sexes become more common.
- The antisocial fellow groups may increase antisocial behavior.
- Close friendships help the youth in the development process of an own identity, separate from that of a child in the family (2, 5).

The present paper is an actuality maintenance assessment of current health in adolescence, through the study of the physical development as a direct indicator of health state.The study that underlies the work continues the research of physical development of young people, performed in Romania at an interval of 7 years and started in 1950 (7) and research in the Banat area (3, 4).

METHODOLOGY AND MATERIAL

Research was conducted on a representative population of 2908 high school students from Timis county, urban area, 51.5% girls and boys 48.5% during 2003-2005.

METHOD

It was used the anthropometric method for determining the two main somatometric indicators: height and weight (8). The individual measurements were statistically processed by computer, depending on the measured indicator, by age and sex criteria. It was calculated the weighted arithmetic mean (Xp) and standard deviation (τ). There were appreciate: the level of increase of height and weight in both sexes; growth speed levels of somatometric indicators in the range 15-19 years old. Average height and weight values of the population taken in the study were compared with the guidance means for Romania, 1999, and the guidance means for Banat, 1998.

RESULTS AND DISCUSSIONS

1. Physical development level (Tables I, II)

Table I. Physical development level in boys of 15-19 years old

Age (years)	Height (cm)		Weight (kg)	
	$\bar{X}p$	τ	$\bar{X}p$	τ
15	171.0	7.0	60.3	6.8
16	173.0	6.8	62.6	7.0
17	175.0	7.1	65.5	7.2
18	177.0	6.5	68.0	6.9
19	177.0	6.8	68.4	7.0

Table II. Physical development level in girls of 15-19 years old

Age (years)	Height (cm)		Weight (kg)	
	$\bar{X}p$	τ	$\bar{X}p$	τ
15	164.0	6.1	53.6	6.6
16	164.0	6.7	54.9	6.7
17	165.0	6.2	54.6	6.2
18	165.0	6.5	54.3	7.0
19	166.0	6.6	55.9	6.7

In the investigated age range, 15-19 years old, height and weight are characterized by growth in both sexes. The average values for boys are always higher than the values corresponding to girls. In boys, the average height increased by 6 cm, and average weight by 8.1 kg. In girls, average height increased by 2 cm and average weight by 2.3 kg.

2. The pace of growth in height and weight of boys and girls (Table III)

Table III. Growth speeds of height and weight means in boys and girls of 15-19 years old

Age (years)	Growth speed of height means (cm)		Growth speed of weight means (kg)	
	Boys	Girls	Boys	Girls
15/16	2.0	0	2.3	1.3
16/17	2.0	1.0	2.9	-0.3
17/18	2.0	0	2.5	-0.3
18/19	0	1.0	0.4	1.6

Increased speeds of height in boys were of 2 cm per year between 15 and 18 years old, and between 18 and 19 years old there hasn't been an increase. In girls, the increase was only 1 cm between 16 and 17 years old respectively 18 and 19 years old; between 15-16 years old and 17-18 years old there hasn't been an increase.

Increased speeds of weight in boys ranged from 2.3 kgr and 2.9 kg per year aged 15-18 years old, between 18-19 years old, the increase was only 0.4 kg. In girls, growth velocities were 1.3 and 1.6 kg between 15-16 years old and 18-19 years old; at 16 years comporative to 17 years and 17 years comporative to 18 years, weight averages were lower.

Specific aspects of male and female gender in the investigated group, overlapping general growth trend in the postpuberty human body: an increase of height and weight to the male gender, compared with female gender, growth speeds are relatively low, which orientates towards achieving the future height and weight of future adults in postpuberty, earlier in girls compared with boys.

3. Correlation of average values for height and weight with age and sex (Table IV)

Tabel IV. Distribution of percentage difference between the average values of height and weight in boys and girls of 15-19 years old, on age groups

Age (years)	Percentage difference* for height, boys-girls (%)	Percentage difference* for weight, boys-girls (%)
15	+4.2	+12.5
16	+5.4	+14.2
17	+6.0	+19.9
18	+7.2	+25.2
19	+6.6	+22.3

* Percentage difference: 100x [height (weight) boys / height (weight)girls - 1]

Between 15-19 years old, percentage differences, calculated to compare the height and weight corresponding to boys and girls, boys are positive and indicate an increased growing level to boys versus girls. This period is after the second cross correlation curves of the media age and sex between 14 and 15 years old. The largest percentage difference in favor of boys, were calculated for weight, towards height, and for both anthropometric indices, in the age range 17-19 years old. The research confirms the human ontogeny.

4. Comparing the average growth level in the studied population, with the national and regional means (Table V, VI)

Table V. Distribution of percentage difference between the average values of height and weight of the inspected consignment compared to the guidance for Romania, urban area, 1999, by age and sex criteria

Age (years)	Percentage differences of means			
	height (%)		weight (%)	
	Boys	Girls	Boys	Girls
15	+1.0	+1.3	+10.0	+4.0
16	+0.1	+0.7	+4.1	+2.8
17	+0.1	+1.1	+3.6	+0.9
18	+0.4	+1.0	+3.8	-0.1

* Percentage difference: 100x [height(weight)boys / height(weight)girls - 1]

Table VI. Distribution of percentage difference between the average values of height and weight of the inspected consignment compared to the guidance for Banat, urban area, 1998, by age and sex criteria

Age (years)	Percentage differences of means			
	height (%)		weight (%)	
	Boys	Girls	Boys	Girls
15	+0,4	+0,1	+7,4	+1,7
16	-0,5	0	+0,4	+1,8
17	-0,6	+0,1	+0,7	-2,5
18	+0,1	+0,4	-0,8	-4,5

* Percentage difference: 100x [height(weight)boys / height(weight)girls - 1]

For both investigated anthropometric indicators, averages calculated by age and sex were higher compared to the national indicatives, study stage 1999, expressing the reality of an superior physical development in adolescents of 15-19 years old in western Romania. Compared with the height and weight means from Banat, calculated in a national study conducted 10 years ago, prevail the higher values from the current study; the lowest mean values recorded in one third of the current results show a reduction in the rate of physical development at the adolescents from Timis county in the last decade.

CONCLUSIONS

Rapid changes that occur in adolescence relates to height and weight augmentation, defined by the term growth, and development towards adult status defined by the term maturation.

If growth and maturation takes place in parallel to the same person, they can be very different from one individual to another. Chronological unfolding of maturation differs from a healthy child to another, from genetic considerations, under the influence of environmental factors and health care. It is difficult to distinguish between normal variability by genetic and hormonal changes of adolescence, and associated changes in environmental factors (6).

Periodic surveillance of the somatometric indicators in the basic and periodic medical examinations from the children and adolescents collectivities, attaches a particular importance to anthropometry in monitoring the state of health and nutrition (8).

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CORELAŢIA UNOR INDICI ANTROPOMETRICI CU VÂRSTA ŞI SEXUL ÎN PERIOADA DE POSTPUBERTATE

REZUMAT

Postpubertatea, cu limite între 15-19 ani, este dominată de reducerea vitezelor de creştere ale taliei şi greutăţii, odată cu instalarea pubertăţii. S-a luat în studiu o populaţie reprezentativă 2908 adolescenţi timişeni de 15-19 ani. Metoda de lucru a fost antropometria. Rezultatele studiului au evidenţiat o creştere taliei şi greutăţii la ambele sexe; valori medii superioare la băieţi, comparativ cu fetele la ambii indicatori somatometrici; valori medii mai mari pentru talie şi greutate, la ambele sexe, faţă de mediile orientative naţionale şi parţial, pentru cele zonale. Supravegherea periodică a indicatorilor somatometrici se constituie în metodă de monitorizare a stării de sănătate şi de nutriţie în adolescenţă.

Cuvinte cheie: postpubertate, dezvoltare fizică, stare de sănătate

LYCOPENE INVOLVEMENT IN HEPATIC LIPID DISORDERS DUE TO HYPERTHYROIDISM IN RATS

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ABSTRACT

Recent studies showed the relationship between oxidative stress and thyroid disorders. Effect of lycopene, the main carotenoid in tomato, was assessed on oxidative stress and on lipid disorders generated by excess in L-Thyroxin in hepatic tissue. Experiments were performed on white, male, Wistar rats. Lipids were extracted with methanol and chloroform, according to the Folch procedure. Fatty acids analysis was performed using gas chromatography coupled with mass spectrometry. Lipid peroxides level was assessed by measuring the production of thiobarbituric reactive substances, according to the method of Buege and Aust. A diet rich in lycopene led to an increase in the fatty acids content, especially in the ratio between unsaturated fatty acids and saturated fatty acids, showing its antioxidant effect. Lipid peroxides increased in rats treated with L-Thyroxin, due to the oxidative stress exerts by the thyroid hormone, and decreased in rats fed a diet rich in lycopene. Lycopene supplementation revealed a reduction in lipids susceptibility to oxidative injury due to the L-Thyroxin administration. Carotenoids association to classic treatment of the hyperthyroidism could be recommended.

Key words: lycopene, oxidative stress, thyroid hormones, lipid disorder, liver

INTRODUCTION

In recent years, it was noted an increasing prevalence and incidence of thyroid disease. Our understanding of the effects of thyroid hormones under physiological circumstances, as well as in pathological conditions, has increased dramatically during the last two centuries and it has become clear that overt thyroid dysfunction is associated with significant morbidity and mortality. Both hypo-and hyperthyroidism and their treatments have been linked with increased risk from cardiovascular disease and the adverse effects of thyrotoxicosis in terms of osteoporosis risk are well established (6, 25).

Thyroid hormones play a crucial role in the regulation of mitochondrial oxidative metabolism; the synthesis and degradation of proteins and vitamins, such as vitamin E, vitamin A, and ß-carotene; the sensitivity of tissues to catecholamines; and the regulation of antioxidant enzyme levels (2, 9). Overt hyperthyroidism and hypothyroidism represent opposite clinical conditions characterized respectively by enhanced oxidative metabolism and reduced lipid and lipoprotein plasma levels and by reduced oxidative metabolism and markedly increased lipid and lipoprotein plasma levels. The hypermetabolic state that characterizes hyperthyroidism should accelerate free radical production in the mitochondria and induce changes in the antioxidant defense system (2).

Accumulating evidence has suggested that the hyper metabolic state in hyperthyroidism is associated with increased reactive oxygen species production and lipid peroxidation products in some of the rat's tissues. The response of the antioxidant systems to hyperthyroidism is unclear. Changes in the nonenzymatic antioxidants levels (α-tocopherols, retinol, β-carotene, coenzyme Q) and the antioxidant enzymes activities in various tissues were found to be imbalanced and often opposite (24).

Lycopene, the main carotenoid in tomato, has been shown to be a potent antioxidant in vitro and in vivo. In vitro studies showed that lycopene has the highest ability to quench singlet oxygen and trap peroxyl radicals (20). In human and animal body, lycopene is able to protect cellular biomolecules against oxidative damages; therefore, it is proved to have beneficial effects in wide range of ROS mediated diseases, including cancer (1). Steel, there is a lack of information regarding the no enzymatic antioxidants effect in hyperthyroidism.

The purpose of our study was to investigate the lycopene involvement in lipid disorders generated by an excess in L-Thyroxin, in rat hepatic tissue.

MATERIALS AND METHODS

Animals and housing conditions

Wistar rats, male, 90 days old, weighting 180-330g, were maintained under pathogen-free conditions in a temperature-controlled (23 ± 1^{0}C, 50–70% relative humidity) and light-controlled (illuminated from 0600–1800 h) room. None of the animals died unexpectedly.

Experimental groups

Three groups of animals, each group consisting in 10 rats, were investigated, as follows:

- Group I: untreated animals (control) fed a standard diet
- Group II: L-Thyroxin injected rats L-thyroxin (10µg/100 g body weight/day), intraperitoneal administered fed a standard diet
- Group III: L-Thyroxin injected rats fed a standard diet rich in lycopene (tomatoes powder).

Distilled water was available to all animals ad libitum.

Experiments were performed for 7 days.

All animal studies were done according to the local guidelines for animal research and

principals of the European Convention for the Protection of Vertebrate Animals

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Used for Experimental and Other (published in the Official Daily N.L. 358/1-358/6, 18, December 1986) and according to The UFAW Handbook on the Care and Management of Laboratory Animals published by Blackwell Science (www.tiny.cc/9y7Sa).

Experimental procedures

Animals were sacrificed by decapitation under ether anesthesia in the 8 th day of the experiment. The liver was quickly excised and placed into Petri dishes containing ice-cold isolation medium. Lipid extracts were obtained with methanol and chloroform, according to the Folch procedure (12). Fatty acids analysis was performed using gas chromatography coupled with mass spectrometry (7). Level of lipid peroxides was evaluated fluorometrically as thiobarbituric reactive substances (TBARS), according to the Satoh method (22).

Quantitative lycopene determination

Tomatoes powder is a natural product made by Künding-Food Company as nutritive supplement. Quantitative lycopene determination was assessed by extraction procedure, followed by spectrophotometer measurement. Average lycopene content in tomatoes powder was 125g-lycopene/2g tomatoes powder. Rats received 0, 4 g tomatoes powder / 100 g body weight / day.

Lipid peroxidation assay

Level of lipid peroxides was evaluated fluorometrically as thiobarbituric reactive substances (TBARS), according to the Satoh method (22). Fluorescent reaction products were estimated spectrofluorometrically at 515 nm excitation and 553 nm emissions using a Kontron SFM25 spectrofluorometer. Results were expressed as µmoles malondialdehyde (MDA) per milligram of protein (23).

Protein extract assay

Liver samples were washed with physiological serum buffered with potassium phosphate (10 mM), pH: 7, 4. Proteins were extracted using a potassium phosphate solution (50 mM), pH: 7, 35 (7).

Total protein amount was photometrically assessed, using Robinson-Hogden method for plasma, applied for animal tissues (7).

Statistical analysis

Data are reported as mean ± SEM (standard error of the mean). Data were analyzed by Student "t" test. Statistical significance was defined as P<.05.

RESULTS

Lipid peroxidation

Malondialdehyde levels in hepatic tissue of the thyroxin treated rats were found significantly increased (P<0.001) as compared to control values. Administration of both thyroxin and lycopene (group III) led to a significant reduction (P<0.001) of the lipid peroxides as compared to control values (Table I).

Table I. Lipid peroxides level (µmols malondialdehyde/mg protein) in rat liver

Experimental groups	MDA
C (n=10)	4.01 ± 2.25
T (n=10)	10.85 ± 1.85 *
TL (n=10)	5.99 ± 3.84 **

Legend: MDA malondialdehyde; C control group; T thyroxin treated group; TL thyroxin and lycopene co-treated group; * p< 0.001 thyroxin vs control; ** p< 0.001 thyroxin vs thyroxin+lycopene

Fatty acids content of the lipid extract in the rat liver

Regarding the lipid extract from the hepatic tissue, thyroxin administration determined a significant increase (P<0.001) in the total fatty acids level in rat liver as compared to control group. Also, lycopene supplementation led to a significant increase (P<0.001) of the same parameter as compared to thyroxin treated animals (Table II).

Table II. Fatty acids content (mg/g tissue) in the rat liver

Experimental groups	Total fatty acids (mg/g tissue)
C (n=10)	3.95± 1.75
T (n=10)	34.03 ± 3.66 *
TL (n=10)	12.81± 2.66**

Legend: C control group; T thyroxin treated group; TL thyroxin and lycopene co-treated group; * p< 0.001 thyroxin vs control; ** p< 0.001 thyroxin vs thyroxin+lycopene

Dynamic of unsaturated fatty acids/ saturated fatty acids ratio in hepatic tissue

Lipid peroxidation is assessed, also, by measuring the ratio between unsaturated fatty acids and saturated fatty acids. This ratio increased in lycopene group due to its antioxidant effect, as compared to control and thyroxin treated rats (Figure1).

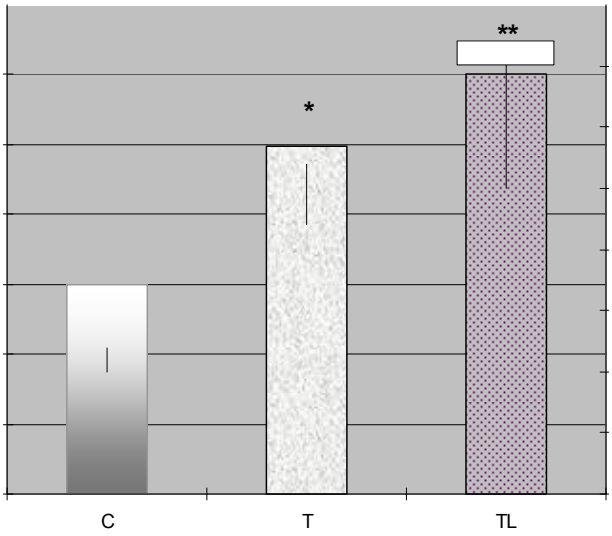

Fig. 1. Dynamic of unsaturated fatty acids/ saturated fatty acids ratio in rat hepatic tissue

Legend: UFA unsaturated fatty acids; SFA saturated fatty acids; C control group; T thyroxin treated group; TL thyroxin and lycopene co-treated group; * p< 0.001 thyroxin vs control; ** p< 0.001 thyroxin vs thyroxin+lycopene

Fatty acids percentage from lipid extract in rat hepatic tissue

Palmitoleic, linoleic, arachidonic and eicosatrienoic acids content was significantly higher (P<0.001) in both groups: thyroxin, respectively thyroxin and lycopene treated rats as compared to control. Regarding the oleic acid content, it was noticed a significant increase determined by thyroxin and noticeably decrease (P<0.001) due to lycopene administration as compared to control and thyroxin treated groups. (Table III)

Table III. Fatty acids percentage from lipid extract in rat hepatic tissue

Legend: C control group; T thyroxin treated group; TL thyroxin and lycopene co-treated group;

	Experimental groups		
Fatty acids (%)	C (n=10)	T (n=10)	TL (n=10)
Palmitoleic acid	1.5±0.75	4±2.25 *	7.5±2.25 **
Linoleic acid	7.5±3.5	20±3.50 *	25±3.75 **
Arachidonic acid	10±2.75	18.5±2.25 *	23±1.5 **
Eicosatrienoic acid	2±0.25	8±1.75 *	4±1.75 **
Oleic acid	13±3.25	22±3.75 *	7.5±4.5 **

* p< 0.001 thyroxin vs control; ** p< 0.001 thyroxin+lycopene vs control

DISCUSSION

Acceleration of the basal metabolic rate and the energy metabolism of tissues in several mammalian species represent one of the major functions of the thyroid hormones (26). The liver is the place where tetra-iodothyronine provides the metabolic thyroid hormone: tri-iodothyronine through deiodination by deiodase type I (18).

Much of the reactive oxygen species production occurs in mitochondria, via oxidative phosphorilation. Because the mitochondria contains specific receptors for the thyroid hormones, being one of the "favorite" target for them, the concept about a possible relationship between reactive oxygen species production and thyroid pathology has increasing importance (15,16). When the thyroid hormones production increases, hepatic tissue is, also, subjected to oxidative stress because of their action on liver mitochondria and on Kupffer cells (8).

'Moreira and coworkers (17) evaluated the influence of tomato powder in diets differing in energy level on antioxidant status in blood and liver of rats. Food intake and thiobarbituric acid-reactive substances contents in liver and plasma were significantly decreased by tomato powder at both energy levels. After tomato powder supplementation, the hepatic levels of ubiquinol 9, alpha-tocopherol, lycopene and beta-carotene were significantly enhanced. In plasma, only the contents of lycopene and beta-carotene were enhanced. The erythrocytic and hepatic activities of catalase were lower, while those of glutathione peroxidase were higher after the ingestion of tomato powder. Total and reduced glutathione contents in liver showed lower levels in cafeteria-fed rats compared to the hypo energetic diet. This data suggest that the lycopene and beta-carotene component in the tomato power supplement might be beneficial for the prevention of oxidative damage in rats fed both types of energetic diets.

The effects of tomato product supplementation, containing lycopene, on biomarkers of oxidative stress and carcinogenesis were investigated in human clinical trials. Supplementation of tomato products, containing lycopene, has been shown to lower biomarkers of oxidative stress and carcinogenesis in healthy and type II diabetic patients, and prostate cancer patients, respectively. Processed tomato products like tomato juice, tomato paste, tomato puree, tomato ketchup and tomato oleoresin have been shown to provide bioavailable sources of lycopene, with consequent increases in plasma lycopene levels versus baseline. Dietary fats enhance this process and should be consumed together with food sources of lycopene. The mechanisms of action involve protection of plasma lipoproteins, lymphocyte DNA and serum proteins against oxidative damage, and anticarcinogenic effects, including reduction of prostate-specific antigen, upregulation of connexin expression and overall decrease in prostate tumor aggressiveness. There is limited in vivo data on the health benefits of lycopene alone. Most of the clinical trials with tomato products suggest a synergistic action of lycopene with other nutrients, in lowering biomarkers of oxidative stress and carcinogenesis. So, consumption of processed tomato products, containing lycopene, is of significant health benefit and can be attributed to a combination of naturally occurring nutrients in tomatoes. Lycopene, the main tomato carotenoid, contributes to this effect, but its role per se remains to be investigated (4).

Our results could be related to the mentioned data: thyroxin administration increased notably the malondialdehyde concentration in hepatic tissue. Lycopene, a well-known antioxidant, exerted a protected effect against oxidative stress induced by an excess in thyroxin.

Thyroid hormones interfere with lipid biosynthesis, recruitment and degradation, the last one being more potent than the first one. The specific effects of the thyroid hormones on lipid metabolism consist in intensifying the triglycerides biosynthesis and recruitment from the adipose tissue, increasing the free fatty acids level and decreasing the lipoprotein-lipases activity (19). According to the Heimberg and his coworkers, the fat acids biosynthesis, cetogenesis and, paradoxically, the fat acids oxidation are intensified in hyperthyroidism. Also, the fatty acids esterification to triglycerides is diminished (14).

The most significant peroxidation substrate is represented by the polyunsaturated fatty acids from membranes. Lipid peroxidation is assessed, also, by measuring the ratio between unsaturated fatty acids and saturated fatty acids (10). In our experiment, this ratio decreased in thyroxin treated rats, due to lipid peroxidation intensifying, but it increased in lycopene group due to its antioxidant effect.

Regarding the lipid extract from the hepatic tissue, thyroxin administration determined a significant increase in the total fatty acids level as compared to control group. Also, lycopene supplimenation lead to a significant increase of the same parameter as compared to thyroxin treated animals. These alterations are in concordance with the results of Blenneman and coworkers: thyroid hormones enhance fatty acids biosynthesis in the hepatic tissue (5).

Also, Thyroxin administration increased fatty acids biosynthesis, and lycopene protected them against oxidation. By cumulating of these two effectes, it could be explained the noticeable increased in fatty acids content: 8 times over the control. Also, the lycopene protective effect is shown by the way in which the ratio between unsaturated fatty acids and saturated fat acids was changed.

Fatty acids content varies accordingly to the thyroid hormones doses and to the administration period. For instance, Faas and Carter showed that high doses of triiodothyronin (over 25 µg/100g body weight), administered in rats for three weeks, determined a significant increase in stearic and arachidonic acid content and a decrease in palmitic, palmitoleic, linoleic and eicosatrienoic acid content of the hepatic tissue. At lower triiodothyronin doses, the content in palmitic, palmitoleic and linoleic acid slightly increased. These disorders regarding the fatty acids content are caused by a decreased in D-6-desaturase activity, an enzyme involved in nonsaturated fatty acids synthesis, with more than 25% (11).

In our study, low doses of L-Thyroxin administration (10 µg/100g body weight) for a week lead to a markedly increased in palmitic, oleic, linoleic and eicosatrienoic acids content and to a noticeably decrease in stearic acid in rat liver. Venditti and coworkers (24) studied the lipid content of the hepatic mitochondria and microsoms in hyperthyroid rats. The phospholipids content increased with 14% in mitochondria and decreased with 23% in microsoms. It did not notice changes regarding the microsomal phospholipids content in fatty acids but it decreased the content in linoleic acid and increased the level in arachidonic acid in mitochondria. The most noticeable alterations were shown regarding the cardiolipids: a 32, 4% increase in palmitic and stearic acid content concomitantly with a decrease in linoleic acid level. Other researchers (31) investigated the alteration of the lipid pattern in rat-liver mitochondria and microsomes induced by triiodothyronine administration. In mitochondria, a 25% total cholesterol decrease and a 14% phospholipids increase have been detected. In these hyperthyroid rat liver organelles, a strong decrease in the total cholesterol/phospholipids molar ratio occurs. On the contrary, in microsomes from the same animals, a decrease of about 23% has been measured for both total cholesterol and phospholipids; hence, in this fraction, the total cholesterol/phospholipids molar ratio is unaffected by hyperthyroidism. The liver mitochondrial phospholipids composition, unlike the microsomal composition, is altered

significantly in hyperthyroid rats; a 7.4% phosphatidylcholine decrease is accompanied by a similar additive percentage increase of both phosphatidylethanolamine and cardiolipin. In regard to total phospholipids fatty acid composition in liver microsomes from hyperthyroid rats, no variation has been observed compared with the control rats, whereas in mitochondria from the same animals, a meaningful linoleic acid decrease with a similar arachidonic acid increase has been found. In addition to fatty acid alteration, the separated mitochondrial phospholipids classes also exhibit some increase in stearic acid. Among phospholipids, cardiolipin changes the most of the esterified fatty acids in hyperthyroid rat liver. In this compound, a strong increase in the percentage of both palmitic and stearic acid and a 32.4% decrease of linoleic acid has been found.

CONCLUSIONS

This study demonstrated the oxidative stress and lipid disorders occurrence in rat hepatic tissue, caused by thyroxin administration. The lycopene rich diet increased the fatty acids content as compared to thyroxin administration. Also, the lycopene enhanced the ratio between unsaturated fatty acids and saturated fatty acids, showing its antioxidant effect.

Lycopene supplementation revealed a reduction in lipids susceptibility to oxidative injury due to the L-Thyroxin administration. Carotenoids association to classic treatment of the hyperthyroidism could be recommended.

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EFECTUL LICOPINEI ASUPRA TULBURARILOR LIPIDELOR LA NIVEL HEPATIC INDUSE DE HIPERTIROIDISM LA SOBOLANI

REZUMAT

Cercetari recente au evidentiat legatura dintre stresul oxidativ si disfunctia glandei tiroide. In acest studiu s-a urmarit efectul licopinei, principalul carotenoid din tomate, asupra stresului oxidativ si tulburarilor lipidelor generate de excesul de tiroxina la nivel hepatic. S-au luat in studiu sobolani albi, sex masculin, rasa Wistar. Dieta bogata in licopina a determinat cresterea continutului in acizi grasi , in special a ratiei dintre acizii grasi nesaturati si cei saturati, subliniind efectul antioxidant al acesteia. Concentratia peroxizilor lipidici a crescut in urma administrarii tiroxinei datorita stresului oxidativ indus de hormonul tiroidian si a scazut la animalele ale caror dieta a fost imbogatita cu licopina. Suplimentarea dietei cu licopina a diminuat susceptibilitatea lipidelor la atacul oxidativ indus de administrarea tiroxinei. In concluzie, recomandam asocierea unei diete bogate in carotenoizi la tratamentul clasic al hipertiroidismului.

Cuvinte cheie: licopina, stres oxidativ, hormoni tiroidieni, lipide, ficat

LARYNGEAL PAPILLOMATOSIS MANAGEMENT

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ABSTRACT

Objective: Juvenile recurrent laryngeal papillomatosis (RLP) is a viral disease caused by Human Papilloma Virus type 6 and 11, with an incidence of 4-7 cases/1 million people characterized by multiple recurrences of benign tumours of the larynx mucosa and significant morbidity on paediatric patient and strain on their families. It represents the most frequent benign tumour of the larynx with a high morbidity, difficult to treat due to its recurrences and respiratory tract dissemination. The aim of this study was to evaluate the results of combined treatment with CO_2 laser microsurgery and interferon (IFN) alpha 2b in children suffering from laryngeal papillomatosis.

Materials and Method: In the study were included, during a 13 years period (1996-2008), 28 patients aged from 2,9 to 9 years diagnosed with laryngeal papillomatosis. CO2 laser microsurgery (microsurgical excision and progressive vaporization of papillomas) followed by IFN adjuvant therapy was the management applied. Tracheotomy was not necessary in any of the cases.

Results: Clinical examination revealed regression of papillomas in all patients. 19 patients (67.85%) had complete regression after primary CO2 laser microsurgery and additional IFN treatment, and 9 patients (32.15%) had partial response to the combined treatment. In these cases second intervention was needed. We encountered no perioperative or postoperative complications or severe side-effects.

Conclusions: The method of choice in laryngeal papillomatosis in children is the combination of CO_2 Laser microsurgery and additional IFN treatment, improving the prognosis of this recurrent viral disease. Reachable functional results, good tumour control, short hospitalization makes it a favourable treatment in paediatric patients.

Key words: juvenile laryngeal papillomatosis, CO_2 laser treatment, IFN alpha 2B

INTRODUCTION

In 1871, MacKenzie noted the frequent association of skin warts and laryngeal papillomas. Ullmann in 1923 was the first to verify an infectious etiology by injecting homogenized papillomata from a child's larynx into his own forearm and observing the development of papillomata there (1).

It was not, however until 1956 that a paediatrician made an association between maternal condylomata and the risk of childhood infection (2). In 1973 an intranuclear icosahedral virus was identified in lesions by electron microscopy (3), and in 1980 human papillomavirus (HPV) DNA was identified in papillomata (4). Surgical debulking was advanced with the use of the CO2 laser and suspension microlaryngoscopy in 1972 (5) which remains the state of the art. Current areas of promise include the use of drugs to slow the disease progression. Originally called juvenile laryngeal papillomatosis, the disease has been increasingly recognized in adults and generally goes by the name recurrent respiratory papillomatosis (RRP). The human papilloma virus (HPV), has more than 60 subtypes identified; 20 of these subtypes affect the epithelium of the genital tract. Condylomata acuminata (subtypes 6 and 11) and cervical dysplasia (subtypes 16, 18, 31, 33 and 35) are the most common clinical manifestations of HPV infections (6,7,8).

The human papillomavirus is a naked, double-stranded, icosahedrally-shaped virus with circular supercoiled DNA that belongs to the Papovavirus family. "Papova" is an acronym for the three types of viruses in the family – papillomavirus, polyomavirus, and simian vacuolating virus. There are only three papovaviruses pathogenic to humans: HPV, JC and BK viruses which are polyoma viruses (JC virus has been implicated in progressive multifocal leukencephalopathy and BK virus has been isolated from urine of kidney transplant recipients).

Juvenile recurrent laryngeal papillomatosis (RLP) is extremely aggressive and resistant to treatment, usually surgical (9,10). It typically involves the trachea, but may spread to the oesophagus and bronchi, and rarely, to the lung where it actually destroys tissue, dramatically worsening the prognosis. Although rare, it is the most common benign tumour of the larynx (11, 12, 13).

The incidence of RLP has been reported as 1:1500 live births. RLP has a bimodal age distribution and presents most commonly in children younger than 8 years (juvenile-onset recurrent respiratory papillomatosis [JORRP]) or in persons in the fourth decade of life (adult-onset recurrent respiratory papillomatosis [AORRP]). JORRP is more common and more severe than AORRP.

MATERIALS AND METHOD

Between 1.01.1996 – 31.12.2008, 28 patients with Juvenile recurrent laryngeal papillomatosis (RLP) have been treated in the ENT Department Timişoara. The mean age at diagnosis was 4,9 years. The proportion of Juvenile recurrent laryngeal papillomatosis cases diagnosed between 2,9 and 3,9 years old was 13,04% (4 cases), from 4 to 4,9 years old 65,21% (17 cases), from 5 to 5,9 years old 8,69%

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(3 cases), respectively from 6 to 8 years old 13,04% (4 cases) (Figure 1).

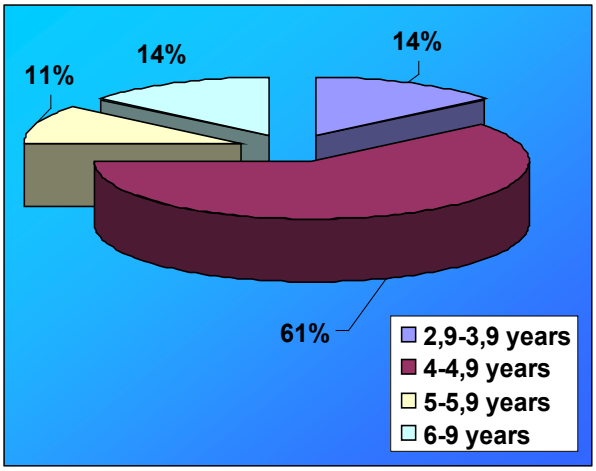

Fig. 1. Age distribution

The Juvenile recurrent laryngeal papillomatosis (RLP) affects males and females in approximativelly equal number: 15 cases (52,17%) were females, and 13 males (48,83%) (Figure 2).

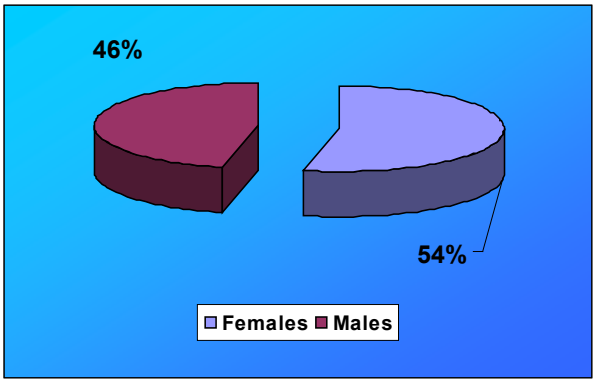

Fig. 2. Gender distribution

The most common presentation of RLP was hoarseness 22 cases (95,65%), voice changes occurred in 1 case (4,34%), young children also presented weak cry 11 cases (47,82%), choking episodes 9 cases (39,13%), foreign body sensation in the throat 18 cases (78,26%), chronic cough 15 cases (65,21%), dyspnoea 11 cases (47,82%), inspiratory wheeze 20 cases (86,95%) and stridor 13 cases (56,52%) (Figure 3).

The diagnosis was made upon the visualization of warty excrescences and confirmed by biopsy. Histological there was an epithelial projection with a fibrovascular core, and there is associated parakeratosis, koilocytosis, and acanthosis.

Regarding localisation of the papillomas 21 cases (75%) presented papillomas at the level of vocal cords, and anterior commisure, 4 cases (14%) at the level of vocal cords and subglottic region and 3 cases (11%) at the level of vocal cords, anterior and posterior commisure, ventricular folds and laryngeal surface of the epiglottis. (Figures 4, 5, 6, 7)

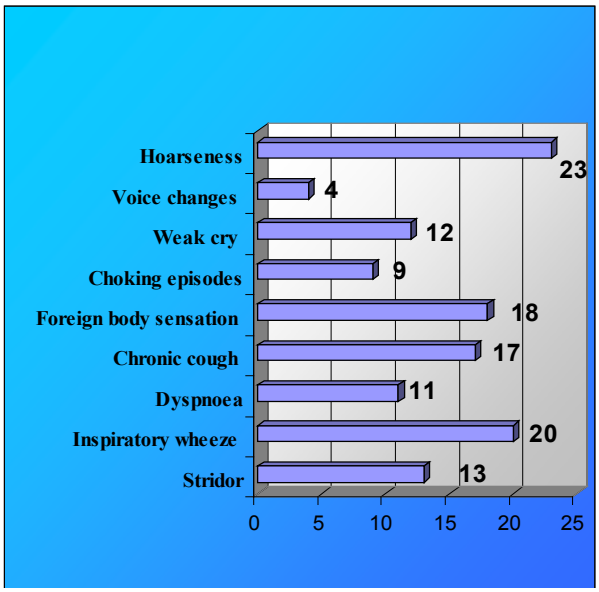

Fig. 3. Presentation symptoms

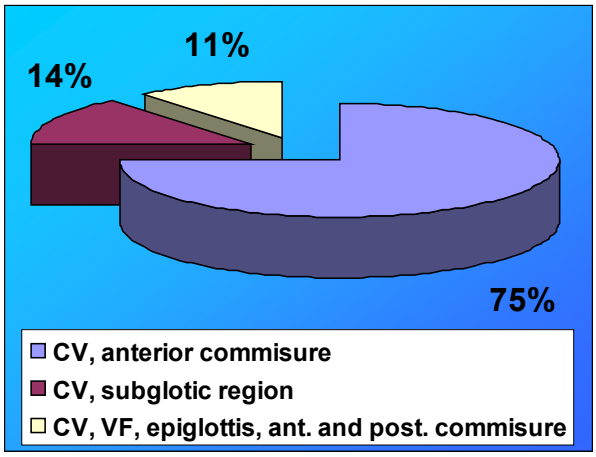

Fig. 4. Papillomas Localisation

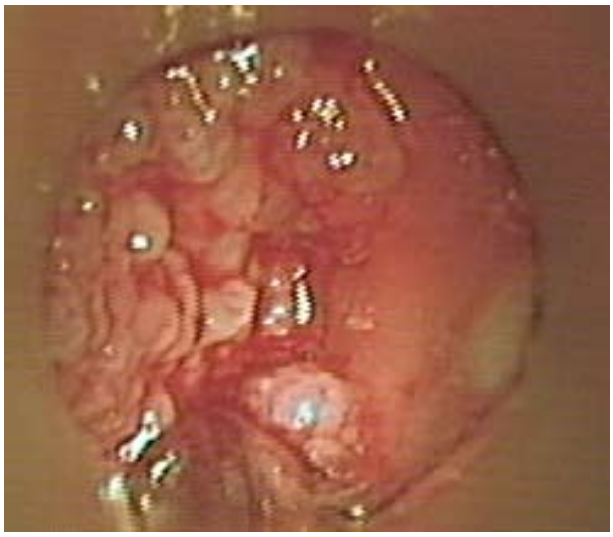

Fig. 5. Intraoperative view

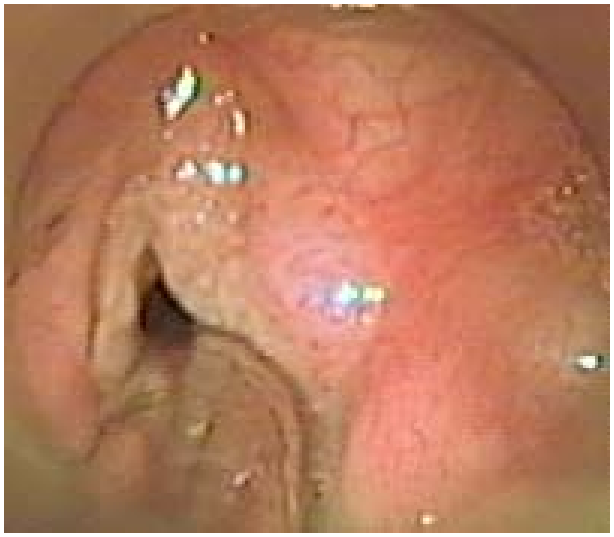

Fig. 6. Intraoperative view

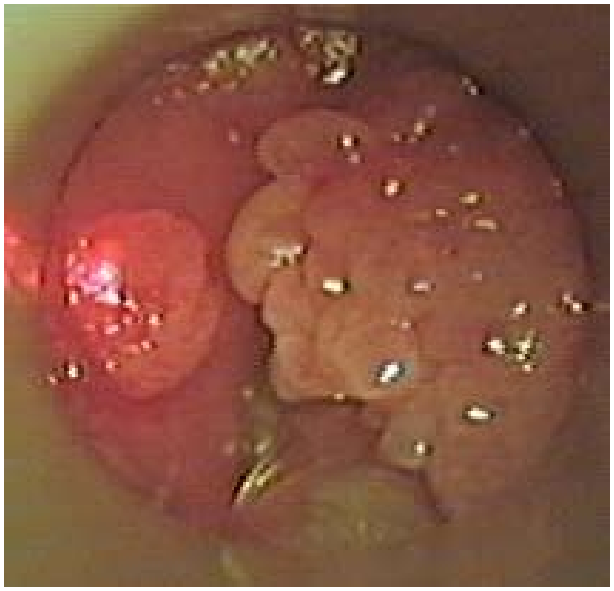

Fig. 7. Intraoperative view

Case management consisted of CO2 laser microsurgery followed by adjuvant therapy with IFN alpha 2b. All patients underwent microsurgical excision and progressive vaporization of papillomas with the CO2 laser under general anaesthesia with good exposure of the anterior and posterior commisure. It was very important to preserve the anterior commisure in order to prevent anterior stenoses, which are not rare. Tracheotomy was not necessary in any of the cases. The dosage of IFN alpha 2b was 100,000 IU/kg/day, 5/7 days during a period of 11 month followed by 50,000 IU/kg/day, 5/7 days for 1 month. Functional aim was to preserve a functional glottis and to protect the laryngeal fundamental functions, for increased quality of patient's life. Follow-up period varied between 4 and 48 month (average 14 months). Followed up data were available for all patients. All were followed up using a precise postoperative protocol.

RESULTS

One goal is to eradicate disease without damaging normal structures. Traditionally this has been done with either cold steel or with the CO2 laser. Other goals of therapy are to relieve airway obstruction, improve voice quality, and facilitate remission. When the papillomas formations were disseminated on a large surface and small in dimension (21 cases) localised at the level of vocal cords and anterior commisure each formation was vaporised with CO2 laser, taking care to not involve the vocal cord mucosa. In case of large and obstructive papillomas formations (4 cases; at the level of vocal cords, anterior and posterior comisure, ventricular folds and laryngeal surface of the epiglottis), we used a forceps, without touching the mucosa or free edge of vocal cord. Regarding posterior commisure and subglottic area (3 cases), the papillomas vaporisation was performed without intubation tube. This area is vaporised last, under a good haemostasis. (Figures 8, 9, 10).

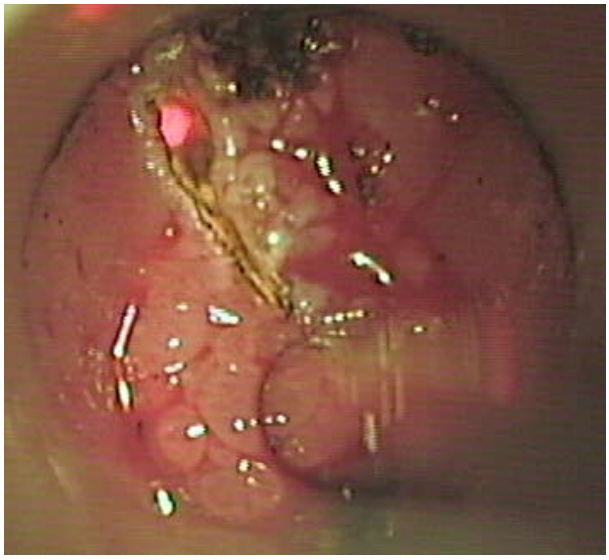

Fig. 8. Intraoperative view

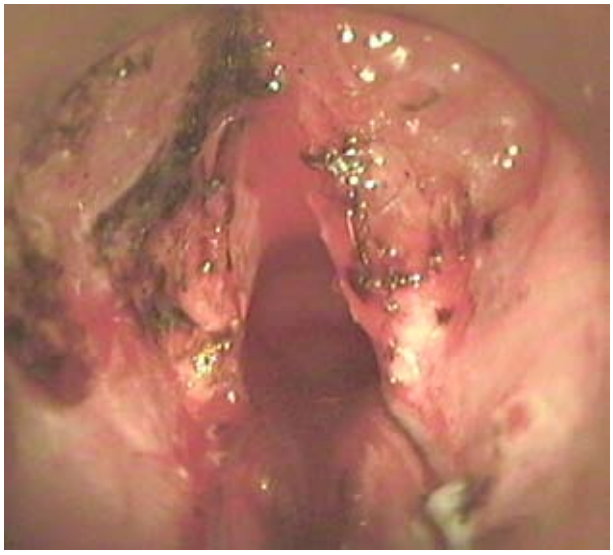

Fig. 9. Postoperative view

Treatment involves repeated debulking of the warty growths by laser surgery. Care must be taken especially around the anterior and posterior commisures to avoid the formation of webs. It is better to be more conservative on one side and leave behind disease than to be aggressive and develop a web. Care must be taken to protect the operating room personnel as papillomata have been demonstrated in the laser plume. Good suction of smoke and laser operating masks are usually

sufficient. Eye protection must be used to avoid laser damage to the globe.

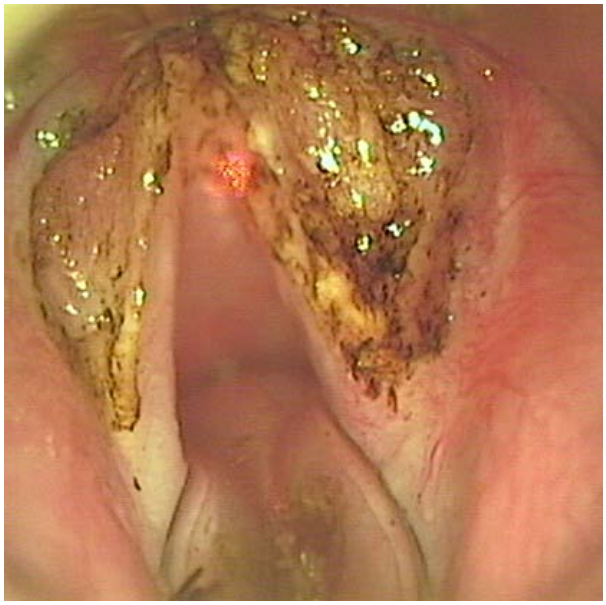

Fig. 10. Postoperative view

Interferon is a product of human leukocytes, although it is now produced via recombinant technology. Interferon treatment appears to slow the rate of growth without curing the disease. Although some antiviral agents (egg, cidofovir, acyclovir) also may slow the rate of regrowth of lesions, they are not curative. Eventually, some patients may enter remission.

Side effects include flu-like symptoms, elevation of hepatic enzymes, renal insufficiency, anorexia, seizures, GI distress, and transient numbness. There is some suggestion that neutralizing antibodies may blunt its beneficial effect and that this may vary by brand. Results may vary considerably from patient to patient.

The surgical interventions were performed under general endotracheal anaesthesia, concerned about the anesthesiological safety aspects of laser surgery. We didn't notice any accidents or complications regarding CO2 laser procedures during these interventions. No intra and postoperative haemorrhages occurred.

We used a minimal postoperative aftercare: antibiotic prophylaxis for 5 days with ampicilin 2g per day im to prevent a bronchopneumonia or a local infection; mucolytics; minor antalgics in the first 24-48 hours to prevent local pain.

We have no postoperative complication. None of the 28 patients had impairment of voice. Clinical examination revealed regression of papillomas in all patients. Functional results were very good at 24 patients (85.71%) and good at 4 patients (14.28%). The results of these 28 patients are:

• Free of disease in 19 cases, 67.85%

• 9 patients (32.14%) had partial response to the combined treatment, in these cases second intervention was needed. No perioperative or postoperative complications and no severe side-effects were noted.

Time interval from surgical intervention to recurrences was on the average 12 months, between 3 and 28 months. In cases of recurrence, the patient underwent CO2 laser removal of the papillomas formations and adjuvant therapy with IFN alpha 2b.

Average hospitalisation was 5 days, between 3 days to 12 days for the patient with bronchopulmonar complication. Treatment failures, recurrences are due to its normal evolution of the disease.

DISCUSSIONS

Since the introduction of the endoscopic CO2 laser microsurgery into clinical otolaryngological procedures, laser surgery for RLP has been gaining increasing importance. RLP represent the most common larynx benign tumour.

The typical course is of recurrent lesions requiring frequent debulking. However, every patient is different and treatment must be tailored to the individual. Some people require such frequent debulking that tracheotomy is necessary for airway protection. This unfortunately frequently leads to lesions around the tracheotomy site (in essence, an iatrogenic squamociliary junction), but traditional thinking has been that this predisposes to distal tracheal disease. A recent study of children, however, suggests that those who require tracheotomy have more aggressive disease and have distal disease prior to tracheotomy, and thus recommends that no child be subjected to a tenuous airway merely to avoid tracheotomy (14). Tracheotomy has two inconvenients: first it facilitates the spread of papillomas and second: produces moisture changes which also facilitate the spread of the virus.

Interestingly, virus can be detected in the normal mucosa adjacent to lesions. It is thought that this provides a reservoir for regeneration of new papillomata (15).

The natural history of the disease includes spontaneous remission. It is impossible to know to whom or when this will occur. The causes of remission are not known, and while the exact incidence is not known, it is thought that approximately one-third will remit by age forty. This makes clinical trials somewhat difficult inasmuch as it confounds the ability to tell who remitted due to treatment and who remitted due to the natural history of the illness.

A recent survey of otolaryngologists showed that 92% favoured the laser (16). The KTP laser can be used for more distal disease.

Management of the airway is controversial. In the apnoea technique the patient is intubated and administered 100% oxygen for a period of time. The tube is then removed for a period of time while the surgeon works. The patient is then reintubated and reoxygenated. This may be advantageous in paediatric airways in which there are not much room to work around a tube. Other methods include use of a laser-safe tube and spontaneous ventilation. Another common method is jet ventilation. Although this is generally felt to be safe, there is concern that this method may lead to distal inoculation of the virus. In patients with an existing tracheotomy, a metal tracheotomy tube can be placed to allow laser surgery to be carried out safely. A recent survey of otolaryngologists found the percent who favoured the various techniques as follows: laser-safe tube 46%, jet ventilation 25%, apnoeic 16%, and spontaneous 12% (16).

Due to the nature of the disease adjunctive measures and alternative treatments have been sought out. In the absence of an effective antiviral agent, use of drugs that will augment host defence is a reasonable approach. Seventy-five to 80% of patients respond to interferon, with a complete response in about 30%. Interferon does not eradicate the virus, and relapse may occur after discontinuation of treatment. Therapies which have been explored but rejected include steroids, estrogens, cryotherapy, cautery, ultrasound, radiation, vaccines, resin of podophyllum, transfer factor, levamisole, suction diathermy, lymphokines, escarotics, calandine, magnesium, and antibiotics (17). Newer therapies which have been tested include alfa-interferon, indole-3-carbinol, acyclovir, retinoic acid, ribavirin, methotrexate, cidofovir, and photodynamic therapy. Case reports suggest that combined treatment with acyclovir or retinoic acid may be beneficial in patients with recurrent disease during interferon treatment.

Some studies and uncontrolled observation in patients with RRP indicate that a diet high in cruciferous vegetables (egg, cabbage, cauliflower, broccoli, Brussels sprouts) may have a favourable effect. Researchers hypothesize that indole 3-carbinol is the active agent in these vegetables; its role is under study.

Cidofovir (Forvade, Vistide) - Currently approved for treatment of CMV retinitis in AIDS. Cidofovir is the first member of a group of antivirals known as acyclic phosphonate nucleotide analogs. In infected cells, nucleotide analogs such as cidofovir inhibit viral DNA polymerase, which is responsible for replication of new viral RNA and DNA. Because HPV is the causal agent for RRP, eradication of the virus offers the potential for cure. Intralesional use is beneficial.

The etiologic link between maternal condyloma at delivery and JORRP in the infant was first recognized by Hajek (2) in a case report in 1956. This observation was supported by additional case reports (4, 18, 19). Subsequent virologic studies fully substantiated the link between genital condylomas and JORRP. HPV types 6 and 11 which are responsible for 80-90% of the condylomas are documented in nearly 100% of JORRP (20,21,22). Transmission of the virus from mother to infant is believed to occur predominantly intrapartum (18). Cases of JORRP rarely give a history of caesarean birth, an indication that caesarean delivery decreases the risk of acquiring JORRP. (23) Adult-onset RRP (AORRP) is also caused by infection with HPV-6 and HPV-11 but very probably, the infection is not acquired at birth (24).

Genital tract infection with HPV-6 and HPV-11 is common, but JORRP is rare. Data are not available to make a reliable estimate of the risk of transmission from an infected mother to a child but this risk is perceived to be low (23,24).

The guideline on perinatal care of the American College of Obstetricians and Gynaecologists states that "caesarean delivery is not recommended solely to protect the neonate from HPV infection". We have estimated that the risk of transmission of JORRP from a condylomatous mother to an infant may be 1-3%, (25) and could be as high as 8% for first-born children of teenage mothers (Bishai et al., unpublished data). The assumptions on which these estimates are made, as well as other considerations (e.g., maternal morbidity due to caesarean delivery, cost-benefit analysis of caesarean delivery), need to be debated before a population-wide policy regarding the prevention of JORRP by caesarean deliveries is instituted, but personal choice is a different matter. It is highly probable that some women at risk would be willing to personally incur the extra expense and operative risk of caesarean delivery to eliminate a 1%-8% chance of JORRP in their child. Several new approaches toward prevention and treatment of condylomas are promising (26,27). Any treatment that would reduce the HPV viral burden in the genital tract during labour, or diminish fetal contact with maternal virus, would likely decrease the incidence of JORRP (28,29).

CONCLUSIONS

In conclusion, RRP is a disease which causes a substantial human and financial cost to the public. HPV has been shown to be the aetiology. It affects people of all ages. Life-threatening airway obstruction may develop. The natural history is poorly understood but is characterized by spontaneous remission in some patients. Treatment is essentially palliative with surgical debulking. Various adjunctive drugs have been developed which slow but do not eradicate the progression of disease in some patients. Currently there is ongoing research aimed at improving the treatment of this insidious disease.

Because the disease is uncommon and requires direct laryngoscopy for diagnosis, children usually have symptoms for a year before a physician makes the diagnosis. The morbidity of this disease has been studied more completely for JORRP, in which the average number of surgical procedures required is 4.4 per child per year, and the average number of procedures per child's lifetime is more than 20.

Although they can be found anywhere in the aerodigestive tract, there appears to be a predilection for areas where there is a junction of squamous and cilliary epithelium. This includes the limen vestibuli (junction of the nasal vestibule and the nasal cavity proper), nasopharyngeal surface of the soft palate, midzone of the laryngeal surface of the epiglottis, upper and lower margins of the ventricle, undersurface of the vocal folds, and the carina and bronchial spurs.

Because the disease is rare, large-scale trials of medical therapies have not been possible; however, several agents are available that appear to increase the intervals at which surgical debulking is required. These include systemic and intralesional interferon, intralesional cidofovir, indole 3-carbinol, and photodynamic therapy. Agents that demonstrate variable effects include cimetidine, acyclovir, and retinoic acid.

The carbon dioxide laser is the preferred method for resection of papillomas because it affords good haemostasis and minimizes potential thermal injury of surrounding healthy tissues.

Combined treatment with Endoscope CO_2 laser Microsurgery and Interferon alpha 2 b is considered to be the election method in RLP, offering long remission periods and sometimes healing. CO2 laser ablation permits the protection of vocal cords, allowing a normal breathing, and especially a normal voice. The associated treatment with IFN alpha 2b has largely improved the prognosis of this viral disease. The good functional results, with the local control of the tumour for a long period of time and a short hospitalization represent the most important advantages of this treatment method.

This combined treatment is a method of choice in laryngeal papillomatosis in children, causing longer remission of the disease. Surgical ablation with laser CO2 is the elective treatment and its combination with IFN improved the prognosis of this recurrent viral disease. Reachable functional results, good tumour control, short hospitalization makes it a favourable treatment in paediatric patients.

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MANAGEMENTUL PAPILOMATOZEI LARINGIENE

REZUMAT

Obiective: Papilomatoza laringiană juvenilă (PLJ) este o afecțiune de o etiologie virală cauzată de Virusul Papilomatozei Umane tip 6 și 11, cu o incidență de 4-7 cazuri/1 milion, caracterizată prin multiple recidive ale formațiunilor tumorale benigne ale mucoasei laringiene, care determină o morbiditate semnificativă în patologia pediatrică ORL. Reprezintă cea mai frecventă tumoră benignă a laringelui, cu o morbiditate ridicată, dificil de tratat datorită recidivelor și a diseminării de-a lungul tractului respirator. Scopul studiului este de a evalua rezultatele tratamentului combinat prin microchirurgie endoscopică cu laser CO2 și Interferon alpha 2b la copiii cu papilomatoză laringiană.

Material și Metodă: In acest studiu au fost incluși, în perioada 1996-2008, 28 de pacienți cu vârste cuprinse între 2,9-9 ani diagnosticați cu papilomatoză laringiană. Tratamentul a constat în microchirurgie endoscopică cu laser CO2 (excizia și vaporizarea formațiunilor tumorale), urmată de tratament adjuvant cu Interferon alpha 2b. Traheotomia nu s-a practicat la nici un caz.

Rezultate: Examinările endoscopice postoperatorii au constatat regresia formațiunilor papilomatoase la toți pacienții. La 19 pacienți (67,85%) s-a constatat regresia completă a afecțiunii, după microchirurgia cu laser CO2, ca metodă primară, și tratamentul adjuvant cu Interferon. Răspunsul parțial la tratamentul combinat s-a constatat la 9 pacienți (32,15%). In aceste cazuri a fost necesară reintervenția. Nu s-au înregistrat complicații intraoperatorii și postoperatorii, precum și efecte adverse severe ale tratamentului cu Interferon.

Concluzii: Tratamentul combinat al papilomatozei laringiene reprezintă terapia de elecție cu perioade de remisiune lungi. Acest tratament a îmbunătățit prognosticul acestei afecțiuni virale. Rezultatele funcționale bune, cu controlul local al formațiunilor tumorale papilomatoase, spitalizarea de scurtă durată, reprezintă elementele favorabile ale acestei metode de tratament la copiii cu papilomatoză laringiană.

Cuvinte cheie: papilomatoză laringiană juvenilă, tratament cu laser CO2, tratament cu IFN alpha 2B

URBAN NOISE AND ENVIRONMENTAL COMPLAINTS IN TIMISOARA

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ABSTRACT

Urban road noise has always been an important environmental problem for man from ancient history. But despite the fact proven by measurements and calculations that road traffic is the main source of noise in urban areas, citizens have the tendency to accept the situation as part of modern way of life and as a "necessary evil". This paper compares the results of noise measurements and the noise related complaints of the citizens from Timisoara.
Keywords: urban noise, noise maps, complaints, environmental protection

INTRODUCTION

Noise has always been an important environmental problem for man. In ancient Rome, rules existed as to the noise emitted from the ironed wheels of wagons which battered the stones on the pavement, causing disruption of sleep and annoyance to the Romans. In Medieval Europe, horse carriages and horse back riding were not allowed during night time in certain cities to ensure a peaceful sleep for the inhabitants. However, the noise problems of the past are incomparable with those of modern society. An immense number of cars regularly cross our cities and the countryside. There are heavily laden Lorries with diesel engines, badly silenced both for engine and exhaust noise, in cities and on highways day and night. Aircraft and trains add to the environmental noise scenario. In industry, machinery emits high noise levels and amusement centers and pleasure vehicles distract leisure time relaxation.

In comparison to other pollutants, the control of environmental noise has been hampered by insufficient knowledge of its effects on humans and of dose-response relationships as well as a lack of defined criteria. While it has been suggested that noise pollution is primarily a "luxury" problem for developed countries, one cannot ignore that the exposure is often higher in developing countries, due to bad planning and poor construction of buildings. The effects of the noise are just as widespread and the long term consequences for health are the same. In this perspective, practical action to limit and control the exposure to environmental noise are essential. Such action must be based upon proper scientific evaluation of available data on effects, and particularly dose-response relationships. The basis for this is the process of risk assessment and risk management.

The extent of the noise problem is large. In the European Union countries about 40% of the population is exposed to road traffic noise with an equivalent sound pressure level exceeding 55 dBA daytime and 20% are exposed to levels exceeding 65 dBA. Taking all exposure to transportation noise together about half of the European Union citizens are estimated to live in zones which do not ensure acoustical comfort to residents. More than 30% are exposed at night to equivalent sound pressure levels exceeding 55 dBA which are disturbing to sleep. The noise pollution problem is also severe in cities of developing countries and caused mainly by traffic. Data collected alongside densely traveled roads were found to have equivalent sound pressure levels for 24 hours of 75 to 80 dBA (2).

For these reasons, in this paper, the noise level measurement are put side by side along the Timisoara City Hall's noise maps and the peoples response to noise issues recorded as written complaints to the environmental authorities of the city of Timisoara

MATERIALS AND METHODS

During 2001 and 2006 a number of 630 daytime and nighttime measurements were conducted in randomly selected measurement points in the urban area of Timisoara by the environmental authority of Timisoara. The measurement points were classified according to the function of the selected urban area in traffic, residential, recreational, protected (medical treatment & care) and industrial areas (5).

The measurements were made with a Bruel&Kjaer 2238 Mediator (Class 1 Integrating Sound Level Meter with logging software) fitted with an outdoor kit (free-field microphone with windscreen, cable preamplifier extension and tripod).

The microphone was set at a height of 1.3 m form the ground level, according to Romanian noise measurement standards (5).

Traffic area measurements were made at the edge of the pathway (25m from the axis in the railways case), industrial and protected area measurements were made along the specific perimeter, recreational area measurements were made inside the area, as central as possible and in the residential area measurement were conducted at a distance of 3m form the façade of the buildings, facing the noise sources, according to Romanian measurement standards (4).

Measurement data included LAeq (A-weighted equivalent noise level) L90 (background noise) and L10 (maximum noise). Lowest sound pressure levels and peak levels were also recorded. The measurement unit was the dBA (A-weighted decibel) (4).

Computed Data in the process of completing the first stage of city's noise mapping by the Timisoara City Hall according to European Commission 2002/49/EC Noise Directive were used to asses the percentage of population affected by various type of noise: road traffic, public rail transportation, industrial and air traffic noise (1,7).

Last but not least, a number of 1523 written complaints addressed during 2001-2006 to the environmental authority were processed and those referring to environmental noise issues were studied in order to find possible links between the data provided by noise measurements, noise maps predictions and citizen's opinions.

RESULTS

Daytime and nighttime measurements of the A-weighted equivalent noise level indicated that the main cause of the urban noise is the road traffic. This type of noise source is combined with the public rail transportation traffic (trams and trains).

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No matter of the type of the zone in which the measurements took place (residential, recreational, industrial or protected) the overall noise level was more or less accurate defined by the technical category of the neighboring roads (6): 1st category roads at the city limits with measured LAeq between 75 dBA and 80 dBA, 2nd category roads – the city's main boulevards and roads with measured LAeq between 60 dBA and 74 dBA, 3rd and 4th category roads – connecting street between main roads, usually with less traffic and lower speed limits (3) with measured LAeq between 49 dBA and 59 dBA. The majority of roads cross the residential area of the town so the impact upon the population of road traffic generated noise is significant.

According to Timisoara City Hall noise mapping, completed in 2008 (7), 52.8% of the population from Timisoara's residential area is affected by daytime road traffic noise levels over 65 dBA; 15.0% of the population is exposed to noise levels exceeding 75 dBA.

The above values should be compared with the 5.5% of the population exposed to tram and railway generated noise (in the 55 dBA to 65 dBA domain) and 0.0% of the population exposed to air traffic generated noise.

Industrial activities exposes about 0.6% of the population to noise levels between 55 dbA and 65 dBA.

The overwhelming impact of road traffic on noise level in the residential area is evident.

These numbers are calculated projections of the Lden which is an indicator of the overall noise level during the day, evening and night which is used to describe the annoyance caused by exposure to noise, according to EC noise mapping specifications (1).

As for the citizens' complaints regarding different environmental issues, the study of the 2001-2006 archives of the Environmental Authority in Timisoara led to the following results:

- from the total number of written complaints (1523), 31.5% are referring to environmental noise;
- the rest of the complaints refer to air pollution (44.1% - smoke, dust, gas, smell) and water and soil pollution (24.4% - dejections, waste, oil and derivates, pesticides and chemicals/poisons)

The noise complaints are divided as:

- 35.2% are reporting daytime annoyance from noise resulted in the activity of small and medium enterprises (car services, car washes, metal works, bakeries, warehouses, construction yards, saw-mills, factories);
- 33.3% are reporting nighttime annoyance from high level music played inside neighboring bars and restaurants;
- 10.8% are reporting nighttime annoyance from high level music played inside neighboring clubs and discotheques;
- 5.4% are reporting nighttime annoyance from noise generated by air conditioners and chillers;
- 4.5% are reporting daytime and nighttime annoyance from noise generated by football supporters, football matches and celebrations held in/around the city's main football arena;
- 8.1% are reporting daytime and nighttime noise from various sources (road/water/sewer/gas maintenance work, dogs, background noises like electric transformer buzzing or pipes hissing, dogs barking, peddlers and illegal motorcycle racings);
- 2.7% are reporting daytime and nighttime noise generated by the road traffic, including public rail transportation (trams and trains).

CONCLUSIONS

Measurements conducted in hundreds of randomly selected points of the urban area of Timisoara indicate the road traffic as the main cause of the urban noise. Public rail transportation (trams and trains) although affects a much smaller residential area and only for short periods of time, generate high levels of noise that could result in serious annoyance especially during night time.

Noise maps calculated values are confirmed by field measurements and indicate a semnificative impact on more than half of the city's population.

Despite these affirmations and despite all the experimental data, only a small percentage of the population reports road traffic to the authorities as being a serious annoyance.

This attitude is due to several factors: the increase in demographic density, the increase in the number of per capita vehicles, the fact that society is getting used to higher noise levels and accepts road traffic as a "necessary evil", and the widespread ignorance regarding the consequences of noise as well as its remedies.

However, it is interesting to remark that citizens that dwell in residential areas exposed to heavy daytime and nighttime road traffic have a much lower "noise acceptance threshold" and will promptly forward complaints for any newly arrived noise source in the residential area, such as a loud music playing restaurant, but will continue to accept or ignore the main noise generator: the road traffic.

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ZGOMOTUL URBAN SI SESIZARILE LEGATE DE PROTECTIA MEDIULUI IN MUNICIPIUL TIMISOARA

REZUMAT

Zgomotul rutier urban a fost o problema de mediu inca din antichitate. In pofida faptului ca masuratorile si calculele indica traficul rutier ca sursa principala a zgomotului urban, cetatenii au tendinta sa accepte situatia ca parte a modului de viata modern si zgomotul generat de traficul rutier ca pe un "rau necesar". Acest articol compara rezultatele masuratorilor de zgomot cu sesizarile legate de zgomot facute de cetatenii orasului Timisoara.

Cuvinte cheie: zgomot urban, harti de zgomot, sesizari, protectia mediului

BOOK REVIEW

STRESSOLOGY, ADAPTOLOGY AND MENTAL HEALTH

SORIN RIGA AND DAN RIGA

CARTEA UNIVERSITARA PUBLISHING, BUCHAREST, 2008, XII + 266 PAGES

Stressology, Adaptology and Mental Health", published by Cartea Universitara Press, Bucharest, 2008, was written by Sorin Riga, MD, PhD, DHc, and Dan Riga, MD, PhD, DHc, Directors of the Department of Stress Research and Prophylaxis, from "Al Obregia" Clinical Hospital of Psychiatry, Bucharest. It is a monograph of excelence in a growing field of great interest, based on the authors experience and work in the stress and anti-aging medicine.

The first part, "Stressology" starts with presenting Hans Selye's concepts about stress, adaptation and health. From a physiological and adaptive perspective, stress can be defined as the aggression by the environment on the human body, together with the specific adaptation mechanisms and nonspecific, neuro-endocrine ones, leading to increase the individual's resistance and to prevent morphological and physiological abnormalities, which may generate pathological states. Under normal circumstances the psycho-neuro-endocrine systems permit the individual to react appropriately to stressors, allowing the adaptation to the conditions induced by the stressors. From a global and comprehensive perspective, stress encompasses the socio-psycho-medical dimension regarding the impact of stress in society, the relationship mental health – stress, the ontogenetic dimension and the field of prevention/therapy. The book presents relevant data regarding the international progress in stressology, as well as aspects of the advanced Romanian researches, both in stress and anti-aging medicine. The authors scientific contribution in the area of psychopharmacotherapy of stress and adaptation is impressive and well recognized internationally. The recent brain researches - at macromolecular, metabolic, subcellular, cellular and tissual levels, through advanced neuroscientific techniques – are the background for the concepts, innovations and anti-stress therapies, as well as of improved adaptation and longevity, areas well presented by the authors. The phenomenon of stress is evaluated from various perspectives: the stressors, stress responses, the psychiatric and psychosomatic stress-dependent disorders. Numerous scales, questionnaires and clinical algorithms, useful in stress research, are discussed in stress diagnosis and evaluation, in both medical practice and in health systems services. Acute and chronic stress-related disorders are connected with clinical descriptions and guidelines of international classifications (ICD-10, WHO, Geneva, 1992 and DSM-IV-TR, APA, Washington, DC, 2000). In the end of this section, the authors analyze psychosomatic disorders and medicine.

The second part of the monograph, "Adaptology" reviews the binomial relationship distress – eustress, homeostatic concepts, and adaptation mechanisms, to formulate growth strategies of the bio-psycho-social resistance and the individual's capability to adapt. It also presents allostasis, vitality and vulnerability of human being. The General Adaptation Syndrome and its pathology, the individual's ability to cope (coping/anti-stress mechanisms), the behavior with its typology and abnormalities are aspects approached with the most recent information in the field, filtered through the authors' vast knowledge and experience. Their original contribution, the entropic cascade (distress – impairment – aging – polypathology) is the foundation for a

new paradigm in medicine – both prophylactic and therapeutic, leading to new perspectives in lifestyle, health and longevity management. The authors have valuable researches and results in oxidative stress, diseases of the free radicals, senescence: anti-stress, anti-impairment and anti-aging therapy (Antagonic-Stress®).

Last part, "Mental Health", presents aspects of mental health policies, their institutionalization and organization at national and international levels, through specialized structures. The concept of mental heath is defined and analyzed in an integrative and dynamic vision, as part of the "health-mental health" binomial, as expression of the quality of individual life and quality of the society at economic, administrative and social planes. In the chapter on health and longevity management, the authors offer solutions, elaborating pro-health and pro-longevity strategies, ways for the individual to optimize his/her relationship with himself/herself and with the outside world (physical and psychical activation and rebuilding, emotional-cognitive-volitive-behavioral therapy, learning healthy lifestyles, anti-stress and anti-aging programmes).

The book is a premiere performance in Romanian medicine, a reference

work in the field, from theoretical, fundamental, experimental to applied clinical perspectives and medical sociology. In a concise, impressive and very well structured presentation, with an actual bibliography for each chapter, the authors offer new concepts and strategies, modern studies in depth, of great use in teaching and research, as well as numerous diagnostic and therapeutic tools, useful in medical practice. Therefore, drs. Riga's contribution represents a progress in the future medicine and personalized health. This monograph is an asset for physicians, psychologists, psychotherapists, sociologists, specialists in mental and public health, for all those interested and involved in promoting healthy life patterns.

Assoc. Prof. Dr. Daniela Motoc