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PO BOX 135
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e-mail: carmen.tatu@umft.ro

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REVIEW

THE INFLUENCE OF EXTERNAL AUDITORY STIMULI ON ELECTRICAL BRAIN ACTIVITY

MARIUS GEORGESCU1, ALINA FLORINA SERB2, CARMEN TATU2, DANIELA PUSCASIU2, DANIEL GEORGESCU1*, DENISA ENESCU-BIERU3, MARIA IANCAU1

1University of Medicine and Pharmacy, Craiova, Romania; 2University of Medicine and Pharmacy "Victor Babes" Timisoara, Romania; 3University of Craiova, Romania

ABSTRACT

Cognitive neuroscience, through its various neuroimaging techniques, enables us to look at the living brain, providing us with the tools necessary to investigate the neural underpinnings of developmental behavioral change. One of these methods relies on the non-invasive and painless recording of electrical brain activity measured by electrodes placed on the scalp-electroencephalography (EEG). EEG patterns of brain activity have been shown to modify under numerous variables, one of which is the range of auditory stimuli – including music. Understanding the neurobiological, neurophysiological and neuropsychological bases of the musical experience is important not only from the perspective of promoting basic research and knowledge, but also in view of the potential application of music therapy in clinical practice. In this regard, the present review provides a brief overview of currently available literature data concerning the influence of music on electrical brain activity while also examining the complex interplay of diverse neurobiological factors involved in musical processing in the brain, and the potential clinical applications of music.

Keywords: EEG, music, brain, primary auditory cortex, Mozart effect, music therapy

INTRODUCTION

During the more than 100 years of its history, EEG has undergone massive development. The existence of electrical currents in the brain was discovered in 1875 by the English physician Richard Caton. The first report on electrical brain activity in humans was published in 1929 by Berger, allowing clinicians and scientists to peek into the skull and watch the brain in action for the first time in a meaningful way. Over the years, due to the improved recording and the significant progress in signal processing and data acquisition systems, EEG analysis has become important in diagnosing various brain related diseases and in designing prosthetic devices and became more popular as it discovered critical facts about the working of the brain. Nowadays EEG atlases are provided, filled with different brain-wave patterns and respective diagnoses. However, further understanding of brain functioning is still very actual for widening horizons of both theoretical and practical knowledge.

Throughout history, several studies were concentrated on the effect of external stimulation on the cortical EEG, predominantly in photic driving response (response to visual stimulation) (1, 2). Interest in using visual and auditory rhythmic stimulation as a means of inducing relaxation and hypnosis was raised in the middle of the last century (3). More recently, devices using light and sound at specified frequencies were used to "drive" the EEG towards certain frequencies. In the last decades audio-visual stimulation has been reported as an effective method in various fields -from relieving dental anxiety through inducing hypnagogic states helping and relieving tension and migraine headaches to therapeutic effects on premenstrual syndrome (4).

Music has been proven to be a valuable a tool for the understanding of human cognition, human emotion, and their underlying brain mechanisms. Music is part of the human nature: throughout human history, in every human culture, people have played and enjoyed music. Some researchers theorize that human musical abilities played a key phylogenetical role in the evolution of language and that music-making behavior engaged and promoted evolutionarily important social functions (such as communication, cooperation, and social cohesion (5)). Since early times, music has been used for healing and stimulating emotions; the beneficial influence of music on epileptiform activity in patients with seizures (6), as well as in individuals with hearing loss and early childhood autism (7) were reported within the last decades. However, EEG investigations of brain activity while influenced by music are scarce. The following sections will review research findings on the influence of music as external auditory stimulus on electrical brain activity, synthesizing current knowledge of this field of neurophysiology.

BIOLOGICAL EFFECTS OF MUSIC

Musical stimuli have been shown to activate specific pathways in several brain areas associated with emotional behaviors, such as the insular and cingulate cortex, hypothalamus, hippocampus, amygdala, and prefrontal cortex, and improve plasticity and neurogenesis (8).

In addition, neurochemical studies have suggested that several biochemical mediators may play a role in the musical

Received 20th of March 2012. Accepted 15th April 2012. Address for correspondence: Daniel Georgescu, Department of Functional Disciplines, UMF Craiova, Petru Rareş no. 2-4, 200349, Craiova, Romania, e-mail: d_g_home@yahoo.com

experience. Pleasant music releases dopamine in the nucleus accumbens, while listening to slow music decreased the level of norepinephrine (a neurotransmitter that regulates arousal) (9). Another neurotransmitter that responds to music is serotonin. Pleasant music increases the release of serotonin - responsible for good moods - in the brain, while unpleasant music reduced the level of serotonin (10). Similarly, pleasant music elevates endorphin level (11), which induces a feeling of well-being and relaxation. Suda et al. demonstrate that major music (Mozart's Allegro con spirito, K448, which induces happiness) reduced stress and cortisol level more than minor music (Beethoven's fur Elise, which induces sadness). ''Techno music' increases the level of many stress hormones including cortisol, while classical music (Beethoven Symphony 6) reduces cortisol level (12).

In regards to the immune system, studies show that certain types of music can modify the level of certain immunological components such as immunoglobulin A (IgA) and natural killer cells (13). Recreational music improves mood and modulates the activity of natural killer–cells and the level of the cytokines IL-6 and IL-10, which are immunological 'stress markers' (14).

Regarding heart activity, studies show that music (Mozart, Symphony # 40, Ligeti, String Quartet # 2) can increase or decrease heart rate and blood pressure (15). Musical stimuli and physiological processes alike (heartbeat, respiratory rate, blood pressure, temperature) are composed of vibrations that occur in a regular, periodic manner and consist of oscillations. Musical stimuli, specifically rhythm and tempo, can be used as a synchronizer to influence changes in physiological responses (i.e. heartbeat, respiration, blood pressure) through entrainment.

MUSIC PERCEPTION

Music perception involves acoustic analysis, auditory memory, auditory scene analysis, processing of interval relations, of musical syntax and semantics, and activation of (pre) motor representations of actions (16). Music perception begins with the decoding of acoustic information. Acoustic information is translated into neural activity in the cochlea, and progressively transformed in the auditory brainstem, as indicated by different neural response properties for the periodicity of sounds, timber (including roughness, or consonance/dissonance), sound intensity, and interaural disparities in the superior olivary complex and the inferior colliculus (16). It appears, notably, that the dorsal cochlear nucleus projects into the reticular formation (16). By virtue of these projections, loud sounds with sudden onsets lead to startle-reactions, and such projections perhaps contribute to our impetus to move to rhythmic music. Moreover, the inferior colliculi can initiate flight and defensive behavior in response to threatening stimuli (even before the acoustic information reaches the auditory cortex (AC)). From the thalamus (particularly over the medial geniculate body) neural impulses are projected mainly into the primary auditory cortex (PAC, corresponding to Brodmann's area (BAs) 41) and adjacent secondary auditory fields (corresponding to BAs 42 and 52). These auditory areas perform a more fine-grained, and more specific, analysis of acoustic features compared to the auditory brainstem. PAC is also involved in the transformation of acoustic features (such as frequency information) into percepts (such as pitch height and pitch chroma). Moreover, with regard to functional differences between the left and the right PAC, as well as neighboring auditory association cortexes, several studies suggest that the left AC has a higher resolution of temporal information than the right AC, and that the right AC has a higher spectral resolution than the left AC (17). Finally, the AC also prepares acoustic information for further conceptual and conscious processing. For example, regarding the meaning of sounds, just a short single tone can sound, for example, "bright," "rough," or "dull."

The auditory sensory memory is connected with both working memory (WM) and long-term memory. Structure building requires WM as well as a long-term store for syntactic regularities, and processing of information meaning is presumably tied to a mental "lexicon" (containing conceptual– semantic knowledge), as well as to a musical "lexicon" containing knowledge about timbers, melodic contours, phrases, and musical pieces (16). The knowledge about musical long-term memory is still rather limited. Functional neuroimaging data suggest that access to musical semantic memory involves the (left) middle temporal gyrus, and that musical semantic representations (that is, parts of a musical lexicon) are stored in (left) anterior-temporal areas (18). In addition, Watanabe et al. (19) reported that retrieval of musical information involves the hippocampal formation and the inferior frontal gyrus.

Functional neuroimaging studies using chord sequence paradigms (20) and melodies (21) suggest that music-syntactic processing involves the pars opercularis of the inferior frontal gyrus (corresponding to BA 44) bilaterally, but with right- hemispheric weighting. The assumption of an intimate connection between music and speech is corroborated by the reviewed findings of overlapping and shared neural resources for music and language processing in both adults and children. Spoken language has rhythm, melody, and timber, and the illusory transformation from speech to song shows that humans can perceive spoken language also as song (although individuals often do not realize this in everyday life). Both speech and music perception involve premotor coding and both music and language give rise to affective processes. These findings suggest that the human brain, particularly at an early age, does not treat language and music as strictly separate domains, but rather treats language as a special case of music (16).

Moreover, music perception potentially elicits emotions, thus giving rise to the modulation of emotional effector systems such as the subjective feeling system, the autonomic nervous system, the hormonal, and the immune system (22). Interestingly, effects on the immune system have been suggested as tied to motor activity such as singing or dancing. With regard to music perception, it is important to note that there might be overlap between neural activities of the late stages of perception and those related to the early stages of action (such as premotor functions related to action planning. Action induction by music perception is accompanied by neural impulses in the reticular formation (in the brainstem; for example, for the release of energy

to move during joyful excitement). It is highly likely that connections also exist between the reticular formation and structures of the auditory brainstem (as well as between reticular formation and the AC), and that the neural activity of the reticular formation therefore also influences the processing of (new) incoming acoustic information (16).

THE MOZART EFFECT

The "Mozart effect" refers to an enhancement of performance or change in neurophysiologic activity associated with listening to Mozart's music. Neurophysiologic changes while listening to Mozart were mainly observed using EEG power and coherence (Coh) measures.

A recent study (23) using induced event-related desynchronization/synchronization (ERD/ERS), and event-related coherence (ERCoh) indicated that Mozart's music-with no regard to the level of induced mood, musical tempo and complexity-influenced the level of arousal. It was suggested that modulations in the frequency domain of Mozart's sonata have the greatest influence on the reported neurophysiologic activity comparatively with Brahm's and Haydn's music. In another study (24) it was found that auditory background stimulation (Mozart's sonata K. 448) can influence visual brain activity, even if both stimuli are unrelated. Students who solved a simple visual task while listening to Mozart's music displayed (mainly in the γ band) more coherent brain activity, whereas a decoupling of brain areas in the γ band was observed while respondents solved the same task in silence.

Listening to Mozart's music increases the coupling of specific brain areas and thus facilitates the selection and "binding" together of pertinent aspects of sensory stimulus into a perceived whole. It can be further assumed that if such a pattern of activated brain areas coincides with the pattern needed for task completion, an increase in task performance could be the result. This hypothesis was also supported by a recent study (25). Using ERD/ERS and ERCoh, it was shown that Mozart's music had a beneficial influence on respondents' performance of spatial rotation tasks, and a slightly negative influence on the performance of numerical tasks as compared with the silence condition. On the physiological level a general effect of Mozart's music on brain activity in the induced γ band was observed, accompanied by a more specific effect in the induced lower- 2 α band which was only present while respondents solved the numerical tasks.

Further data (26) showed that all conditions involving Mozart's music (either prior to (MS), or after the training session (SM), or prior to and after the training session (MM) listened to music) had a beneficial influence on the solution of the spatio-temporal rotation tasks. On the physiological level a measurable influence on EEG activity (analyzed using the methods of ERD/ERS and approximated entropy (ApEn)) was only observed for respondents of the MM group who prior to and after training listened to music. The displayed pattern of brain activity (lower α and γ ApEn, more α and γ band ERS) of the respondents who prior to and after learning listened to Mozart's music is similar to findings reporting neurophysiological differences in brain activity related to verbal and performance components of intelligence, creativity and emotional intelligence (27).

The beneficial influence of Mozart's sonata K. 448 on epileptiform activity in patients with seizures was reported by Hughes et al. (28). In a follow-up study analyzing the music of Haydn, Liszt, Bach, Chopin, Beethoven and Wagner it was found that Mozart's music continued to score significantly higher than the selections from the other six composers (6).

THE PATTERNS OF BRAIN ELECTRICAL ACTIVITY DURING LISTENING TO MUSIC

1. The Influence of music on brain electrical activity in neurologically healthy newborns

Auditory capability is one of the earliest discriminative abilities of the foetus. Music is an intentional auditory stimulus with organized elements including melody, rhythm, harmony, timbre, form and style. Music soothes, and the existence of lullaby is evidence of the innate acceptance that music quietens babies. Informal observation of infants' and young children's musical behaviors strongly implied the presence of musical cognitive processes even in earliest infancy. Some level of cognitive activity which organizes sense input into some coherent units exists from birth (29). In order to gain some understanding of the musical cognitive process shortly after birth Hefer et al. (30) investigated the reponses of neonates (37–40 weeks of gestational age) to random sounds and to music (a segment from Mozart's Piano Concerto No. 8 in C major) respectively. The methodology included: video recordings and physiological measurements collected by a polysomnograph and also recordings of body and facial expressions carried out by a mini digital video camera; EEG was recorded continually during the test using 6 electrodes. The analysis of EEG recordings evidenced that musical excerpts have induced a harmony in the infants' brain waves, which were unlike those registered during the silence and the random sounds. Investigation of the kinaesthetic behavior of the newborns revealed that they used cyclical motions that matched the musical phrases of the Mozart excerpts while during the 'random' excerpt there was a near total absence of cyclical movements and a sharp decrease in movements in general. Thus, the EEG results supported the observation that random material is perceived differently than organized sounds, i.e. music, and therefore does not evoke cognitive activity associated with the processing of music.

It has been suggested that recorded music is a valuable resource in the neonatal intensive care unit (NICU) to reduce stress and increase physiological stability, to provide better growth rates and to facilitate neurosensory maturation (31). The amplitude-integrated electroencephalography (aEEG) is now increasingly used in NICUs with demonstrated clinical value in the monitoring of the newborn infant's brain function. The method is based on filtered and compressed EEG that enables evaluation of long-term changes and trends in electrocortical background activity by relatively simple pattern recognition. In aEEG, sleep–wake

cycling (SWC) appears as a sinusoidal pattern with varying bandwidth and continuity, more discontinuous during quiet sleep (QS) than during wakefulness and active sleep. The average duration of QS periods is 24– 28 min between 32 and 36 post-conceptional weeks. In this view, using aEEG, Olischar et al. (32) examined the possible effects of music on QS in neurologically healthy newborns (≥32 gestational weeks). Ten subjects were exposed to music (Music for Dreaming- Brahms' Lullaby) using a CD player (50–55 decibel A), and 10 more subjects represented the control group. aEEG activity was recorded before and after musical stimulation, in order to obtain data for one SWC before and two to three SWCs after musical stimulation. Background pattern, presence and quality of SWCs were assessed before and after exposure to music. All 20 subjects showed a normal continuous background activity and well-developed SWCs. SWC is present in healthy term newborns and is a sign of neurological integrity and the presence of SWC pattern on aEEG is therefore considered a good prognostic sign. The obtained results showed that music tends to change the appearance of SWC by lowering minimum amplitude (5.8 μV vs. 7.7 μV for controls). SWC became more sinusoidal through reduction in minimum amplitude and therefore more easily discernable. The observed trend to more mature SWC in subjects who were exposed to music when compared to controls could suggest that music might be a positive stimulus to the newborn brain.

2. The Influence of music on brain electrical activity in neurologically healthy adults

The data in literature concerning changes in patterns of brain electrical activity during listening to music is contradictory (33, 34). This concerns both the power spectra and the synchronism of brain potentials. Some authors point out that listening to music increases the power of the θ and α frequencies of the human EEG; others have found that the initial α rhythm changes to activity in the Δ , θ , and β bands.

Most frequently, researchers classify musical compositions only as classical or rock music. However, the effect of music on a listener is largely determined by its intensity. Changes in the EEG spectral power (SP) depend on the intensity and style of music: listening to classical music (of low and moderate intensity) produces an increase in the SP of the high-frequency EEG (α 2, β 1, β 2, and γ bands) widely generalized over the brain cortex, while listening to rock music (of moderate and high intensity) produces an increase in the SP predominantly in the θ and α 1 frequency bands (35). Different musical styles and intensities produce characteristic patterns of changes in the correlation of neocortical biopotentials (36): classical music of three intensities (low, medium and high) and rock music of medium and high intensities were shown to produce asymmetry in the pattern of coherence (Coh) of the cortical activity of listeners: intrahemispheric Coh in the right hemisphere increased, while a decrease in the EEG synchronization prevailed in the left hemisphere. A focus of Coh integration, most pronounced for the γ frequencies, was formed during listening to both classical and rock music (except rock music of a low intensity). Listening to rock music of a low intensity substantially increased the probability of a decrease in intra- and interhemispheric Coh in both hemispheres, which was pronounced in all frequency bands. Listening to rock music changed interhemispheric Coh in a greater percent of cases than listening to classical music. Other data showed that listening to music increases Coh of biopotentials between the right frontal and left temporoparietal areas as well as between distant hemispheric regions (frontal and occipital) (37).

Natarajan et al (38) analyzed the EEGs under music stimulation (rock and classical music) using various nonlinear parameters - Correlation Dimension (CD), Largest Lyapunov Exponent (LLE), Hurst Exponent (H) and approximated entropy (ApEn). EEG signal can be used as a reliable indicator of the state of the mind. The importance of ApEn lies in the fact that it is measure of the disorder in the EEG signal. Obtained results evidenced an ApEn fall indicating the decrease in the disorder of the EEG signal due to rock/classical music. This indicates that α wave tends to become more predominant due to the influence of the music. Similarly, CD, LLE and H also decrease after exposure to music. This suggests that when the subjects are undergoing auditory stimuli, the number of parallel functional processes active in the brain is lessened and the brain goes into a more relaxed state.

Using recent regional brain activation/emotion models as a theoretical framework, Schmidt et al. (39) examined whether the pattern of regional EEG activity distinguished emotions induced by musical excerpts which were known to vary in affective valence (i.e., positive vs. negative) and intensity (i.e., intense vs. calm). The obtained data showed that the pattern of asymmetrical frontal EEG activity distinguished valence of the musical excerpts: subjects exhibited greater relative left frontal EEG activity to joy and happy musical excerpts and greater relative right frontal EEG activity to fear and sad musical excerpts. Although the pattern of frontal EEG asymmetry did not distinguish the intensity of the musical emotions, the pattern of overall frontal EEG activity did, with the amount of frontal activity decreasing from fear to joy to happy to sad excerpts, consistent with behavioral ratings of intensity. This appears to be the first distinction made between valence and intensity of musical emotions on frontal electrocortical measures.

Music could change our emotions, could have an influence on our mood, and could affect our health. Music therapy is one of the oldest methods used for treating some diseases. Real-time emotion recognition from EEG is a new area of research which can be combined with music therapy in order to obtain an algorithm which would allow adapting the therapy to the predefined time of the treatment and adjusting the music therapy session to the current emotional state of the user in the way an experienced music therapist works. In this way, using the International Affective Digitized Sound system and also excerpts from Oscar films' soundtracks (40), four emotions states were induced: positive/aroused (joy), positive/calm (pleasure), negative/calm (sadness) and negative/aroused (anger) after 5 and 30 seconds exposure to music, respectively, followed by 10 seconds of silent/calm state. The subjects' current emotion is recognized from his/her EEG in

real time by applying Fractal dimension (FD) in real-time EEG signal processing and the music therapy is adjusted automatically based on the feedback. Using this algorithm, three types of music therapies were developed to reduce the anxiety level of the patient, to improve the mood of the depressed patients and to improve the tolerance to the pain.

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INTERACTINEA DINTRE CELULELE STEM MEZENCHIMALE SI EFECTORII CELULARI AI SISTEMULUI IMUN

REZUMAT

Neurostiinta cognitiva ofera, prin tehnicile sale neuroimagistice, instrumentele necesare pentru investigarea substratului neural al dezvoltarii modificarilor de comportament. Una dintre aceste tehnici se bazeaza pe metoda non-invaziva si nedureroasa de masurare a activitatii electrice a creierului, cu ajutorul electrozilor plasati pe scalp – electroencefalografia (EEG). Modelele de activitate cerebrala EEG se modifica sub actiunea a numeroase variabile – printre care se numara si stimulii auditivi, inclusiv muzica. Intelegerea substratului neurobiologic, neurofiziologic si neuropsihologic al experientei muzicale este important nu doar din perspectiva promovarii cercetarii si cunostiintelor, dar si in vederea aplicatiilor potentiale ale terapiei muzicale in practica medicala. Review-ul prezent ofera o scurta prezentare generala a datelor din literatura legate de influenta muzicii asupra activitatii electrice a creierului, examinand simultan potentialele aplicatii clinice ale muzicii precum si interactiunea complexa a diversilor factori neurobiologici implicati in prelucrarea muzicii la nivel cerebral.

Cuvinte cheie: EEG, muzica, creier, cortex auditiv primar, efectul Mozart, meloterapie

MODULATORY EFFECT OF GREEN TEA ON LIPID METABOLISM AND BRAIN NEUROTRANSMITTERS OF OBESE MICE MODEL

HAMDY A. MANSOUR AND KHALED A. ABDEL-SATER *
Pharmacology Department, Al-Azhar Faculty of Medicine – Cairo, Egypt
*Physiology Department, Al-Azhar Faculty of Medicine – Assiut, Egypt

ABSTRACT

Objective: The main goal of the present work was to study the relationship between weight reducing effect of green tea and its effect on GABA and 5-HT neurotransmitters in obese mice. Furthermore, the antilipidemic activity of green tea was also evaluated.

Methods: Adult male albino mice weighting (20 to 35 gm of both obese and non-obese) were used. The newborn mice were injected SC with MSG for the first 5 postnatal days. A control group was injected SC with physiological saline solution. The animals were weaned at the age of 4 weeks and kept on a commercial chow and tap water. At the age of 8 weeks, female mice were excluded from the study. At age of 12 weeks, each group was further subdivided into two different groups: group (a) that was not allowed to take green tea and group (b) that was allowed to take green tea (1% in diet for 2 weeks). Now, mice groups are four: control group without tea intake (Ia), control group with tea intake (Ib), obesity group without tea intake (IIa) and obesity group with tea intake (IIb). Serum cholesterol, triglyceride, HDL cholesterol and LDL cholesterol were measured by enzymatic calorimetric method using kits. Brain tissue GABA and 5-HT were determined by spectrophoto-fluorometric method.

Results: The obtained data revealed that; green tea caused a significant decrease in body weight, Lee index and food intake of obese mice group (IIb). There was also a significant decreased level of serum cholesterol, TAG and LDL and increased level of HDL in group (Ib). The study showed a significant increased level of serum cholesterol, TAG and LDL and decreased level of HDL in group (IIa). There was no significant difference between GABA concentration in group (Ia) and group (IIa). Treatment with green tea caused non-significant changes in brain GABA and significant elevation of the brain levels 5-HT. The present work clearly demonstrated that; obese mice have been shown reduced 5-HT brain levels compared to non-obese animals.

Conclusion: It is concluded that the green tea has antiobesity effect might be mediated by modulation of serum lipid profiles and brain 5-HT neurotransmitter.

Key words: Grean tea, Mice, Antioxidant, Lipid metabolism, Neurotransmitters.

INTRODUCTION

Tea (Camellia sinensis, Theaceace) is the second most popular beverage in the world next to water and has been extensively studied for its putative disease preventive effects (1). A large part of the research on green tea has focused on its effects related to the prevention of cancer, and encouraging knowledge regarding efficacy, safety and potential mechanisms of action has accumulated in this area (2). Furthermore, the anti-inflammatory (3), anti-arthritic (4), anti-angiogenic (5), anti-oxidative (6), anti-bacterial, anti-viral (7) and neuroprotective effects (8)of green tea and isolated green tea constituents have been investigated.

Many of the putative health benefits of tea are attributed to the high polyphenol (also called catechins) content of this beverage. There are four kinds of catechins in green tea: epicatechin, epigallocatechin, epicatechin-3- gallate and epigallocatechin-3-gallate (EGCG). EGCG is the most abundant catechin present in green tea and accounts for approximately 30–50% of the catechin content (9).

Obesity is caused by chronic imbalance between energy intake and energy expenditure. Discrete areas of the hypothalamus act as regulatory centers for obesity where energy homeostasis is regulated through food intake (hunger and satiety), energy expenditure and the secretion of hormones that regulate the use and storage of substrates (10).

It is currently well established that neurotransmitters play a key role in regulation of appetite and food intake. Norepinephrine, serotonin (5-HT) and gamma aminobutyric acid (GABA) are among the most important mediators in the brain that have important role in appetite and food intake control as demonstrated by numerous investigators (11). The modern pharmacological approach concentrates on drugs that interfere with monoamine neurotransmitter effects (e.g. sibutramine) and act as appetite suppressants, drugs which increase thermogenesis (e.g. β_3-adrenoreceptor agonists), decrease fat absorption by inhibiting the pancreatic lipase (orlistat) or drugs which act on brain peptides (10). The sympathetic nervous system (SNS) plays a major role in the regulation of energy expenditure and lipolysis. Therefore, there are a number of approaches to increase SNS activity either directly (by β-adrenergic agonists) or indirectly (by norepinephrine releasers and reuptake inhibitors) (12).

The dearth of information on the possible role of GABA on the appetite and food intake control has tempted us to carry

Received 16th of April 2012. Accepted 16th of May 2012. Address for correspondence: Khaled A. Abdel-Sater Eliwa, Department of Physiology, Faculty of Medicine, Al-Azhar University –Egypt, King Abdul Aziz University-Rabigh, KSA, phone/fax: +699502470699, e-mail: Khaled_71111@yahoo.com.

out the present work. The main goal of the present work was to investigate the relationship between weight reducing effect of green tea and its effect on GABA and 5-HT neurotransmitters in obese mice. Furthermore, the antilipidemic activity of green tea was also evaluated. There are no reports especially with neurotransmitters after green tea intake in obese animals up to date.

MATERIALS AND METHODS

A. Materials

Green tea was obtained from Saudi Arabia local market in the form of granules and stored at room temperature. Monosodium glutamate (MSG) was brought from Fluka Chemie, Switzerland. MSG was dissolved in 0.9% saline and kept in dark glasses. It was used in volume of 0.1 ml/mouse (13). GABA and 5-HT was obtained from the MP Biomedicals, Eschwege, Germany. Lipid profiles kits were obtained from Boehringer-Mannheim, Germany.

B. Experimental Animals

Adult male albino mice were obtained from the animal house of the Faculty of Medicine in Assiut University. The body weight range of both obese and non-obese mice was 20 to 35 gm and their age was 12 weeks. Mice were housed in boxes, 6 animals each, under standard conditions of temperature and humidity and a 12-hours light/dark cycle. The mice were fed a standard diet of commercial chow and tap water and left to acclimatize to environment for two weeks prior to inclusion in the experiment. All experiments were performed during the same time of day, between 8 a.m and 2 p.m to avoid variations due to diurnal rhythms. Manipulations throughout test were carried out by the same person (14).

C. Procedures

1. *Induction of Obesity*

The newborn mice were injected subcutaneously with MSG 0.1 ml/mouse (3 mg/gm body weight), per day for the first 5 postnatal days (13). A control group of newborn mice was injected subcutaneously with physiological saline solution (0.1 ml /mouse) per day for the first 5 postnatal days. The animals were weaned at the age of 4 weeks and kept on a commercial chow and tap water. At the age of 8 weeks, female mice were identified and excluded from the study. A mouse is considered obese when the Lee index is more than 340.

$$\text{Lee index} = \sqrt[3]{\text{body weight(gm)}} \div \text{nasoanal length(cm)} \times 1000$$

At age of 12 weeks, each group was further subdivided into two different groups: group (a) that was not allowed to take green tea and group (b) that was allowed to take green tea (1% in diet for 2 weeks). Now, mice groups are four: control group without tea intake (Ia), control group with tea intake (Ib), obesity group without tea intake (IIa) and obesity group with tea intake (IIb).

2. *Assessment of the Antiobesity Effect*

The weight (gm), nasoanal length (mm) and Lee index of the animals were measured on the first and last day of the experiment. The food intake (gm) per day was determined for each rat (total food at beginning of day – remaining food after 24 hours divided on 6) (15).

3. *Collection of Blood and Brain Samples*

The animal was anaesthetized with i.p. sodium pentobarbital (20 mg/kg body weight). Blood was collected (5ml of blood for each) from the retro-orbital plexus, using heparinized capillary tube inserted in the medial canthus of the eye globe. To obtain serum, the blood was collected into a dry clean graduated centrifuge tube and left for clotting. Then it was set to centrifuge at 3000 r.p.m. for 15 minutes. Serum was sucked out into eppendorf tubes and stored frozen at -20°C until required.

Brains were rapidly removed and dissected. Then each brain was put in a test tube containing 5 mL of acidified butanol. The mixture was homogenized in a conical tube immersed in ice. The homogenate was centrifuged at 1000 r.p.m. for 5 min. then 2.5 mL of the supernatant was transferred to a test tube for determination of GABA and 5-HT by spectrophoto-fluorometric method.

4. *Lipid Profiles Measurements*

Serum cholesterol, triglyceride, HDL cholesterol and LDL cholesterol were measured by enzymatic calorimetric method using kits (16, 17).

5. *Determination of Brain GABA by Spectrophoto-Fluorometric Method*

The method depends on that as a result of the reaction of ninhydrin with GABA and glutamic acid in the octanolic milieu, a fluorescent product, presumably a copper-II–chelate–complex is formed (18).

6. *Determination of Brain 5-HT Spectrophoto-Fluorometric Method*

The method depends on those 5-HT form highly fluorescent complexes with O-phthalaldehyde. The addition of L-cysteine to the reaction mixture improves the sensitivity of the method (19).

7. *Statistical Analysis*

Statistical analysis was done using the computer software program prism (Comshare's version of a decision support system = DSS) version 3.3. The quantitative data were presented in the form of mean ± standard error (M ± S.E). Student's t-test was done to compare between each two means. Also correlation coefficients were done. A probability less than 0.05 ($P < 0.05$) was considered significant for all analyses.

RESULTS

1. Assessment of the Antiobesity Effect of Green Tea

The obtained data revealed that; the induction of obesity in newborn mice caused stunting of growth of mice which became obese adult animals. The Lee index was significantly increased with obesity mice compared with control mice. Green tea caused a significant decrease in body weight, Lee index and food intake of obese mice group (IIb) compared with group (IIa). The nasoanal length was not significantly changed after intake of green tea in group (IIb) compared with group (IIa) as shown in Table I.

Table I. Effect of green tea intake on body weight, nasoanal length, Lee index and food intake in obese mice (14 weeks/old)

Parameters Groups	Body weight (gm)	Nasoanal length (mm)	Lee index	Food intake (gm)
Ia	30.7±1	90.8±1*	330±2.3*	4.3
Ib	28.2±1.5	89.0±0.5*	387±5.9*	3.9
IIa	30.3±1.05	80.7±3.41	414±9.9	7.1
IIb	18.2±0.79*	78.0±1.44	379±4.8*	4.8

Data represent mean ± S.E. of 6 observations.
* Significant difference at P< 0.05 from group IIa.

2. Lipid profiles levels in the studied groups

The obtained data showed a significant decreased level of serum cholesterol, triglycerides (TAG) and LDL and increased level of HDL in group (Ib) as compared to (Ia) group. The obtained data showed a significant increased level of serum cholesterol, TAG and LDL and decreased level of HDL in group (IIa) as compared to groups (Ia) and (IIb), Table II.

Table II. Effect of green tea intake on lipid profiles in obese mice (14 weeks/old)

Parameters Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Ia	228.8±19.04*	14.05±0.79*	48.4±6.5*	234.25±10.8*
Ib	154.2±15.04*	10.8±1.1*	41.9±2.5*	218.25±10.8*
IIa	262.8±22.4	22.62±2.09	70.9±10.5	292.82±14.4
IIb	235.3±17.0*	16.6±0.09*	42.1±8.8*	170±5.4*

Data represent mean ± S.E. of 6 observations.
* Significant difference at P< 0.05 from group IIa.

3. Effect of Green Tea on Brain GABA and 5-HT in Obese Mice

There was no significant difference between GABA concentration in group (Ia) and group (IIa). Induction of obesity did not significantly change the levels of brain GABA in mice. However, obesity caused a highly significant decrease in brain 5-HT in mice in group (IIa) compared with group (Ia). Treatment with green tea (group IIb) caused non-significant changes in brain GABA and significant elevation of the brain levels 5-HT compared with group (IIa) (Table III).

Table III. Effect of green tea intake on GABA and 5-HT in obese mice (14 weeks/old)

Parameters Groups	GABA concentration (μg/gm brain tissue)	5-HT concentration (ng/gm brain tissue)
Ia	800±41.2	502±30.22*
Ib	920±50.1	609±28.1
IIa	850±31.5	310±26.97*
IIb	996±71.3	650±68.89

Data represent mean ± S.E. of 6 observations.
* Significant difference at P< 0.05 from group IIa.

DISCUSSION

Various studies suggested different mechanisms responsible for MSG-induced obesity. Fernandez–Tresguerres (20) indicated that MSG injection causes degeneration and necrosis of several nuclei of the hypothalamus which may be responsible for obesity of animals after puberty.

In the present work, intake of green tea leads to significant decrease in Lee index of obesity in obese mice. A number of mechanisms have been proposed to explain the antiobesity effects of green tea. One reported by several groups is related to modulation of dietary lipid digestion and absorption by green tea treatment. Catechins were reduced gastric and intestinal fat digestion-mediated by direct inhibition of gastric and pancreatic lipases as well as a reduction of lipid emulsification process (21). Also, green tea increases fecal lipid content in high fat-fed rats. Similar findings have been observed in high fat-fed mice (22).

Other possible mechanisms including; increased tissue thermogenesis, increased fat oxidation and decreased appetite, may also play roles in the antiobesity effects of green tea. It can inhibit catechol *O*-methyltransferase (COMT), an enzyme involved in the degradation of norepinephrine. As a consequence, once released, norepinephrine remains in the synaptic cleft longer and provides a prolonged stimulation of adrenergic receptors (23). Caffeine also inhibits the phosphodiesterase, "enzyme that induce degradation of intracellular cyclic AMP (cAMP)" (24). After consumption of green tea, cAMP concentration rises and SNS activity will be increased and inactive hormone-sensitive lipase (HSL) will be activated, which promotes lipolysis that result in an increase in energy expenditure and fat oxidation (25).

Stimulation of thermogenesis and fat oxidation by the green tea was not accompanied by an increase in heart rate (26). In this respect, the green tea extract is distinct from sympathomimetic drugs; whose use as antiobesity thermogenic agents is limited by their adverse cardiovascular effects, and hence, are particularly inappropriate for obese individuals with hypertension and other cardiovascular complications (12).

The mechanism of hyperlipidemia in obesity could either be an increase in splanchinic production of very low density

lipoprotein or a defect in the removal of the very low density lipoproteins and/or chylomicron. The decreasing of HDL in obesity may be a result of an increased triglyceride load in the HDL particle that is acted upon by hepatic lipase, which hydrolyzes the triglycerides. The loss of the triglyceride results in a small HDL particle that is filtered by the kidney, resulting in a decrease in apolipoprotein A and HDL concentrations. The improving effect of green tea on obesity is mostly due to decreasing lipogenesis (27). In addition, its polyphenols were found to modulate the hypercholesterolaemia in the experimental animals by upregulating the liver LDL receptor, inhibiting cholesterol synthesis and increasing fecal excretion of cholesterol, total fatty acids and bile acid (28). Catechins were reducing triglyceride absorption, although the mechanisms of these reductions are not yet clear. A possible explanation is that; tea polyphenols may modify dietary fat emulsification in the gastrointestinal tract (29). In particular, green tea catechins might affect lipid metabolism by interfering with the micellar solubilization of cholesterol in the digestive tract, which in turn, decreases cholesterol absorption (30).

Brain levels of GABA are similar in brain of obese. However, the present work clearly demonstrated that; obese mice show reduced 5-HT brain levels compared to non-obese animals. The antiobesity effect of the green tea was remarkably associated with significant increase in the brain levels of 5-HT. This suggests that the antiobesity effect of the green tea might be mediated, in part, by increasing levels of 5-HT in certain brain regions.

The possible mechanism through which 5-HT exerts its body weight losing effect could be a result of an action on the hypothalamus to cause anorexia, weight loss and increased thermogenesis. This serotonergic activity is possibly mediated via inhibition of hypothalamic neurons that express the powerful appetite-stimulating peptide NPY (neuropeptide Y) (31).

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EFECTELE MODULATOARE ALE CEAIULUI VERDE ASUPRA METABOLISMULUI LIPIDIC SI NEUROTRANSMITATORILOR INTR-UN MODEL ANIMAL DE SOARECI OBEZI

REZUMAT

Obiective: Scopul principal al acestui studiu a fost investigarea relatiei dintre efectul scaderii in greutate indus de ceaiul verde si efectele acestui compus asupra neurotransmitatorilor GABA si 5-HT la soarecii obezi. Mai mult, a fost evaluata activitatea anti-lipemianta a ceaiului verde.

Metode: Au fost utilizati soarecii adulti din rasa Albino, cantarind 20 pana la 35 gm, atat pentru grupul de soareci obezi, cat si non-obezi. Soarecii nou-nascuti au fost injectati cu MSG in primele 5 zile de viata post-natala. Grupul de control a fost injectat s.c. cu solutie fiziologica salina. Animalele au fost hranite cu hrana speciala pana la varsta de 4 saptamani, avand apa at libitum. La varsta de 8 saptamani, au fost excluse din grupurile de studi femelele. La varsta de de 12 saptamani, fiecare grup a fost subdivizat in 2 grupuri distincte: grupul (a) caruia nu i s-a permis sa bea ceai verde si grupul (b), care a fost supus tratamentului cu ceai verde (1% din dieta, timp de 2 saptamani). Astfel, au fost formate 4 grupuri: grup control fara aport de ceai verde (Ia), grup control cu aport de ceai verde (Ib), grupul obez fara aport de ceai verde (IIa) si grupul obez cu aport de ceai verde (IIb). Au fost determinati urmatorii parametri prin metode de calorimetrie enzimatica: colesterol, trigliceride, HDL colesterol si LDL colesterol. GABA si 5-HT au fost determinati din tesutul cerebral prin metode spectro-fluorimetrice.

Rezultate: Datele obtinte au relevat ca ceaiul verde a indus o scadere semnificativa a greutatii corporale, a indexului Lee si a aportului de hrana la grupul de soareci obezi (IIb). A fost relevata si o scadere semnificativa a nivelului de colesterol, TAG si LDLsi cresterea nivelului HDL in grupul (Ib). Studiul a aratat un nivel semnificativ crescut al colesterolului seric, TAG si LDL si un nivel scazut al HDL in grupul (IIa). Nu a fost constatata o diferenta semnificativa intre concentratia GABA in grupul (Ia) si grupul (IIa). Tratamentul cu ceai verde a determinat modificari nesemnificative statistic GABA cerebral si cresteri semnificative ale nivelurilor cerebrale ale 5-HT. Studiul prezent demonstreaza clar ca soarecii obezi prezinta un nivel scazut al 5-HT comparativ cu soarecii non-obezi.

Concluzie: Se poate concluziona ca ceaiul verde are efecte anti-obezitate care ar putea fi mediate prin modularea profilului lipidic seric si a neurotransmitatorului 5-HT.

Cuvinte cheie: ceai verde, soareci, antioxidant, metabolism lipidic, neurotransmitatori

GLUTATHIONE S-TRANSFERASE P1 (GSTP1): PREDICTIVE BIOMARKER FOR PSA BIOCHEMICAL RECURRENCE IN PROSTATE CANCER MEN FOLLOWING RADICAL PROSTATECTOMY

DUMACHE RALUCA[1], PUIU MARIA[2], MALIȚA IOANA[6], TUDOR ANCA[3], NEGRU ŞERBAN[4], BUMBĂCILĂ BOGDAN[5]

[1]Department of Biochemistry;
[2]Department of Medical Genetics;
[3]Department of Medical Informatics;
[4]Department of Medical Oncology;
[5]Department of Clinical Pharmacy;
[6]Department of Pharmacology;
University of Medicine and Pharmacy "Victor Babes", Timisoara, Romania

ABSTRACT

Purpose: In our study,we evaluated the association between serum preoperative glutathione S-transferase P1(GSTP1) hypermethylated gene, in men with clinically localized prostate cancer (PCa) who underwent radical prostatectomy, with prostate-specific antigen (PSA) recurrence on a period of 36 months (3 years), as a predictive biomarker of recurrence in PCa men following radical prostatectomy.
Materials and methods: We included a number of 87 men with clinically localized PCa, who underwent radical prostatectomy at the Urology Clinic from County Emergency Hospital Timisoara, and 48 men with negative prostate biopsy. All serum samples were collected before prostate biopsy or at least 4 months after prostate biopsy. PSA recurrence was defined as a single postoperative PSA level ≥0.2 ng/ml. To analyze the methylation status of gene GSTP1 before the surgery intervention we used the methylation-specific polymerase chain reaction(MSP) method.
Results: From the 87 men who underwent radical prostatectomy included in our study, 26 (29.8 %) experienced PSA recurrence within the study period. From the 48 men with negative prostate biopsy only 2(4%) presented positive GSTP1 hypermethylation.
Conclusions: Our study suggests that preoperative serum GSTP1 may be a useful prognostic biomarker for men with clinically localized PCa treated with radical prostatectomy.
Key words: prostate cancer (PCa); methylation-specific polymerase chain reaction (MSP); prostate-specific antigen (PSA);glutathione S-transferase P1 (GSTP1)

INTRODUCTION

Since its approval by the US FDA in 1986, prostate-specific antigen (PSA) has been employed to early detection of PCa and monitoring men with a diagnosis of PCa. In 1994, PSA was approved for use in PCa screening and after, it has been used worldwide. Due to the limited specificity of PSA for the disease, novel biomarkers are needed for early detection of PCa and for determining which cancers need to be treated. Early occult dissemination of PCa cells from the primary tumor through the bloodstream into secondary organs is a critical step in tumor progression (1).

One of the treatments modalities in men with clinicaly localized PCa is radical prostatectomy. It has been demonstrated that about two thirds of patients treated by radical prostatectomy, remain disease free following radical prostatectomy,but there is one third of patients who will present PSA recurrence within the first 2-3 years after surgery,and these patients will develop metastatic lesions (2).

Epigenetic changes represent changes in gene expression, which are not caused by alterations in the primary sequence of the nucleotides that compose the gene. It has been demonstrated that, one of the most common epigenetic change and the most frequent molecular alterations in human cancers is DNA hypermethylation (3). Gluthatione S-transferase P1(GSTP1) is the most common somatic genome abnormality in human PCa (4). The somatic GSTP1 hypermethylation has been observed in more than 90% of PCa cases, whereas it has not been observed in normal or benign prostate (5). Analyses of body fluids(whole blood, serum,plasma or urine) from men with the diagnosis of PCa showed that DNA contains GSTP1 hypermethylation, thus suggesting a role of this epigenetic gene as a biomarker in detection of PCa recurrence (6).

Aim and objectives:

In our study, using the methylation-specific PCR (MSP)

Received 20th of April 2012. Accepted 15th May 2012. Address for correspondence: Raluca Dumache, M.D, PhD, Department of Biochemistry, University of Medicine and Pharmacy "Victor Babes", Eftimie Murgu Square, No.2, 300.041, Timisoara, Romania; E-mail: raluca.dumache@umft.ro

method, we evaluated the methylation status of GSTP1 gene in the preoperative serum of men with clinically localized PCa following radical prostatectomy, to determine its association with PSA biochemical recurrence (7).

MATERIALS AND METHODS

For this study, we enrolled a number of 87 patients who received a histological diagnosis of PCa after needle biopsy between December 2007 and January 2009 at the Urology Clinic from The Clinical Emergency County Hospital from Timisoara, and 48 patients with benign urological diseases.

We collected all clinical and pathologic data for all the patients included in our study, such as : age, pre-radical prostatectomy PSA, clinical and pathologic stage, Gleason grade, surgical margins status. All of the patients were followed for at least 3 years.

Biochemical progression (recurrence) was defined as PSA values > 0.2 ng/ml and higher. None of the patients included in the study group received adjuvant or hormonal treatment at the time of the study. Routine PSA follow-up consisted of a measurement of post-prostatectomy PSA levels 3 months after radical prostatectomy, and annually thereafter.

All of the men studied had an undetectable serum PSA level 3 months after radical prostatectomy. The clinico-pathological characteristics of the patients included in our study are presented in Table 1.

The study was conducted in accordance with The World Medical Association Declaration of Helsinki statements and written informed consent was obtained from each patient.

In this study, we considered preoperative and postoperative PSA recurrence predictors as follows: preoperative serum PSA, clinical stage, biopsy Gleason score, pathologic Gleason score, extraprostatic extension, pathologic organ confined status.

Table I.

Patient characteristics of 87 men with clinically localized prostate cancer treated with radical prostatectomy	
Study cohort, n = 87	
Age (y), mean (range)	58.9 (40 – 71)
Preoperative PSA (ng/mL), mean (range)	7.68 (0,9 – 38)
Pathologic Gleason score (%)	
5 – 6	43 (49.5%)
7	15 (17.2%)
8 - 10	29 (33.3%)
Pathologic stage (%)	
Organ confined	22 (25.3%)
Nonorgan confined	37 (42.5%)
Extraprostatic extension	8 (9.2%)
Seminal vesicle invasion	8 (9.2%)
Positive surgical margins	7 (8%)
Pathologic lymph node positive	5 (5.8%)

1. *Sample collection and DNA extraction*

All serum samples were collected before prostate biopsy, or at least 4 months after prostate biopsy and stored at -80º C. DNA was extracted from 600 µl of serum from each patient using the Qiagen Blood Mini Kit , and diluted after in a total volume of 25 µl. All of the men studied had an undetectable serum PSA level 3 months after radical prostatectomy.

2. *Bisulfite treatment*

Sodium bisulfite conversion of unmethylated cytosine residues to uracil of genomic DNA obtained from patient serum samples was done as described in the manufacturer's instructions.

We used 4µl of serum DNA for the c hemical treatment. After, the DNA samples were purified with the Wizard purification resin (Promega, Madison, WI), treated with sodium hydroxide , precipitated with EtOH, and resuspended in 200µl water and stored at -80ºC.

3. Methylation-specific polymerase chain reaction (MSP)

The modified DNA was used as a template for the MSP . The PCR conditions were as follows: hot start at 95ºC for 5 minutes (to fully denaturate the bisulfite-modified genomic DNA), 35 amplification cycles (94ºC for 30 seconds for denaturation, 58ºC for 30 seconds for primer annealing and 72ºC for 60 seconds for extension), and a final full extension at 75ºC for 5 minutes.

The primers used for amplification were as follows:

Forward primer:
5'-TTCGGGGTGTAGCGGTCGTC-3' (methylated)
5'-GATGTTTGGGGTGTAGTGGTTGTT-3'(unmethylated)
Reverse primer:
5'- GCCCCAATACTAAATCACGACG-3' (methylated)
5'- CCACCCCAATACTAAATCACAACA-3' (unmethylated)

The reaction was set up on a Master cycler Gradient thermal cycler (Eppendorf, Hamburg, Germany). Leucokyte DNA collected from healthy individuals was used as negative control.

To detect the preoperative serum methylation levels of gene GSTP1 in patients undergoing radical prostatectomy and in control subjects, we have separated electrophoretically the MSP products on a 2% Seakem agarose gel (Lonza, Basel, Switzerland) as presented in Figure no.2, and visualized them under an ultraviolet (UV) transluminator (Vilbert Lourmat®, Marne-la-Valee',France) after staining with ethidium bromide.

Statistical methods

The major statistical end point of our study was time to progression (TTP). Progression included PSA elevations of >0.2 ng/ml , metastasis and death. The event time distributions for this end point were estimated with the method of Kaplan and Meier.

RESULTS

We evaluated the methylation status of GSTP1 gene in the preoperative serum from 87 PCa patients by MSP method, before radical prostatectomy and determined the association

of serum GSTP1 hypermethylation and PSA recurrence on a period of 36 months.

After analyzing GSTP1 methylation status from all serum DNA by MSP method, we found that only 2(4%) of the 48 serum samples from men with negative prostate biopsy results presented GSTP1 hypermethylated levels. These men were folowed-up for further investigations. During the study period of time, 26 men who followed radical prostatectomy presented serum PSA levels > 0.2 ng/ml. Before radical prostatectomy, all 26 men presented hypermethylated levels of serum GSTP1.

From Figure 1, we can see that the probability of presenting PSA biochemical recurrence after 12 months post-prostatectomy is about 0.5, comparing with the probability of 0.2 which is present after 18 months post-prostatectomy. After 24-36 months the probability of PSA recurrence decreases significantly to zero.

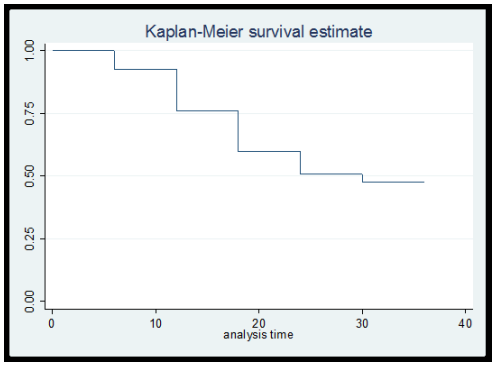

Fig. 1. Kaplan-Maier analysis surveilence curve in association with early PSA recurrence after radical prostatectomy for prostate cancer

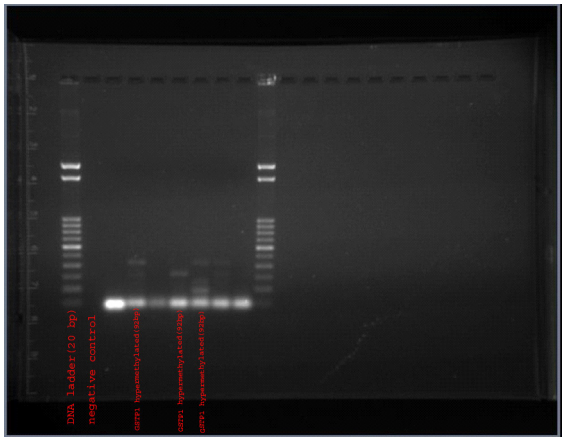

Fig.2. Analysis of glutathione S-transferase P1(GSTP1) gene on 2% agarose gel

DISCUSSION

It has been shown that circulating DNA-based biomarkers present some advantages compared with those using RNA or protein, because DNA is more stable than RNA and is easier to manipulate in laboratory (8,9).

In our study we have demonstrated that, men with clinically localized PCa who have a positive preoperative serum analysis for GSTP1 hypermethylation, present the risk of PSA recurrence within the first 2-3 years following radical prostatectomy. We have choosen gene GSTP1 for our study, because it presents the ability to differentiate between benign prostatic hyperplasia (BPH) and PCa (10), and in many studies it has been found that it correlates with clinicopathologic characteristics in PCa (11).

To our knowledge this study represents the first and elaborate study from Romania, regarding the monitoring of men who underwent radical-prostatectomy and present the risk of disease recurrence within the first 2-3 years after surgery.

CONCLUSION

Our obtained results suggest that hypermethylation of GSTP1 gene is a promising DNA-based predictive biomarker for men who underwent radical prostatectomy. Its use in the clinical management of men with PCa may improve the abillity to identify men who are less cured by surgery.

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GLUTATION S-TRANSFERAZA P1 (GSTP1): BIOMARKER PREDICTIV AL RECURENȚEI BIOCHIMICE A PSA ÎN CAZUL BĂRBAȚILOR PROSTATECTOMIZAȚI

REZUMAT

Obiectivele studiului: În acest studiu am evaluat asocierea dintre metilarea genei GSTP1 din serul preoperator al pacienţilor cu cancer de prostată(CaP) localizat supuşi prostatectomiei radicale cu recurenţa antigenului-specific prostatic (PSA) , pe o perioada de 36 luni(3 ani), ca biomarker predictiv al recurenţei malignitatii în cazul acestor pacienţi.

Material şi metodă: În studiul nostru am inclus un număr de 87 bărbaţi cu CaP, cărora li s-a făcut prostatectomie radicală, şi un număr de 48 bărbaţi cu afecţiuni urologice benigne şi rezultatul negativ al biopsiei prostatice. Probele de ser au fost recoltate înainte de efectuarea biopsiei prostatice sau la interval de cel puţin 4 luni după biopsia prostatică. Recurenţa biochimică a PSA seric a fost considerată ca fiind creşterea valorii PSA o singură dată > 0.2 ng/ml post-prostatectomie. Pentru analiza metilării genei GSTP1 am folosit metoda metilării-specifice PCR(MSP).

Rezultate: Din totalul de 87 pacienţi incluşi în lotul de studiu pe perioada celor 36 luni, 26 (29.8%) au prezentat recurenţă biochimică a PSA, iar dintre cei 48 pacienţi cu rezultatul biopsiei negativ, 2(4%) au prezentat hipermetilare a genei GSTP1 în ser.

Concluzie: Rezultatele obţinute de noi în acest studiu, indică faptul că, determinarea statusului metilării genei GSTP1 din serul preoperator al pacienţilor cu CaP localizat, care vor urma prostatectomie radicală, poate fi folosit ca biomarker predictiv al recurenţei biochimice a PSA seric.

Cuvinte cheie: cancer de prostată (CaP); glutation S-transferaza P1 (GSTP1); metilare-specifică a reacţiei de polimerizare în lanţ (MSP)

ANALYSIS OF ENDOTHELIAL DYSFUNCTION IN ASYMPTOMATIC SUBJECTS AT CARDIOVASCULAR RISK

BÂIBÂȚĂ EDS, IONESCU VA, MARIUS BĂDĂLICĂ-PETRESCU, SIMONA DRĂGAN, SILVIA MANCAŞ

University of Medicine and Pharmacy "Victor Babeş" Timişoara

ABSTRACT

Background: Vascular dysfunction, including the endothelial dysfunction and arterial stiffness are abnormalities indicating systemic vulnerability for major cardiovascular events. Identifying these changes in the vascular structure and function during preclinical stages, as well as establishing their appropriate use for risk stratification in clinically manifested disease, will bring benefits for both individuals and populations.
Our study aims: To analyze the behavior of arterial function using the arteriography method in asymptomatic subjects with cardiovascular risk profile assessed using the Framingham and SCORE prediction equations.
Materials and method: 35 consecutive asymptomatic subjects from the Cardiovascular Rehabilitation Clinic- Institute of Cardiovascular Diseases Timisoara were included in the study. They were evaluated from several perspectives : global cardiovascular risk- SCORE risk charts, Framingham risk charts; cardio-metabolic risk: traditional cardiovascular risk factors, metabolic syndrome-the IDF criteria; hemodynamic profile- systolic blood pressure, diastolic blood pressure, mean blood pressure, pulsed pressure; arterial wall functional profile: pulse wave velocity (PWVAo) and endothelial dysfunction by the systolic augmentation expressed by the Arteriography (Aix).
Results: We found significant positive correlations between: systolic augmentative index (Aix) and pulse wave velocity (PWVAo) ($p < 0.001$, $r = 0.28$), Aix and MBP (mean blood pressure) ($p < 0.005$, $r = 0.46$), and PWVAo and DBP (diastolic blood pressure) ($p < 0.001$, $r = 0.62$). The risk for fatal cardiovascular events in the next 10 years was significantly positively correlated with the endothelial dysfunction: Aix and risk Score ($p = 0.021$). The 10-year risk Framingham score for coronary artery disease was positively and significantly associated with Aix ($p = 0.034$) and PWVAo ($p = 0.029$). The association between the endothelial dysfunction and the metabolic syndrome factors was statistically insignificant.
Conclusions: We appreciated endothelial dysfunction and arterial stiffness using non invasive methods and we found statistical correlations between endothelial dysfunction and hemodynamic variables of the cardiovascular risk. Metabolic syndrome variables were not statistically significant associated with endothelial dysfunction degree appreciated by Aix. We also found a statistically significant positive correlation between endothelial dysfunction /arterial stiffness and risk of fatal cardiovascular event over the next 10 years.
Keywords: index ambulatory systolic augmentation, pulse wave velocity, pulse pressure, risk stratification of asymptomatic patients, arterial stiffness.

INTRODUCTION

Vascular dysfunction, including endothelial dysfunction, arterial stiffness and preclinical morphologic changes in the arterial vascular bed, are abnormalities indicating systemic vulnerability for cardiovascular major events.

Discovering these structural changes and vascular function during preclinical stages, and appropriate use for risk stratification during the disease stage, can bring benefits for both the individuals and population (1). The early functional disorder primarily involves the resistance vessels (small arteries, arterioles) leading over the years to the loss of elasticity of medium and large arteries (high stiffness), then to macro-vascular atherosclerotic changes and finally, to the formation of the atherosclerotic plaques (2,3).

In this advanced stage of atherosclerosis, the first sign of macro-vascular atherosclerosis is represented by the decreasing of the aortic arterial wall elasticity, leading to the development of an high aortic stiffness (characteristic to the increase of PWVAo) (4,5).

MATERIALS AND METHODS

Patients: 35 consecutive asymptomatic patients, all presenting an accumulation of cardiovascular risk factors, were included in the study. They were hospitalized in the Cardiovascular Rehabilitation Clinic Institute of Cardiovascular Diseases Timisoara in order to study their arterial function and for the assessment of the cardiovascular risk. Inclusion and exclusion criteria are presented in Table No. I.

Table I. Inclusion and exclusion criteria

Inclusion criterias	Exclusion criterias
• Asymptomatic • Normotensive • Hypertensive • EF>35	• Arrhythmias • Valvulopathies • Congenital cardiac diseases • EF<35

Clinical Evaluation: for each patient were followed:

• anamnesis;

• family history of cardiovascular disease;

Received 18th of February 2012. Accepted 15th of March 2012. Address for correspondence: Emilia Dana Bâibâţa, University of Medicine and Pharmacy "Victor Babeş" Timişoara, Eftimie Murgu Square No. 2A, RO-300041, Timisoara, phone: +40745615654; +40737034932, e-mail: danaemilia@yahoo.com

• quantified cardiovascular risk factors from SCORE risk prediction equations (age, sex, systolic blood pressure, smoking, total cholesterol) or the Framingham risk charts (age, sex, total cholesterol, HDL-C, smoking, systolic blood pressure).

Assessment of the absolute risk for cardiovascular events

We started the evaluation of the 10-year fatal cardiovascular risk using SCORE nomogram, in accordance with European Guidelines for Cardiovascular Disease Prevention www.heartscore.org. According to SCORE risk, patients were divided into two categories: asymptomatic with high cardiovascular risk and asymptomatic with low cardiovascular risk. We used the SCORE electronic version as well in order to take into account the HDL-c, noted as Score-HDL. We rated the group of patients by calculating the 10-year risk of coronary heart disease according to the Framingham model, using the interactive electronic diagram calculation: http://www.framinghamheartstudy.org/risk/coronary.html.

Cardio-metabolic risk assessment

The lipid profile analysis was performed as recommended by guidelines of the European Society of Cardiology. The framing of the subjects in each risk category was made according to the recommendation of the European Society of Cardiology Guidelines. The metabolic syndrome was defined according to IDF criteria if they were associated with at least 3 of the 5 criteria:

Table II. Metabolic Syndrome: defining criteria (IDF)

CRITERIA	NCEP-ATP III	IDF
Waist circumference (cm)	>88 (F),>102 (M)	>80 (F),>94 (M)*
Systolic blood pressure (mmHg)	≥ 130/80	≥ 130/80
Fasting glucosa (mg/dl)	> 110	> 100
Triglycerides (mg/dl)	>150	>150
HDL-c (mg/dl)	<50(F),<40(M)	<50(F),<40(M)

If BMI ≥ 30 kg/m^2, waist measurement is not required in accordance with IDF

Hemodynamic risk assessment

Based on the results of the 24h automatic recording (performed by the BTL-08 ABPM), the analysis of the arterial blood pressure was done. Measurements were performed every 15 minutes during the day (7 am-23 am) and every 30 minutes during the night (23-7 am). The monitoring was repeated when there were more than 30% artifacts. During the monitoring, all medication was stopped. From the ambulatory blood pressure monitoring protocol, we used the following parameters: 24-hour SBP, 24-hour DBP, PP 24 hours, 24 hours MAP (3). We have defined the following categories: normal BP <130/85, hypertension: SBP ≥ 140 mmHg and / or DBP ≥ 90 mmHg or antihypertensive treatment, which corresponds to an average 24-hour BP> 120/80 mmHg (3). Mean 24-hour pulse pressure> 60 mm Hg was considered pathological.

Assessment of arterial function

We followed the exact recommendations of the evaluation protocol using the arteriography method as described by the bibliographic references (2). We analyzed the following parameters: arterial augmentation index (Aix) as a marker of endothelial dysfunction and aortic pulse wave velocity (PWVAo) as a marker of arterial stiffness. During the evaluation of hemodynamic and arterial functions, all medication was stopped and all the recommendations regarding physical rest and the abuse of toxic substances (alcohol, energy drinks, coffee) were followed 12 hours prior the exam. Statistical analysis of data: data normality was assessed using Shapiro-Wilk test. Data were analyzed using parametric tests-One Sample t test for comparing the mean values of two groups, and One Way ANOVA for comparing the mean values obtained in more than three groups. The testing of the association between qualitative variables was performed using Chi square test, and for testing correlations between numerical variables, we used Pearson correlation coefficient. For statistical analysis, P.A.S.W. statistics 18.0 was used.

RESULTS

We studied 35 consecutive patients, asymptomatic in terms of cardiovascular, but presenting the accumulation of cardiovascular risk factors, hospitalized in Clinic of Cardiovascular Rehabilitation-Institute of Cardiovascular Diseases Timisoara, of which a higher percentage (68.6%) was represented by women, the average age of the sampled patients was 49.51 (± 17.19) years, 11.4% of patients had *diabetes mellitus*, 20% of patients had a SCORE risk ≥ 5% , 5.71% had a Framingham risk ≥ 20 and 48.57% were smokers.

Table III. Features

Characteristics	Mean	Standard Deviation
Age	49.51	17.19
Height	166.743	8.79
Weight	72.43	14.12
Abdominal circumference	86.2	12.26
Systolic blood pressure	134.63	19.22
Diastolic blood pressure	84	11.25
Mean blood pressure	100.857	13.27
Aix	27.78	27.61
PWVAo	9.02	2.17
Heart rate	69.257	10.50
Cholesterol total	171.54	25.22
HDL-C	47.463	11.31
LDL-C	103.5	25.23
Triglycerides	116.77	50.57
Fasting plasma glucose	88.775	10.55

Endothelial function behavior according to hemodynamic profile of the sampled patients

We have obtained a positive and significant correlation between the aortic augmenting index and the two variables that characterize the hemodynamic profile of SBP (p <0.003, r= 0.48) and MAP (p <0.005, r = 0.46) (Table IV).

Table IV. Augmentative arterial index variation according to hemodynamic profile

dependent variable	independent variables	Pearson correlation coefficient	statistical significance
Aix	SBP	r=0.48	p<0.003
	MAP	r=0.46	p<0.005
	PWVAo	r=0.28	p<0.001

The correlation between *endothelial dysfunction* and arterial *wall stiffness*

We found a significant positive correlation between Aix and PWVAo (r =0.28, p <0.001) (Table III). To demonstrate any significant differences between patients groups at risk and the patients groups without cardiovascular risk, we created 3 new variables, dividing into categories of patients the variables Aix, PWVAo and PP, resulting in the following:

- Aix quartile with four categories: 1 (-79.00-10.40), 2 (10.41-27.4), 3 (27.5-49.6), 4 (49.6 77.0 = 4);
- PWV quartile with four categories: 1 (6.1-7.2), 2 (7.3-8.9), 3 (9.0-9.8), 4 (9.9-6.7);
- PP category with two categories: 1 (30 thru 59.9 = 1), 2 (60 thru 85.8 = 2) (cut off value considered at risk: 60).

To analyze whether there are statistically significant differences between the numeric variables in the new created categories, we used non-parametric Kruskal-Wallis test for variables with 4 categories (Aix quartile and PWV quartile) and Mann-Whitney test for variables with two categories (PP categ).

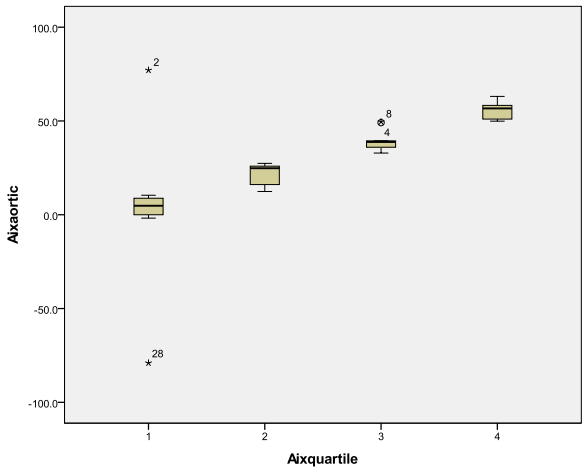


Fig. 1. Aix aortic in Aix quartile

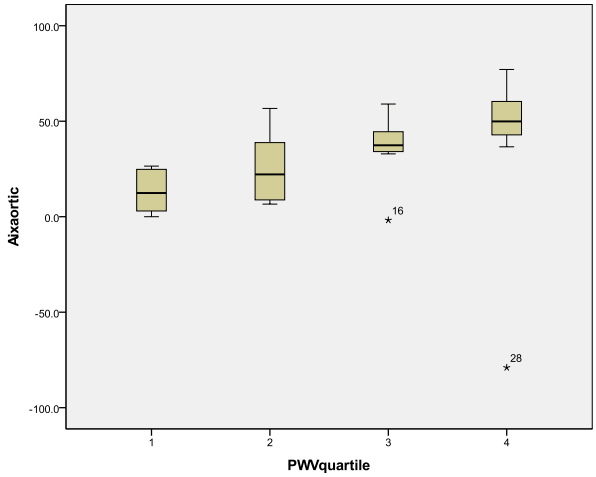


Fig. 2. Mean aortic Aix in PWVAo quartiles

Tabel V. Aix aortic in PWV quartile

Aixaortic

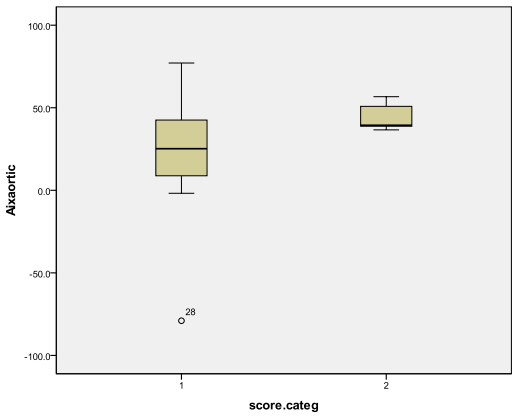
PWVquartile		Mean	N	Std. Deviation
-- ---- ------- -----	1	13.256	9	10.4401
	2	25.960	10	17.9917
	3	36.150	8	17.6154
	4	38.038	8	48.7565
	Total	27.783	35	27.6170

We demonstrated that in the 4 categories of PWVAo quartiles there are significant differences for mean age (p = 0.001), BMI (p = 0.008), Aixaor

tic (p =0.009),PWVAop <0.001), SBP (p = 0.007), DB P (p = 0.001) and MAP (p = 0.001). We obtained significant differences of mean aortic Aix in the PWVAo1 quartiles (p = 0.009) and PWVAo3(p=0.009) and between PWVAo 1 and PWVAo 4 (p = 0.009).The Aix average was lower in quartile 1 versus quartile 3 and in quartile 4 versus quartile 1, therefore PWVAo increased with the increasing of the Aix.

The correlation between endothelial dysfunction and risk of fatal cardiovascular event over the next 10 years

We obtained significant differences of mean aortic Aix in the SCORE risk categories (p =0.021). Aix increased with the increasing of the SCORE risk.



Aortic Aix

SCORE categ.		Mean	N	Std. Deviation
dimension1	1	23.586	28	29.2369
	2	44.571	7	8.0205
	Total	27.783	35	27.6170

Ranks

	SCORE categ.	N	Mean Rank	Sum of Ranks
Aortic Aix	1	28	16.00	448.00
	2	7	26.00	182.00
	Total	35		

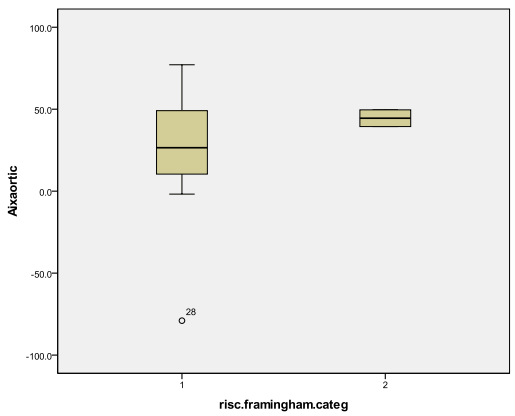
Fig. 3. Mean aortic Aix in SCORE categories

We found statistically significant association between Aix aortic and Framingham risk (p = 0.034, Chi2 test 95% CI), but we didn't find significant differences between mean Aix aortic and Framingham risk categories (p = 0.255 Man-Whitney test 95% CI).

Table VI. Mean aortic Aix in Framingham risk categories

Aortic Aix

Framingham risk categ		Mean	N	Std. Deviation
dimension1	1	26.770	33	28.1108
	2	44.500	2	7.2125
	Total	27.783	35	27.6170



Ranks

	Framing-ham risk. categ	N	Mean Rank	Sum of Ranks
Aix-aortic	1	33	17.52	578.00
	2	2	26.00	52.00
	Total	35		

Fig.4. Aix aortic behavior in Framingham risk categories

DISCUSSION

Our study demonstrates the association relationship between endothelial dysfunction assessed by arteriography and arterial stiffness (PWVAo), between endothelial dysfunction and hemodynamic parameters (SBP, DBP, MAP, PP) and between endothelial dysfunction , age and risk of cardiovascular events in 10 years (SCORE risk , SCORE- HDL risk , Framingham risk).

The study conducted by Hansen TW and colleagues (1) has demonstrated the necessity of measuring arterial stiffness's (PWVAo) for asymptomatic people without cardiovascular disease, proving that the rate of aortic pulse wave propagation alone, without keeping track of the independent classical risk factors, is an effective predictor of cardiovascular mortality based on a follow-up period of 9 years (7,8).

In our study, we have demonstrated the existence of correlations between arterial stiffness, hemodynamic parameters and SCORE risk. There is a relationship between endothelial dysfunction (Aix) and arterial stiffness assessed by PWV Ao, where the increase of the arterial stiffness increases the augmentation index that is the endothelial dysfunction degree.

With or without being associated with the traditional risk factors, PWV represents a very important predictor of cardiovascular events (9).

The predictive value of Aix for cerebrovascular events should not be considered only in terms of hemodynamics, but also in terms of cardio-metabolic. G. Leoncini and co. have studied the association between Aix and the metabolic profile of 156 non-diabetic, hypertensive patients, and concluded that the prevalence of increased Aix was higher in patients with metabolic syndrome.

Aortic PWV measurement supplies information, primarily on the properties of the aortic wall. The more rigid and impervious the aortic wall is, the faster the pulse wave travels along it.

We must take into account situations where there is an increase of the ejected blood volume in the aorta (e.g. hypertension, tachycardia, increased cardiac output), circumstance that determines an increase in aorta diameter, therefore increasing the parietal blood pressure, resulting in an increase in propagation velocity of wave pulse.

In conclusion, aortic PWV provides a prognostic value only if it was measured in isobaric conditions, that means in normotensive conditions (9).

The correlation between the arterial stiffness and the cardiovascular risk was conclusively demonstrated in the study conducted by Guerin and co. in 2001, performed on a group of patients in end-stage kidney disease. They effectively decreased the blood pressure and followed the changes of aortic PWV in parallel. In the group that besides lowering blood pressure, the aortic PWV was decreased as well, all patients survived the tracking period. Instead, the group in which the lowered blood pressure was not accompanied by a decrease in the aortic PWV, all patients died by the end of follow-up period of 51 months (5,9, 10).

This shows, that in this second group, contrary to the first group, the increased aortic PWV was not caused by parietal tension due to hypertension, but rather by morphological vessel lesions.

CONCLUSIONS

• We assessed endothelial dysfunction and arterial stiffness using noninvasive methods and we found statistical correlation between endothelial dysfunction and hemodynamic variables of cardiovascular risk, but not with metabolic profile

• We also found statistically significant positive correlation between endothelial dysfunction / arterial stiffness and risk of fatal cardiovascular event in the next 10 years.

• It is essential to detect real individual risk, even in early reversible stages of atherosclerosis, when most patients are still asymptomatic and without subjective complaints, therefore we have the chance to favorably influence the process thru lifestyle changing or with a simple cost-effective therapy.

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ANALIZA DISFUNCTIEI ENDOTELIALE LA SUBIECTI ASIMPTOMATICI CU CUMUL DE FACTORI DE RISC CARDIO-VASCULAR

REZUMAT

Disfuncţia vasculară, incluzând disfuncţia endotelială şi rigiditatea arterială, precum şi modificările morfologice preclinice în patul vascular arterial, sunt anomalii sistemice semnalând vulnerabilitatea pentru evenimente cardiovasculare majore. Identificarea acestor modificări de structură şi funcţie vasculară în stadiile preclinice, precum şi utilizarea lor adecvată pentru stratificarea riscului în stadiul de boală clinic manifestă, pot aduce beneficii atât la nivel individual, cât şi populaţional .

Obiective: Studiul nostru isi propune sa analizeze comportamentul functiei arteriale prin metoda arteriograf la subiecti asimptomatici cardiovascular si cu profil de risc apreciat prin ecuatiile de predictie Score si Framingham.

Materiale si metode: Au fost incluși in studiu 35 pacienţi consecutivi , asimptomatici cardiovascular, dar cu cumul de factori de risc cardiovascular, internaţi in Clinica de Recuperare Cardiovasculară a Institutului de Boli Cardiovasculare Timișoara pentru evaluarea riscului cardiovascular și studiul funcţiei arteriale . Riscul cardiovascular global a fost evaluat prin ecuaţiile SCORE și risc Framingham - alături de prezenţa sindromului metabolic (criteriile IDF); profilul hemodinamic prin utilizarea Tensiomed Arteriograf s-au urmarit parametrii: TAS, TAD, TAM, presiunea pulsului precum și profilul functional al peretelui arterial: velocitatea undei pulsatile aortice(PWVAo) și disfuncţia endotelială prin indicele de augmentaţie sistolică (Aix).

Rezultate: am găsit corelaţii pozitive si extrem de semnificative intre: Aix si PWVAo ($p<0.001$, $r=0.28$) , Aix si TAM ($p<0.005$, $r=0.46$), PWVAo si TAD ($p<0.001$, $r=0.62$). Riscul pentru eveniment cardiovascular fatal la 10 ani s-a corelat pozitiv si semnificativ cu disfuncţia endotelială: Aix și risc Score ($p=0.021$). Scorul Framingham pentru boală coronariană la 10 ani s-a asociat pozitiv și semnificativ cu Aix ($p=0.034$) si cu PWVAo ($p=0.029$). Relaţia de asociere a disfuncţiei endoteliale cu factorii sindromului metabolic nu a fost semnificativă statistic.

Concluzii: Am apreciat disfuncţia endotelială și rigiditatea arterială folosind metode noninvasive și am găsit o corelaţie statistică semnificativă intre disfuncţia endotelială şi variabilele hemodinamice ale riscului cardiovascular. Variabilele sindromului metabolic nu s-au asociat semnificativ statistic cu gradul disfuncţiei endoteliale apreciată prin Aix. Am constatat, de asemenea o corelaţie pozitivă semnificativă statistic între disfuncţia endotelială și riscul de eveniment cardiovascular fatal in urmatorii10 ani.

Cuvinte cheie: indicele ambulatoriu de augmentare sistolică, velocitatea undei pulsate, presiune pulsata, stratificarea riscului, asimptomatic la risc, rigiditate arterială.

DIAGNOSTIC ASPECTS OF THYROID DISEASE IN ADULT PATIENTS WITH TYPE 1 DIABETES

ADRIANA GHERBON, LAVINIA NOVEANU, GEORGETA MIHALAŞ
Department III-Functional Sciences, Physiology, "Victor Babes" University of Medicine and Pharmacy Timisoara

ABSTRACT

Patients with diabetes have an increased prevalence of thyroid disease compared with non-diabetic population. In type 1 diabetes, it is often associated with endocrine disorders and systemic disease with autoimmune etiology of type: Graves-Basedow disease, Hashimoto thyroiditis, Addison's disease, celiac disease, pernicious anemia, myasthenia gravis, vitiligo, etc. A particular association of type 1 diabetes with hypo-or hyperthyroidism is characteristic to polyglandular autoimmune syndrome.

The purpose of this study is to determine the main aspects of thyroid diseases diagnosis in adult patients with type 1 diabetes.The studied adults group was represented by 60 cases, aged 18-80 years. Gender distribution was net in favor of women being represented by 55 women and 5 men. They used clinical, imaging, biochemical, hormonal and immunological parameters.

Keywords: type 1 diabetes, thyroid disease, diagnosis, thyroid ultrasound, thyroid hormones, antithyroid antibodies

INTRODUCTION

The thyroid disorders are common in the general population; their prevalence increases with age. The screening for thyroid disorders is indicated in certain high-risk groups as new born and elderly people (16).

The hypothyroidism is the most common thyroid disorder in adults and older women. Its origin is usually autoimmune, presenting as atrophic primary hypothyroidism or Hashimoto thyroiditis. It may be also secondary to radioactive iodine treatment or thyroid surgery. In rare cases, occurs secondary hypothyroidism to hypothalamic or pituitary disease (16).

By contrast, the hyperthyroidism is less common, with a ratio female/male 9/1. The Grave's disease is most common and usually affects young adults. The toxic multinodular goiter usually occurs in older people (16).

The patients with diabetes mellitus (DM) have an increased prevalence of thyroid disease compared with non-diabetic population (16).

Because the patients with a specific organ autoimmune disease are at risk to developing other autoimmune diseases, and the thyroid disorders are more common in women, it is not surprising that 30% of the women with type 1 diabetes have thyroid disease. The rate of postpartum thyroiditis in diabetic patients is three times higher than in healthy women (16).

The thyroid disorders into the general population have a prevalence of approximately 6.6%. In patients with diabetes, the thyroid disease prevalence is between 10.8% and 13.4%, consisting of: clinical hypothyroidism (36%), sub clinical hypothyroidism (41%), hyperthyroidism (12%) and postpartum thyroiditis (11%) (16).

Type 1 diabetes is commonly associated with endocrine and systemic diseases with autoimmune etiology of type: Graves-Basedow disease, Hashimoto's thyroiditis, Addison's disease, celiac disease, pernicious anemia, myasthenia gravis, vitiligo, etc. (7).

A particular association of type 1 diabetes with hypo- or hyperthyroidism is characteristic to polyglandular autoimmune syndrome. There are three polyglandular autoimmune syndromes.

1. **Polyglandular autoimmune syndrome type I (PAS - I)** is an autosomal recessive transmitted disease caused by a mutation in the short arm of chromosome 21, characterized by the triad: cutaneous-mucosal candidacies, hypoparathyroidism and Addison's disease.

2. **Polyglandular autoimmune syndrome type II (PAS - II)** (4, 14) is the most common immuno-endocrinopathies. It occurs in adulthood, especially affecting women. It is characterized by the appearance into the same person of two or more of the following diseases: Addison disease, Graves-Basedow disease, thyroiditis of autoimmune etiology, type I diabetes mellitus, primary hypogonadism, myasthenia gravis, and celiac disease.

3. **Polyglandular autoimmune syndrome type III (PAS - III)** (2) is a PAS II syndrome but without adrenocortical involvement. This syndrome is associated with the following diseases: celiac disease, hypogonadism, myasthenia gravis, sarcoidosis, Sjogren syndrome, rheumatoid arthritis, gastric cancer, malabsorption due by pancreatic exocrine deficiency, and can be classified into three subcategories:

- PAS III A - autoimmune thyroiditis with DM type1
- PAS III B - autoimmune thyroiditis with pernicious anemia
- PAS III C - autoimmune thyroiditis with vitiligo and/or alopecia and/or other autoimmune diseases

The diagnosis of thyroid dysfunction in patients with diabetes, based only on clinical manifestations, can be difficult. An unsatisfactory glycemic control can cause similar symptoms with

Received 15th of March 2012. Accepted 20th of April 2012. Address for correspondence: Adriana Gherbon, MD, PhD, Department of Physiology, "Victor Babes" University of Medicine and Pharmacy Timisoara, Romania, Eftimie Murgu Square No. 2A, 300041, Timisoara, phone/fax: +40256220479; e-mail: adrianagherbon@yahoo.com

the hyperthyroidism, such as weight loss despite increased appetite, and fatigue. On other hand, the severe diabetic nephropathy can be omitted in patients with hypothyroidism because these patients may present edemas, fatigue, and pallor and weight gain (16).

To further complicate the diagnostic process, unbalance DM, with or without complications, can cause alterations of thyroid function tests, which can occur in patients without associated thyroid disease. The typical changes are represented by the decrease of T_3, determined by the decrease of T_4 conversion in T_3, decreased serum T_4, due to lower fixing on protein and lower serum TSH (16).

The most sensitive test to detect the thyroid dysfunction is the determination of TSH, which allows the certain diagnosis of the hypo-and hyperthyroidism. It also allows the diagnosis of sub clinical thyroid dysfunction, characterized only through the change of the TSH level's, with T_3 or T_4 in the normal range; the patients with this type of dysfunction are usually asymptomatic (16).

The thyroid dysfunction can produce significant biochemical and clinical changes. Thus, the sub clinical hypothyroidism may increase LDLc and aggravating pre-existing dyslipidemia, increasing the risk of arteriosclerosis. On other hand, the sub clinical hyperthyroidism may increase the risk of cardiac arrhythmias and can exacerbate a pre-existing coronary heart disease. Because the patients with diabetes are an increased risk for cardiovascular disease, the diagnosis and treatment of associated thyroid disease is important (16).

The presence on antiperoxidase thyroid antibodies (AB antiTPO) has a role in predicting occurrence of autoimmune thyroid disease, particularly of the hypothyroidism. Auto antibodies may occur long before obvious clinical manifestations of autoimmune thyroid disease and predict the appearance of clinical disease. The patients with polyglandular autoimmune syndrome may present auto antibodies against the same antigen. At the patients with present AB antiTPO must determinate their annual screening for early detection of thyroid dysfunction and establishment of an appropriate treatment (9).

Also, at all the patients with diabetes should be performed a screen for adrenal auto antibodies, celiac disease, steroid, gastric parietal and thyroid even at diagnosis. Also, at the patients who have one of the diseases listed above must be confirmed existence of diabetes by determining the ICA, GAD, IA_2, in absence of characteristic clinical manifestations (9).

At the family members of patients with diabetes must also determine auto antibodies, especially if the diabetic patient has thyroid auto antibodies. Identifying of the auto antibodies presence should be followed by hormonal assessment and the treatment of thyroid disease (9).

Therefore, thyroid function regular screening is indicated in all the patients with diabetes for establishment of early treatment of sub clinical thyroid dysfunction (9). The best screening test is the TSH determination. In patients with type 1 diabetes is useful to determining the presence of anti-TPO antibodies. If they are present, it is indicate the annual determination of TSH. In their absence, the TSH determination should be performed at every 2-3 years (9).

In the case of the patients with thyroid disease is obligatory annual determination of glycemia. In the case of elevated blood glucose levels, according to the values obtained, it is necessary employment in one of the category: type 1 diabetes, type 2 diabetes or IGT (9). If the thyroid disease is autoimmune it is better to determine the DM specific antibodies to identify the presence of type 1 diabetes (9).

MATERIAL AND METHOD

Investigated population

The study included adult subjects with diabetes mellitus, which in time present thyroid disease, or adult subjects with thyroid disease that subsequently present diabetes mellitus.

The study group comprised 60 cases, aged 18-80 years. Gender distribution was net in favor of women being represented by 55 women and 5 men.

Methods of investigation

Methods of investigation were the clinical data - history, present status, and imaging - thyroid ultrasound, biochemistry - carbohydrate metabolism parameters: fasting blood glucose, urine glucose, glycosylated hemoglobin and thyroid hormones investigations and some immunological parameters.

Glucose determination was performed by enzymatic techniques with glucose oxidase. Were considered normal fasting blood glucose between 70-110 mg%, diabetes mellitus - fasting blood glucose values above 126 mg%, impaired glucose tolerance - fasting blood glucose values between 110-126 mg% and the oral glucose tolerance test (OGTT) at 2 h between 140-200 mg% and fasting impaired glucose tolerance - fasting blood glucose values between 110-126 mg% and OGTT at 2 h under 140 mg%.

Determination of glycosylated hemoglobin (HbA1c) was achieved through the DiaStat program for glycosylated hemoglobin HbA1c that measures the ratio of glycated hemoglobin to total HbA.

Determination of serum levels of TSH, free fraction of serum level of triiodothyronine (FT_3), free fraction of thyroxin (FT_4) were ARCHITECT quantitative method, which is an immunologic determination by chemiluminescence's with small Chemilumnescent Micro particle Immunoassay (CMIA). The following values were considered normal: TSH = 0.465 - 4.68 mIU/ml, FT_3 = 3.69 -10.4 pmol/l, FT_4 = 10 - 28.2 pmol/l.

Immunological parameters were represented by some markers of thyroid autoimmunity – antiperoxidase (antiTPO) and antithyroglobulin (antiTG) antibodies (AB). To determine the serum titers of antiTPO AB, AxSYM antiTPO kit was used, the method is enzyme immunoassay with micro particles, Meia (Micro particle Enzyme Immunoassay). It was considered normal: antiTPO AB < 35 IU / ml. To determine the serum titers of antiTG AB, AxSYM antiTG kit was used, the method is enzyme immunoassay with micro particles, Meia (Micro particle Enzyme Immunoassay). It was considered normal: antiTG AB < 55 IU/ ml.

Thyroid ultrasound performed in all cases is a non-invasive method of exploration that allows measurement of thyroid volume, thyroid study report with cervical anatomical structures and thyroid parenchyma changes.

RESULTS AND DISCUSSION

In the case of the type 1 diabetes group, **family history** was present in 10% cases. In terms of **clinical symptoms**, the patients with Graves-Basedow disease presented the specific thyreotoxic disease syndrome.

In the case of *autoimmune chronic thyroiditis (ACT)*, some patients were asymptomatic, and some presented typical symptoms of hypothyroidism. In the case *of the nodular goiter*, they were discovered incidentally at ultrasound examination (incidentalomas) or by clinical symptoms due by compression. In the case of *the malignant tumors*, the symptoms were very different: firm goiter, loco-regional adenopathy, quick growth of the formation spontaneous or by thyroxin suppression therapy. In the case of *the euthyroid diffuse goiter*, were no symptoms and the diagnosis was based on clinical examination and ultrasound. In the case of *the sub acute thyroiditis*, the symptoms occurred after an upper respiratory tract infection, framing local pain, inflammatory syndrome, functional disorders (transient hyperthyroidism, followed by transient hypothyroidism). To assess the goiter was used inspection and palpation and were taken into account the WHO goiter staging criteria. Based on these criteria, at the studied group with type 1 diabetes we have obtained the results given in Table I.

A percentage of 23.33% cases presented the goiter stage 2, 6.66% had goiter stage 1 a, 3.34% stage 1 b and 1.67% stage 3, with the relatively firm consistency. Most patients (65 %) had no goiter on clinical examination (Figure 1).

Table I. The goiter stages distribution in patients with type 1 diabetes

Thyroid disease type	Goiter stages									
	0		1 a		1 b		2		3	
	n	%	n	%	n	%	n	%	n	%
Total (n = 60)	39	65	3	5	3	5	14	23.33	1	1.67
Graves-Basedow disease (n = 6)	3	50	-	-	1	16.66	2	33.34	-	-
Euthyroid diffuse goiter (n = 1)	-	-	-	-	-	-	1	100	-	-
Differentiated thyroid carcinoma (n = 3)	3	100	-	-	-	-	-	-	-	-
ACT (n = 50)	33	66	3	6	2	4	11	22	1	2

The most common causes of goiter are iodine deficiency. At patients who don't come from regions with iodine deficiency, the cause is obscure (10). The incidence of sporadic goiter in North America is estimated at 5%. Usually does not occur before puberty and don't present a peak of incidence. Generally, the goiter development increases with age. The prevalence of palpable nodules is approximately 5-6% in people aged 60 years, but at the ultrasound examination, the incidence of impalpable nodules reaches 50% in people aged 60 years (10).

WHO, UNICEF and ICCIDD believes that in the absence of iodine deficiency, the goiter prevalence is below 5%, mild iodine deficiency is associated with a prevalence of 5-20%, moderate iodine deficiency with a prevalence of 20-30% and severe iodine deficiency with a prevalence of 30% (10). Goiter predominance was found in women, the ratio being 1.2 - 4.3/1 for F/M (10). Some studies have shown that the treatment with T_4 of sporadic goiter decreased the thyroid volume to 58% of patients compared to 4% of patients treated with placebo. In the case of the treatment with T_4 must consider the risks of sub clinical hyperthyroidism which is associated with various metabolic and visceral complications (10). The diffuse goiter, with firm consistency, with rapid growth, with compressive symptoms and regional adenopathy are sometimes suspicious of malignancy.

Thyroid ultrasound allows the accurate assessment of thyroid volume and thyroid parenchyma appearance appreciation.

As consequence of iodine intake study in the Banat plain, especially in the Timisoara, the normal thyroid volumes were within the following values: women - 10.27 ± 2.09 ml and in males - 12.18 ± 2.52 ml.

In the case of our study, the goiter has been diagnosed with thyroid volume exceeded 3 standard deviation (SD) (females > 16.54 ml and men > 19.74 ml).

In the case of the type 1 diabetes group, the biggest thyroid volume (TV) was found in a single patient with euthyroid diffuse goiter (32.08 ml) and in patients with Graves-Basedow disease (25.26 ± 15.28 ml). The smallest TV was found in patients with ACT (11.11 ± 5.61 ml). The results about the TV value and hypoechogenity intensity of thyroid parenchyma, according to sex and type of thyroid disease are shown in Table II.

Table II. The classification of cases according to the TV and the type of thyroid parenchyma hypoechogenity

Thyroid disease type	TV type				Hypoechogenity type					
	normal		increased		mild		moderate		intense	
	n	%	n	%	n	%	n	%	n	%
Graves-Basedow disease (n=6)	2	33.33	4	66.67	1	16.66	5	83.34	-	-
Euthyroid diffuse goiter (n=1)	-	-	1	100	1	100	-	-	-	-
Differentiated thyroid carcinoma (n=3)	3	100	-	-	3	100	-	-	-	-
ACT (n=50)	50	100	-	-	7	14	35	70	8	16

All the thyroid carcinoma cases received surgery, the TV being evaluated also intraoperatory. In the case of the type 1 diabetes group, all cases presented hypoechogenity (100%). The moderate hypoechogenity was present in 46.66% cases; intense hypoechogenity was present in 11.66% cases. Mild hypoechogenity appearance was present only in 3.33% cases.

It is noted that for thyroid carcinoma (100% cases) and

euthyroid diffuse goiter (100% cases) prevailed mild hypoechogenity (+ and +/++), while for the Graves-Basedow disease and ACT prevailed moderate (+ + and ++/+++) (83.34% cases for Graves-Basedow disease and 70% for ACT) and intense (+ + + and over + + +) (16% for ACT) hypoechogenity (Figure 1).

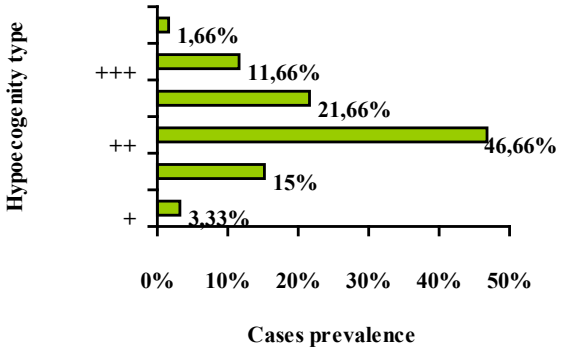

Fig.1. The intensity of the thyroid parenchyma hypoechogenity in adults group with type 1 diabetes

Regarding the homogeneity of the thyroid parenchyma it is noted that the heterogeneity of the thyroid parenchyma echogenity (88.33% cases) was slightly higher to the thyroid parenchyma homogeneity (11.67% cases) divided by the 60 members of the group.

Most authors found association of intense hypoechogenity with hypothyroidism in ACT, this aspect (hypoechogenity) representing a prognostic index for the development of subsequent hypothyroidism (12).

The thyroid functionality evaluation was performed by determining serum levels of TSH and FT_4.

Depending on the thyroid disease and the type of the changes in glycemic balance, we obtained the following results:

Table III. Evaluation of thyroid functionality

Parameters	TSH (mIU/ml)	FT_4 (pmol/l)
Graves-Basedow disease	0.2 ± 0.12	64.05 ± 29.85
Euthyroid diffuse goiter	1.14	15
Differentiated thyroid carcinoma	2.23 ± 1.53	13.86 ± 3.37
ACT	9.7 ± 11.77	9.6 ± 6.84

The Graves-Basedow disease was found in 6 patients with type 1 diabetes. Depending on the TSH value was performed functional classification of the cases. All cases with Graves-Basedow disease were accompanied by thyrotoxicosis. Between TSH and HbA1c value we found a very weak correlation (r = 0.22, p = 0.001) in all cases with Graves-Basedow disease and diabetes. The Graves-Basedow disease complications were represented by cardiothyreosis, the most common complications that occur as a consequence of cardiac overload and metabolic abnormalities in myocardium. This takes the form of heart failure secondary tachyarrhythmia, with increased flow and with resistance to tonicardiac treatment.

The euthyroid diffuse goiter met in a patient with type 1 diabetes. Depending on the TSH value was performed functional classification of cases. All cases with diffuse goiter functionally were euthyroid.

The thyroid carcinoma was found in 3 patients with type 1 diabetes. Depending on the TSH value was performed functional classification of cases. All cases of thyroid carcinoma functionally were initially euthyroid. All cases received treatment required (total thyroidectomy, treatment with I^{131}, thyroid suppressive therapy).

The ACT was found in 50 patients with type 1 diabetes. In the case of the type 1 diabetes group, 1.44% (n = 22) patients presented normal values, and 56% (n = 28) cases increased values of TSH. It is noted that in all changes of glycemic balance prevailed ACT with clinical hypothyroidism (48%), followed by ACT with euthyroidism (44%) and then ACT with sub clinical hypothyroidism (8%).

A study of 771 U.S. patients with type 1 diabetes showed that 7% had hypothyroidism and 38% hyperthyroidism (1). The correlation coefficient between serum TSH and HbA1c in all cases with DM type 1 and ACT was not significant (r = -0.17, p = 0.40). It is generally accepted that evolution of ACT is stages. The risk of hypothyroidism is higher in women over 45 years, presenting slightly increased serum TSH concentrations, and elevated titers of antiTPO AB. The thyroxin therapy induces a decrease in goiter volume at 50-90% cases, corrects the thyroid function and after some decreased the antibodies titers.

Humoral immunity was assessed by determining thyroid serum antibodies titers: antiTPO AB and antiTG AB at all patients with type 1 diabetes.

About 10% of normal subjects present antithyroid antibodies (ATAB) in serum in insignificant titers. ATAB incidence increases with age, affecting 33% of women aged over 70 years (13). A study of 243 Taiwanese patients with type 1 diabetes showed that 21.8% of them presented positive antiTPO AB. They were correlated with female gender, with older age and longer duration of disease (5).

Another study conducted in France on 258 adults with type 1 diabetes showed the presence of antiTPO AB at 17% of patients. Their prevalence was not influenced by gender, disease duration, screening age and diagnosis age of diabetes, positive family history (11). Another study in the Czech Republic on 210 patients with type 1 diabetes and 83 patients with type 2 diabetes showed that antithyroid antibodies were present in 30% of patients with type 1 diabetes and 27% of patients with type 2 diabetes (3).

They found elevated levels of antiTPO AB to 90% of cases with ACT. AntiTPO AB was normal in 10% of cases with ACT.

Table IV. Comparative data (p) between positive antiTPO AB cases and negative antiTPO AB cases in adults with type 1 diabetes group

Parameters	Negative antiTPO AB	Positive antiTPO AB	p
Cases number (%)	23	27	0.42
Age (years)	54.43 ± 20.14	40.81 ± 18.19	0.01
Onset age of DM (years)	43.43 ± 23.06	18.18 ± 19.46	0.0001
DM duration (years)	11 ± 9.33	22.62 ± 14.22	0.001
BMI (kg/m^2)	26.33 ± 3.37	24.33 ± 3.2	0.03
HbA1c (%)	12.21 ± 3.81	10.3 ± 4.11	0.09
Serum glucose (mg %)	224.13 ± 96.4	189.55 ± 97.62	0.21
TSH (mIU/ml)	3.51 ± 2.75	14.98 ± 13.86	0.0002
FT_4 (pmol/l)	12.5 ± 4.5	7.14 ± 7.57	0.003

In the case of the adults group with diabetes type 1 and ACT with positive antiTPO AB we see that they had a younger onset age of diabetes (18.18 ± 19.46 years vs. 43.43 ± 23.06 years), a longer duration of diabetes (22.62 ± 14.22 years vs.11 ± 9.33 years) and TSH values more increased (14.98 ± 13.86 mIU/ml vs. 3.51 ± 2.75 mIU/ml) than those with negative antiTPO AB (Table IV).

Significant differences ($p < 0.05$) between the two groups we have obtained regarding the current age, onset age of diabetes, diabetes duration, BMI, TSH and FT_4. We did not obtain significant differences regarding blood glucose and glycosylated hemoglobin.

In the case of antiTG AB they were present in higher titers in 80% cases (n = 40) and in normal titers in 20% cases (n = 10). The higher titers of antiTG AB were detected in ACT, with a maximum incidence of 25%. Small titers can be observed in thyroid carcinoma, various thyropaties, and some immunopaties and even in healthy subjects. In our study, in adult patients with type 1 diabetes and ACT the number of subjects with significant titers of antiTPO AB was higher than that of antiTG AB (90% vs.10%) ($X^2 = 64$, $p < 0.001$). At 35 patients were identified both antibodies (70% cases). Autoimmune thyroid disease is frequently found in patients with type 1 diabetes.

The prevalence of positive antiTPO AB in patients with type 1 diabetes and increased TSH was reported to be 80% and in those with diabetes type 1 and normal TSH was 10-20% (8). ATAB values by age are shown in Table V. Some studies have found that ATAB titers significantly increase parallel to increasing age of the patients (8). Takaichi's studies in patients with ACT found that 2% of them have only positive antiTPO AB titers, 19% of cases only positive antiTG titers and 79% of subjects have positive both types of TAB (15).

Table V. ATAB titers by age in patients with type 1 diabetes and ACT

AGE	n	AntiTPO AB (mIU/ml)	AntiTG AB (mIU/ml)
18-19 years	8	253.45 ± 217.46	315.58 ± 302.67
20-29 years	6	625.1 ± 611.16	498.27 ± 424.68
30-39 years	7	455.01 ± 279.53	33.85 ± 7.25
40-49 years	7	241.42 ± 279.02	199.28 ± 355.07
50-59 years	2	367 ± 436.56	519.95 ± 678.89
60-69 years	14	149.27 ± 313.48	165.06 ± 329.14
70-79 years	6	262.9 ± 401.05	168.6 ± 39.53

In the case of ACT, according to clinical form, we obtained two lots:

- ACT with hypothyroidism
- ACT with euthyroidism

At every group we appreciated the onset age of diabetes, current age, diabetes duration, BMI and gender (Table VI and Table VII). We found significant differences between the two groups regarding BMI, onset age of diabetes and diabetes duration.

Table VI. Comparative data between ACT with hypothyroidism and without hypothyroidism in adults with diabetes type 1 studied group

Parameters	ACT with hypothyroidism	ACT with euthyroidism	p
Cases number	28	22	0.23
Subject gender			
- male	2	1	0.41
- female	26	21	0.30
BMI (kg/m^2)	21.68 ± 3.43	26.48 ± 3.00	0.01
antiTPO AB (mIU/ml)	381.3 ± 338.49	199.00 ± 383.15	0.08
Onset age of DM type 1 (years)	21.35 ± 22.49	40.54 ± 23.15	0.005
Current age (years)	43.07±18.91	52.18 ± 20.87	0.11
DM type 1 duration (years)	21.71±14.19	11.63 ± 10.15	0.005

Table VII. Characteristics of different forms of ACT in adults with diabetes type 1 studied group

Characteristics	ACT with hypothyroidism		ACT with euthyroidism	
	Without goiter	With goiter	Without goiter	With goiter
Cases number	21	7	12	10
Subject gender				
- male	2	-	1	-
- female	19	7	11	10
Age (years)	41.09±20.08	49±14.5	49.46±20.84	46±22.96
Onset age of DM (years)	22.14±23.79	19±19.51	47.75±21.42	31.9±23.19
DM duration (years)	18.95±13.26	30±14.6	9.58±8.28	14.1±12.01

AntiTPO AB presence is associated with an increased risk of developing hypothyroidism. Colin et al. (2001) in one of the studies on the incidence of ATAB which included 1420 patients with type 1 diabetes showed that 75% of patients had positive titers of ATAB with euthyroid status, 20% had sub clinical hypothyroidism and 5% had clinical hypothyroidism (6).

CONCLUSIONS

In conclusion, thyroid damage is common in the patients with diabetes and may cause significant metabolic changes. In the case of the type 1 diabetes was predominant autoimmune etiology (ACT, Graves – Basedow disease).

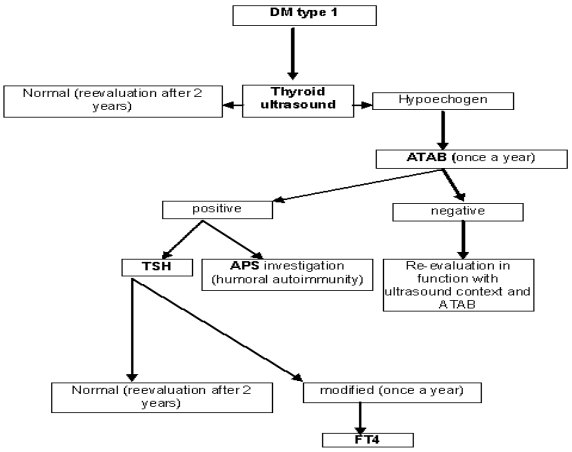

Fig. 2. Diagnostic algorithm for thyroid diseases at patients with type 1 diabetes

Chronic autoimmune thyroiditis (ACT), most commonly associated with type 1 diabetes, presented hypertrophic (34%) and atrophic (66%) forms. From the functional perspective, was revealed euthyroid or hypothyroid with different severity. Detection of this disease requires monitoring by thyroid ultrasound, thyroid function tests and determination of the serum ATAB titers at all type 1 diabetics.

Dispensary of the patients with diabetes and associated thyroid disease (including those euthyroid) needs annual monitoring of TSH to detect early hypothyroidism. If thyroid hypofunction has not major implications for glycemic balance, atherosclerotic complications of mixedema adversely affect long term prognosis of the association between diabetes mellitus and hypothyroidism.

In the case of the patients with autoimmune endocrinopathies, it is recommended annual TSH determination, possibly also antithyroid antibodies determination in patients with type 1 diabetes (especially in the presence of significant family history) to determine the onset of the thyroid disease and to institute an appropriate treatment. In the case of the pre-existing thyroid disease it is necessary glucose monitoring to determine the onset of diabetes and to institute an appropriate treatment.

In conclusion, we propose the following algorithm to diagnose the thyroid disease at patients with type 1 diabetes:

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ASPECTE DIAGNOSTICE ALE BOLII TIROIDIENE LA PACIENTII ADULTI CU DIABET ZAHARAT TIP 1

REZUMAT

Pacienţii cu diabet zaharat prezintă o prevalenţă crescută a afecţiunilor tiroidiene comparativ cu populaţia nediabetică. În cazul diabetului zaharat tip 1, acesta se asociază frecvent cu boli endocrine şi sistemice de etiologie autoimună de tipul: boala Graves–Basedow, tiroidita Hashimoto, boala Addison, boala celiacă, anemia pernicioasă, miastenia gravis, vitiligo etc. O asociere particulară a DZ tip 1 cu hipo- sau hipertiroidia este caracteristică sindromului poliglandular autoimun.

Scopul acestui studiu este de a determina principalele aspecte de diagnostic ale afec ţiunilor tiroidiene la pacienţii adulţi cu DZ tip 1. Lotul de adulţi studiat a fost reprezentat de 60 cazuri, cu vîrste cuprinse între 18-80 ani. Distribuţia pe sexe a fost netă în favoarea femeilor, fiind reprezentată de 55 femei, şi de 5 bărbaţi. Au fost utilizaţi parametrii clinici, imagistici, biochimici, hormonali şi imunologici.

Cuvinte cheie: diabet zaharat tip 1, afecţiuni tiroidiene, diagnostic, ecografie tiroidiană, hormoni tiroidieni, anticorpi antitiroidieni

REVIEW

NEW INSIGHT INTO THE RHEUMATOID VASCULITIS

M. COJOCARU[1], INIMIOARA MIHAELA COJOCARU[2], ISABELA SILOŞI[3], CAMELIA DOINA VRABIE[4]

[1]"Titu Maiorescu" University, Faculty of Medicine, Department of Physiology, Center for Rheumatic Diseases, Bucharest
[2]"Carol Davila" University of Medicine and Pharmacy, Clinic of Neurology, Colentina Clinical Hospital, Bucharest
[3]"University of Medicine and Pharmacy, Department of Immunology, Craiova
[4]"Carol Davila" University of Medicine and Pharmacy, Clinical Hospital of Emergency "Sfantul Ioan", "Victor Babes" National Institute for Pathology and Biomedical Sciences, Bucharest

ABSTRACT

Vasculitis in rheumatoid arthritis (rheumatoid vasculitis) has a heterogeneous clinical presentation that includes skin disorders, neuropathy, eye symptoms and systemic inflammation. Rheumatoid vasculitis (RV) is an unusual complication of longstanding, severe rheumatoid arthritis (RA). While RA affects the body's joints, vasculitis is a condition in which blood vessels become inflamed. RV occurs in approximately 2 to 5 % of patients who have RA. The blood vessels most often involved are arteries that bring blood to the skin, nerves, and internal organs. Veins can also be involved. Rheumatoid vasculitis is skin condition that is a typical feature of RA, presenting as peripheral vascular lesions that are localized (purpura, cutaneous ulceration, and gangrene of the distal parts of the extremities). The cause of RV is unknown, but given the presence of immune components and the pathologic changes in involved blood vessels, an autoimmune process is suggested. Compared to other forms of vasculitis, there has been relatively little research in recent years on the specific entity of RV. There is some evidence that the incidence of RV has decreased over the past several decades, perhaps because of better treatment of the underlying RA. In the present review, we discuss pathologic features, the pathogenesis and laboratory tests in RV.

Key words: rheumatoid vasculitis, pathologic features, pathogenesis, laboratory tests

INTRODUCTION

Rheumatoid vasculitis (RV) is arguably the most serious systemic disease manifestation of rheumatoid arthritis (RA). Rheumatoid vasculitis is a rare but serious complication of RA. Rheumatoid vasculitis occurs in approximately 2 to 5 % of patients who have RA. Males with RA are more likely (2 to 4 times more likely) than females with RA to develop RV. Rheumatoid vasculitis most often occurs in people with at least 10 years of severe disease. The active vasculitis occurs in about 1% of this patient population. Fortunately, recent reports have noted declines in the prevalence of RV. Rheumatoid vasculitis is a manifestation of "extra-articular" (beyond the joint) RA and involves the small and medium-sized arteries in the body. RV can affect a person from any ethnic background, either gender, and from any age group. However, more often than not, the typical patient has long-standing RA with severe joint deformities from the underlying arthritis. Although the arthritis has usually led to significant joint damage, at the onset of RV the joint disease is paradoxically quiet (1-3).

PATHOLOGIC FEATURES

Rheumatoid vasculitis has many potential signs and symptoms. The consequences of vasculitis depend on the size, site and number of blood vessels involved. Patients with RV nearly always have rheumatoid nodules Vasculitis is also associated with most of the extra-articular manifestations described in rheumatoid arthritis. The manifestations of RV can involve many of the body's different organ systems, including but not limited to the skin, peripheral nervous system (nerves to the hands and feet), arteries of the fingers and toes causing digital ischemia, and eyes with scleritis. Scleritis (inflammation of the white part of the eye) commonly occurs in the setting of RV. In addition, generalized symptoms such as fever and weight loss are common. As is true with other forms of vasculitis that involve the skin, cutaneous lesions can erupt on various areas of the body in RV, with a predilection for the lower extremities. Typical findings include ulcers concentrated near the ankles. Small nail fold infarcts (small spots around fingernail) can occur in rheumatoid arthritis but these do not necessarily signify the presence of systemic vasculitis and do not necessitate a change in rheumatoid arthritis treatment. Nerve damage can cause foot or wrist drop, known in medical terminology as "mononeuritis multiplex". The images below show a patient with a right wrist drop and a patient with right foot drop. This condition, which may be significantly disabling, is often preceded by a change in sensation in the same area (numbness, tingling, burning, or pain). These abnormal sensations can progress to muscle weakness, focal paralysis, and eventually to muscle wasting. Recovery from this condition, caused by nerve infarction, can take months. In some cases, recoveries from mononeuritis multiplex are incomplete. Early recognition and treatment with immunosuppressive drugs is usually effective (2-4).

Received 20th of April 2012. Accepted 15th of May 2012. Address for correspondence: Dr. Manole Cojocaru, Titu Maiorescu University, Faculty of Medicine, Department of Physiology, Center for Rheumatic Diseases, Bucharest; Thomas Masaryk No. 5 Sector 2 Postal Code 020983, Bucharest, Romania, e-mail: manole.cojocaru@yahoo.com

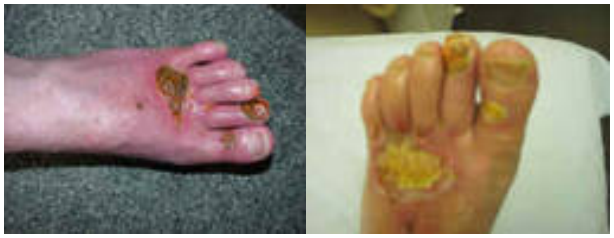

Fig. 1. Rheumatoid vasculitis with skin ulceration

PATHOGENESIS

The molecular mechanisms underlying RV are not fully understood; however, the importance of a chronic imbalance of the cytokines and chemokines involved in orchestrating inflammatory responses is well established in patients with rheumatoid arthritis, and similar deregulation of these mediators has been suggested to occur in patients with RV. Rheumatoid vasculitis refers to patients with RA, a chronic disease with painful inflammation of the joints, who also develop inflammatory disease in small and medium-sized blood vessels. RV leads to necrosis. RV usually develops at a time when the inflammatory arthritis is "burned out," (i.e., when the erosive process that led to joint destruction has become less active). RV most commonly occurs in the skin as venulitis or capillaritis, meaning the very smallest blood vessels are affected by inflammation from the disease. The reason why RV develops in some patients with RA and not others is not clear. Genetic factors may be involved. Viral infections and drug reactions have been suggested as causes of RV, but there is little research to support this. Some research suggests that long time use of drugs such as corticosteroids, gold compounds, penicillamine and azathioprine that are used to treat RA can cause the development of RV. However, this may not be true and difficult to determine because more use of these drugs is probably because of more severe or long standing RA, both of which may also be associated with the development of RV. The association of RV with rheumatoid factor (RF) and anti-tissue antibodies (e.g., anti–cyclic citrullinated polypeptide [anti-CCP], anti-nuclear antibodies [ANA]) suggests that immune complex disease may be causative. Immune complexes may be found in affected tissue, and most patients with RV have circulating autoantibodies. However, many patients with RA who have circulating or tissue-deposited immune complexes and high levels of autoantibodies do not develop vasculitis. The relationship of these immune complexes to RA and vasculitis leaves many unanswered questions and is far from definitive. Evidence linking the immune system to vasculitis includes: High levels of RF, the presence of other proteins in the blood (called immune complexes), lower levels of proteins in the blood (called complement), which are used up when inflammation occurs, the appearance of inflamed blood vessels under the microscope, which shows immune cells within the wall of the vessel. The most devastating form of RV is characterized by a medium-sized vasculitis reminiscent of (and histological identical to) polyarteritis nodosa (3-5).

LABORATORY TESTS

Laboratory tests may support, but do not confirm, a diagnosis of RA or RV. Rheumatoid vasculitis occurs almost exclusively in patients with sero-positive nodular RA. An abnormally active immune system (the body's defense system) appears to play an important role in blood vessel inflammation. Laboratory findings may include anaemia of chronic inflammation, elevation of erythrocyte sedimentation rate or C-reactive protein, polyclonal hypergammaglobulinemia, and RA-associated autoantibody. Complement levels may be dynamically decreased during active disease and, along with inflammatory parameters, may provide useful follow-up information. Most laboratory findings in RV – for example, elevations in the erythrocyte sedimentation rate or C-reactive protein – are non-specific, and reflect the presence of a generalized inflammatory state. Hypocomplementemia, ANA, and atypical anti-neutrophil cytoplasmic antibodies (ANCA) are common. Rheumatoid factor levels are usually extremely elevated. However, there is no definitive laboratory test for RV short of a tissue biopsy. The diagnosis must usually be made using a combination of history, physical examination, pertinent laboratory investigations, specialized testing (e.g., nerve conduction studies), and sometimes a tissue biopsy.

An atypical perinuclear ANCA was found in 48% of patients with RV. Specific enzyme immunoassay studies for anti-myeloperoxidase and anti–proteinase-3 should be performed and are typically negative in RV.

Because the treatment implications for RV are major, any diagnostic uncertainty must be met with definitive approaches to establishing the diagnosis. This usually involves biopsy of an involved organ. Deep skin biopsies (full-thickness biopsies that include some subcutaneous fat) taken from the edge of ulcers are very useful in detecting medium-vessel vasculitis. Nerve conduction studies help identifies involved nerves for biopsy. Muscle biopsies (e.g., of the gastrocnemius muscle) should be performed at the same time as nerve biopsies, to increase the chance of finding changes characteristic of vasculitis. Imaging studies have no consistent role in the evaluation of RV, although sometimes angiography of the gastrointestinal tract is useful. Biopsy of the skin or other symptomatic organs is sometimes necessary.

Some studies have shown an association between elevated RF or anti-CCP antibodies and RV. However, the high prevalence of these antibodies in RA without vasculitis lends little to a positive predictive value for the diagnosis of RV. A blood test for specific antibodies that are directed against the inner layer of blood vessels (endothelial cells) are present in approximately 75% of patients with RV compared to only 15 to 20% of those with RA alone. Therefore, this blood test may be checked regularly in patients with any of these new or worsened symptoms. Characteristic histopathology confirmation of vasculitis is generally necessary for a diagnosis of RV (4-7).

CONCLUSION

Systemic vasculitis is a rare but serious complication of rheumatoid arthritis and may be considered one of the most serious extra-articular consequences of this disease. Rheumatoid vasculitis is among the most serious complications of RA.

Rheumatoid vasculitis, as clinical-pathologic manifestation of RA, is characterized by tissue damage or ischemia verified pathologically by vasculitis. Rheumatoid vasculitis may involve virtually any organ of the body. In general, people who get vasculitis have many joints with pain and swelling, rheumatoid nodules, high concentrations of RF in their blood, and sometimes smoke cigarettes. Rheumatoid vasculitis manifests almost exclusively in patients with rheumatoid autoantibodies. Biopsy is typically required to ensure a definitive diagnosis of RV,

COMPETING INTERESTS

The authors declare that they have no competing interests.

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ASPECTE NOI ÎN VASCULITA REUMATOIDĂ

REZUMAT

Vasculita din cadrul poliartritei reumatoide (vasculita reumatoidă) se prezintă clinic polimorf, cuprinde modificări cutanate, neuropatie, simptome oculare şi inflamaţie sistemică. Vasculita reumatoidă (VR) este o complicaţie neobişnuită a poliartritei reumatoide (PR) severe cu evoluţie îndelungată. În timp ce PR afectează articulaţiile, vasculita este o stare în care vasele sanguine sunt inflamate. VR se întâlneşte la aproximativ 2-5 % dintre pacienţii cu PR. Vasele sanguine cel mai adesea afectate sunt arterele care vascularizează pielea, nervii şi organele interne. Venele pot, de asemenea, să fie afectate. Vasculita reumatoidă este modificarea cutanată tipică în PR, se prezintă cu leziuni vasculare periferice care sunt localizate (purpura, ulceraţii cutanate, gangrena extremităţilor). Cauza VR este deocamdată neelucidată, din cauza prezenţei componentelor imune şi modificărilor patologice la nivelul vaselor sanguine afectate s-a sugerat un proces autoimun. Comparativ cu alte forme de vasculite, există puţine cercetări în ultimii ani referitoare la VR. Unele rapoarte evidenţiază că incidenţa VR a scăzut în ultimele decade datorită tratamentului mai eficient în PR. În acest referat, autorii discută despre trăsăturile clinice, patogenia şi testele de laborator în VR.

Cuvinte cheie: vasculita reumatoidă, trăsături clinice, patogenie, teste de laborator

PERIODONTAL DISEASE AND GLYCEMIC CONTROL IN PATIENTS WITH DIABETES

CIPRIAN-ALEXANDRU DINA[1], RALUCA DINA[2], IULIA VLADU[3], RALUCA VINTILESCU[1], TAISESCU CITTO[1], TUDOR ADRIAN BĂLŞEANU[1], MARIA IANCAU[1]

[1]University of Medicine and Pharmacy Craiova – Discipline of Physiology
[2]Clinic of Diabetes, Nutrition and Metabolic Diseases, Clinical County Emergency Hospital Craiova
[3]Clinic of Nephrology, Clinical Emergency County Hospital Craiova

ABSTRACT

Periodontitis is a common complication in patients with diabetes mellitus. Their classification is complex and it is based on the clinical presentation, rate of disease progression, age at diagnosis and local and systemic factors that may multiply the risk. **Background and objectives.** The existence of correlations between the glycemic values, HbA1c and the two main stages of periodontal disease, the study of the effect of periodontal treatment on HbA1c. **Material and method:** The study was conducted in the Clinic of Stomatology, Jean Verdier Hospital in Paris, on a group of 58 patients with diabetes, who underwent periodontal control. **Results and Discussion.** Both advance chronic stages of periodontitis (superficial and profound) were correlated with fasting glucose ($p < 0.05$), postprandial glucose ($p < 0.05$) and HbA1c ($p < 0.05$). Following systemic antibiotics, plus local periodontal treatment, HbA1c decreased in the 20 patients between 0.1 to 0.5%. **Conclusion.** The relationship between these two diseases appears bidirectional, insofar that the presence of one disease tends to promote the other, and that the meticulous management of either may help the treatment of the other. Some authors believe that this disease should be included in the "classical"complications of diabetes.

Key words: periodontal disease, diabetes mellitus, gingivitis

INTRODUCTION

Periodontal infection is a complication, that can be responsible of damaging systemic physiology in diabetic patients. Periodontitis may be more than a confined oral infection, the consequences have been assumpt to be far-reaching. Chronic and severe forms of this condition can follow a systemic response of the bacteria and bacterial result products, that are outspread due to the collapse of the periodontal apparatus (that is composed from the ligament attachment around the tooth, the gingival tissues and bone). The interdependency between periodontal disease and diabetes is an example that a systemic disease can predispos to oral infection, and then once that infection is settled, the oral infection can augment the progression of systemic disease (10,11).

Periodontal diseases are the most common diseases known to humanity. The two major stages of periodontal diseases are gingivitis and periodontitis. In the early stage of gingivitis, the inflammation is located to the gingiva, and is reversible, can usually be treated with good oral hygiene. The second stage involves the extension of inflammation and results in tissue destruction and alveolar bone resorption, stage called periodontitis (8).The examination of the available data have proved that diabetes is an important risk factor for periodontitis and gingivitis, and that the level of glycemic control is an important determinant in this relationship (3-5).

Background and objectives. The existence of correlations between the glycemic values, HbA1c and the two main stages of periodontal disease, the study of the effect of periodontal treatment on HbA1c.

MATERIAL AND METHOD

The study was conducted in the Clinic of Stomatology, Jean Verdier Hospital in Paris, on a group of 58 patients with diabetes, 45 females and 13 males, with ages between 23 and 82 years. In the study were included patients with diabetes who underwent periodontal control: depth of periodontal pockets, gingival bleeding, supra- and subgingival calculus. Following data were recorded: demographic (age, sex), anthropometric parameters (height, weight, WC, HC, BMI) laboratory data (fasting glucose, postprandial glucose, HbA1c, cholesterol total, LDL cholesterol, HDL cholesterol, triglycerides, creatinine, AST, ALT, gamaGT, C-reactive protein). All the patients underwent periodontal treatment plus local and systemic antibiotics, but only in the patients which had not changed the antidiabetic therapy (20 patients) the HbA1c was recorded at 3-4 months of consultation.

RESULTS

Following the statistical analysis, the first stage of periodontal disease, gingivitis, was not correlated with fasting glucose ($p=0.410$) (Figure 1), postprandial glucose ($p=0.310$) (Figure 2) and HbA1c ($p=0.339$) (Figure 3).

Both advance chronic stages of periodontitis (superficial and profound) were correlated with fasting glucose ($p < 0.05$) (Figures 4 and 9), postprandial glucose ($p < 0.05$) (Figures 5 and 7) and HbA1c ($p < 0.05$) (Figures 6 and 8).

Received 20th of April 2012. Accepted 15th May 2012. Address for correspondence: Ciprian Alexandru Dina, MD, PhD student, Discipline of Physiology, University of Medicine and Pharmacy Craiova, Petru Rareş no. 2-4, RO-200349, Craiova, Romania, e-mail: dina_ciprian@yahoo.com

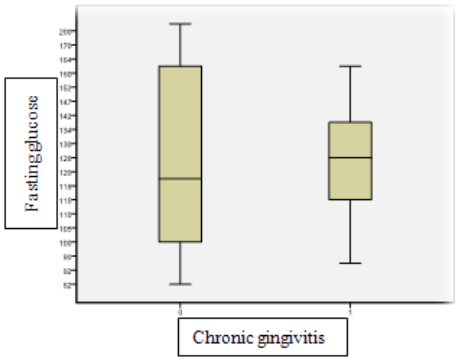

Fig.1. Correlation between chronic gingivitis and fasting glucose (p=0.410)

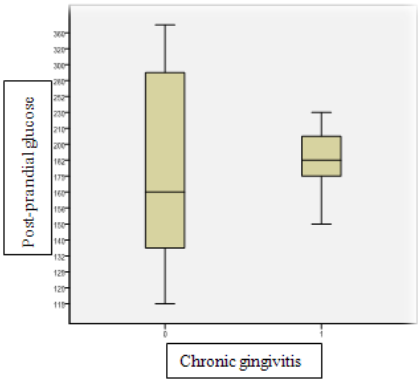

Fig.2. Correlation between chronic gingivitis and postprandial glucose (p=0.310)

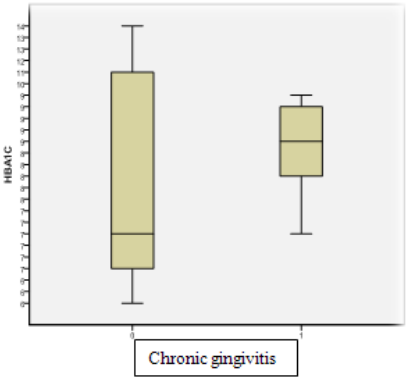

Fig.3. Correlation between chronic gingivitis and HbA1c (p=0.339)

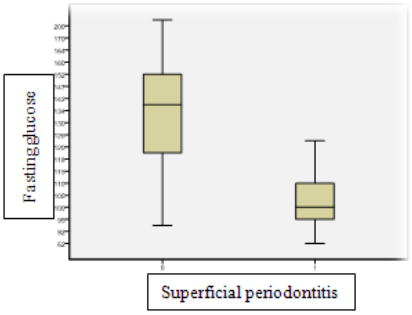

Fig.4. Correlation between superficial periodontitis and fasting glucose (p < 0.05)

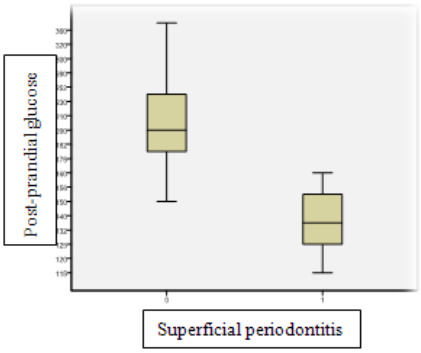

Fig.5. Correlation between superficial periodontitis and postprandial glucose (p < 0.05)

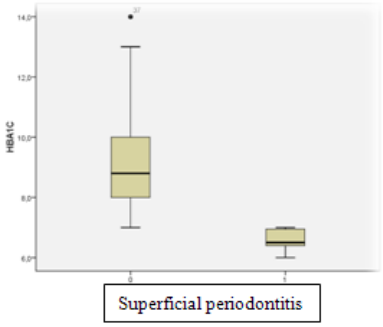

Fig.6. Correlation between superficial periodontitis and HbA1c (p < 0.05)

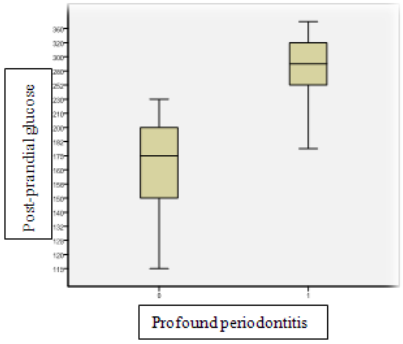

Fig.7. Correlation between profound periodontitis and postprandial glucose (p < 0.05)

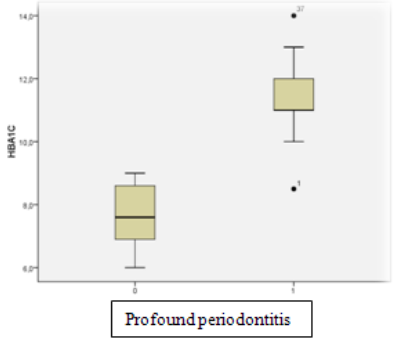

Fig.8. Correlation between profound periodontitis and HbA1c (p < 0.05)

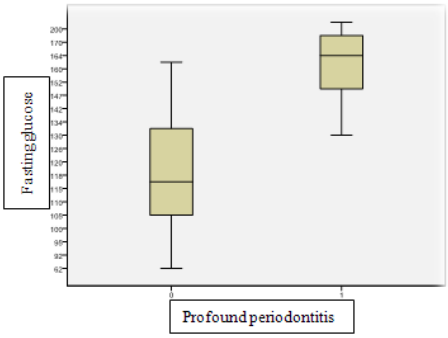

Fig.9. Correlation between profound peridontitis and fasting glucose ($p < 0.05$)

Following systemic antibiotics plus local periodontal treatment HbA1c decreased in the 20 patients between 0.1 to 0.5% (15% of patients with 0.1, 25% with 0.2, 20% with 0.3; 15% with 0.4 and 25% with 0.4) (Figure10).

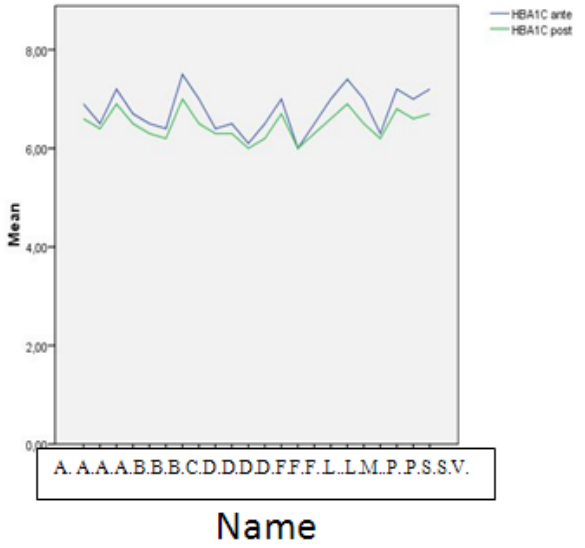

Fig.10. HbA1c before and after treatment

DISCUSSIONS

Our results are consistent with the literature, both advance chronic stages of periodontitis (superficial and profound) were correlated with fasting glucose ($p < 0.05$), postprandial glucose ($p < 0.05$) and HbA1c ($p < 0.05$), the relationship between diabetes and periodontal disease is well studied and known for a long time. Although the relationship between periodontal and diabetes condition is not enough questioned in the current literature, the effect of periodontal treatment on metabolic control in diabetic patients and the effect of the metabolic control of diabetes on periodontal condition remains controversial (1,2). In general, periodontal treatment is a important priority in patients for whom periodontal condition may pose a health risk and this includes diabetic subjects (9-12). In our study group the treatment of periodontal disease whith systemic antibiotics and local mechanical treatment decreased HbA1c between 0.1 to 0.5%. A number of studies have evaluated whether the use of systemic antibiotics ameliorate the periodontal prognosis and metabolic control in diabetic subjects. Mechanical periodontal therapy, with 100 mg doxycycline, has reduced HbA1c with 0.6% in type-2 diabetic subjects (13-16). Tetracyclines and their derivatives appear to have an important role in the inhibition of tissue destruction enzymes. Hence, doxycycline appears to be a powerful modifier of the response to periodontal treatment in diabetic subjects, by inhibiting non-enzymatic glycosylation of extracellular proteins, indicating that it has a equal effect on haemoglobin glycosylation (18). Treatment of periodontitis, using a association of mechanical therapy, scaling and root planning, plus systemic tetracycline antibiotics has been demonstrated to have important reductions in HbA1c values- indicative of improved glycaemic control. Also, a research study by Miller and colab. demonstrated that mechanical periodontal therapy, associated with adjunctive systemic doxycycline resulted in a decreasing with 0.6% HbA1c values, in a group of Hispanic patients with type 2 diabetes (17). Iwamoto and colab. associated mechanical periodontal therapy with local application of minocycline gel to each periodontal pocket, every week for a month, in a group of patients with type 2 diabetes; following this treatment, the number of bacteria, existing in periodontal pockets, was highly reduced, serum TNF-α levels were decreased with 0.49 pg/mL and glycemic control, quantified by HbA1c values, was also improved (20-22). Grossi and colab. reported results of periodontal therapy in a group of Pima indians with type 2 diabetes. An association of ultrasonic root debridement with systemic doxycycline, 100 mg per day for two weeks, decreased Porphyromonas Gingivalis to nondetectable levels, in all patients who tested positive at baseline, after three months of treatment. In addition, there was a important improvement in periodontal attachment and a 1% decrease in the level of HbA1c, three months after the ending of the treatment. These results are in contrast with a number of researches, cited by Grossi, in which diabetic patients treated with mechanical periodontal therapy, scaling and root planning, without adding adjunctive systemic antimicrobials, failed to improve glycemic control. Therefore, a mechanical periodontal therapy associated with systemic doxycycline seems to ameliorate glycemic control, in many diabetic subjects with periodontitis. This is in contrast to the periodontal treatment of patients without diabetes, where systemic antimicrobials are not usually used and are not necessary for a successful treatment. Also, it should be noted that the treatment with systemic tetracyclines, alone, have been proven to suppress or inhibit activity of collagenase in the gingival crevicular fluid of diabetic subjects with periodontitis. Therefore, a limited number of interventional studies have demonstrated that treatment of periodontal infection and the consequent reduction in bacterial load and pro-inflammatory molecules may have as result an improved glycemic control, in diabetic patients. Also, these data indicate that other periodontal therapies designed to consistently free the patient of periodontal pathogens should bring similar benefits (19).

CONCLUSIONS

1. In our study group the first stage of periodontal disease, gingivitis, was not correlated with fasting glucose (p=0.410), postprandial glucose (p=0.310), and HbA1c (p=0.339).

2. Both advance chronic stages of periodontitis (superficial and profound) were correlated with this glycemic parameters (p<0.05). The relationship between these two diseases appears bidirectional insofar, that the presence of one disease tends to promote the other, and that the meticulous management of either may help the treatment of the other.

3. Mechanical treatment of periodontitis, scaling and root planing, associated with short-term administration of tetracycline, can temporarily ameliorate glycemic control in diabetic subjects, especially in those with advanced stages of periodontitis and low glycemic control before treatment. In our study group, after the treatment of periodontal disease whith systemic antibiotics and local mechanical treatment HbA1c decreased between 0.1 to 0.5%.

4. The study initiated by us has sought to complete the data from literature, which is mostly limited to highlight the predilection of diabetics to develop periodontal lesions. In the research conducted, we brought objective arguments about the evolutionary interrelationship between the two diseases.

5. Based on the data presented we suggest that periodontal patients with diabetes should be consulted and treated by a periodontist (22).

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BOALA PARODONTALĂ SI CONTROLUL GLICEMIC LA PACIENȚII CU DIABET ZAHARAT

REZUMAT

Parodontita este o complicaţie frecventă la pacienţii cu diabet zaharat. Clasificarea bolilor parodontale este complexă şi se bazează pe prezentarea clinică, rata de progresie a bolii, vârsta la diagnostic şi factorii locali şi sistemici, care pot multiplica riscul. **Premise si Obiective:** Existenţa unor corelaţii între valorile glicemiei, respectiv HbA1c şi cele 2 stadii principale ale bolii parodontale; studierea efectului tratamentului parodontal asupra valorilor HbA1c. **Materiale şi metode**: Studiul a fost efectuat în Clinica de Stomatologie a Spitalului Jean Verdier din Paris, pe un grup de 58 de pacienţi cu diabet la care a fost efectuat un examen parodontal. **Rezultate şi Discuţii:** Ambele stadii avansate cronice de parodontită (superficială şi profundă) s-au corelat pozitiv cu glicemia a jeun ($p < 0,05$), glicemia postprandială ($p < 0,05$) şi cu HbA1c ($p < 0,05$). În urma tratamentului parodontal local plus antibioterapia sistemică HbA1c a scăzut, la cei 20 de pacienţi, între 0,1 - 0,5 % (la 15% dintre pacienţi cu 0,1; 25% cu 0,2; 20% cu 0,3; 15% cu 0,4; 25 % cu 0,4). **Concluzii.** Relaţia dintre aceste două boli pare bidirecţională, în măsura în care prezenţa unei boli tinde să o agraveze pe cealălaltă si de asemenea, tratamentul uneia tinde să o amelioreze pe cea de-a doua. Unii autori consideră ca această boală ar trebui să fie inclusă în cadrul complicatiilor „clasice" ale diabetului zaharat.

Cuvinte cheie: boala parodontala, diabet zaharat, gingivita

DIAGNOSTIC AND TREATMENT METHODS FOR KERATOCONUS

ROŞCA COSMIN
OPTILENS Ophthalmology Clinic Cluj - Napoca

ABSTRACT

Keratoconus is a rare disease that appears at teenagers. This condition is diagnosed frequently and at early stages in the last years by the means of modern diagnostic methods (pachymetry, corneal topography). New and efficient treatments for this disease such as corneal cross-linking and intrastromal rings are available.

Keywords: keratoconus, corneal topography, intrastromal corneal rings, cross-linking

INTRODUCTORY AND EPIDEMIOLOGICAL DATA

Keratoconus is a noninflammatory pathology characterised by progressive central and paracentral corneal thinning with asymmetric conical ectasia and irregular myopic astigmatism.

The prevalence varies between 10 and 600 cases/100,000 population, many authors agreeing over an average prevalence of 54.5 cases per 100.000 population (6). On the contrary, the incidence (the number of new cases per year) varies between 50 and 230 cases/100.000 population, approximately 1/2000 (6).

In the past the prevalence was deemed higher in women, but more recent studies show that a greater number of men are affected and they have a more rapid disease evolution. Other studies found no significant differences between men and women (6).

According to most authors, the diagnosis age is 20 to 30 years. In more rare cases, keratoconus may appear later and evolve at older ages (6).

Keratoconus starts in one eye usually and extends to the other eye after 2-5 years only. The first diseased eye is the most affected throughout the entire disease course. A single eye is affected very rarely, bilaterality being evidenced in 96% of cases; in many cases the merely incipient pathology of the second eye is visible by corneal topography only (7).

With the advent of modern diagnostic means, particularly the topography and the pachymetry, the number of bilateral keratoconus cases increased due to easier diagnosis in early or advance stages, even in the absence of some important subjective deficiencies.

Regarding the importance of inheritance in the onset of keratoconus, the percentage of familial cases described in the literature is of 6-23%. Autosomal dominant inheritance with incomplete penetrance has been proposed. Prevalence of keratoconus in first degree relatives of the patients is 3.34%, 15 to 67 times greater than in general population (0.05- 25%) (8).

As the ethnic differences concerns, some authors consider keratoconus is more frequent in Whites, while others deem Asians have three times greater incidence with earlier onset and more rapid evolution towards transplant (9).

Keratoconus is in most cases an isolated state, but sometimes these patients experience systemic and/or ophthalmologic problems, i.e. diseases in which a defect of collagen metabolism appears: Down syndrome, Turner syndrome, Ehlers-Danlos syndrome, Marfan syndrome, atopy, osteogenesis imperfecta, mitral valve prolapse, psychoastenia (10).

The most frequent ophthalmologic disorders associated with keratoconus are allergic conjunctivitis, Leber congenital amaurosis, pigmentar retinitis, blue sclerae, and aniridia and ectopia lentis.

CLINICAL SEMIOLOGY

As regards the clinical semiology, the first symptoms are the following:

- Poor visual acuity corrected with eyeglasses
- Visual tiredness, headache
- Myopia and/or astigmatism with abnormal evolution
- Lens adjustment difficulties
- Night driving difficulties

Early signs of disease:

- oil droplet reflex evidenced by direct ophthalmoscopy from a distance of 30 cm (Fig. 1)
- fine vertical deep stromal striae (Vogt's striae) evidenced through biomicroscopy (Fig. 2)
- irregular corneal astigmatism evidenced at keratometry, leading to marked alteration of visual acuity

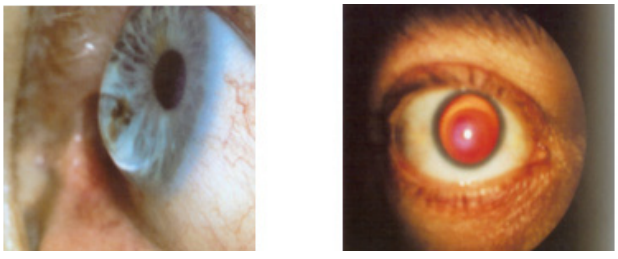

Fig. 1. "Oil droplet " corneal reflex

Received 25th of March 2012. Accepted 15th April 2012. Address for correspondence: Dr. Cosmin Rosca, OPTILENS Ophthalmology Clinic Cluj – Napoca, phone: 0740168910, e-mail: roscacosmin@yahoo.com

Fig. 2. Deep stromal striae (Vogt lines)

Late signs of disease:

In advanced cases of keratoconus, the simple external inspection - looking from a side - reveals the conical shape of cornea with anterior protrusion.

When the patient looks downward, an accentuation of the lower eyelid margin curvature caused by corneal protrusion (Munson sign) can be seen (Fig. 3).

Epithelial deposits of iron surrounding the base of the cone (Fleischer's ring) are revealed by biomicroscopy. These deposits are best visualised with cobalt blue filter.

Acute hydrops is an acute influx of aqueous humour into the cornea caused by the rupture of Descemet's membrane (fig. 4). This leads to sudden loss of vision accompanied by discomfort and weeping. The oedema disappears within 6-10 weeks and the cornea may remain with stromal scars.

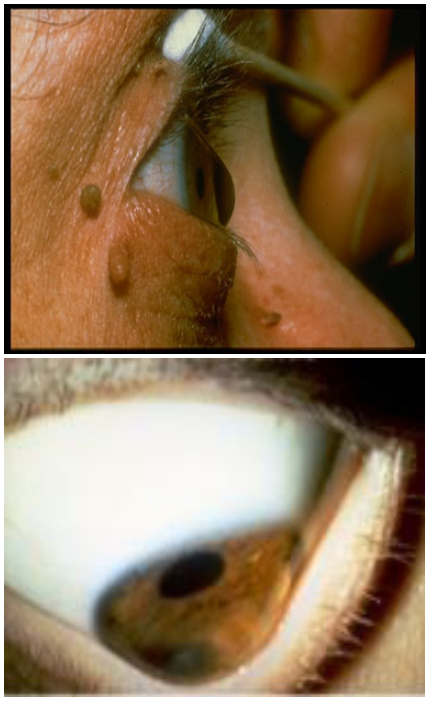

Fig. 3. Bulging of the lower lid in down-gaze (Munson sign)

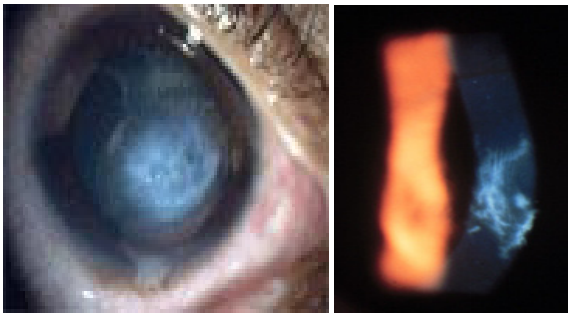

Fig. 4. Acute hydrops is caused by a rupture in Descemet membrane- after healing stromal scarring may develop

INSTRUMENTAL DIAGNOSIS

The instrumental diagnosis consists of the following techniques:

1. keratometry – detection of irregular astigmatism suggesting the possibility of keratoconus.

2. retinoscopy – light reflected through patient's eye causes a scissor reflex, i.e. two bands moving toward and away from each other like the blades of a pair of scissors.

3. manual keratoscope – known as "Placido's disc", allows the simple non-invasive visualisation of corneal surface by projecting a series of light circles on the cornea (Fig. 5).

4. corneal topography (fig. 6) – used in order to obtain a positive diagnosis by automatic projection of a light pattern onto the cornea, thus obtaining a topographic map showing all the corneal distortions. Keratoconus appears as a curvature increase usually located under the central line of the eye. This technique allows the detection of the disease in the first stages, when symptoms are not yet present.

5. ultrasonic pachymetry – is the method through which the thickness of cornea can be assessed; this is critical in therapeutic indication, especially cross-linking and Ferrara intrastromal implants, as these techniques are contraindicated when cornea thickness is under 400 microns.

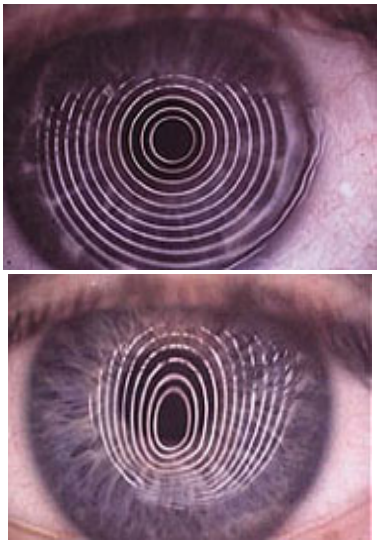

Fig. 5. Manual keratoscope (Placido disk)

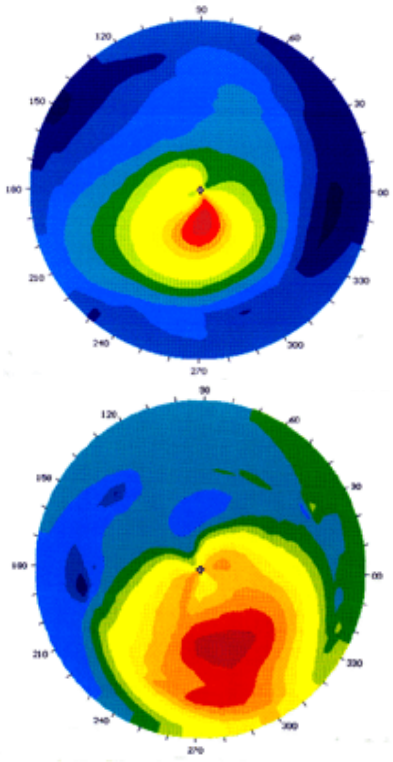

Fig. 6. Corneal topography

KERATOCONUS STAGES

Depending on the keratometric measurements, there are three severity stages:

- stage 1: k < 45D, average
- stage 2: 45 <k <52D, advanced
- stage 3: k > 52D, severe

Depending on the cone morphology, the following forms:

- nipple form- small and near-central
- oval form – larger, below-centre
- globus form – more than 75% of cornea is affected.

DIFFERENTIAL DIAGNOSIS OF KERATOCONUS

a) Keratoglobus
b) Pellucid marginal degeneration
c) Posterior keratoconus

KERATOCONUS TREATMENT

1. **Aerian and contact lens correction**

In the first two stages, the vision correction can be achieved by the means of spherical-cylindrical lens (deformation of cornea leads to myopic astigmatism with oblique axes), soft toric lenses or combination of soft spherical contact lenses and eyeglasses.

In the third stage, wearing of rigid, oxygen permeable contact lens is attempted, which would regularise the ocular surface more than the soft contact lenses that mould to the shape of cornea. These lenses are more difficult to tolerate, but they may ensure a satisfactory vision and succeed in delaying the surgical intervention time.

2. **Parasurgical therapy by cross-linking**

The cross-linking or corneal collagen cross-linking method consists of photopolymerisation of stromal fibers in order to increase their rigidity and resistance to keratectasia through the combined action of a photosensitising substance (riboflavin – vitamin B2) and ultraviolet light irradiation with a solid-state illuminator.

This method is preferred in stages 1 and 2 that have negative progression, cannot be optically corrected and are candidates for perforating or lamellar keratoplasty.

Exclusion criteria:

- patients under 18 and over 60 years of age;
- cornea thickness under 400 microns;
- previous herpetic keratitis;
- corneal scars;
- severe forms of dry eye;
- ongoing corneal infections;
- concomitant autoimmune diseases.

As regards the pathophysiology, the cross-linking causes the increase of inter-fiber bonds and collagen fiber diameter in the anterior or intermediate corneal stroma to 300 microns depth without damages in the endothelium.

The cross-linking method proved to be technically simple and low cost, leading to collagen stabilisation and blocking of melting processes without clinically visible side effects (2).

3. **Keratoplasty**

Corneal transplantation is an outpatient procedure generally performed under local anaesthesia consisting of the circular "cutting-out" of the "diseased" corneal area and its replacement with a "healthy" donor cornea. This is one of the ophthalmological surgeries that heal very slowly. As the cornea has no blood vessels, postoperative wound healing and graft integration into the new "host" is extremely difficult. At late postoperative times we look for signs of rejection (which unfortunately is a lifelong risk), ocular tension, dioptres and visual acuity, possibly eyeglasses correction.

4. **Ferrara intrastromal corneal rings**

The underlying principle of this procedure is the flattening of keratoconus distorted cornea and the selection of two different diameter segments in order to reduce the asymmetric astigmatism responsible for the decline in visual acuity. Ferrara intrastromal rings are represented by two 160 degrees semicircular segments of 5 and 6 mm diameter, with a thickness between 0.150 and 0.350 mm, having triangular shape with 600 μm basis. They are made of Perspex CQ Acrylic (PMMA) similar to crystalline implants, this material being perfectly tolerated by the organism, without risk of rejection (fig. 7).

Ferrara intrastromal rings are actually rigid elements that fill the space and act mechanically on the cornea by separating the lamellae and thus:

- modify the arc length of the anterior curvature
- flatten cornea from periphery to centre
- restores the shape of cornea, reducing the astigmatism

- reduces keratoconus progression

Implantation is performed at 70% depth of corneal stroma preserving the integrity of Bowman's and Descemet's membrane, according to the nomogram that shows the size of the rings to be implanted, depending on patient's refraction.

Before surgery, visual acuity with and without correction is assessed and corneal topography, keratometry and ultrasound pachymetry are performed.

The great advantage of this method is that central optical zone of the cornea remains untouched and it is reversible – in case of failure, the two circle arcs segments can be explanted without complications.

The patient has to be examined as early as possible after surgery, and follow-ups are needed at 24 hours; 7 days; 30 days; 3 months; 6 months; 9 months; 12 months.

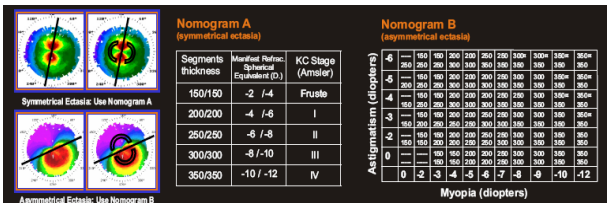

Fig. 7. Nomogram for the intracorneal ring implantation

CONCLUSIONS

1. Keratoconus is a common pathology nowadays, more and more patients being diagnosed with this disease;

2. Age of diagnosis is between 20 and 30 years, usually being bilateral;

3. Due to modern examination techniques (corneal topography and ultrasound pachymetry), keratoconus can be diagnosed in early stages;

4. By stabilising and delaying the conical progression, modern treatments (cross-linking and intrastromal rings implantation) bring great advantages to patients, as well as a low rate of complications, thus allowing rapid integration into daily activities.

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METODE DE DIAGNOSTIC SI TRATAMENT IN KERATOCON

REZUMAT

Keratoconul este o afecţiune relativ rară care apare la tineri, diagnosticată mult mai frecvent şi în stadii incipiente în ultimul timp datorită metodelor moderne de diagnostic (pahimetria, topografia corneană) şi beneficiind de tratamente noi şi eficiente precum cross-linking, implantare de inele corneene intrastromale.

Cuvinte cheie: keratoconus, corneal topography, intrastromal corneal rings, cross-linking