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CONTENTS

CUPRINS

INTERACTIONS BETWEEN MESENCHYMAL STEM CELLS AND CELLULAR EFFECTORS OF IMMUNE SYSTEM

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ABSTRACT

Mesenchymal stem cells represent a rare subset of stem cells that reside in the bone marrow, where they interact with hematopoietic stem cells, guiding their growth and differentiation, and they serve as a reservoir of renewal of various mesenchymal tissues. They also possess remarkable immunosuppressive properties, inhibiting the proliferation and function of most major immune cell populations. This review provides information regarding the current research on the interactions of mesenchymal stem cells with immune cells, the possible mechanisms of these interactions, the *in vivo* use outcomes, as well as other potential clinical implications, and future research directions.

Key words: mesenchymal stem cells, immune system, immunosuppression, clinical applications

INTRODUCTION

In vitro interactions of mesenchymal stem cells (MSCs) with multiple sets of immune cells have shown that MSCs possess remarkable immunosuppressive properties, being able to inhibit the proliferation and modulate the function of the major immune cell populations, including T cells, B cells and natural killer (NK) cells. They have also been shown to modulate the activities of dendritic cells (DCs) and to induce regulatory T cells. The immunomodulatory effect of MSCs is mediated by non-specific anti-proliferative mechanisms, dependent on cell–cell contact or secreted soluble factors.

These unique properties make MSCs ideal candidates for clinical application as immunosuppressants, in pathologies such as graft-versus-host disease, solid organ transplants, or autoimmune diseases.

MSCs and T cells

The correlation between MSCs and T cells starts in the early ontogenic stage, both in the bone marrow (BM) and in the thymus. MSCs derived from the mesothelium invade the vasculature and localize in the BM stroma, where they provide support for the hematopoietic stem cells [1]. Primitive T cells migrate to the thymus, where they undergo positive and negative selection, leading to their maturation. It has been shown that marrow stromal cells can support T-cell maturation even in the absence of the thymus, suggesting that the bone marrow stroma can function as an extrathymic site for T cell development [2]. This leads to the conclusion that MSCs may play an important role in the process of hematopoiesis.

In vitro and *in vivo* studies have shown multiple effects of MSCs on T cells. However, they differ, depending on the concentration of the MSCs. A high MSC/lymphocyte ratio is associated with an inhibitory effect of MSCs, while a low MSC/lymphocyte ratio is often accompanied by enhanced T cell proliferation [3]. MSCs may have dual roles, depending on the environment to which they are exposed. They have been shown to inhibit the proliferation of activated T cells, but they can also support the survival of quiescent T cells (as they are in the hematopoietic stem cell niche) [4]. Under quiescent conditions, MSCs can promote T cell survival, and stimulate CD4+ T cell activation and proliferation, independently of cell-cell contact [5].

Some of the mechanisms of immunosuppression have been elucidated, but the underlying molecular mechanisms remain uncertain. This immune inhibition seems to be mediated by cell-cell interactions, but also by the release of cytokines [6]. Apparently, species-dependent mechanisms are involved: in rodents cell-cell contact is enough [3], but in humans soluble factors are necessary for the suppressive effect [6].

MSCs can inhibit T cell proliferation by different stimuli, such as alloantigens, cognate antigen stimuli, and non-specific mitogen stimuli. The inhibition affects different aspects of proliferation, such as the expression of activation markers, cytotoxic T (Tc) cell formation (they can also inhibit the cytotoxic effects of antigen-

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primed Tc cells), cytokine production (interferon (IFN)-γ by Th1 cells, interleukin (IL-4) by Th2 cells) [7]. The suppressive effect is present on both naïve and memory T cells, CD4+ and CD8+ T cells [3]. It does not require major histocompatibility complex restriction and can be mediated by allogeneic MSCs [8]. The accumulation of cells in the G0/G1 phase of the cell cycle may mean that MSCs act through the inhibition of cell division [9].

Several MSC-derived molecules have been proposed which are believed to exert immunomodulatory effects on T cells: transforming growth factor (TGF)-β1 [10], hepatocyte growth factor (HGF) [3], indoleamine 2, 3-dioxygenase (IDO) [5], prostaglandin E2 (PGE2), and human leukocyte antigen-G5 (HLA-G5) [3].

MSCs treated with IFN-γ express functional IDO, which degrades tryptophan, resulting in kynurenine synthesis, which in turn suppresses lymphocyte proliferation [11].

If MSCs are co-cultured with T cells, a high level of PGE2 can be found in the medium, and treatment with PGE2 inhibitors reduces the MSC-mediated immune modulation [12]. The mechanisms of this process are still uncertain.

The secretion of human leukocyte antigen-G5 (HLA-G5) by MSCs is believed to be essential for the suppression of T cell and NK cell function, the shift of the allogeneic T cell response towards a T helper type 2 (Th2) cytokine profile, and the induction of CD4+CD25^{high}forkhead box P3 (FoxP3+) regulatory T cells (Tregs) [3].

MSCs may also function through suppressive T cells. Apart from Th cells and Tc cells, there is a third type of T cells – the regulatory T cells, which negatively regulate immune responses. Three subsets of Tregs have been identified so far: CD41+CD25^{high}foxP3+ (naturally occurring, acting in an antigen-non-specific manner), CD4+CD25+/-, and CD8+CD28- (both are induced, and they both act in an antigen-specific manner) [13].

MSCs and B cells

B cells originate in the bone marrow and are in close contact with marrow stromal cells during their development. After going through negative selection and clonal deletion, the majority of them (in mice, 90%) die without ever reaching the circulating pool [1]. The negative selection process takes place with the help of stromal cells, through a classic immune cell-mediated mechanism. Self-antigens present on stromal cells cross-link self-antibodies of mature B cells, leading to their negative selection. MSCs have been involved in the negative regulation of B cell development through an additional mechanism: the LY-6A/E protein (or Sca-1, present on MSCs) increases GM-CSF production, which inhibits B cell development [14].

The B cell inhibition seems to be mediated mostly by cell-cell interactions, but also by the release of soluble cytokines, which leads to the arrest of the cell cycle in the G0/G1 phase [6].

MSC-derived molecules can inhibit B cell proliferation and differentiation, precluding the necessity of HLA matching of donor and host [15]. They have also been shown to downregulate chemokine receptors on B cells, decreasing their migration to inflammation sites [16].

MSCs can inhibit the proliferation and differentiation into antibody-secreting cells of B cells activated with anti-Ig antibodies, soluble anti-CD40L antibody and cytokines (IL-2, IL-4), while also impairing their chemokine receptor (CXCR4, CXCR5 and CCR7) production and secretion [3]. These are receptors for CXCL12 and CXCL13, which are chemokines responsible for chemotaxis, and playing an essential role in B cell positioning in secondary lymphoid organs. MSCs were unable to affect the expression of other cytokines and co-stimulatory molecules by the B cells [16].

This inhibition process took place only in the presence of IFN-γ (produced by activated B cells) [17], leading to the conclusion that IFN-γ causes MSCs to produce IDO, which suppresses lymphocyte proliferation through the tryptophan pathway, through an uncertain mechanism.

As with T cells, MSC have different actions on B cells, depending on their concentration: low numbers of MSCs have a stimulatory effect, while MSCs in excess are immunosuppressive. A possible explanation might be that the stimulatory pathway becomes overloaded when the MSCs are in excessive quantities [10].

MSCs can also assist the estrogen and androgen suppressive effects on B cells. The effect of androgen is mediated by androgen receptors expressed on the MSC surface [1].

TGF-b produced by MSCs has also been shown to inhibit B-cells, through the downregulation of IL-7 [18].

However, there is still no *in vivo* evidence of the suppressive effect of MSC on B cells.

MSCs and NK cells

As with T and B cells, NK cell progenitors originate in the BM, where they establish close interactions with marrow stromal cells [19].

The molecular mechanisms underlying the immunosuppressive effects of MSCs on NK cells are still uncertain.

Different studies have shown variable results. Some have shown that IL-2 and IL-15-induced NK cell proliferation and IFN-γ production are inhibited by MSCs [20], but according to others MSCs only partially inhibit the proliferation of activated NK cells [21], or they are lysed by IL-2-activated NK cells [22].

The MSC effect on NK cell toxicity seems to differ depending on experiment set-ups. For example, there was no inhibition of NK cell-mediated lysis when freshly isolated NK cells were used in order to lyse allogeneic, HLA class I negative or positive targets, in the presence of MSCs [21]. However, when cultured NK cells were used (4-5 days culture, with IL-2) to lyse K562 cells in the presence of MSCs, they were less efficient than NK cells that were not exposed to MSCs [23]. This suppressive effect might be due to the IFN-γ produced by NK cells.

Allogeneic and syngeneic MSCs are vulnerable to lysis by activated NK cells, due to their low expression of HLA class I molecules and to the MSC expression of several ligands, such as ULBP, PVR and nectin-2, which are recognized by activated NK cell receptors (NKp30, NKG2D and DNAM-1) [3]. If the MSCs are incubated with IFN-γ, the HLA expression is up-regulated, leading to a decreased MSC susceptibility to NK cell-mediated lysis [19].

MSCs are also capable of inhibiting the surface expression of NK cell receptors, such as NKp30 and NKG2D, which are involved in NK cell activation and target cell lysis [6].

MSCs may exert an effect on NK cell cytokine production. After co-culture with MSCs, IL-2-activated NK cells produced decreased amounts of IL-15-induced cytokines – IFN-γ, IL-10, TNF-α [19].

Like the case with T or B cells, immunosuppressive soluble molecules, such as PGE2, IDO, or TGF-β1 (less important), have also been proposed to play a role in the inhibition of NK cells [19]. Synthesis of IDO in MSCs seems to be induced both directly (through MSC exposure to IFN-γ), and indirectly, after autocrine stimulation with PGE2, which induces de novo expression of IDO in MSCs. Moreover, IFN-γ and TNF-α, secreted by NK cells after IL-2 activation, may induce the production of PGE2 [24].

MSCs and DCs

Dendritic cells (DCs) have an essential role in naïve T cell stimulation during the primary immune response. They are also involved in the activation of B cells, either directly through soluble molecules, or indirectly through Th cells. This means that DCs are critical to both cell-mediated immunity and humoral immunity.

MSC-induced inhibition of DCs seems to be mediated by soluble molecules, such as PGE2 [25], released upon cell-cell contact.

MSCs are able to inhibit the differentiation, maturation and activation of co-cultured DCs. They can inhibit the differentiation of monocytes to DCs [26], downregulating the expression of several differentiation markers, such as CD1a, CD86 and HLA-DR [4], and they can also inhibit the maturation of DCs, through the downregulation of CD83 expression, deflecting their phenotype towards an immature status [1]. The immature DCs secrete lower amounts of TNF-α, and higher amounts of IL-10 [12].

DCs may also be involved indirectly in the immunosuppression of T cells by MSCs, through several different possible mechanisms. MSCs decrease the IL-12-secreting capacity of DCs, which is essential in cell-mediated immunity, where it activates naïve T cells and differentiates them towards a Th1 phenotype [27]. MSCs also secrete TGF-β1, which is a soluble factor able to inhibit DC activation and maturation. Also, DC maturation may be delayed through the preferential activation of CD4+CD25+ Treg cells [28]. MSCs may also lead to a state of immunotolerance through the decrease of TNF-α secretion in mature DC1s, and through the increase of IL-10 secretion in mature DC2s. IL-10 has an inhibitory effect on the functions of antigen presenting cells, downregulating the IL-12 secretion, and the expression of several surface markers, such as CD40, CD80, CD83 and CD86. Therefore, DCs that secrete IL-10 have little or no stimulatory effect when co-cultured with T cells, inhibiting their proliferation [1].

MSCs have been shown to impair the differentiation of monocytes into DCs by inhibiting their response to maturation signals, and decreasing their expression of co-stimulatory molecules [27]. This inhibitory effect may be mediated by soluble molecules, and it may be dose-dependent. After co-culture with MSCs, the cell cycle of DCs was arrested in the G0/G1 phase [28].

MSCs have different effects on different DC populations. On myeloid DCs, the effect is to induce a decreased production of TNF-α, while on plasmacytoid DCs the effect is to increase IL-10 production, leading to decrease IFN-γ by Th1 cells, increased IL-4 secretion by Th2 cells, and an increased number of Tregs [19].

A scheme of the main important interactions between MSCs and the cells of the immune system can be seen in Figure 1.

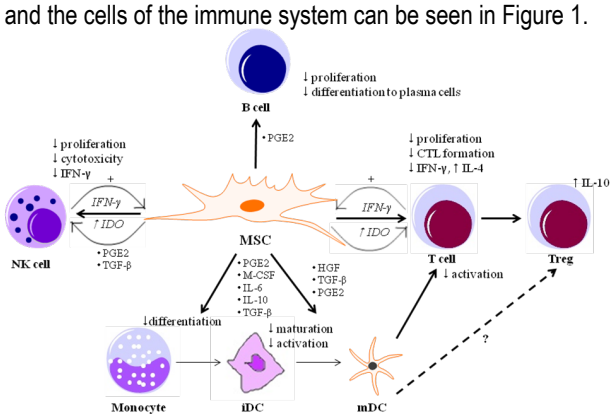

Fig.1. Immunomodulatory effects of MSCs [25]

MSCs and GVHD

GVHD (graft-versus-host-disease) is a condition that causes considerable morbidity and mortality after HSCT (hematopoietic stem cell transplantation). The main targets for acute GVHD are the skin, the gastrointestinal tract, and the hematopoietic system. If the graft is T-cell depleted, the GVHD is reduced, but when using non-manipulated grafts, it needs to be prevented with immunosuppressive drugs, such as cyclosporine, methotrexate, high-dose steroid therapy, anti-IL-2 receptor antibodies, monoclonal anti-CD3 antibodies, anti-TNF-α antibodies, and others. There are different grades of GVHD, and grade IV is life-threatening [29].

During the past 15 years, there have been promising results in patients treated with MSCs. Patients with HSCT undergoing MSC infusion have shown significantly improved clinical outcomes without significant adverse effects [30]. Also, all patients who received MSCs have shown sustained hematopoietic engraftment, and no adverse reactions [26]. When there is HLA disparity in the donor/recipient pair, it seems that co-transplantation of HSCs (hematopoietic stem cells) and MSCs modulate the host alloreactivity, and promote better cell engraftment.

Where GVHD prophylaxis is concerned, study results have shown that the co-transplantation of HSCs and third-party or same-donor MSCs is safe and that it reduces nonrelapse mortality [31]. These results should be interpreted with caution, though, due to the small numbers of subjects and the lack of control cohorts. Also, further studies are required to investigate the MSC effects on GVL (graft-versus-leukemia).

Chronic GVHD is another major cause of morbidity and mortality after allogeneic HSC. It has been shown that MSCs may ameliorate sclerodermatous chronic GVHD, as well as the

cutaneous, hepatic, and gastrointestinal manifestations of the disease [32]. Further research is needed in order to establish whether the GVL effect is preserved in patients with MSC-treated chronic GVHD.

There are still on-going clinical trials trying to establish the number of infusions to be performed, the optimal dose of MSCs per infusion, and the possible interactions with other therapies.

Besides the concerns regarding the preservation of the GVL effect, there is also concern that MSCs may function as a sanctuary for leukemic blast cells, promoting disease relapse [31]. Apoptosis has been shown to be inhibited in leukemic cells that interact with MSCs, which also transiently arrest the cell cycle of hematopoietic and non-hematopoietic tumor cell lines [33]. One should also keep in mind the possibility of tumorigenicity, ectopic activity, infectious agent transfer, and other potential adverse effects.

Further research is needed, both preclinical and correlative in the context of carefully controlled trials, to help address these important issues.

MSCs and solid organ transplants

Organ transplantation has become standard therapy in such cases as kidney, heart, or liver failure, being one of the most remarkable achievements in modern surgery. Even though organ transplants are highly successful, there usually is ongoing need for immunosuppression, which results in significant morbidity due to infections and malignancies.

The goal of immunosuppression in solid organ transplants is different than in HSCT, aiming to prevent the immune response of the host organism against the graft. It is not necessary to balance this against the graft-versus-tumor effect. MSCs may be more effective at allowing specific targeting of the immunoinhibitory effect. A key difference between HSCT and solid organ transplant rejection is that in solid organ transplantation there is also involvement of immune humoral responses. Hyperacute graft rejection has become rare since the introduction of routine crossmatching before transplantation, but antibody-mediated rejection is still an important cause of acute and chronic allogeneic graft dysfunction.

Animal studies have shown that MSCs migrate to cardiac allogeneic heart grafts in patients with chronic graft rejection [26], delivering localized immunosuppression, and thereby minimizing non-specific, systemic immunosuppressive complications. For example, a single dose of intravenous donor MSCs had a modest, but significant improvement in a baboon skin graft model [34], while systemic administration of MSCs in a rat cardiac allograft model also had a significant positive effect on survival [35]. However, in another mismatch cardiac model, no MSC effect has been found on allograft outcomes [36]. In the majority of the studies allogeneic MSCs were co-infused at the time of organ transplantation. The reasons for these differences are uncertain, but improved survival was not associated with tolerance in the studies, suggesting that MSCs alone are probably not sufficiently immunosuppressive for vascularized transplants.

Another potential use for MSCs might be to promote a state of immunological chimerism, as well as long-term graft tolerance by the host immune system, after co-infusion of MSCs at the moment of the solid organ transplantation. When immunologic chimerism is established (through simultaneous transplantation of solid organ and bone marrow from the same donor), there is long-term graft survival in the absence of immunosuppression [37].

Most of the immunomodulatory functions and properties of MSCs have been shown *in vitro*, and further animal studies are necessary in order to evaluate their potential use in clinical solid organ transplantation. However, despite the lack of clinical studies, MSCs remain attractive for the therapy of solid organ rejection, because of their immunoregulatory effects on the host, promoting graft tolerance, their trophic functions, which help to minimize ischemic and inflammatory injuries to the graft.

MSCs and autoimmune disease

MSCs have also been considered for the therapy of autoimmune diseases. To that purpose, they have been tested in various animal models of autoimmune diseases, such as experimental autoimmune encephalomyelitis [38], diabetes [39], systemic lupus erythematosus [40], and rheumatoid arthritis [41]. Autoimmune diseases involve aberrant recognition of host tissues by the immune system, which mounts an immune response against its own tissues. The clinical manifestations affect many organs, such as the central nervous system, pancreas, joints, or multiple systems (like in systemic lupus erythematosus). Even though the underlying immunopathological causes are similar, one cannot always extrapolate from one disease to others.

Mice with experimental autoimmune encephalomyelitis (EAE) constitute a model for human autoimmune diseases. Mice that received MSCs at the onset and at the peak of the disease showed lower levels of demyelination, axonal loss, and inflammation than control mice, and their proliferation of T cells from the spleen and lymph nodes was reduced, as well as the production of proinflammatory factors (TNF-α and IFN-γ). MSCs were able to improve disease symptoms, as well as to decrease relapses. The therapeutic effect was maximal when the MSCs were administered at the onset of the disease, but there was no effect after disease stabilization [38]. These findings could not be replicated in a clinical study on patients with multiple sclerosis who had received a single dose of intrathecal MSCs. Some patients showed some clinical neurological improvement, but this could not be documented at radiological investigations. Only one of the patients showed a quantitative improvement in the central nervous system plaque, on magnetic resonance imaging [42].

In type II collagen-induced arthritis, administration of allogeneic MSCs prevented the irreversible immune destruction of cartilage and bone [43]. However, another study showed that MSCs in fact worsened the clinical parameters [26]. A possible reason for this is that MSCs were administered at different times after disease induction. As MSCs can also be used in bone and cartilage replacement, they might have multiple therapeutic

effects in arthritis.

In a mouse model of systemic lupus erythematosus, MSCs were able to reconstruct the osteoblastic niche, and to restore immune homeostasis, conferring significant therapeutic effect [40]. In a clinical study, patients showed improvement in serologic markers and renal function [44]. However, it appears that in patients with systemic lupus erythematosus, the source of MSCs has some importance. Autologous MSCs had no beneficial effect on disease activity, but allogeneic bone marrow or cord blood MSCs produced an improvement in clinical and laboratory parameters. This suggests that, at least in some forms of systemic lupus erythematosus, MSC functions could be impaired by the underlying disease.

In a chemically-induced model of autoimmune colitis, the symptoms were significantly improved by systemic infusion of MSCs, and clinical studies on patients with perianal fistulae due to Crohn's disease showed encouraging results. Administration of MSCs has induced healing of inflammatory ulcers and improved nutritional status [45]. So far, inflammatory bowel diseases appear to be a responsive target for the immunomodulatory effects of MSCs.

MSCs and other pathologies

MSCs derived from the early human embryo can be transformed into epidermal cells *in vitro* and *in vivo* [46]. This finding has led to the question whether MSCs can be used in order to accelerate wound healing. Bone marrow derived MSCs differentiate into dermal tissue after subcutaneous implantation in immunocompromised mice. In a clinical study, patients with treatment-refractory dermatopathies showed significant improvement of non-healing wound areas after autologous MSC transplantation [47]. In diabetic foot wounds, injections of autologous skin fibroblasts and MSCs into the wound edges decreased wound size, while increasing the vascularity of the dermis [48].

MSCs implanted into devitalized muscle grafts have been shown to support peripheral nerve regeneration, while MSCs injected into patients with chronic spinal injury transdifferentiated into neural stem cells, improved their electrical and functional symptoms [24].

In patients with metachromatic leukodystrophy and Hurler's syndrome, infused MSCs induced significant improvement in nerve conduction velocities [49].

In a pig model, MSCs from the apical papilla of tooth co-transplanted with periodontal ligament stem cells were able to generate a root periodontal complex which could support a porcelain crown, leading to the recovery of tooth strength and appearance [24].

MSCs injected into infarcted rat myocardium engrafted and improved cardiac function and structure, through myogenesis and angiogenesis, while also enhancing the survival of existing myocytes. If the MSCs are combined with erythropoietin treatment, there is improvement of capillary density, as well as reduction of infarct size and fibrotic areas [50].

MSCs regenerated segmental femur defects in sheep and other large animal models, restoring bone morphology [51, 52].

In diabetes, MSCs may induce the regeneration or the proliferation of resident insulin-producing cells, contributing to the repair of damaged islets, while also aiding in the long-term complications of diabetes, such as neuropathy, nephropathy, and cardiomyopathy [53]. They can also transdifferentiate into functional hepatocytes, inducing liver regeneration and offering potential cell replacement therapy in the treatment of liver failure [54].

MSCs have also shown promise in the replacement and rebuilding of damaged structures, with the aid of three-dimensional (3-D) scaffolds made from biocompatible materials. The ideal scaffold should be biocompatible and biodegradable, it should mimic the extracellular matrix structurally and functionally, while also offering a suitable environment for the growth and differentiation of MSCs.

The systemic inflammatory response syndrome (SIRS) develops in response to severe sepsis, surgery, or trauma, due to the dysregulated activation of the immune system. It causes multiple organ dysfunctions, with a mortality rate of up to 40% [37]. *In vitro* studies have shown that MSCs can modify the immune response of SIRS [55]. Animal studies with MSCs have been performed, in models of endotoxemia [56], acute respiratory distress syndrome (ARDS) [57], and intra-abdominal sepsis [58]. They showed a reduction of local and systemic inflammation, improved hemodynamics and organ function, reduced organ injury, and improved survival.

CONCLUSIONS

The current data suggest that MSC may constitute a promising alternative strategy in the treatment of a wide range of immune-mediated diseases, through their immunomodulatory effects, but also as systems of drug or gene delivery to diseased tissues or organs.

They can prove to be useful in GVHD, autoimmune diseases, organ transplantation, neurodegenerative diseases, cardiac failure, burns, bone defects, etc.

However, the clinical immunological outcome may not always be predictable, because it depends on environmental factors that cannot be reproduced *in vitro*.

Directions for future research should include:

• Standardization and validation of MSC isolation and expansion methods;

• Animal studies to determine the underlying mechanisms of the MSC immunosuppressive effects;

• *In vivo* tracking of MSCs in patients;

• Multicenter randomized clinical trials, to further assess MSC safety and efficacy.

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INTERACTINEA DINTRE CELULELE STEM MEZENCHIMALE SI EFECTORII CELULARI AI SISTEMULUI IMUN

REZUMAT

Celulele stem mezenchimale reprezintă un subpopulaţie rară de celule stem cu sediul în măduva hematogenă, unde interacţionează cu celule stem hematopoietice, ghidându-le creşterea şi diferenţierea, şi unde servesc drept rezervă pentru diferite ţesuturi mezenchimale. Acestea prezintă şi proprietăţi imunosupresive remarcabile, înhibitând proliferarea şi funcţiile majorităţii populaţiilor de celule imune. Acest raport oferă informaţii cu privire la cercetările actuale în ceea ce priveşte interacţiunile celulelor stem mezenchimale cu celulele sistemului imunitar, posibilele mecanisme prin care se desfăşoară aceste interacţiuni, rezultatele utilizării in vivo, precum şi alte potenţiale implicaţii clinice, şi direcţiile viitoare de cercetare.

Cuvinte cheie: celule stem mezenchimale, sistem imunitar, imunosupresie, aplicaţii clinice

RELATIONSHIP BETWEEN SERUM LEVELS OF INTERLEUKIN-2 AND LIVER INJURY IN CHRONIC HEPATITIS C VIRUS INFECTION BEFORE INTERFERON TREATMENT

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ABSTRACT

Background: The pathogenetic role of immune-mediated mechanisms in chronic hepatitis C virus (HCV) infection has not yet been elucidated. **Objectives**: To investigate the immune response to HCV through expression of IL-2 in the serum of chronically HCV-infected patients compared to normal controls and its association with histological inflammatory indicators. **Materials and methods**: Fifteen patients with chronic HCV infection (6 male, 9 female, mean age 47.35±10.78 years) and 14 healthy subjects (6 male, 8 female; mean age 35.00±15.45 years) were included in this study. The diagnosis of the patients with chronic HCV infection was established on the basis of clinical, laboratory, ultrasonographic and histopathologic findings. The healthy subjects had negative hepatitis serology, normal liver function tests and normal ultrasonographic findings. **Results**: Serum levels of IL-2 were increased in HCV patients as compared to healthy controls (385±41.2 vs.126±15.4 pg/mL; p<0.001). There was correlation between serum IL-2 levels, histological activity index scores (r=0.746; p<0.001) and serum ALT levels (r=0.714; p<0.001). **Conclusion**: IL-2 is the main mediator of the inflammatory responses to chronic viral infection and in our study serum level of IL-2 in HCV infected patients before interferon treatment was higher than healthy subjects. Serum IL-2 levels were increased due to chronic HCV infection, and the amount of increase corresponds to the degree of inflammation.

Key words: chronic hepatitis C, IL-2, physiopathology of chronic hepatitis C virus infection, T lymphocytes

INTRODUCTION

Hepatitis C virus (HCV) is a ubiquitous virus infection. It has been estimated that about 3% of the world's population have chronic HCV infection. It is a major cause of chronic liver disease. Chronic hepatitis C has been a public health concern during the last decade in most developed countries (1). Host immune mechanisms are involved in chronic infection. The reason for the hepatocellular injury in chronic HCV infection is still unknown. Several studies have suggested that T-cell immunoregulatory cytokines play a key role in both HCV viral persistence and in extent of liver damage. Factors involved in the progression to liver disease in HCV infected patients are not well characterized. Little is known about the production of IL-2 in chronic HCV infection. The IL-2 production by T cells is part a complex network in which a discrete alteration is capable of disrupting the whole system (2, 3).

In this study, we aimed to investigate the immune response to HCV through expression of IL-2 in the serum of 15 chronically HCV infected patients compared to normal controls and also its association with inflammatory indicators.

MATERIAL AND METHODS

Fifteen patients (6 male, 9 female, mean age: 47.35±10.78 years) with chronic HCV infection and 14 healthy subjects (6 male, 8 female; mean age: 35.00±15.45 years) were included in this study. Patients were consecutively recruited from the" Matei Bals" Institute Infectious Diseases Division from April 2010-October 2010. All of the patients and controls provided written informed consent. All of the patients underwent a complete medical and laboratory evaluation and a liver biopsy. The inclusion criteria for this study were as follows: a confirmed HCV infection, a positive PCR for HCV-RNA, increased levels of alanine aminotransferase (ALT) and a liver biopsy confirming chronic HCV infection. Measurement of serum IL-2 was performed for the cases and controls using ELISA (R&D Systems). Serum samples were tested twice for IL-2. Comparisons of proportions between the patients and controls were assessed by Fisher's exact test. Continuous data were compared by Mann-Whitney rank sum test. The Spearman's rank coefficient was used to assess correlation between the continuos variable. The results were statistically analyzed by Student t test and a p<0.05 (95% CI) was considered statistically significant.

RESULTS

The mean of alanine aminotransferase (ALT) levels were increased (the range was 63 to 160 IU/L). The majority of patients (80%) were infected with HCV genotype I and 86% had an HCV RNA viral load greater than 850.000 IU/mL. An increased expression of IL-2 has been documented in the serum of chronically HCV infected patients before interferon therapy (385±41.2 vs.126±15.4 pg/mL; p<0.001). The serum levels of IL-2 were higher in male compared with female but the differences were not significant (p>0.05). The highest level of IL-2 was detected in patients lower than 32 years old. However, these differences were not significant (p>0.05). There was correlation between serum IL-2 levels and

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histological activity index scores (r=0.746; p<0.001) and serum ALT levels (r=0.714; p<0.001).

DISCUSSION

Host immune response is important in determining the outcome of chronic HCV infection. Results of our study showed elevated serum levels of ALT (4).

The immunopathogenesis of chronic hepatitis C virus (HCV) infection is a matter of great controversy. It has been demonstrated that T cells play a role in HCV clearance in HCV infected patients. Helper T cells (Th) help in the functions of the immune system as the major regulator and also help to destruct antigen and to reinforce antibody production. We found that there was a greater expression of IL-2 compared to the controls. IL-2 reflects the degree of histological and biochemical activity of chronic HCV infection (5, 6).

Immune-mediated mechanisms are believed to play an important pathogenetic role in HCV infection. It is thought that cytotoxic T lymphocyte responses early in infection may be important for viral clearance. Several cytokines and chemokines induced by viral infection play directly or indirectly roles in antiviral defense. Cytokines are key mediators of inflammation, apoptosis, necrosis and fibrosis and they are actively involved in the regeneration process of liver tissue after injury. Th1 cytokines, such as IL-2, interferon gamma (IFN-γ), and tumor necrosis factor-alfa (TNF-α) are required for host antiviral immune responses. IL-2 is considered to be a Th1 type cytokine and is involved in enhancing the proliferation and activation of most T lymphocytes, NK cells and B-lymphocytes. Liver sinusoidal and inflammatory cells have been reported to be sources of IL-2 and no consensus exists on the predictive value of this cytokine (7). Apparently, expression of IL-2 is associated with a more advanced stage of disease. There was a correlation between ALT levels and IL-2 expression. The level of expression of type 1 cytokines, such as IL-2 and IFN-γ, is correlated with the degree of histologic injury. IL-2 levels may contribute to the role of innate immunity in stimulating the adaptive immune responses (8, 9).

Further studies are warranted on the role of IL-2 in chronic HCV infection.

CONCLUSION

IL-2 may exert a pro-inflammatory activity. Serum IL-2 levels increase due to chronic HCV infection, and the amount of increase corresponds to the degree of inflammation.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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RELAȚIA DINTRE NIVELURILE SERICE ALE INTERLEUKINEI-2 ŞI DISTRUGEREA HEPATICĂ ÎN INFECȚIA CRONICĂ CU VIRUSUL HEPATITEI B

REZUMAT

Introducere: Rolul patogen al mecanismelor mediate imunologic în infecţia cronică cu virusul hepatitei C (HCV) nu a fost încă elucidat. **Obiective**: Investigarea răspunsului imun faţă de HCV prin concentraţia IL-2 în serul pacienţilor infectaţi cronic cu HCV comparativ cu subiecţi aparent normali şi asocierea cu indicatorii inflamaţiei la nivel tisular. **Pacienţi şi metode**: Cincisprezece pacienţi cu infecţie cronică HCV (6 bărbaţi, 9 femei, vârsta medie 47, 35±10, 78 ani) and 14 subiecţi aparent sănătoşi (6 bărbaţi, 8 femei, vârsta medie 35, 00±15, 45 ani) au făcut parte din acest studiu. Diagnosticul pacienţilor cu infecţie cronică cu HCV a fost stabilit pe baza datelor clinice, de laborator ultrasonografice şi histopatologice

Subiecţii din lotul martor au avut serologia negativă pentru hepatită, testele pentru funcţia ficatului normale, datele de la examenul ultrasonografic normale. **Rezultate**: Nivelurile serice IL-2 erau crescute la pacienţii cu infecţie cronică HCV comparativ cu lotul martor (385±41, 2 vs.126±15, 4 pg/mL; p<0, 001). Există corelaţie între nivelurile serice IL-2 şi scorul de activitate tisulară (r=0, 746; p<0, 001) şi nivelurile ALT în ser (r=0, 714; p<0, 001). **Concluzie:** IL-2 este un mediator important al răspunsului inflamator faţă de infecţia cronică cu virus, iar nivelul seric IL-2 la pacienţi cu HCV înaintea tratamentului cu interferon a fost mai crescut comparativ cu lotul martor. Nivelurile IL-2 în ser erau crescute datorită infecţiei cronice cu HCV şi au corespuns gradului de inflamaţie.

Cuvinte cheie: hepatita cronică C, IL-2, fiziopatologia infecţiei cronice cu virusul hepatitei C, limfocite T

THERAPEUTIC PARTICULARITIES IN ADULT PATIENTS WITH THYROID DISEASE AND DIABETES

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ABSTRACT

Diabetes mellitus and thyroid diseases are two endocrinopathies frequently in general population. Because insulin and thyroid hormones are involved in cellular metabolism, excess or deficiency of one of these determinates functional disorders of another. The purpose of this study is to determine the main therapeutic particularities in adult patients with thyroid disease and diabetes. The study group was represented by 650 cases, aged 18-80 years. The gender distribution was net in favor of women being represented by 588 women and 62 men. Was used clinical, radiological, biochemical, hormonal and immunological parameters.
Keywords: diabetes, thyroid disease, and treatment

INTRODUCTION

The thyroid disorders are common in the general population; their prevalence increases with age. **The hypothyroidism** is the most common thyroid disease in adults and older women. Its origin is usually autoimmune presenting as atrophic primary hypothyroidism or Hashimoto's thyroiditis. It may be also secondary to radioactive iodine treatment or thyroid surgery. In rare cases, occurs secondary hypothyroidism to hypothalamic or pituitary disease (17).

The clinical hypothyroidism should be treated through hormone replacement therapy. The most used replacement therapy is with L-thyroxin. The usual dose is 1.6 μg L-thyroxin/kg. Often, in the case of mild thyroid failure, the initial dose may be lower and it is adjusted according to TSH value at every 23 months. With the normalization of TSH levels and titrating L-thyroxin dose, TSH should be monitored annually. In the case of aggravation of the thyroid failure is necessary to increase the dose of thyroxin. The L-thyroxin therapy in subjects with diabetes may exacerbate pre-existing coronary heart disease by increasing myocardial contractility and heart rate. Therefore, it is better to start with a low dose, around 25 μg/day, followed by a slow growth, monthly, with 25 μg/day, monitoring of clinical status and TSH level (17).

The subclinical hypothyroidism should be treated in patients who present increased LDLc, which is worsened by hypothyroidism, which present the antithyroid peroxydase antibodies (antiTPO AB) detectable in serum (which means a possible transition to clinical hypothyroidism) and in patients with clinical manifestations (17).

By contrast, **the hyperthyroidism** is less common, with a ratio female/male 9/1. The Graves' disease is most common and usually affects young adults. The toxic multinodular goiter usually occurs in older people (17).

The hyperthyroidism can cause adverse effects such as worsening of glycemic control and coronary artery disease. For this reason, it is indicated definitive treatment with iodine radiotherapy, whenever is possible. There are no contraindications for antithyroid medication at the diabetic patients, but ***the Graves-Basedow disease*** long-term remission is less than 40%. The patients with ***toxic multinodular goiter*** or with ***autonomous thyroid nodules*** should be treated definitive by iodine radiotherapy or by surgery (17).

The patients with diabetes mellitus (DM) have an increased prevalence of thyroid disease compared with non-diabetic population (17).

Diabetes type 1 is commonly associated with endocrine and systemic diseases with autoimmune etiology of type: Graves-

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Basedow disease, Hashimoto's thyroiditis, Addison's disease, celiac disease, pernicious anemia, myasthenia gravis, vitiligo, etc. (3). In patients with type 1 diabetes, as treatment is indicated diet and insulin in various doses and regimens.

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 characterized by the triad: cutaneous-mucosal candidiasis, hypoparathyroidism and Addison's disease.

2. Polyglandular autoimmune syndrome type II (PAS - II) (11, 2) is the most common immuno-endocrinopathies, characterized by the appearance into the same person of two or more of the following diseases: Addison's disease, Graves-Basedow disease, thyroiditis of autoimmune etiology, type I diabetes, primary hypogonadism, myasthenia gravis, and celiac disease.

3. Polyglandular autoimmune syndrome type III (PAS - III) (1) is a PAS II syndrome but without adrenocortical involvement. This syndrome is associated with the following diseases: celiac disease, hypogonadism, myasthenia gravis, sarcoidosis, Sjogren's syndrome, rheumatoid arthritis, gastric cancer, malabsorption due by pancreatic exocrine deficiency, and can be classified into three subcategories:

-PAS III A - autoimmune thyroiditis with type 1 diabetes

-PAS III B - autoimmune thyroiditis with pernicious anemia

-PAS III C - autoimmune thyroiditis with vitiligo and/or alopecia and/or other autoimmune diseases

The key of the successful management in patients with autoimmune endocrinopathies is to identify and treat autoimmunity before it cause significant morbidity and mortality. The treatment of organ insufficiencies is identical whether they occur isolate or in the polyglandular autoimmune syndrome. The replacement therapy remains the cornerstone of the clinical management (8).

Educating patients regarding the nature of the disease is often critical for early recognition of associated autoimmune diseases, and as in any chronic disease, the psychosocial support should be evaluated individually (8). The genetic counseling is also justified at affected family members who required carrying out specific tests (8).

Emergency identification should be performed in all patients with polyglandular autoimmune syndrome, because use of high doses of corticosteroids in acute stress usually prevents adrenal crisis in the patients with Addison's disease, in those with adrenal autoantibodies and increased risk of adrenal insufficiency. Some authors believe that exogenous supplement with glucocorticoids administered during acute stress is indicated also at asymptomatic individuals with biochemical changes in adrenocortical disease (8).

From all endocrine components of the polyglandular autoimmune syndrome, only diabetes has not a favorable prognosis after hormonal replacement therapy and appropriate monitoring (8).

All patients with PAS I and PAS II require hormone replacement therapy, vitamins and minerals. As a rule, they can and they are advised to continue normal activities, except for certain professions incompatible with the disease (eg. airline pilot who has type 1 diabetes).

Because most diseases have in common endocrine autoimmunity, and there are many genetic studies at animal models, the perspective is gene therapy, as the immunostimulatory or immunomodulatory therapy.

A number of studies also indicate an increased prevalence of thyroid disease in patients with type 2 diabetes; the hyperthyroidism is the most common disorder seen (3). In the case of association of impaired glucose tolerance (IGT) and fasting impaired glucose tolerance (IFG) with thyroid disease, they usually occur as a result of excess of thyroid hormones.

In patients with type 2 diabetes the treatment is diet, diet and oral antidiabetic medication, or if is necessary, diet and insulin. In patients with IGT and IFG the treatment is diet.

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 650 cases, aged 18-80 years. Gender distribution was net in favor of women being represented by 588 women and 62 men.

Methods of investigation

Methods of investigation were the clinical data-history, present status, and imaging-thyroid ultrasound, biochemistry-carbohydrate metabolism parameters: fasting bloods 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 (HbA_{1c}) was achieved through the DiaStat program for glycosylated hemoglobin HbA_{1c} 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 to 4.68 mIU/ml, FT_3 = 3.69 -10.4 pmol/l, FT_4 = 10 to 28.2 pmol/l.

Immunological parameters were represented by some markers of thyroid autoimmunity-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 <35IU/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

Adult group was subdivided in function of the type at glycemic balance in 4 subgroups (Figure 1):

− group with type 1 diabetes represented by 60 cases (9.23%)

− group with type 2 diabetes accounted for 290 cases (44.61%)

− group with impaired glucose tolerance (IGT) accounted for 183 cases (28.15%)

− group with fasting impaired glucose tolerance (IFG) accounted for 117 cases (18%)

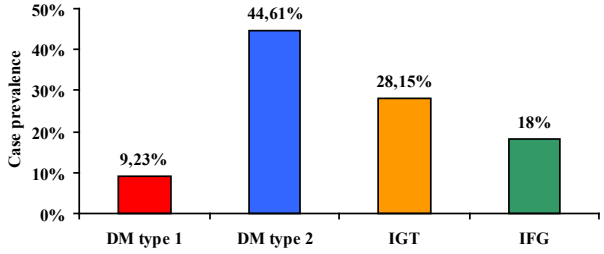

Fig.1. Distribution of cases by type of changes in glycemic balance

The prevalence of the glycemic type treatment is shown in Table I.

Table I. Distribution of cases by glycemic type treatment

Treatment type	DM type 1		DM type 2		IGT		IFG	
	n	%	n	%	n	%	n	%
Insulin	60	100	16	5.51	-	-	-	-
OAD	-	-	148	51.03	-	-	-	-
Diet	-	-	126	43.44	183	100	117	100

Legend: OAD – oral antidiabetic medication

In the case of adults with diabetes type 1, all patients were treated with insulin, in different regimens. In the case of adults with type 2 diabetes, a rate of 51.03% of patients were treated with OAD, 43.44% with diet and 5.51% with insulin. In the case of adults with IGT and IFG, all patients were treated with diet. Diet is the crucial element in the treatment of diabetes. It is essential to all forms of disease and it is only indicated therapy in some patients. Over one third of patients with newly discovered diabetes can initially be balanced with diet.

The objectives of the nutritional therapy in diabetes are the following (7):

− maintaining blood glucose levels as close to normal

− normalization of lipid profile

− ensuring adequate caloric intake to achieve and maintain an optimal weight for adults

− preventing and treating acute complications of diabetes (hypoglycemia, diabetic ketoacidosis) and chronic complications (diabetic nephropathy, autonomic neuropathy, hypertension, cardiovascular disease)

− improved quality of life through proper nutrition

The treatment with OAD is individualized. At the beginning of therapy it is account of blood glucose level, body weight, severity of clinical symptoms. In some situations it is necessary to associate two or more OAD. Sometimes it is necessary the therapy with insulin or the combination between insulin and OAD. In the case of insulin therapy, intensive insulin therapy is preferred, with 3 or 4 injections of insulin/day. At the choice of the regimen it must take account of blood glucose level, patient age and its life expectancy.

A study in France showed that blood glucose self-monitoring in patients with type 2 diabetes improve glycemic control and reducing appearance of complications (6). The treatment of adult hypothyroidism is thyroid hormone replacement therapy (HRT). Substitution is made for life. The treatment will take into account: patient age, duration of untreated disease, cardiovascular complications, etc. The benefits of the treatment appear after 7 days and reached maximum after 4-6 weeks. In elderly patients, in patients with ischemic heart disease it starts with ¼-½ of the usual dose, with incremental doses. The total or optimal replacement dose can be achieved after 2-3 months of therapy. The efficacy is measured by clinical and laboratory criteria. It is monitor patients for symptoms of overdose, which include tachycardia, palpitations, irritability, headache, tremors and possible seizures angina. The clinical and laboratory reassessment is carried out every 6 months (15). The therapy with thyroxin in patients with present antiTPO AB is more or less controversial in euthyroid or subclinical hypothyroid patients. Several authors recommend the establishment of replacement therapy in patients with antiTPO AB and subclinical hypothyroidism when TSH values>10 mIU/ml, aged over 45 years. It is showed that 24% of patients who have HRT for 1 year, after discontinuation replacement therapy had a euthyroid status undetermined period of time. Remission could be explained by the disappearance of AB blockers of TSH or AT receptor and secondary of discontinuation treatment with cytokine, lithium, and amiodarone. The treatment depends also by goiter size. The results of drug therapy can be evaluated with ultrasound, measuring at different time intervals the thyroid volume.

At 90% of patients, after the therapy with thyroxin, the goiter fell by 30% after a period of 6 months. Under TSH, the titers of antithyroid antibodies became smaller. In the cases with present antibodies it is necessary the annual thyroid investigation, the screening of TSH in thyroid functionality evaluation is preferred (14). In the case of the hyperthyroidism, the main treatment is administration of synthesis antithyroid drugs (SATD). They are initially administered at doses of attack (6-8 tab/day, for 4-6 weeks), followed by the maintenance dose (approximately 1/3 of the attack dose). The duration of therapy is 2 years. If the treatment is correct, 20-40% of the cases have lasting remissions. The criteria for the therapy efficacy are: clinical (thyrotoxicosis remission, goiter reducing), hormones (thyroid hormone values normalization) and immunological (disappearance of TSI from plasma). The growth of goiter reflects an excess of TSH by overdose. In this case we can associate thyroid hormones for short periods of time. The surgical treatment consists in subtotal thyroidectomy and it is applied to cases of Basedow disease with high goiter that does not respond adequately to SATD, toxic adenoma and hyperthyroid goiter.

Treatment with I^{131} is applied to persons over 40 years, those with recurrence after thyroidectomy and those in which surgery is contraindicated. It is not applicable to those with large goiter. The disadvantage of this treatment is iatrogenic hypothyroidism, which affects about 70% of cases in the first 10 years after radiation treatment (5). The treatment of thyroid disease at patients with type 1 diabetes is shown in Table II.

Table II. Distribution of cases by thyroid disease treatment in patients with type 1 diabetes

Thyroid disease	Treatment type							
	None		Suppression		Inhibition		Surgery	
	n	%	n	%	n	%	n	%
GravesBasedow disease (n=6)	-	-	-	-	6	100	-	-
Euthyroid diffuse goiter (n=1)	-	-	1	100	-	-	-	-
Thyroid differentiated carcinoma (n=3)	-	-	3	100	-	-	-	-
ACT (n=50)	10	20	40	80	-	-	-	-

Legend: ACT = autoimmune chronic thyroiditis

In the case of the patients with Graves-Basedow disease, all were treated with Thyrosol (inhibition treatment), with an appropriate response. In the case of the patients with thyroid carcinoma, all patients were treated with thyroid hormone (suppression treatment), all cases were operated, treated with I^{131} and presenting iatrogenic hypothyroidism. In the case of the patients with euthyroid diffuse goiter, all cases have received suppression treatment with thyroid hormones. In the case of the patients with ACT, 20% of cases did not require any treatment, and 80% of cases received replacement therapy with thyroid hormones.

The treatment of thyroid disease at patients with type 2 diabetes is shown in Table III.

Table III. Distribution of cases by thyroid disease treatment in patients with type 2 diabetes

Thyroid disease	Treatment type									
	None		Suppression		Substitution		Inhibition		Surgery	
	n	%	n	%	n	%	n	%	n	%
Graves-Basedow disease (n=60)	-	-	-	-	-	-	57	95	3	5
Autonomous thyroid adenoma (n = 3)	-	-	-	-	-	-	-	-	3	100
Iodine induced hyperthyroidism (n = 4)	-	-	-	-	-	-	4	100	-	-
Euthyroid diffuse goiter (n = 125)	28	22.4	87	69.6	-	-	-	-	10	8
Nodular euthyroid goiter (n =7)	3	42.85	4	57.15	-	-	-	-	-	-
Differentiated thyroid carcinoma (n=10)	-	-	10	100	-	-	-	-	-	-
ACT (n=77)	15	19.48	62	80.52	-	-	-	-	-	-
Sub acute thyroiditis (n=4)	4	100	-	-	-	-	-	-	-	-

In the case of the patients with Graves-Basedow disease, 93.34% were treated with Thyrosol (inhibition treatment). Among the cases under inhibition treatment, 11 (18.33%) were presented postoperative recurrences.5% were associated with large goiter, with compressive phenomena; postoperative recurrences were treated with SATD. In the case of the patients with autonomous thyroid adenoma, all 3 cases required surgical treatment. In the case of the patients with thyroid carcinoma, they have received suppression treatment with thyroid hormones, all cases were operated, treated with I^{131} and presenting iatrogenic hypothyroidism. In the case of the patients with euthyroid diffuse goiter, 22.4% of cases did not require any treatment. A percentage of 69.6% received suppression treatment with thyroid hormones and 8% of cases requiring surgery due to the presence of large goiter, with the compression phenomena. In the case of the patients with ACT, 19.48% of cases did not require any treatment, and 80.52% of cases received replacement therapy with thyroid hormones. In the case of the patients with iodine-induced hyperthyroidism, all cases were treated with Thyrosol (inhibition treatment). In the

case of the patients with sub acute thyroiditis, it was practiced classical treatment: anti-inflammatory, and then small doses of T_4. In the case of the patients with nodular euthyroid goiter, 42.85% of cases did not require any treatment and will be evaluated periodically (ultrasound evaluation - thyroid cyst). A percentage of 57.15% of cases have received suppression treatment with thyroid hormones.

The treatment of thyroid disease at patients with IGT is shown in Table IV.

Table IV. Distribution of cases by thyroid disease treatment in patients with IGT

Thyroid disease	Treatment type									
	None		Suppres-sion		Substitu-tion		Inhibition		Surgery	
	n	%	n	%	n	%	n	%	n	%
Graves-Basedow disease (n=43)	-	-	-	-	-	-	40	93.02	3	6.97
Au-tonomous thyroid adenoma (n=3)	-	-	-	-	-	-	-	-	3	100
Euthyroid diffuse goiter (n= 81)	10	12.34	61	75.3	-	-	-	-	10	12.34
Nodular euthyroid goiter (n=2)	1	50	1	50	-	-	-	-	-	-
Differ-entiated thyroid carcinoma (n=2)	-	-	2	100	-	-	-	-	-	-
ACT (n=52)	4	7.7	48	92.3	-	-	-	-	-	-

In the case of the patients with Graves-Basedow disease, 93.02% were treated with Thyrosol (inhibition treatment). A percentage of 6.97% requiring surgery, after treated with the thyroid hormone replacement therapy. In the case of the patients with autonomous thyroid adenoma, all cases received surgical treatment. In the case of the patients with thyroid carcinoma, all patients were treated with thyroid hormones (suppression treatment), all cases were operated, treated with I^{131} and presenting iatrogenic hypothyroidism. In the case of the patients with euthyroid diffuse goiter, a percentage of 12.34% of cases did not require any treatment, a rate of 75.3% cases received thyroid hormones (suppression therapy) and 12.34% cases required surgery due to the presence of a large goiter, with the phenomena of compression. In the case of the patients with ACT, 7.7% of cases did not require any treatment, and 92.3% of cases received thyroid hormone replacement therapy.

The treatment of thyroid disease at patients with IFG is shown in Table V.

Table V. Distribution of cases by thyroid disease treatment in patients with IFG

Thyroid disease	Treatment type									
	None		Suppres-sion		Substi-tution		Inhibition		Surgery	
	n	%	n	%	n	%	n	%	n	%
Graves-Basedow disease (n=38)	-	-	-	-	-	-	38	100	-	-
Autono-mous thyroid adenoma (n=2)	-	-	-	-	-	-	-	-	2	100
Euthy-roid diffuse goiter (n= 39)	4	10.25	30	76.92	-	-	-	-	5	12.82
Nodular euthy-roid goiter (n=2)	2	100	-	-	-	-	-	-	-	-
Differ-entiated thyroid carci-noma (n=6)	-	-	6	100	-	-	-	-	-	-
ACT (n=29)	7	24.13	22	75.87	-	-	-	-	-	-
Sub acute thyroidi-tis (n=1)	1	100	-	-	-	-	-	-	-	-

In the case of the patients with Graves-Basedow disease, all were treated with Thyrosol (inhibition treatment). In the case of the patients with autonomous thyroid adenoma, all have benefited from surgery. In the case of the patients with thyroid carcinoma, all patients were treated with thyroid hormone (suppression treatment), all cases were operated, treated with I^{131} and presenting iatrogenic hypothyroidism. In the case of the patients with euthyroid diffuse goiter, 10.25% of cases did not require any treatment. A percentage of 76.92% of cases have received treatment with thyroid hormone (suppression treatment), and 12.82% of cases requiring surgery due to the presence of large goiter, with the phenomena of compression. In the case of the patients with ACT, a rate of 24.13% of cases did not require any treatment, and 75.87% of cases received thyroid hormone replacement therapy. In the case of the patients with sub acute thyroiditis, all patients did not require any treatment. In the case of the patients with nodular euthyroid goiter (thyroid cyst), all cases did not require any treatment and will be evaluated periodically.

If the thyroid disease was the first, excess thyroid hormone or administrations of the thyroid hormone led to an imbalance of pre-existing type 2 diabetes or unmasking latent one, and also to appearance of IGT and IFG. Appeared type 2 diabetes was

easy type, requiring only treatment with diet.

If the diabetes preceded thyroid disease with hyperfunction, its appearance has led to imbalance of the DM. Thus, the cases of type 2 diabetes treated with diet initially require treatment with OAD and who received OAD initially required a combination of several OAD or insulin therapies.

In the case of the patients with type 1 diabetes, was a major imbalance in 9 cases, manifested by diabetic ketoacidosis (3 cases - mild, 4 cases - moderate and two cases - advanced). The main thyroid disease that caused significant metabolic imbalances in the type 1 diabetes case was Graves-Basedow disease. Thus, insulin requirement increased from an average of 0.65 U/kg to 1.3 U/kg in Graves-Basedow disease, at 0.9 U/kg for thyroid carcinoma and to 0.78 U/kg for ACT.

A number of studies show the influence of thyroid disease on the metabolic balance. Thus, studies performed in Spain, Japan and the U. S. stresses the role of thyrotoxicosis in the development of diabetic ketoacidosis, it is meeting in these conditions not only in patients with type 1 diabetes but also in those with type 2 diabetes (12, 10, 9, 4, 13). Also, studies in the Czech Republic show the influence of the various endocrine disorders association on metabolic control. The most severe consequences are increased occurrence of hypoglycemia in hypothyroidism and appearance of the diabetic ketoacidosis in case of thyrotoxicosis (16).

CONCLUSIONS

Thyrotoxicosis unmasks latent diabetes and worsens preexisting diabetes. In the patients with diabetes and hyperthyroidism, the thyroid function returns to normal after antithyroid treatment, which also improves glycemic balance.

The Graves-Basedow disease and diabetes is a particular pathogenic entity, the presence of uncompensated thyrotoxicosis adversely affecting the evolution and prognosis of diabetes. Because the Graves-Basedow disease presents a chronic evolution, it is relatively difficult to stabilized with antithyroid medication, especially its association with type 1 diabetes requires the correction of hyperthyroidism by thyroidectomy definitive (by surgical or radiation).

The hypothyroidism can cause reduction of requirements for insulin or oral antidiabetic medication in patients with diabetes. The treatment with thyroid hormones in patients with diabetes and hypothyroidism, improves the thyroid function, but worse the metabolic balance. It also causes an increased prevalence of dyslipidemia, aggravating arteriosclerosis.

In the case of the thyroid carcinoma, although its diagnosis imposed the therapeutic maneuvers (total thyroidectomy, treatment with I^{131} and suppressive therapy with high doses of thyroid hormones), that causes deterioration of glycemic balance, it is recommended to continue the treatment with thyroid hormones and to adapted diabetes treatment for prevent the occurrence of local recurrence.

The diffuse and nodular euthyroid goiter imposed a suppression treatment with thyroxin to induce a subclinical hyperthyroidism. In the case of a difficult control of diabetes it is required thyroidectomy.

The polyglandular autoimmune syndrome including type 1 diabetes, various thyroid diseases, CSR deficiency, ovarian failure, severe systemic disease, worsen the glycemic balance, caused mainly by associated medication: estroprogestative, corticosteroids, immunosuppressive medication.

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

From a therapeutic standpoint, the replacement therapy remains the cornerstone of clinical management, the treatment of organ deficiency are identical, whether they occur isolate or in the polyglandular autoimmune syndrome (PAS). Of all the endocrine components of the PAS, only DM has not a favorable prognosis even with hormone replacement therapy and appropriate monitoring. As adjunctive therapy it is required the psychosocial counseling and genetic counseling, and at affected family members would be useful to determine specific antibodies.

For perspective, the immunomodulating therapy remains; this must be considered experimental and should be prescribed only in controlled trials. Following the identification of new auto antigens and better knowledge of the disease pathogenesis, selective therapies will be introduced that does not cause generalized immunosuppression. Also, in the future it is provided curative organ transplantation. Transplantation of pancreas or pancreatic islands is used in patients with diabetes and renal impairment.

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PARTICULARITATI TERAPEUTICE ALE PACIENTILOR ADULTI CU AFECTIUNI TIROIDIENE SI DIABET

REZUMAT

Diabetul zaharat şi afecţiunile tiroidiene reprezintă două endocrinopatii frecvent întâlnite în populaţie. Deoarece insulina şi hormonii tiroidieni sunt implicaţi în metabolismul celular, excesul sau deficitul unuia determină modificări funcţionale ale celuilalt. Scopul acestui studiu este de a determina principalele particularităţi terapeutice la pacienţii adulţi cu afecţiuni tiroidiene şi diabet zaharat.
Lotul de adulţi studiat a fost reprezentat de 650 cazuri, cu vîrste cuprinse între 18-80 ani. Distribuţia pe sexe a fost netă în favoarea femeilor, fiind reprezentată de 588 femei, şi de 62 bărbaţi. Au fost utilizaţi parametrii clinici, imagistici, biochimici, hormonali şi imunologici.
Cuvinte cheie: diabet zaharat, afecţiuni tiroidiene, tratament

PELOIDOTHERAPY MODULATES CORTISOL`S AND THYROTROPHIN-STIMULATING HORMONE`S BALANCE

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ABSTRACT

The main aim of the study is to evaluate the dynamic of serum level of cortisol and thyrotrophin-stimulating hormone during mud therapy. Blood samples were harvested as follows: before the first treatment, at 24 hours after the first mud application and at the end of the balneal treatment. According to requirements of evidence-based medicine were applied normality tests of distribution and homogeneity of variance values and t-test for comparison of sampling.

For plasma levels of cortisol, the comparative analysis (t-test) of the groups pointed out that final value for cold application shows a statistically significant decrease with 31.27% ($p=0.04<0.05$). The TSH variations after cold mud ointment increases significantly statistic from the baseline ($p=0.03<0.05$) with 36.8%. This results show that the cold mud application ask more adaptive reserve than mud bath. Under the action of peloid therapy cortisols and TSH balance is improved.

Key words: peloid therapy, cortisol, thyrotrophin-stimulating hormone

INTRODUCTION

„The couple“ human body - peloid can be compared to a cybernetic system with automatic adjustment. Such a system shows reverse connection mechanisms (feedback) for functional correction. Keystone of the neuroendocrine system is the mutual morphological and functional relation of hypothalamic-pituitary humoral control of hypophysis function, which secretes hormones for“ subsequent“ glands. Stress conditions (including and especially cold thermal stimulation) generates the“ information“ of the hypothalamus about the situation in the periphery and the respond with increased hormone secretion addressed to the pituitary gland. This“ information“ modulates, among other hormones, the pituitary hormone secretion (which in turn adjust the cortisol) and the thyroid-stimulating hormone secretion (involved in modulating metabolic and energetically cells functions) (1, 2).

In this summary presented context, it seems logical and important to investigate the plasma levels of cortisol and thyroid-stimulating hormone comparing the contrasting cure with the thermo neutral cure.

So, the main objectiv of the study is to evaluate the dynamics of serum level of cortisol and thyrotrophin-stimulating hormone during mud therapy and the secondary objectiv is to compare the two forms of peloidotherapy.

MATERIALS AND METHOD

The study was performed on 25 hospitalized patients in Balneal and Rehabilitation Sanatorium from Techirghiol durring the summer of 2010. The inclusion and exclusion criteria were applied.

Inclusion criteria in study plot:

• rheumatic deseas by degenerativ type and posttraumatic sequels of limbs who had correct indications for balneal cure, apparently healthy based on clinical examination and laboratory tests

Exclusion criteria:

• any contraindication for balneal cure

• chronic inflamatory deseases, heart failure and/or hight blood pressure, endocrine, neurological disorders;

• skin lesions witch forbid balneal cure and adjunctive procedures.

Study plot presentation. The average age of patients was 50.20±8.75 yrs. Age average for the group with thermo neutral application (MB) was 49.41±9.43yrs, and for the group with cold application (CO) was 49.36±8.98 yrs. The distribution of the patients by type of application is balanced: 12 patients (48%) performed cold mud applications and 13 patients (52%) performed the neutral thermic applications (mud bath and salt bath). The distribution of the patients by age, sex, pathology

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type and the presence/absence of the comorbidities are balanced (Table I).

Tabel I. Study batch presentation

Age group	Sex		Pathology type		Comorbidities	
	Men	Women	Degenerative	Post traumatic	Present	Absent
30 -39	1	1	0	2	0	2
40 -49	2	9	8	3	3	8
50-59	1	5	1	5	3	3
>60	2	4	6	0	4	2
Total	6	19	15	10	10	15

Most of the patients belongs to the age group 40-49 yrs, patients in activity, and preoccupied with their health, and the less belong to age group 30-39 yrs, presenting post-traumatic pathology and without further sufferings.

In the age group <60 yrs was not present any patient suffering post-traumatic conditions, and in the age group 30-39 yrs was not present any patient suffering degenerative disease.

Treatment applied. Cold mud application is a therapeutical complex composed of successive contrast hot-cold, witch is developed in two phases: first phase for about 20 minutes includes sun exposure on the warm sand from the solarium/beach (heating), followed by the mud ointement (cold stimulation), and the second phase involves for about 30 minutes the exposure to sunlight in order to install a mild hyperthermia followed by a cold bath in the lake's water. After several days of treatement patients extend the two phases by introducing at least one additional immersion into the lake, before and / or after mud ointment. Patients are instructed that the correct method of application requires a single mud ointment.

During the study, we followed carefully the correctness of the therapeutic application, being presents at the solarium every day. Our presence at the solarium was required, because despite of all the indications they received, patients are tempted, at least in the second week, to apply the mud twice.

Samples procurement. Blood harvesting was performed in the morning, before the breakfast and before the start of the treatment, in three moments of the cure:

⇒ at the beginning of the treatment (noted „in");
⇒ at 24 hours after the first mud application (noted „24");
⇒ at the end of the balneal treatment (noted „fin").

RESULTS

1. Serum level of cortisol during peloidotherapy

Normal value for plasma level of cortisol is between 5 - 20 mg/day. Results obtained were tabled for systematization and statistical study (Table II). In accordance with the requirements of evidence-based medicine were applied normality tests of distribution and homogeneity of variance values which showed that obtained distribution of values are normal for all the investigated moments and homogenous in the groups. Comparative analysis (t-test) of the groups pointed out that only the final value for cold application shows a statistically significant difference ($p=0.04<0.05$).

Tabel II. Statistic presentation of plasma level cortisol values

	Before the treatment (in)	24 hours after the first mud application (24)	At the end of the balneal treatment (fin)
Average plot ± DS	13.68±6.34	13.94±6.00	11.52±4.10
Average group MB±DS	11.87±6.41	11.98±3.06	12.66±3.52
Average group CO ±DS	15.35±6.88	15.75±7.49	10.48±4.45
t-test p<0.05			
MB/CO		0.11	0.19
MB in/MB 24, fin		0.95	0.68
CO in/CO, 24, fin		0.89	0.04

The values obtained for the whole group in dynamic, at the determination of plasma cortisol shows:

• statistically insignificant slight increase immediately after the first application
• statistically insignificant slight decrease at the end of cure (Figure1)

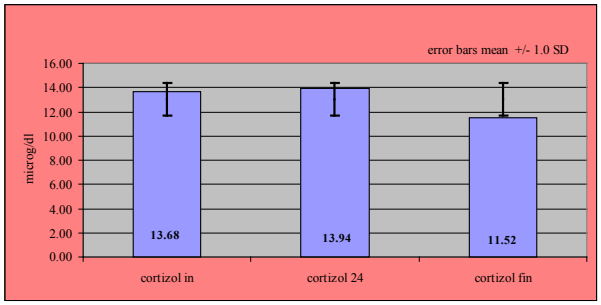

Fig.1. Plasma cortisol values at the entire group

Graphic appearance is suggestive of the low level of variation, accounted for the entire lot:

The cortisol slight increase observed at the whole study batch in the first twenty-four hours respectively after the first therapeutic application is the immediate adaptive response to new conditions of stress generated by pain, travel, new surroundings, and different climate parameters from those in the area of origin, medical consultation, and therapeutic application (2).

The comparative analysis of the cortisol levels by the type of peloid therapy (Figure 2) shows major differences between them:

• cold application produces intense stimulation
• thermoneutral application is mild

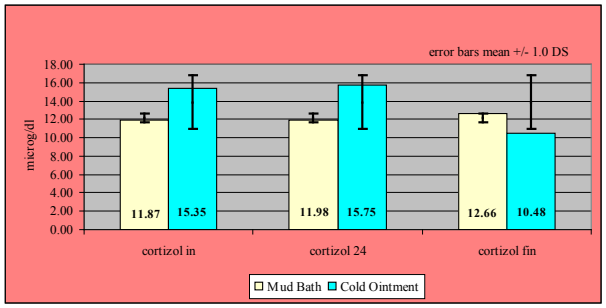

Fig.2. Variation of plasma cortisol during different type of peloid therapy

Cold application is a contrasting thermal stres request (thermal contrast is performed 4-5 times during the 1.5 - 2 hours how long the application is) and that add to the other stressed factors of the balneal cure (3). Daily stimulation leads to increased consumption of plasma cortisol during the first days of treatment, which require adaptation of the secretory system. This is illustrated by the slight growth of plasmatic level of cortisol in the determination made at twenty -four hours after the first application. Add to this the fact that under the combined action of heliotherapy, the immersion in the lake's water and other therapeutically applications (massage, electrotherapy, physiotherapy) possible subclinical joint inflammations, rheumatic pain, and many other factors are reduced or even removed in the second part of the diet and thus secretory demand is lower.

Under the action of contrasting complex the thermoregulatory function is optimized in the" hypothalamic thermostat" level and adapted secretory with prompt responses, so endocrine secretory activity is reduced at the end of the cure. It should also consider optimizing the sweat secretion at the end of the cure, which also means more efficient energy consumption, including glucose.

Under the influence of heat contrast and information from thermal receptors the function of the variable thermal effect is improved in the peripheral thermoregulation zone by modulating local flow, segmentation, and finally general and optimizing the release of autocoids factors.

In addition to contrasting thermal therapeutic application should be noted that the summer climate parameters are disruptive for the functioning of the body: high temperature and high humidity of air increases energy consumption due to a hard to stabilize balance between breeze (favorable evaporation of sweat factor) and high humidity (unfavorable evaporation of sweat factor).

2. TSH variation on patients undergoing peloid therapy

The metabolic response of local and systemic effects of peloid therapy is endocrine coordinated mainly by the adrenal glands and thyroid (2). Normal value for TSH is 0.27-4.2 UI/ml. In order to apply criteria for evidence based all values obtained were recorded in tables (Table III). Statistical analysis of obtained values shows that the distribution of values is normal for all investigated times and homogeneous in the groups.

Tabel III. Plasma TSH values and statistic analysis

	Before the treatment (in)	At 24 hours after the first mud application (24)	At the end of the balneal treatment (fin)
Average plot ± DS	1.98±1.72	2.32±1.15	3.14±1.79
Average group MB±DS	2.20±2.20	2.65±1.26	3.39±2.22
Average group CO±DS	1.78±1.17	2.02±0.98	2.90±1.31
t-test (p<0.05)			
MB/CO		0.18	0.52
MB in/MB 24, fin		0.55	0.20
CO in/CO, 24, fin		0.58	0.03

Comparative analysis (t-test) of the groups pointed out that for the cold mud ointment the values obtained at the end of the application differ significantly from the baseline and those obtained from application thermo neutral (p=0.03<0.05). The aspect is suggestive of how reacts the secretion of hormone at the adaptive needs of the body on balneal cure: the growth of metabolic needs as the thermoregulatory causes a progressive increase of the thyrotrophin-stimulating hormone till the end of the cure (Figure 3).

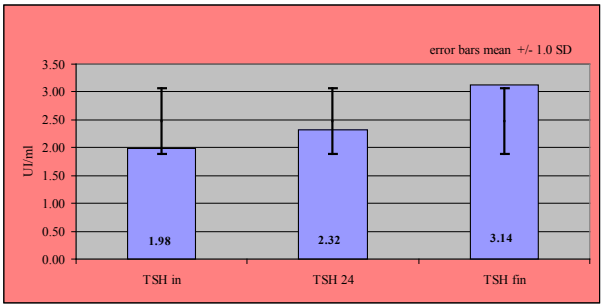

Fig.3. Plasma TSH variation during peloid therapy

TSH variation during cold application shows the favorable effect of cold ointment on thermoregulatory function by reducing thyrotrophin-stimulating hormone releasing (Figure 4). During the cold phase of the application (mud temperature 20^0 - 24^0C and immersion in the lake's water at approximately the same thermal parameters) is necessary to maintain skin temperature almost unchanging (3). The thermo neutral application does not require significant thermoregulation, on the contrary is gentle, soothing (during application the bath temperature remains almost constant). Thermal comfort during thermo neutral bath stabilizes the hypothalamic thermostat does not require major neuroendocrine responses, but rather addresses to the physiological area where are minimal responses. The thermoregulatory answer depends on the" error" referred to the periphery from the hypothalamic set-point. When the amount of" error" is reduced or the number of" errors" produced in reduced time is low then the adaptive response is reduced (1).

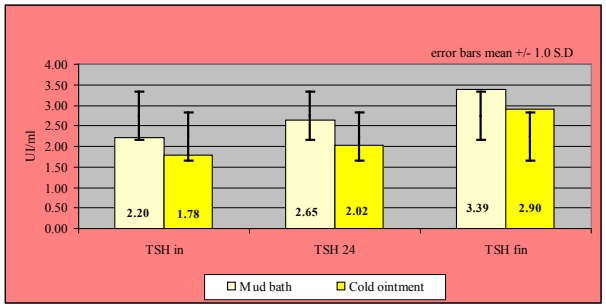

Fig.4. Plasma TSH variation during different type of peloid therapy

The final results at the end of contrasting balneal cure are consistent with other studies performed under the same conditions but with other types of mud: respectively the reduction of the plasmatic cortisol level.

Analysis of data from the application of cold mud treatment highlights the contraindication of the contrasting cure in patients with thyroid hyper function and those with thyroid at the limit of the compensation because the risk of decompensating is major owing to statistically significant increase in plasma levels of TSH. Increase of serum level of TSH during thermo neutral application is statistically insignificant, and safety for the patient.

CONCLUSIONS

1. Cortisol level during peloidotherapy shows different respose according to the type of application and to the intensity of thermal stimulation.

Thermoneutral application produces slight increase of plasma level, statistic insignificant. The values obtained have minimal differences, within physiological frame, showing that the therapeutic application of mud is a safe procedure for patients.

Cold mud ointment produces statistically significant decrease of serum levels of cortisol with 31.27% at the end of the cure. This results might show:

• increased peripheral consumption of cortisol in the first days of cure produced by the hight level of stres;

• decreased needs of cortisol în the second part of the cure due to diminution of pain, inflamation and accomodation to new conditons.

So, during peloidotherapy consumption/need ratio of cortisol appear to be rebalanced. Our results are similar with other references on mud of different type.

2. Variation of serum level of TSH is concordant with cortisol evolution.

Thermoneutral application generates minimal differences concordant with the low level of thermal stimulation. Cold mud application produces statistic significant increase of thyroid stimulating hormone with:

• 11.89% after first cold mud application;

• 36.8% at the end f balneal cure.

Increasing level of thyroid stimulating hormone is produced by diminution of tiroxine and triiodotironine (negativ feedback) due to modulation of cells metabolic activity. Interaction between cortisol and TSH is also, negativ feed-back type. Peloidotherpy does not brokes this type of interaction, only modulates it. So, serum level of cortisol and TSH varies according with the type of thermal stimuation. Cold mud ointment is stronger than mud bath but both are safe procedure for patients.

Abbreviations: TSH - thyrotrophin-stimulating hormone; MB – mud bath; CO – cold ointment with mud.

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PELOIDOTERAPIA MODULEAZA ECHILIBRUL CORTIZONIC SI AL TSH

REZUMAT

Scopul principal al acestui studiu a fost evaluarea nivelului seric al cortizolului si al TSH in timpul terapiei cu namol. Probele sanguine au fost recoltate in diferite puncte cheie ale terapiei: inainte de prima aplicare, la 24 de ore de la prima aplicare a peloidoterapiei si la finalul tratamentului balneal. In conformitate cu cerintele medicinei bazate pe dovezi, au fost aplicate teste statistice de normalizare a distributiei si omogenizare a valorilor variantelor, precum si testul „t“ pereche pentru compararea probelor.

Pentru evidentierea nivelului de cortizol a fost efectuata analiza comparativa (testul“ t“ pereche) a grupurilor studiate, care a relevant ca valorile finale pentru procedurile de aplicare la rece prezinta o scadere semnificativa din punct de vedere statistic cu 31, 27% (p=0, 04<0, 05). Variatia TSH dupa aplicarea procedurilor peloidoterapeutice la rece a prezentat o crestere semnificativa statistic fata de control cu 36, 8% (p=0, 03<0, 05) with 36.8%. Aceste rezultate arata ca procedurile terapeutice de apicare a namolului la rece solicita rezerva adaptativa a organismului intr-o masura mai mare decat baile de namol. Sub actiunea peloidoterapiei, echilibrul cortizonic si al TSH au fost imbunatatite.

Cuvinte cheie: peloidoterapie, cortizon, TSH

MYASTHENIA GRAVIS: CARDIAC AND PHARMACOLOGICAL CONSIDERATIONS

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ABSTRACT

Myasthenia Gravis (M.G.) is an autoimmune neuromuscular disease caused by antibodies that react with the nicotinic acetylcholine receptor (nAChR) in the postsynaptic region of the neuromuscular junction. It is already known that some M.G patients, have also antibodies that bind in a cross-striational type to skeletal and heart muscle tissue (striational autoantibodies).This article summarizes what is known about the cardiac involvement in myasthenia, starting from molecular characterization of striational autoantibody types, as well as the clinical presentation via some pharmacological particularities of the main therapeutical agent used in this disease.

Key words: myasthenia, cardiac involvement, striational antibodies, structure, immunopathology, anti-cholinesterases

STRIATIONAL MUSCLE ANTIBODIES RELATED TO MYASTHENIA GRAVIS. MOLECULAR CHARACTERISTICS AND CLINICAL PRESENTATION

1. Titin

Also known as connectin, is a giant protein (3000 kDa), that function as a molecular spring, being responsible for the passive elasticity of muscle. Titin is also important in the contraction of striated muscle. It connects the Z line to the M line in the skeletal and cardiac sarcomere. Molecules of Titin are arranged so that they allow mechanical stability and tension in the sarcomere. According to Romi et al., 2005, the main immunogenic region of Titin is called myasthenia gravis titin-30 (MGT-30), and is located near the junction of the A/I band.

Another immunogenic region is located between the N1 and N2 lines. The expression of homologous immunoglobulin domain is different. For example 15 modules are expressed in cardiac muscle, while in the skeletal muscle such modules could be up to 68 (Lubke et al., 1998). In sera containing MGT-30 antibodies, antibodies to the I-band are present. M.G. patients who exhibit antibodies to the I-band epitopes could have also antibodies attached to the main immunogenic region. Anti-Titin antibody were first put in evidence in sera of myasthenic patients, where first discovered by Aarli et al., in 1990.

Clinical features: anti-Titin antibodies are found in 20-40% of all M.G. patients and in 60-80% in patients older than 60.A percentage of 32% were found in patients with non-thymomatous myasthenia, older than 50 years. Myopathic signs in these patients were detected via electromyography.

Immunopathology: T cell proliferative response to MGT-30; capability to activate the complement pathway; association with severe M.G. concomitant with myositis.

2. The Ryanodine Receptor

The ryanodine receptor (RyR) is a calcium-release channel located in the sarcoplasmic reticulum. There are two forms of RyR: skeletal (RyR1) and cardiac (RyR2). The molecular weight of this receptor is about 565 kDa, containing 5035 amino-acids (Romi et al., 2005). The ryanodine receptor is mainly expressed in striated muscle tissue, but is also found in epithelium and neurons. RyR antibodies can generate allosteric inhibition of RyR function in vitro, by inhibiting Ca^{2+}, release from the sarcoplasmic reticulum. From antigenic point of view, cardiac and skeletal muscle RyRs are not the same but, anti-RyR antibodies in MG patients cross-react with both subtypes of the receptor (Mygland et al., 1994).

Clinical features: Found in 13-38% of all myasthenia gravis patients. Mean onset at age 57; M : F = 1:1; more severe than anti-Titin antibodies; bulbar, respiratory and neck involvement; myocarditis and/or myositis.

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Immunopathology: Complement activation; inhibiting the release of Ca^{2+}, from the sarcoplasmic reticulum; activation of autoantibodies to the dihydropyridine receptor and the transient receptor potential canonical type 3.

3. Voltage-gated K^+ Channel

Voltage-gated K^+ channels (VGKCs) have four transmembrane α-subunits that can combine as homo/hetero-tetramers. Kv1.4 is an α-subunit, with a molecular weight of 73kDa, that is mainly located in the brain, peripheral nerves, skeletal and heart muscles.

Clinical features: 12-15% of all MG patients; mean onset at age 49 years; M : F = 1:1; more severe than anti-Titin; bulbar involvement and myasthenic crisis; lethal arrhythmias; myocarditis and/or myositis.

Immunopathology: Different from neuronal VGKC; QT- prolongation on electrocardiogram.

OTHER STRIATIONAL ANTIGENS AND ANTIBODIES

Antibodies to human myosin Antibodies to anti-actomyosin	High titers in patients with MG and thymoma

Anti-Rapsyn antibodies are also found in serum samples of patients with MG, Lupus and Chronic Procainamide associated myopathy (Aigus et al., 1998). Striational autoantibodies are rarely found in AChR antibody-negative MG patients.

CARDIAC CONSIDERATIONS

Although relatively common, cardiac manifestations in patients with M.G. may take several forms. Among these, we mention asymptomatic ECG changes to ventricular tachycardia, myocarditis, heart failure and sudden death, cardiac fibrosis and rhythm disturbances.

Changes in cardiac function via electrocardiogram are already known. This could be a terminal notching of the QRS complex, higher frequency of QT-prolongation, right bundle branch block, sinus tachycardia, inverted T-waves, ST-depression or poor progression of R-waves.

Anyway, the significance of ECG in MG evaluation related to cardiac involvement remains uncertain.

The effect of acetyl-cholinesterase inhibitors on cardiac function

In 2008, Owe and Gilhus demonstrated a normalizing of cardiac tissue velocity and strain after administration of acetyl cholinesterase inhibitors, but previous, in 1992, Johannesen found an increasing peak of diastolic filling rate. Sometimes the rhythm disturbances may be caused by the main medication of the patient and/or the mechanism of the disease itself. Anticholinesterase drugs (also known as acetyl cholinesterase inhibitor) are used to reverse the effects of non-depolarizing neuromuscular blocking drugs. This drug increases the concentration of acetylcholine at the level of neuromuscular junction via an inhibitory activity of acetyl cholinesterase. Frequently in the therapeutic scheme of myasthenia gravis, is used a pharmacological agent with a longer onset than neostigmine: Mestinon.

Mestinon (pyridostigmine bromide tablets) is an orally active cholinesterase inhibitor. Chemically, pyridostigmine bromide is 3-hydroxy-1-methylpyridinium bromide-dimethylcarbamate (Figure 1).

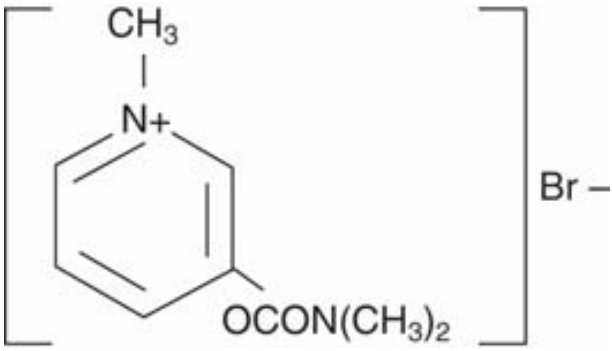

Fig.1. Structural formula of Mestinon

Cardiac unwanted effects and their impact on heart function

a) Bradycardia: occurs due to stimulation of muscarinic receptors in the sino-atrial node. Bradycardia is more common in children and after increased or high doses of drug.

b) Prolonged block due to reduced plasma cholinesterase activity. This can occur due to congenital acquired causes and production of atypical plasma cholinesterase. Duration of prolonged block varies from 30 minutes to several hours.

It has been reported (Rowland et al., 2007) that some acetyl cholinesterase inhibitors are capable to exert severe peripheral cholinergic effects and afterwards serious cardiovascular complications. Parasympathetic activity dominates neural control of heart rate and cardiac function. Moreover, increased level of acetylcholine stimulates GABA-ergic and glycine-ergic inhibitory receptors by acting on vagal neurotransmission, and their vagal activity via muscarinic receptors is translated by a slow heart rate. As we suggested before, the acetyl cholinesterase inhibitors can induce sinus bradycardia, sino-atrial block and aggregate pre-existing sinusal node disease and atrioventricular block. Profound sinus bradycardia or bradycardia related to second or third-degree heart block could have the potential to generate heart failure, syncope or seizures. Some adverse effects of anticholinesterases are illustrated in Table I as well.

Table I. Frequency of cardiovascular adverse effects for anti-cholinesterases drugs

Adverse effect	Frequency (%)
Dizziness and syncope	1-10
Bradycardia, atrial arrhythmias, myocardial infarction, angina, seizures	0.01-1
Sino-atrial and atrioventricular block	0.001-0.1

*Shire Pharmaceuticals Ltd, 2005; Elisai Ltd, 2006; Novartis Pharmaceuticals UK Ltd, 2007

c) Other effects: increased intra-ocular pressure, muscle pain, nausea, increased intra gastric pressure, vasospastic effects.

Biochemical features of acetyl cholinesterase inhibitors

Mestinon. Absorption. Distribution. Metabolism and excretion.

Absorption. Only about 40% of an oral dose of Mestinon is absorbed, afterwards significant quantities being destroyed in the gastrointestinal tract. The presence of permanently charged quaternary ammonium groups confers higher water solubility. Thus, pyridostigmine comprises a great absorption in the duodenum, but overall absorption is poor and variable.

Distribution. Mestinon is mainly distributed in the extracellular fluid. It doesn't enter in the central nervous system. The plasma area under the curve after 4 hours is relatively constant (6000 to 10000 nanogram/ml/minute). Pyridostigmine has been reported to cross the placenta and to decrease fetal plasma cholinesterase activity after increased oral doses.

Metabolism and excretion. Mestinon is highly metabolized and 95% is excreted in the urine. The main metabolite is 3-hydroxi-N-methyl-pyridinium bromide. Neither pyridostigmine nor its metabolites are protein bound. Excretion is by filtration or by hepatic conversion to the glucuronide. Competition for renal transport mechanism by tertiary amines can occur.

Biochemical effects of cortisone in heart muscle

As is known already, cortisone and non-selective non-steroidal anti-inflammatory drugs are widely used to control symptoms in osteoarthritis, rheumatoid arthritis, cardiomyopathies and other diseases with severe inflammatory bursts. With regards to MG cortisone is the principal therapeutic agent (corticotherapy) but sometimes with undesirable side effects. However, the attention should be paid to the effects on exacerbation of congestive heart failure and arrhythmias due to hypopotassemia. All steroids exhibit positive inotropic and also inhibitory effects on the myocardial Na^+-K^+-ATP-ase activity .Modifications in the corticosteroid structure, particularly in the steroid nucleus itself, are able to influence the pharmacodynamic properties and corticosteroid metabolism even in the cardiac muscle.

CONCLUSIONS

In order to have an accurate evaluation of heart involvement in MG a correlation between laboratory findings and clinical cardiac function has to be made. In our point of view any myasthenic patient should be cardiologically evaluated with available modalities and specific tests to detect or to exclude the coexistence of a cardiovascular dysfunction/disease towards with MG.

The detection of striational antibodies can provide information regarding cellular and molecular alteration in the heart muscle tissue, allowing in this way a new classification and management of MG patients. Due to the fact that the anticholinesterases drugs are the main therapeutical tool in this disease, their effects and impact on central organs and their function, should be from time to time evaluated.

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MIASTENIA GRAVIS: CONSIDERATII CARDIACE

REZUMAT

Miastenia Gravis este o boala neuromusculara autoimuna cauzata de anticorpii ce reactioneaza cu receptorul nicotinic pentru acetilcolina (nAChR) din regiunea postsinaptica a jonctiunii neuromusculare. Este deja cunoscut faptul ca unii pacienti cu Miastenia Gravis poseda atat anticorpi care reactioneaza incrucisat atat cu tesutul muscular scheletic cat si cu tesutul muscular cardiac (anticorpi striationali). Acest articol, prezinta pe scurt ce este cunoscut despre implicatiile cardiace in Miastenia Gravis, plecand de la caracteristicile moleculare ale tipurilor de anticorpi striationali, precum si prezentarea clinica, prin unele particularitati farmacologice ale principalului agent terapeutic folosit in această afectiune.

Cuvinte cheie: miastenie, implicare cardiaca, anticorpi striationali, structura, imunopatologie, anticolinesterazic.

REVIEW

LIVER AND IMMUNITY

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ABSTRACT

The immune system is a compilation of cells and molecules that silently guard the body's well-being. Due to the fact that the liver is the largest organ in mammals and has a constant flow of all kind of substances, toxic or nutritious, viruses or microorganism, it needs to have strong protection. Sometimes this can backfire and the protection can turn into self-aggression, when the liver suffers from a type of autoimmune condition. There are many factors that can lead to the many diseases that the liver can suffer from, but there are also a few liver helpers at hand.

Keywords: liver, immunity, autoimmunity, Kupffer cells

INTRODUCTION

The liver's function as an immune organ is beginning to be appreciated more and more. It has one of the largest resident populations of macrophages, natural killer cells, and natural killer T cells, all of which are key components of the innate immune system. The liver is in a permanent contact with allogenic antigens due to the fact that they penetrate the body trough food. This requires a strict control of inflammatory responses to different antigens (13). Autoimmune diseases of the liver are considered rare, but in the last centuries they have become more prevalent. They are the result of a variety of mechanisms that usually have a genetic predisposition as a starting point, and some environmental, nutrition or work-related factors. Reducing exposure to xenobiotics and resolving digestive problems can normalize and maintain a good and healthy immune function (10).This review will focus on recent knowledge concerning the hepatic immune system and the pathologies involving it.

In the present review, the literature search was made using the following keywords: "liver immunity", "liver autoimmunity", "inflammation in liver", and other terms that were needed to complete the subject. The articles were found in the on-line libraries of Pubmed, Springer Publications, the official site of NIH and CDC and Google Scholar.

The components and their role in liver immunity

In the liver, only up to 80% of all cells are hepatocytes. The other 20% are constituted mostly of liver sinusoidal endothelial cells, which represent about half of the non-hepatocytes. The remaining half of non-hepatocytes is made up of cells belonging to the adaptive immune system (T and B lymphocytes, natural killer cells and natural killer T cells), the innate immune system (Kupffer cells and eosinophils) and the humoral factors such as the complement system and interferon. The innate immune system is better represented because of the strong traffic of foreign substances (21, 31).

In the perisinusoidal Disse spaces, the adult liver contains a number of pluripotent hematopoietic stem cells that will eventually produce all lineages of leukocytes and red blood cells. Innate immune lymphocytes, natural killer cells and T cells are present in the liver in high numbers, unlike in other organs or in peripheral blood. In normal conditions all of these cells exist in the sinusoidal space and in the liver parenchyma. Kupffer cells stay tightly attached to the sinusoidal endothelial cells, and natural killer cells come in contact with them modulating the immune response to different harmful agents. This status is altered in most liver conditions (30).

Neutrophils are a type of phagocytic granulocytes or polymorphonuclear leukocytes that have a characteristic staining pattern. They derive from the myeloid stem cell and are the most numerous and important cellular component of the innate immune system. They circulate throughout the body until they are needed to act at sites of infection and inflammation. Cellular infiltration of activated neutrophils, which produce oxygen radicals and secrete other toxic mediators, may increase the inflammatory response, leading to cell injury and death (16, 33).

Kupffer cells are macrophages, and it is in their nature to continuously mature from circulating monocytes that leave the circulation to migrate into tissues throughout the body. They

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have a few important roles: to phagocyte bacteria or cellular debris, to present antigens to the T cells, to release signals and to remove senescent blood cells from the circulation. To fulfill these tasks these cells use scavenger receptors from the cysteine-rich superfamily, and produce interleukins IL-1, IL-6, IL-12, tumor necrosis factor-α, transforming growth factor-β, and reactive oxygen species. IL-12 activates the hepatic natural killer cells and natural killer T cells to produce interferon-γ, which will activate the hepatic T cells. The result is phagocytosis and cytokine production by Kupffer cells. Besides being a foreign body protection mechanism this is also an anti-tumor and anti-metastatic immunity process of the liver, delivered by the activated natural killer cells and natural killer T cells located in the liver. Macrophages and neutrophils are the first line of defense in the liver (11, 14, 16, 28, 30, 31, 34).

Kupffer cells have the ability to eliminate various exogenous and endogenous substances. Recent experiments have shown that Kupffer cells produce mediators that stimulate alcohol metabolism. It was shown that the gut microflora-produced endotoxin stimulates Kupffer cells to produce mediators, which determine a hypermetabolic state of the parenchymal cells, followed by the development of hypoxia in the pericentral regions of the lobule. Free radicals are produced with cell death as a result (33).

Liver sinusoidal endothelial cells, although not immune cells, have among their purposes to present antigens and to create strong bonds with Kupffer cells, leukocytes and platelets in case of inflammation, a bond that will increase the concentration of signals, which in turn will help leukocytes migrate to the injured hepatocytes (6, 34).

Natural killer cells derive from the common lymphoid progenitor, develop in the bone marrow and can circulate in the blood. They are larger than T and B lymphocytes, have distinctive cytoplasmic granules, and are functionally identified by their ability to kill certain lymphoid tumor cell lines *in vitro* without the need for prior immunization or activation. The killing-mechanism used by natural killer cells is the same as the one used by the cytotoxic T cells generated in an adaptive immune response. Cytotoxic granules are released onto the surface of the bound target cell, and the effector proteins they contain penetrate the cell membrane and induce programmed cell death or apoptosis. Natural killer cells are activated by interferon (IFN-α, and IFN-β) or macrophage-derived cytokines (IL-12). In their activated state they are able to contain virus infections, and wait for the adaptive immune response to produce the antigen-specific cytotoxic T cells that can clear the infection. These cells present two types of surface receptors for the cytotoxic activity: activating receptors and inhibiting activation receptors, that won't allow the killing of normal, healthy cells. Even so, they lack the antigen-specificity that is typical to the adaptive immune response. They have a protective action against fibrosis, inhibiting liver fibrogenesis by killing only the activated stellate cells which are known to mass produce collagen in inflammation. They also induce apoptosis or at least a metabolic freeze of the Ito cells using its production of interferon, thus having a liver protective status against fibrogenesis (11, 16).

Hepatic **natural killer T cells**, also known as cytotoxic T cells, are mainly used to kill viruses or intracellular bacteria infected cells, which they recognize by the antigens displayed on the surface of infected cells. Another role of natural killer T cells is to release signals for Kupffer cells. Cytotoxic T cells typically express the molecule CD8 on their cell surfaces that can recognize MHC class I molecules and store specialized cytotoxins, in granules, that are to be released when the infected target cell is met. The cells die by apoptosis induced by the holes created in their cytoplasm by the killer cell, through which it introduces its toxic content (16).

In liver injury cytotoxic T cells were found to balance the local production of pro-inflammatory (Th-1) and anti-inflammatory (Th-2) cytokines. The hepatic natural killer T cells themselves are regulated by Kupffer cell produced cytokines, dietary factors, and certain neurotransmitters, such as norepinephrine (21).

Ito cells, sinusoidal fat-storing cells or hepatic stellate cells represent 30% of the liver immune cells and can be found in the Disse space, between hepatocytes and sinusoidal endothelial cells. They are not actual immune cells but act as such. A great number of stellate cells inhibit T cell proliferation but will lose the inhibition control over B cell proliferation. They can store retinol lipid droplets and regulate retinoid homeostasis. They become myofibroblastic cells in liver injuries, lose the retinol accumulation and overproduce collagen. Ito cells are considered the primary cells to set fibrosis in the liver, and are related to alcoholic liver steatosis and immune responses because they can interact with the liver's immune cells (11, 22, 31). Although it is thought that the innate immune system unspecific recognizes a pathogenic factor, it was demonstrated that it can very well recognize certain types of proteins called pathogen-associated molecular patterns which are located on the surface of the pathogen (e.g. lipopolysaccharides and peptidoglycans) using pattern recognition receptors (secreted, membrane-bound and phagocytic) (11).

Liver conditions

Autoimmune liver disorders are characterized by an inflammation of the liver, increased level and number of circulating auto-antibodies and immunoglobulin G without a clear cause. Autoimmune liver disorders include autoimmune hepatitis and autoimmune sclerosing cholangitis, chronic active hepatitis, primary biliary cirrhosis (1, 24), but there are a few acquired liver conditions that can, at some point, induce autoimmunity or severe degradation of liver and can require a transplant. Such conditions include the hepatic viruses and a syndrome of the small intestine.

Autoimmune hepatitis is characterized by mononuclear, plasma cells and eosinophils invasion in the periportal area. The condition can grow into fibrosis, distortion of the hepatic lobule, the formation of regenerative nodules and cirrhosis. It is a progressive, chronic disease that occurs both in children and adults, predominantly appearing in women (70% of patients), and that's cause is unknown. Blood analysis is characterized by a high level of gamma-globulins and self-antibodies. The

condition responds well to immunosuppressant medication and liver transplants have been successful in patients who have no response to medication. There are two types of autoimmune hepatitis. Type 1 is the most common form, appearing in adolescence or young adulthood. Type 2 autoimmune hepatitis occurs in 2 to 14 year old girls. Most of the time, autoimmune hepatitis is associated with other immune disorders such as: type 1 diabetes, proliferative glomerulonephritis, thyroiditis, Graves' disease, Sjögren's syndrome, autoimmune anemia and ulcerative colitis (3, 15, 19, 25).

Chronic active hepatitis affects hepatocytes and starts with lymphocytes entering the parenchyma trough the portal areas and producing inflammation around the vessel. The presence of toxins or viral and bacterial entities in the liver will determine the leukocytes immune function to activate and the Kupffer cells to produce IL-6 that will subsequently induce the production of acute phase proteins and the complement system activation in the hepatocytes (10, 30). The cause of autoimmune hepatitis is unknown, but the illness presents the same characteristics as any autoimmune liver disease: infiltration of leukocytes, high level of gamma-globulins and the presence in great number of auto-antibodies. Women have an almost four times greater prevalence than men (5, 15).

The **hepatitis A** virus is a picornavirus transmitted by contact or ingestion of contaminated food or water. It is the most common hepatitis virus infection and it usually leads to a total recovery in about three months. It passes undetected in childhood, but when contracted in adulthood can cause severe liver damage in individuals with autoimmune hepatitis. The two conditions together lead to a rapid destruction of the liver, and the presence of one can lead to the easy onset of the other (25, 27, 32, 35, 36).

The **hepatitis B** virus infection is a global health problem affecting almost 400 million people worldwide. Many of the individuals infected progress into chronic hepatitis leading to cirrhosis and tumors. In hepatitis B the blood CD56+ T cells and natural killer cells do not decrease upon reaching the cirrhotic stage, but there are significant increases in CD8+ cells and nonspecific cytotoxic T cells in patients with chronic severe hepatitis B (9, 26, 30, 32, 37).

The **hepatitis C** virus, belonging to the Flaviviridae family, infects about 170 million people from all around the world, and in 80% of cases progresses to chronic liver disease (9, 38). In hepatitis C, cirrhotic status natural killer CD56+ T cells and natural killer CD56+ cells decrease until the majority are lost (27, 30).

Hepatitis D is a contagious liver infection, which spreads trough a virus that needs hepatitis B virus antigen, although it is able to replicate independently of it. It is thought that some immune-mediated mechanisms may be implicated in the progression of the liver damage. There are two types of hepatitis D infections: hepatitis D coinfection, and hepatitis D super-infection. The two differ in the amount of time passed from the moment of contacting the hepatitis B virus until contacting the hepatitis D virus. The first one has a 5% chance of leading to a chronic infection, while the latter has an 80-90% chance. The condition develops rapidly to cirrhosis or liver cancer (12, 27).

Primary biliary cirrhosis is a slowly progressive autoimmune disease of the liver that affects ten times more women than men, and peaks in prevalence around the age of fifty. It is characterized by inflammation around the portal area and by the immune controlled destruction of the hepatic bile ducts. It is a distinctive condition by the fact that it affects mitochondria within liver. Cirrhosis develops at about 10 to 30 years after the onset of the disease (1, 10, 17, 29).

Primary sclerosing cholangitis is characterized by inflammation and fibrosis of both intrahepatic and extrahepatic bile ducts, and is a progressive cholestatic disease that affects children and young adults. It appears more often in men than women, and peaks in prevalence around the age of forty. It manifests itself trough inflammation and destruction of the hepatic bile ducts, both intrahepatic and extrahepatic. It is thought that bacterial antigens are the starting point in genetically predisposed patients. The activation of Kupffer cells leading to the overproduction of tumor necrosis may be connected to bile duct lesions. The total number of circulating T cells in the body is decreased, but is increased in the portal tracts. There are more CD4 then CD8 lymphocytes in the circulation and an increased number of B cells. Leukocytes cannot migrate because of the bile content, but the T lymphocytes manifest an auto-reactivity towards the biliary components and apparently epithelial cells act as autoantigens to lymphocytes. The condition gradually progresses to the sclerosis of major bile ducts ending with cholestasis, fibrosis and cirrhosis. It is usually associated with a few other immune conditions: inflammatory bowel disease, celiac disease, diabetes mellitus, rheumatoid arthritis, Sjögren's disease, systemic sclerosis, chronic pancreatitis, cystic fibrosis, retroperitoneal fibrosis, sarcoidosis, thyroiditis, systemic lupus erythematosus, lupus nephritis, autoimmune hemolytic anaemia, idiopathic thrombocytopenic purpura or Langerhans cell histiocytosis. A liver transplant remains the only alternative for patients in end-stage primary sclerosing cholangitis (1, 2, 4, 20).

Intestinal Permeability, also called leaky gut syndrome, is a key condition to many autoimmune conditions. It has long been recognized that untreated celiac disease, a primary cause of leaky gut, can lead to autoimmune hepatitis and other autoimmune conditions like insulin-dependent diabetes mellitus or Hashimoto's thyroiditis (10).

Kupffer cells, natural killer cells, natural killer T cells and CD8+CD122+ cells cooperatively act not only against bacterial and viral infections, but also against cancers. Endo- or exotoxins entering the liver circulation activate Kupffer cells and stimulate them to produce IL-12. This interleukin determines natural killer cells, natural killer T cells and CD8+CD122+ cells to generate an antitumor activity. These cells can migrate to other organs and inhibit a certain tumor growth there (30).

Possible causing agents

It was proven that **estrogen** treatment in vivo increases the sensitivity of Kupffer cells to endotoxins (33)**.** Many agricultural

chemicals have been shown to have immune-modulating effects because of their estrogenic properties, and many other types of chemicals are now thought to induce, and possibly also exacerbate, autoimmunity (10, 18). Cortisol maintained at a high level for a long period of time can determine adrenal depletion and reduce its level. This will create violent immunoglobulin A reactions followed by an autoimmune reaction (10).

Pesticides, fertilizers, and synthetic substances can trick the immune system, and many of them can act as endocrine disruptors because they mimic the estrogen form and function and, as such, they affect every branch of the immune system: innate cell-mediated and humoral immunity, and host resistance. Vinyl chloride inhibits suppressor T cells and other immune cells. Many other plasticizers and plastics, found in consumer products such as food containers and wraps, are also known as endocrine disruptors and are suspected of contributing to immune dysfunctions (10)

Females show a greater susceptibility to **alcohol**-induced liver injury than males. Additionally, females who consume alcohol regularly and have been obese for 10 years or more are at greater risk for both hepatitis and cirrhosis. Evidence has been presented that Kupffer cells are pivotal in the development of alcohol-induced liver injury. It involves an increase in circulating endotoxin, leading to the activation of Kupffer cells, which causes an injury created by the difference between the presence and the absence of oxygen in the tissue. Ethanol affects some Kupffer cell functions such as phagocytosis, bactericidal activity and cytokine production. Serum tumor necrosis factor-alfa (TNF-a) increases in alcoholics, supporting the idea that Kupffer cells are activated in patients with alcoholic liver disease. TNF-α is produced by Kupffer cell macrophages. During acute exposure to ethanol, carbon uptake by the perfused liver increases about 25%, being a testimony that ethanol activates Kupffer cells (33). Only 10 to 40% of heavy drinkers develop cirrhosis (31).

Fructose and lactose may overdrive a metabolic process called glycation, which can normally appear when a small amount of these sugars is consumed. Glycation will alter the proteins in such a way that the immune system will respond to them as non-self and attack them. This process can be enhanced when these products are ingested from heated foods like browned meats and bakery products, especially those that are high in fructose and lactose. Over-consumption of refined carbohydrates leads to hyperglycemia, which also contributes to a high rate of glycation that determines an overproduction of inflammatory prostaglandins (10).

Anticonvulsants, beta-blockers, sulfonamides, estrogens, penicillin, and interferon have also been associated with autoimmunity (10).

Imbalances of gut bacteria can produce an immune response that triggers an inflammation of the mucosa which can lead to an autoimmune disease (7, 10, 11).

Liver support

In order to treat an inflammatory condition, the first step is to eliminate the cause. Because this is not possible for autoimmune diseases, the next thing to do is to apply a suppression of the response to the antigens, self or non-self.

Autoimmune hepatitis responds well to corticosteroids while primary biliary cirrhosis is manageable with ursodeoxycholic acid. Primary sclerosing cholangitis has no clear treatment, and usually ends in surgery. When nothing has worked and the conditions have evolved to the later stages, there is often a need for a liver transplant. For chronic hepatitis B and C, the viruses need to be inactivated by antiviral agents like nucleoside analogues or interferon.

If foreign substances are ingested, the liver damage can be minimized by removing that substance from the organism, as is the case for drugs or alcohol (15).

Milk thistle or **Silybum marianum,** is a well known detoxifying agent, rich in flavonoids and antioxidants and especially silymarin, extracted solely for this purpose. **Bupleurum** (Bupleurum chinense; B. falcatum) is an anti-inflammatory, as well as a stimulator of the production and release of bile, which facilitates the detoxification process. **Schizandra chinensis** is considered to have liver protective properties as it has the ability to normalize liver enzyme levels (10).

CONCLUSION

It appears that from the last century the incidence of liver disorders is getting higher by the year. Whether the causes are environment, the food we eat or unknown, these conditions are a burden on the suffering and their family and friends. Therefore there is a need for a clear big picture of the problem, in order to act specifically. Scientists may well work miracles and discover treatments, but liver conditions will still be a constant problem if we don't change our lifestyles, in a way that won't affect the environment, so that the environment won't affect us.

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FICATUL ŞI IMUNITATEA

REZUMAT

Sistemul imunitar este o compilaţie de celule şi molecule care, întrun mod silenţios, păzesc bună-starea organismului. Deoarece ficatul este cel mai mare organ la mamifere şi are un trafic continuu de substanţe diverse, fie toxice, fie hrănitoare, de virusuri sau de microrganisme, el are nevoie de o bună protecţie imunitară. Acest lucru poate să fie uneori cu două tăişuri deoarece o protecţie prea puternică poate deveni agresivă cu propriile structuri, fapt întâlnit în bolile autoimune ale ficatului. Există factori numeroşi care pot duce la diversele boli de care poate suferi ficatul, dar există de asemenea şi câteva produse de ajutor la îndemână.
Cuvinte cheie: ficat, imunitate, autoimunitate, cellule Kupffer

PREVALENCE OF CAROTID ARTERY STENOSIS AND MYOCARDIAL ISCHEMIA IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE

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ABSTRACT

The peripheral arterial disease (PAD), caused by localized atherosclerosis in the legs, is an important marker for generalized atherosclerosis and is closely related with CV events. The carotid arteries are among the vessels that are prone to develop atherosclerotic lesions in the presence of risk factors such as cigarette smoking and hypertension.

Our aim was to evaluate the incidence and the risk factors mostly associated with the occurrence of carotid artery stenosis (CAS) and myocardial ischemia in individuals with PAD.

The study enrolled 648 patients, between 2007-2011, aged >50 years, mean age 63±5 years, 53% men and 47% women, from family medicine offices of Timiş County. The median follow-up time was 34±6 months. The study inclusion criteria were defined as age between 50-79 years and the presence or history of PAD and at least one major risk factor for CV diseases. The ABI was performed using a continuous wave Doppler probe and a sphygmomanometer. The PAD was defined as a resting ABI < 0.90 or when post exercise the ABI decreased at least in one leg with >20%. Only 151 patients matched with the study inclusion criteria and were considered for carotid ultrasound and exercise stress test (EST), together with a similar control group. The carotid color Doppler scanning was performed to detect and to quantify the hemodynamic severity of the CAS. An artery lumen stenosis >50% was considered significant.

The values obtained from the ABI measurement ranged from 0.42 to 1.53, with a mean of 0.98. ABI <0.9 was found in 174 patients (27%). CAS > 50% was found in 26 patients (17%) of the study group and in 8 subjects (5.3%) of the control group (OR: 3.71; 95% CI: 1.07-11.13, P = 0.031). The prevalence of CAS increased to 34% and 50% in patients who had three or four risk factors for CV disease. EST was positive in 31 patients (20.5%) from the study group and in 17 (11.3%) control cases (OR: 2.03; 95% CI: 0.56-5.32, P=0.036). The incidence of CAS and positive EST was higher in elderly patients, smokers and in those with hypertension, diabetes or dyslipidemia.

The ABI and the carotid ultrasound proved to be easy-to-perform, cheap and non-invasive investigations, with great importance in the early diagnosis of the atherosclerosis elsewhere in the vascular system and with a significant contribution in predicting future CV events in patients at high risk.

Key words: ankle-brachial index, carotid artery stenosis, peripheral arterial disease, myocardial ischemia, exercise stress test

BACKGROUND

The patients referred to the hospital or outpatient clinic with symptomatic peripheral arterial disease (PAD) often have symptomatic or asymptomatic manifestations of atherosclerosis elsewhere in the vascular system, because atherosclerosis is a generalized and progressive process (1).

The peripheral arterial disease, caused by localized atherosclerosis in the legs, is an important marker for generalized atherosclerosis and is closely related with cardiovascular and cerebrovascular events (4). The incidence of fatal and nonfatal cardiovascular events is two to three times higher in patients with atherosclerosis in the lower limbs (5).

Although it has been shown that the PAD is a strong predictive factor for future cardiovascular events, previous studies have revealed that only a small fraction of patients are early diagnosed, most of the cases evolving asymptomatic (3,6). It is estimated that for every patient with symptomatic PAD, there are approximately three up to four undiagnosed cases (2-7). Both symptomatic and silent PAD proved to have high levels of mortality and cardiovascular morbidity (3, 9). The risk of premature death in a patient with PAD is three times higher than of the one without peripheral vascular damage (5-8). Approximately 50% of the deaths are caused by myocardial ischemia, and 15% are caused by stroke (4, 12). The presence of a co-existing cerebro-cardiovascular disease increases mortality in patients with PAD (10, 11).

The ankle-brachial index (ABI) is currently the most common and useful diagnostic method, used to detect PAD, including

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asymptomatic cases (1, 2). This has come to a widespread use of the ABI measurement in the daily clinical practice and to the identification of a high number of asymptomatic individuals with PAD. In addition to its diagnostic value, the ABI can be used in the evaluation of the generalized atherosclerosis (15, 16). Previous studies have shown that an ABI<0.9 has a specificity > 98% for PAD and > 92% in the prediction of myocardial infarction and stroke (1).

The carotid arteries are among the vessels that are prone to develop atherosclerotic lesions in the presence of risk factors such as cigarette smoking and hypertension (18). It has been shown that with high resolution two-dimensional ultrasound, the vessel wall characteristics of the carotid arteries can be assessed in an effective and accurate way at large-scale (19, 20). This technique facilitates the evaluation of the lumen diameter, the intima-media thickness and the presence and extent of the plaques of the carotid arteries (21).

The prevalence of coronary and cerebrovascular disease in the asymptomatic population has not been investigated previously and for that reason, specific recommendations on the screening for carotid stenosis or coronary artery disease cannot be made at present.

OBJECTIVE

The aim of our study was to evaluate the incidence of carotid artery stenosis (CAS) and of myocardial ischemia in individuals with PAD. We have also investigated which of the patient's characteristics were mostly associated with the occurrence of CAS and myocardial ischemia.

MATERIAL AND METHODS

Study population

The study enrolled between 2007-2011 patients from 7 family medicine offices of Timiş County. The average follow-up time was 34±6 months.

The initial study population included 648 patients aged >50 years, who underwent a complete vascular screening at the time of the enrolment to verify the study inclusion criteria and to study atherosclerosis at the other sites of the vascular bed. They were investigated by medical history, physical examination, vital signs, ECG, laboratory analysis, echocardiography and noninvasive markers for the screening of the atherosclerosis (intima-media thickness and ankle-brachial index measurement). Written and informed consent was obtained from all participants.

The study inclusion criteria were defined as age between 50-79 years, the presence or history of PAD and at least one major risk factor for cardiovascular or cerebrovascular diseases. Hypertension, diabetes mellitus, obesity, dyslipidemia and smoking were considered as CV risk factors.

The exclusion criteria were represented by age over 79 years, the patient refusal to participate in the study or who were incapable of understanding the protocol, and those with problems requiring hospitalization such as various forms of advanced cancer or other life-threatening diseases. The patients with stroke, transient ischemic attack (TIA), carotid endarterectomy, atrial fibrillation or those receiving treatment with drugs that could alter the basal ST segment were also excluded from our study.

Cardiovascular Risk Factors and Events

The patients were evaluated in terms of cardiovascular risk factors and cardiovascular events. The assessment was made considering age, sex, smoking habits, alcohol intake, glucose tolerance, systolic and diastolic blood pressure, total, and high- and low-density lipoprotein cholesterol. Medical history included data about current use of medication, alcohol intake, current and past smoking behavior.

Physical examination included the measurement of height, weight, abdominal circumference and body mass index (BMI). The body mass index was calculated as weight to height squared. The obesity was defined as BMI ≥ 30 kg/m^2. The abdominal obesity was defined according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guide as the abdominal circumference > 88 cm in women and > 102 cm in men.

The blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer in sitting and after three minutes of standing position. The average of two measurements, separated by a count of the pulse rate, obtained at two different visits, was used in the study. Hypertension was defined as a systolic blood pressure > 140 mmHg or a diastolic blood pressure > 90 mmHg or current use of antihypertensive drugs for the indication of hypertension. Diabetes mellitus was considered to be present when a subject was currently receiving oral antidiabetic drugs or insulin treatment or had a fasting blood glucose level > 126 mg/dl.

For the **laboratory analysis** we used a CardioChek-PA analyzer with PTS-Panels test strips (provided by Terapia-Ranbaxy). The blood samples were collected after an overnight fast (fasting was defined as a self-reported interval of >12 hours since the last intake of food) and were drawn for glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and ketones. Dyslipidemia was defined as total plasma cholesterol > 200 mg/dl, triglycerides level > 150 mg/dl or the current use of lipid-lowering medication.

The urinalysis was performed using Arkray AutionCheck II equipment and Arkray AutionSticks EB5 urinary test strips (provided by Sanofi-Aventis). The urine samples were analyzed for detection of glucose, albumin, creatinine, bilirubin, leukocytes, erythrocytes, nitrites. For microalbuminuria these tests have four levels of interpretation: >10 mg/l (negative), >30 mg/l, >80 mg/l >150 mg/l. In our study, microalbuminuria was defined as any different interpretation of the negative. By using this equipment we have also determined the albumin/creatinine ratio.

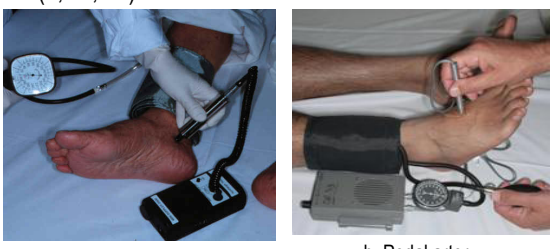
Signs and symptoms suggestive for peripheral arterial disease include: intermittent claudication (pain of the lower limb muscles appeared at walk and disappeared at rest), no pulse in the leg arteries (pedal artery, posterior tibial artery, and popliteal artery), ulcer or gangrene. The asymptomatic peripheral arterial disease was defined as ABI ≤ 0.9 in at least one of the legs, but

in the absence of intermittent claudication.

Ankle-brachial index (ABI)

The presence of atherosclerosis in the lower limbs was evaluated by measuring the systolic blood pressure (SBP) level of the posterior tibial artery and the pedal artery at both sides with an 8 MHz continuous-wave Doppler probe and a sphygmomanometer (Figure 1 a, b). The same test was performed at the brachial arteries (1).

For the calculation of the ABI, we used the highest values obtained (1). The ratio of the SBP at the ankle to the SBP at the arm was calculated for each leg. In patients with leg pain during walking and having a normal resting ABI, we used an exercise test to settle the diagnosis (1, 8). The peripheral arterial disease was considered present when the resting ABI was <0.90 or when, post exercise, the ABI decreased at least in one leg with >20% (8, 11, 17).



a. Posterior tibial artery b. Pedal artery

Fig.1. Technique for measuring blood pressure with Doppler probe

Ultrasonography of the carotid arteries

The carotid color Doppler scanning was performed to detect and to quantify the hemodynamic severity of the carotid artery stenosis. The carotid arterial scanning was made by a certified sonographist with a Sonoscape SSI-8000 high-resolution ultrasonography system, equipped with a 10-Mhz linear array transducer. The transducer aperture was 46 mm.

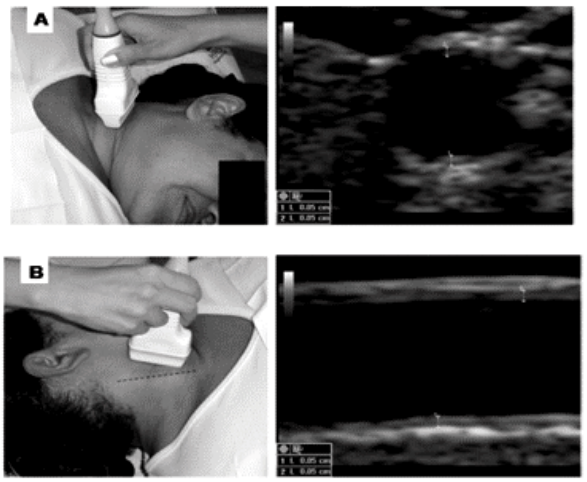
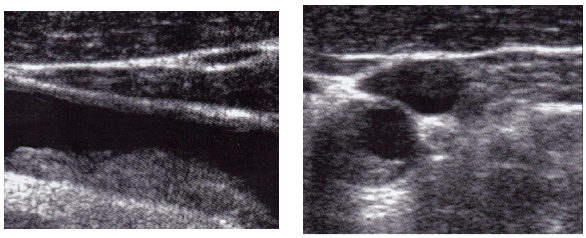


Fig.2. Angles used for carotid artery scanning: a. transversal; b. longitudinal

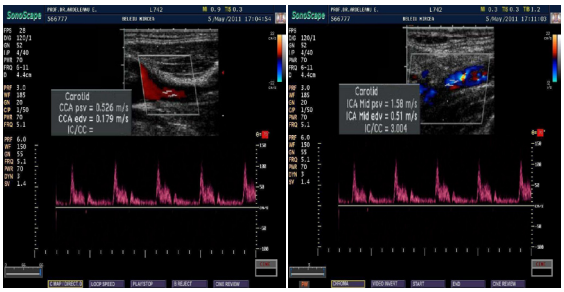
The subjects were examined in a supine position with the head rotated away from the side being scanned (Figure 2 a, b). The evaluation of the carotid plaques (mode B) was performed in longitudinal and transversal planes at the level of the common, bulb, and internal carotid artery. The presence of a plaque was defined as a thickening of the arterial wall>1.5 mm (21). Two measurements (longitudinal/ transversal) were made to determine the grade of the stenosis (Figure 3 a, b).



a. Longitudinal section b. Transversal section

Fig.3. Common carotid artery stenosis

In the stenosed area, the velocity of the flow is directly proportional to the degree of the stenosis. The highest velocity occurs at the level of stenosis or immediately downstream from the place of maximum narrowing. The severity of the stenosis can be assessed by determining, at different times of the cardiac revolution, the blood flow velocity and by calculating the ratios between the determined velocities. An artery lumen stenosis of more than 50% of the cross-sectional diameter was considered significant, and was defined as a peak systolic velocity in the internal carotid artery (ICA PSV) >125 cm/s, an end-diastolic velocity (ICA EDV) >40 cm/s, and an ICA/CCA PSV ratio >2 (Figure 4 a, b) (10).



a. Common carotid artery b. Internal carotid artery

Fig.4. Carotid artery stenosis >50%

To demonstrate the association between PAD and myocardial ischemia, the patients underwent an exercise stress test according to the modified Bruce protocol (7). A test was defined as positive if there was a decrease in the ST segment of at least 1 mm measured at 80 ms from the J point.

Only patients with ABI <0.9 and one control group (one subject for each patient, in ratio 1:1) were considered for statistical analysis.

Statistical analysis

The logistic regression analyses were performed to evaluate

the contribution of the low ABI and of each of the cardiovascular risk factors (age, sex, hypertension, dyslipidemia, diabetes, sedentary lifestyle) to the risk of carotid artery stenosis and of an abnormal exercise stress test.

Differences between patients with and without peripheral arterial disease were represented by using the χ^2 test and unpaired t-student test. P<0.05 was considered statistically significant. All the confidence intervals were calculated at a rate of 95%.

The calculations were performed using the statistical software package Statcalc Epi-Info Version 6.

RESULTS

The study enrolled, between 2007- 2011, 648 patients, aged >50 years, from 7 family medicine offices of Timiş County. The mean age of the study group was 63±5 years. A number of 346 (53.4%) were men and a number of 302 (46.6%) were women. Baseline characteristics of the initial population are presented in Table I.

Table I. Baseline characteristics of the initial population

Characteristic		Total (n=648)	ABI <0.9 (n=174)	ABI >0.9 (n=474)	P
Males (n, %)		346 (53.4)	106 (61.2)	240 (51)	0.01
Age (years)		63±5	65±7	62±4	0.01
Age groups (n, %)	50-59	239 (36.9)	35 (20.3)	204 (43)	
	60-69	260 (40.1)	73 (42)	187 (39.5)	<0.001
	70-79	149 (23)	66 (37.7)	83 (17.5)	
Smokers (n, %)		379 (58.5)	125 (71.8)	254 (53.6)	<0.001
Hypertension (n, %)		403 (62.2)	121 (69.5)	282 (59.5)	0.02
Diabetes (n, %)		221 (34.1)	75 (43.1)	146 (30.8)	0.01
BMI (kg/m^2)		28.5	29.4	28.3	0.05
SBP (mmHg)		150	155	148	0.02
DBP (mmHg)		87	89	87	0.1
LDL-C (mg/dl)		135	144	133	0.02
HDL-C (mg/dl)		44	39	45	0.02
Triglycerides (mg/dl)		148	157	145	0.01
IMT (mm)		0.96	1.11	0.79	0.01
No. of risk factors (n, %)	1	113 (17.4)	13 (7.5)	100 (21.1)	
	2	277 (42.7)	45 (25.8)	232 (49)	
	3	188 (29)	88 (50.6)	100 (21.1)	<0.001
	4	70 (11)	28 (16.1)	42 (8.8)	

Determination of the ankle-brachial index (ABI)

The values obtained from the ABI measurement ranged from 0.42 to 1.53, with a mean of 0.98. Normal ABI values, between 0.9-1.3, were recorded in 454 patients (70%). A number of 20 patients (3%) presented an ABI >1.3, 14 (70%) of whom were diabetics. An ABI <0.9 was found in 174 patients (27%), which presented the study inclusion criteria and were considered eligible for the study (Figure 5).

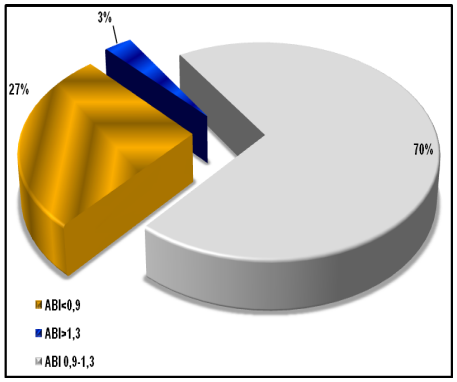


Fig.5. Distribution of the study population according to the ABI values

In 9 patients, the carotid Doppler scanning could not be performed because of logistic reasons and 14 patients did not give their consent to continue the study.

In total, 151 (23.3%) patients were included for statistical analysis. 33 subjects (21.8%) were symptomatic, while in 118 (78.2%) cases we found asymptomatic PAD (Figure 6). 94 (62.2%) of them were males and 57 (37.8%) were females.

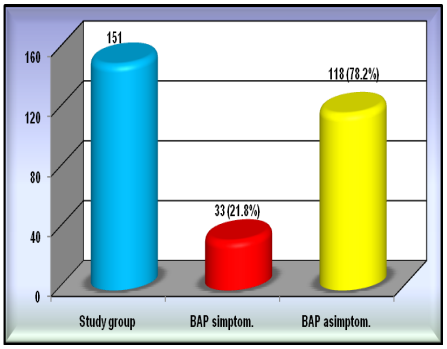


Fig.6. Prevalence of symptomatic and asymptomatic PAD in the study group

In the study group, 48 (32%) patients had one risk factor, 55 (36%) had two risk factors, 36 (24%) had three risk factors and a number of 12 (8%) had even four risk factors for cardiovascular disease.

Table II. Characteristics of the study group and control subjects

Characteristic	Study group (n=151)	Control group (n=151)	P
Hypertension (n, %)	104 (68.9)	96 (63.5)	0.01
BMI (kg/m^2)	29.4	29.2	
SBP (mmHg)	155	144	<0.001
DBP (mmHg)	89	87	
LDL-C (mg/dl)	144	140	
HDL-C (mg/dl)	39	41	
Triglycerides (mg/dl)	157	153	
ABI	0.81	1.11	<0.001
IMT (mm)	1.12	0.77	0.001

Ultrasound of the carotid arteries

Carotid artery plaques were found in 95 patients (63%) compared to 56 patients (37%) of the control group (Figure 7). 26 patients (17%) of the study group presented a carotid artery stenosis (CAS) of 50% or higher on at least one side while in the control group, a significant CAS was found in only 8 cases (5.3%) (Odds Ratio: 3.71; 95% CI: 1.07-11.13, P =0.031).

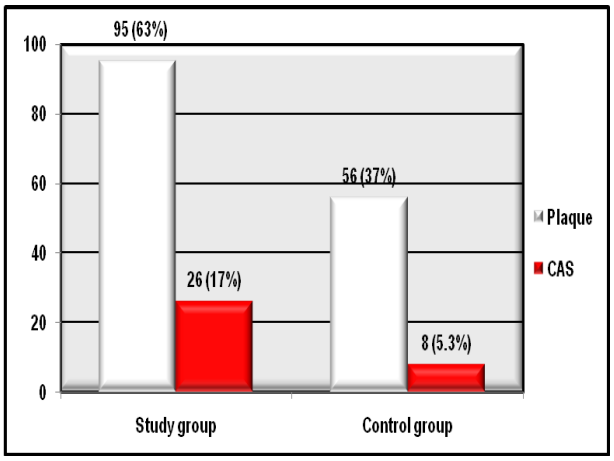


Fig.7. Incidence of plaques and CAS in the study and control groups

In the study group, 9 patients (34.5%) had a CAS of 70% to 99% (2 of them bilateral), and 15 (58%) patients had a CAS of 50% to 69% (3 of them bilateral) at least in one side. One patient had an occlusion of the right internal carotid artery and a CAS of 50% to 69% on the left side, and another patient had an occlusion on the right side and a stenosis less than 50% on the left side (Figure 8).

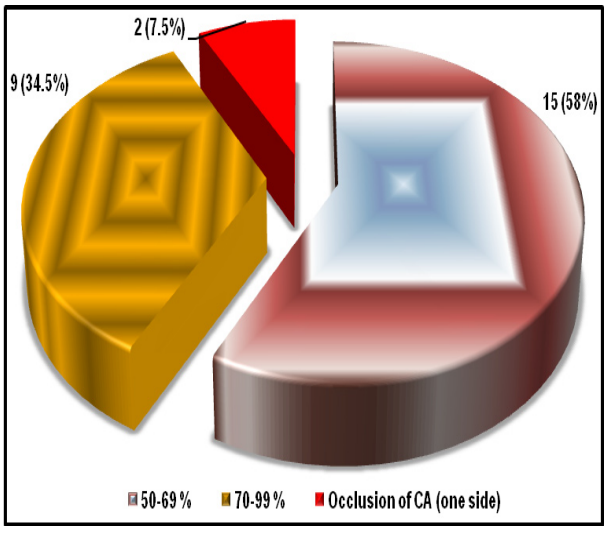


Fig.8. Types of CAS in the study group

The patients with CAS of 50% or higher were more frequently women, older, active smokers, hypertensives, with higher levels of total cholesterol, LDL-cholesterol and plasma triglycerides, presenting angina pectoris and lower ABI (Table III).

Table III. Characteristics of patients with and without CAS

Characteristics	Total study group (n=151)	CAS (n=26)	No CAS (n=125)	P
Males (n, %)	94 (62.2)	16 (61.5)	78 (62.4)	-
Age (years)	63±5	69±4	60±6	0.001
Smokers (n, %)	108 (71.5)	21 (80.7)	87 (69.6)	0.001
Hypertension (n, %)	104 (68.9)	22 (84.6)	82 (65.6)	0.001
Diabetes (n, %)	65 (43)	12 (46)	53 (42.4)	0.05
BMI (kg/m^2)	29.4	27	29.8	-
SBP (mmHg)	155	160	153	0.05
DBP (mmHg)	89	85	89	-
Total Cholesterol (mg/dl)	215	245	199	0.001
LDL-C (mg/dl)	144	161	138	0.001
HDL-C (mg/dl)	39	37	40	0.01
Triglycerides (mg/dl)	157	167	153	0.001
Angina Pectoris (n, %)	27 (18)	7 (27)	20 (16)	0.001
IMT (mm)	1.12	1.26	1.07	<0.001
ABI	0.81	0.72	0.83	<0.001

The prevalence of CAS increased from 17% (prevalence in all patients studied) to 34% (12 cases) and 50% (6 cases), respectively, in patients who had three (24%-36 patients) or four (8%-12 patients) risk factors (Table IV). The prevalence decreased to 11% (6 cases) and 4% (2 cases), respectively, in patients with only two (36%-55 patients) or one risk factor (32%-48 patients).

Table IV. Prevalence of CAS in the study group according to the number of risk factors

No. of risk factors	No. of patients (n, %)	CAS (n, %)	
		yes	no
1	48 (32)	2 (4)	46 (96)
2	55 (36)	6 (11)	49 (89)
3	36 (24)	12 (34)	24 (66)
4	12 (8)	6 (50)	6 (50)
Study group	151	26 (17)	125 (83)

Exercise stress test (EST)

The EST was valid in 135 patients. In 16 subjects, there were basal alterations in the ST segment or a left bundle branch block (8 cases), failure to reach sub-maximum heart rate (5 cases), or problems with the adaptation to the treadmill (3 cases). The EST was positive in 31 (20.5%) patients from the study group and in 17 (11.3%) control cases (OR: 2.03; 95% CI: 0.56-5.32, P=0.036). The prevalence of a positive EST was higher in subjects with older age, hypertension, diabetes, and in those with high triglycerides or low HDL-cholesterol levels (Figure 9).

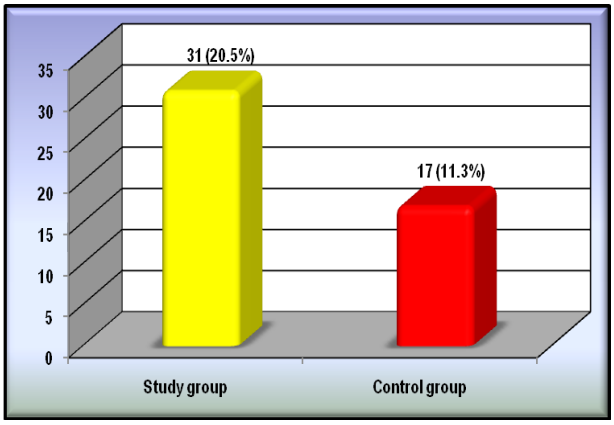


Fig.9. Prevalence of positive stress tests in the study and control groups

DISCUSSIONS

The peripheral arterial disease is widespread among the elderly, presenting an increased risk of major cardiovascular events. In the general population, the incidence of PAD range between 5 and 13% (16).In a population with atherosclerosis and cardiovascular risk factors, the PAD range between 15% and 40% (17).

The prevalence of PAD increased in elderly patients and in those with hypertension, diabetes or smoking habits (12). In our study, the ABI confirmed the diagnosis of PAD in 27% of patients, being very useful in detecting asymptomatic cases. The association of multiple risk factors for atherosclerosis determined an increased incidence of PAD.

Our study showed that the patients aged >60 years with a low ABI and without known vascular disease had a prevalence of CAS 3.7 times higher and of myocardial ischemia 2 times higher than the subjects with a normal ABI. The CAS was present in 17% of patients, mostly in women, smokers, in elderly subjects, in those with hypertension, and in those with elevated levels of triglycerides, total cholesterol or LDL-cholesterol. Similarly, the prevalence of myocardial ischemia was higher in subjects with hypertension, diabetes, advanced age, high triglyceride or low HDL-cholesterol levels.

The prevalence of CAS and of myocardial ischemia found in our study may be used to estimate the efficiency of the screening for cardiovascular disease in this population. The results are relevant given that the ABI is being increasingly used in primary-care health centers as well as in specialized outpatient clinics for improving the stratification of the cardiovascular risk.

The incidence of CAS was higher in patients with three or four cardiovascular risk factors: 34% and 50%, respectively. This characteristic can be used to increase the chance of detecting a CAS in patients with PAD but without known history of cerebrovascular disease (18, 20).

The search for CAS has been recommended in populations in which the prevalence of significant stenosis (>50%) is >4.5% and in which the annual rate of stroke is >3.3% per year (19, 20). For this reason, several clinical guidelines recommend the carotid scanning in patients with symptomatic PAD (19). The early detection of asymptomatic CAS is important to identify the potential candidates for carotid endarterectomy.

The prevalence of a positive stress test was 20.5%. The incidence of myocardial ischemia in the general population is influenced by the techniques used in the evaluation, by the age of the population and the presence of the cardiovascular risk factors. In patients with diabetes, the presence of PAD is the best predictive factor for coronary arterial disease. The subjects with a low ABI have a higher prevalence of segmental wall motion abnormalities, as showed by echocardiography (16).

CONCLUSIONS

In conclusion, our study showed that in subjects aged between 50 and 80 years and with at least one major risk factor for cardiovascular disease, the presence of a low ABI <0.9 is associated with a higher incidence of carotid artery stenosis (OR: 3.71; 95% CI: 1.07-11.13, P =0.031) and of myocardial ischemia (OR: 2.03; 95% CI: 0.56-5.32, P=0.036) than in subjects with a normal ABI.

The prevalence of CAS increased to 34% and 50% in patients who had three or four risk factors for CV disease.

The ankle-brachial index and carotid artery ultrasound proved to be easy-to-perform, cheap and non-invasive investigations, with a great importance in the early diagnosis of the atherosclerosis elsewhere in the vascular system and with a significant contribution in predicting future cardiovascular and cerebrovascular events in patients at high risk. These parameters can also improve the identification of patients at high risk.

Acknowledgement

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PREVALENȚA STENOZEI DE ARTERĂ CAROTIDĂ ŞI A ISCHEMIEI MIOCARDICE LA PACIENȚII CU BOALĂ ARTERIALĂ PERIFERICĂ

REZUMAT

Boala arteriala periferică (BAP), determinată de ateroscleroza localizată la nivelul membrelor inferioare este un important marker pentru ateroscleroza generalizată şi este strâns asociată cu evenimente cardio- şi cerebrovasculare. Arterele carotide se regăsesc între vasele predispuse a dezvolta leziuni aterosclerotice în prezenţa factorilor de risc, cum sunt hipertensiunea arterială sau fumatul.

Scopul lucrării a constat în evaluarea incidenţei şi a factorilor de risc asociaţi cu apariţia stenozei de arteră carotidă (SAC) şi a ischemiei miocardice la persoanele cu BAP.

Studiul s-a desfăşurat între 2007-2011 şi a inclus 648 pacienţi, cu vârsta >50 ani, vârstă medie 63±5 ani, 53% bărbaţi şi 47% femei, dispensarizaţi prin intermediul mai multor cabinete de MF din judeţul Timiş. Perioada medie de urmărire a fost de 34±6 luni**.** Criteriile de includere în studiu au fost reprezentate de vârstă, cuprinsă între 50-79 ani, prezenţa BAP sau istoric de BAP şi cel puţin un factor de risc major pentru boli CV. Acestora li s-a efectuat IGB folosind un dispozitiv Doppler, ei fiind supuşi şi unei evaluări detaliate din punct de vedere al factorilor de risc cardiovasculari şi al patologiei asociate. BAP a fost definită prin prezenţa IGB ≤0,9 în repaus. Criteriile de includere în studiu au fost întrunite de 151 pacienţi care, împreună cu un lot similar de control, au fost examinaţi ecografic la nivelul arterelor carotide şi au fost supuşi unui test de efort. Au fost considerate semnificative stenozele luminale mai mari de 50%. Valorile obţinute la măsurarea IGB au variat între 0,42-1,53 cu o medie de 0,98. IGB <0.9 a fost obţinut la 174 pacienţi (27%). SAC >50% au fost întâlnite la 26 pacienţi (17%) din lotul de studiu şi la 8 indivizi (5,3%) din grupul de control (OR: 3.71; 95% CI: 1.07-11.13, P =0.031). Incidenţa SAC a crescut până la 34% şi, respectiv 50%, la pacienţii care au asociat trei sau chiar patru factori de risc majori pentru boală CV. Testul de efort a fost pozitiv la 31 pacienţi (20,5%) din grupul de studiu şi la 17 indivizi (11,3%) din lotul de control (OR: 2.03; 95% CI: 0.56-5.32, P=0.036). SAC şi ischemia miocardică au fost mai frecvent întâlnite la vârstnici, fumători, diabetici şi hipertensivi.

IGB şi ecografia carotidiană s-au dovedit a fi investigaţii neinvazive, accesibile şi uşor de efectuat, având o mare importanţă în diagnosticul precoce al aterosclerozei, cu o contribuţie semnificativă în predicţia evenimentelor CV viitoare la pacienţii cu risc înalt.

Cuvinte cheie: indice gleznă-braţ, stenoză de arteră carotidă, boală arterială periferică, ischemie miocardică, test de efort