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Oct - Dec

CONTENTS

СОДЕРЖАНИЕ

Обзор

Оригинальные статьи

Клинические исследования

Дентальные исследования

Казуистика

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REVIEW

IMMUNOPATHOLOGICAL CHARACTERISTICS OF CRYPTOGLANDULAR AND CROHN'S ANORECTAL FISTULAS

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ABSTRACT

Anorectal fistulas are quite common proctologic disorders. They can be either of cryptoglandular origin or can be associated with Crohn's disease and chronic ileocolitis. Mechanical obstruction and local infections are prime causes of this pathological condition. Genetic predisposition and inadequate immune response with overproduction of pro-inflammatory cytokines appear prominently in the course of Crohn's disease. Interferon-γ, a Th1 type cytokine, reflecting the engagement of cellular immune mechanisms, is the first to be produced in the intestinal mucosa. The inflammatory process in the colon mucosa induced by the abundant microbial flora is sustained and turned chronic by the gradual elevation of the local TNF-α and regulatory cytokines levels (interleukin-10, transforming growth factor-β). The number of activated local memory T cells CD4+CD45RB^{lo} increases significantly. The regulatory CD4+CD25+ T lymphocytes producing interleukin-10 increase also trying to counterbalance the cytokine reaction. The chronic inflammatory infiltrates of the colon mucosa are represented by lymphocytes, plasma cells, macrophages. The long-term activation of macrophages by the released interferon-γ leads to tissue damage and potentiation of angiogenesis - a risk factor for carcinoma development. Management of anorectal abscesses and fistulas is complex aiming to alleviate the symptoms, prevent relapses, reduce the risk of sphincter damage and improve quality of life. The main approach (surgery) should be combined with antimicrobial infection control and immunomodulation by intravenous or local administration of anti-TNF-α antibodies.

Key words: *anorectal fistulas, Crohn's disease, cytokines, anti-TNF-α antibodies, immunomodulation*

INTRODUCTION

Anorectal fistulas (ARF) are among the most common benign proctologic diseases. Their prevalence as non-specific cryptoglandular ARF is between 8.6 and 18.4 per 100000 people per year, while as associated clinical manifestation they are found in 14-43% of Crohn's disease patients and in as much as 76% of all patients with inflammatory diseases of the ileocolon.[1-3] According to the cryptoglandular theory proposed by Prat and Eisehammer, in their former form, ARF are considered a chronic phase in the development of pathologic inflammatory abscesses in the anal glands in humans.[2] Their mechanical obstruction and local infection are main pathogenetic mechanisms in the occurrence and development of ARF.[3] In a similar way they are accepted as consequence of anorectal abscess (ARA), being along with the ulcerations of colon mucosa and strictures the main complications of Crohn's disease.[4-6] The clinical manifestations of frequently recurring and fistulizing anorectal disease lead to anal incontinence with poor hygiene and overall decreased quality of life.[7] Nowadays, Crohn's disease is considered a chronic granulomatous disease of the intestines in which the intestinal wall is thickened and inflammatory altered as a result of the genetic predisposition and development of immune response to infectious agents.[8] The basis of the process is the immune reaction, presenting with overproduction of pro-inflammatory cytokines further deteriorating the intestinal lesions typical for the disease.[9]

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ARF are classified by different criteria depending on:

- the extent of anal sphincter involvement: intrasphincteric (submucous and subcutaneous), intersphincteric, transsphincteric (low and high), extrasphincteric and suprasphincteric,
- drainage conditions: open (with active drainage) and closed, respectively complete and incomplete,
- localization of their external communication: external (perianal or open into the abdominal wall) and internal (recto-vaginal, ano-vaginal, recto-vesical and recto-urethral),
- the presence of complications: simple and complicated. Complicated fistulas result from: 1. multiple external fistulous openings (fistula orifices); 2. abscess cavities and branches; 3. proctitis.

Complicated ARF are a common finding in Crohn's disease, usually high and associated with general manifestations when abscessing. Their exacerbation is a consequence of colitis with diarrhoea, bloody secretion and anal fissures. Patients with simple (non-complicated) ARF show higher frequency of improvement and clinical recovery in comparison with patients with complicated ARF.[10]

The classical view on the problem is that there is much greater probability of recurrence of ARF in Crohn's disease due to difficulties in the combined (medication and surgical) treatment.

CYTOKINES AS "CONDUCTORS" OF THE LOCAL IMMUNE REACTION IN ANORECTAL FISTULAS

In ARF combined with Crohn's disease, the balance between pro- inflammatory cytokines (interleukin-1 [IL-1], interleukin-6 [IL-6], interferon-gamma [IFN-γ], tumor necrosis factor-alpha [TNF-α]) and regulatory cytokines (interleukin-10 [IL-10], transforming growth factor-beta [TGF-β]), is essential for maintaining the local immunity of the intestinal mucosa.[11] Cytokines are produced from various cells. IFN-γ is synthesized by Th1 lymphocytes, conducting cell-mediated immunity and affecting activation of macrophages. Its production is dependent on TNF-α levels.[12,13]

In ARA and ARF that are concomitant complications of Crohn's disease or other forms of chronic colitis, the local overproduction of pro-inflammatory cytokines IFN-γ and TNF-α along with IL-10 and IL-12, is main pathogenetic factor that sustains the inflammatory process in the intestinal mucosa. The abundant microbial flora in the terminal ileum and the colon is a powerful inducing factor for cytokine synthesis. Studies of experimental mice models show that IFN-γ is the first and the most rapidly increasing cytokine preceding the inflammatory cellular alterations in the intestinal mucosa and the activation of local lymphocytes.[4] Following the changes in IFN-γ and correlating with the severity of inflammation, the local levels of TNF-α, IL-4, IL-5 и IL-10 are raised. The last two cytokines possess modulating effect on later stages of inflammation. Increased local levels of IFN-γ may serve as a prognostic marker of recurrence of the disease. (Fig. 1)

Cytokine concentrations are measured in *in vitro* five-day cell culture supernatant of colon mucosa biopsies acquired by colonoscopy.[14] Enzyme-linked immunosorbent assay (ELISA) is used. Another possibility is immunohistochemical determination of cytokine levels in mononuclear cells from lamina propria.[15]

Our long-term clinical and scientific studies in patients with various inflammatory and infectious processes (gastrointestinal forms of salmonellosis, viral hepatitis B, Mediterranean spotted fever) convincingly show that the determination of serum and local levels of some pro-inflammatory cytokines (IL-1, IL-6, IFN-γ TNF-α) has diagnostic and prognostic significance for the development of the disease.[16-19] We therefore decided that the determination of local cytokine levels from intestinal biopsy material in patients with ARF may be used for assessment of inflammatory reaction development.

OTHER IMMUNOLOGICAL FACTORS TRIGGERING ANORECTAL FISTULAS

The role of T-lymphocytes in Crohn's disease as immune cells in the development of chronic inflammatory diseases of the colon, including complicated ARF, is demonstrated by experiments on mice with chronic ileitis and perianal fistulas.[4] Flow cytometric analysis of lymphocytes from mesenteric lymph nodes of laboratory animals shows decrease in CD4+ T-lymphocytes with naive phenotype ($CD4+CD45RB^{hi}$ population), while the percentage of activated memory cells $CD4+CD45RB^{lo}$, as well as of regulatory CD4+CD25+ T-lymphocytes (T regs), expressing alpha-chain of IL-2, is significantly greater. The increase of cells with activation markers and of T regs with disease progression is a sign of occurrence of memory and activated T-cells. Activated T regs in intestines play an important role in

Folia Medica 2010; 52(4): 5-12

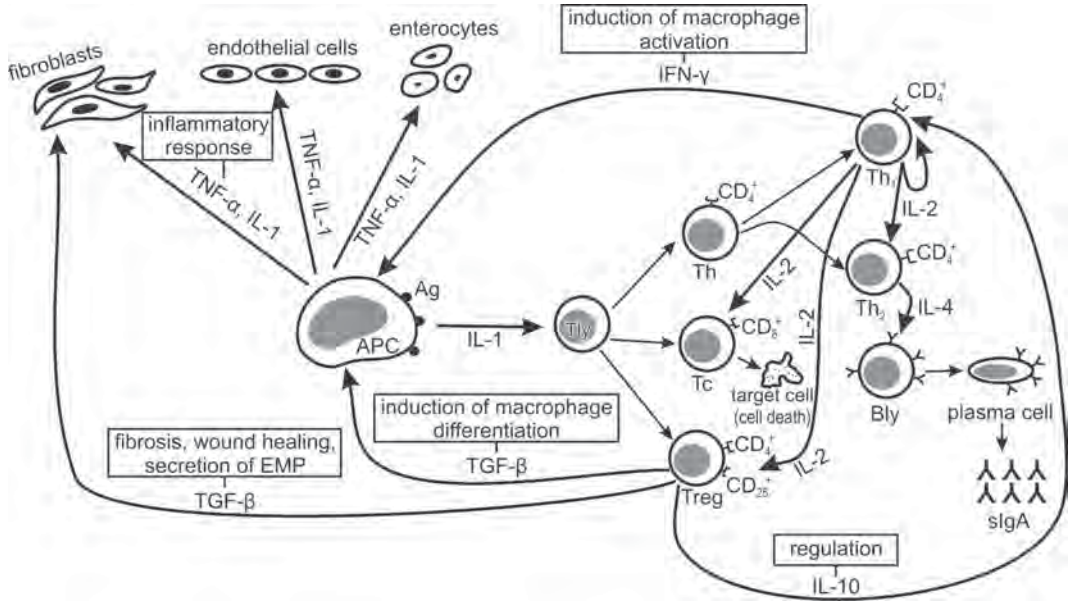

Figure 1. Intercellular interactions through cytokines in the intestinal mucosa in anorectal fistulas with Crohn's disease.

The contact of antigen presenting cells (APC) - dendritic cells and macrophages, with microbial antigens leads to activation of intestinal T lymphocytes in Peyer's patches and lamina propria after stimulation with IL-1. One of the T-helper subpopulations - Th1, produces IL-2 for proliferation and differentiation of T and B cells. Th1 also release IFN-γ, stimulating the activation of intestinal macrophages which begin to synthesize the pro-inflammatory cytokines TNF-α and IL-1 with cytotoxic effect, blood vessels alterations and fever. IL-2 also stimulates other T cells - Th2, which synthesize IL-4, helping the differentiation of B lymphocytes to plasma cells which in turn produce secretory IgA. T-regulatory cells (T reg) play controlling role over the inflammatory process in the intestinal mucosa. They produce regulatory TGF-β and IL-10. TGF-β supports the differentiation of macrophages, the secretion of extracellular matrix proteins (EMP), wound healing and tissue fibrosis. IL-10 inhibits the cell released IFN-γ and neutralizes the inflammatory effect. A small part of cells involved in the inflammatory reaction are cytotoxic T lymphocytes (Tc), which, activated by IL-2, kill the microorganism-infected target cells.

the control of cytokine imbalance.[14] Isolated from intestinal mucosa of animals with inflammatory diseases of the colon, they are considered intestinal inflammation regulators and responsible for induction of immune tolerance in the colon mucosa via IL-10 production.

Continuous inflammatory process in ARA and ARF associated with certain microbial agents is a major factor of immune suppression in patients. Abscess etiology is polymicrobial, being associated in over 70% of the cases with obligate (*Bacteroides fragilis*, *Peptostreptococcus spp*., *Prevotella spp*., *Fusobacterium spp*. etc.) and facultative anaerobic (*S. aureus, Streptococcus spp*., *Escherichia coli*) bacteria.[13] Brook and Frazier[20] report the highest participation of Gram positive bacteria (staphylococci and streptococci), followed by *E. coli* among facultative anaerobes. Our previous studies show that *E. coli* and other gram negative bacteria are isolated in more than half of the patients with abscess forms of ARF, and gram positive bacteria (enterococci, staphylococci) account for one third of the isolated facultative anaerobes.[21] We consider the high percentage of *E. coli* isolated from the investigated purulent material to be a disturbing fact since, according to Hamalainen and Sainio, these cases are prone to chronification with fistulization of the process.[22] Risk of sepsis development is present in considerate share of the patients.[20] Based on the hypothesis that prolonged inflammation caused by infectious agents leads to immunosuppression, our investigations of peripheral blood from ARF patients prove functional insufficiency of phagocytic reaction with imbalance in NBT test, total leucocyte count and neutrophil percentage.[21] Humoral factors of systemic acquired immunity (serum immunoglobulins A, G and M) are however scarcely engaged in the abovementioned inflammatory processes, which implies the predominant role of local immune response.

On the other hand, the overactivation of the immune system in Crohn's disease is related to the occurrence of autoimmune or allergic reactions. In 13 to 100% of the patients with Crohn's disease, including patients with ARF, perinuclear anti-neutrophil cytoplasmic antibodies (p-ANCA) are

found.[23] Allergy to cow milk proteins is considered to be one of the reasons for ARF and anal fissures development in Crohn's disease, since it leads to chronic obstipation and ileoproctitis.[24]

LOCAL IMMUNOHISTOLOGICAL ALTERATIONS IN COLON MUCOSA IN ANORECTAL FISTULAS

The morphological histological alterations in the colon mucosa of patients with Crohn's disease also provide evidence of involvement of immune mechanisms in the development of ARA and ARF as complications of inflammatory diseases of the colon. In these cases the inflammatory reaction is presented by mononuclear and polymorphonuclear cells in lamina propria and neutrophils in the epithelium.[25] Granulomatous alterations with polynuclear giant cells in the colon mucosa - a sign of chronic inflammation, may be found in patients with Crohn's disease. Except for macrophages, lymphocytes and plasma cells also infiltrate the intestinal mucosa. Prolonged activation of macrophages leads to tissue damage due to the released by them substances, one of which - nitric oxide (NO), directly damages DNA, inhibits DNA reparative enzymes, suppresses caspase activity and apoptosis, and stimulates angiogenesis.[26] The inflammatory process generates pro-inflammatory cytokines and prostaglandins, which also potentiate angiogenesis. Cancerogenic products like reactive oxygen species are produced at the site of the chronic inflammatory reaction. That is the reason for increased risk of carcinoma in case of prolonged inflammatory benign alterations in the intestinal mucosa in ARA and ARF, as well as in anal fissures.[26,27] These benign anorectal diseases also enhance the negative role of human papillomavirus (HPV) in cancerogenesis initiation facilitating its access to the anal epithelial cells.

In allergy-associated chronic obstipation in patients with Crohn's disease the immunohistologic alterations of the rectal mucosa are presented with cubic or plasmatic metaplasia of the epithelium, infiltration with plasma cells and lymphocytes and presence of lymphoid nodules in lamina propria.[24]

The findings of local immune alterations in patients with ARF are supported convincingly by experiments with mice with chronic ileitis and fistulizations.[4] The ulcerations of anal mucosa, the fissures near the anus and ARF in the mice are accompanied by occurrence of chronic inflammatory infiltrates of lymphocytes, plasma cells and macrophages in the anus and the rectum, as well as by accumulation of neutrophils in the perianal soft tissues. Loss of normal villous architecture of the intestinal mucosa and thickening (to the extent of hypertrophy) of the muscle layer of the intestinal wall from the distal to the proximal compartment of the ileum are also observed.

NOVEL COMBINED TREATMENT (SURGERY AND IMMUNOMODULATION) OF ANORECTAL FISTULAS IN CROHN'S DISEASE

The modern therapy used for ARFs, especially the type presenting as complications of Crohn's disease or chronic inflammatory diseases of the colon, is still a challenge to surgeons and gastroenterologists, because full recovery and prevention of disease recurrence are hard to achieve while keeping a better quality of life.[25] There are two main approaches in the treatment of ARF in Crohn's disease: surgery and conservative treatment.[28,29] (Table 1). The current trends demand definitively a combined therapy using both approaches.[30,31]

Surgery is the classical therapeutic approach in treating ARA and ARF in Crohn's disease. The goals in this approach are symptom alleviation, ARF-associated draining of ARA, reduction of disease recurrence and danger of damaging the anal sphincters by preventing the development of anal incontinence, even to the point of avoiding the need for proctectomy in some rare cases. Currently, surgical interventions in this field include: opening of the fistula tract by one- or two-stage fistulotomy (in low ARF); seton treatment with non-cutting (draining) ligatures (in higher fistulas with involvement of considerable part of the external anal sphincter), or plastic covering of the internal fistulous opening with endorectal forward-shifted flap (as an alternative to the two classical methods but possible only in patients without macroscopic signs for rectal inflammation).[5,32,33] In ARF, complicated with Crohn's disease, a trend for less aggressive surgical approaches, including drainage by insertion of non-cutting setons, is promoted, since more aggressive surgical procedures increase the risk of anal incontinence.[31] For these complicated cases we agree with most of the authors and prefer to use the most novel surgical techniques for obturation of ARF by instilling fibrin glue into them to attach them, or by occlusion of the lumen by implantation of biological anal fistula plugs, manufactured from porcine small intestinal submucosa - Surgisis®-Anal Fistula Plug.[31,34] The surgical treatment we have been performing for several years in patients with chronic ARF, including instilling of fibrin glues, is safe, easy to perform and effective in more

Folia Medica 2010; 52(4): 5-12

Table 1. Novel combined treatment of anorectal fistulas in Crohn's disease

Approach	Type of intervention or medication	Comments
Surgical	Fistulotomy	In low ARF
	Seton with drainage ligature	In high ARF
	A plastic cover of the internal fistula opening with endorectal flap	Alternative to 1 and 2; in patients with no visible rectal inflammation
	Drainage by inserting non-cutting setons	In complicated ARF with Crohn's disease
	Obturation by instilling fibrin glue or by occlusion of the lumen by implantation of biological anal fistula plugs	In complicated ARF with Crohn's disease
Medications	Standard medications	
	- orally: 5-aminosalycilates	
	- antimicrobial	
	- corticosteroids	
	- immunomodulators	
	Hyperbaric oxygen therapy	In severe perianal complications of Crohn's disease
	Anti-TNF-α antibodies	In patients, resistant to standard therapy; neutralizes TNF-α and lowers IFN-γ

than 70% of the patients, preventing development of anal incontinence and the need of additional therapy.[35,36]

Drug therapy in perianal fistulas in Crohn's disease includes: 1. standard medications, including immunomodulators; 2. hyperbaric oxygen therapy and 3. anti-TNF-α antibodies.

The drug typically used here are: oral 5-aminosalycilates; antimicrobial drugs (metronidazole, ciprofloxacin); corticosteroids for systemic and topical application (prednisone and beclometasone dipropionate), and immunomodulators (azathioprine, cyclosporine, 6-mercaptopurine, methotrexate, tacrolimus).[37] Despite their application, the relapses of ARF are quite frequent and the fistulas are difficult to heal. Furthermore, the immunomodulators applied in these cases have serious side effects such as pancreatitis, hepatitis and severe leucopenias. Hyperbaric oxygen therapy is still not widely accepted alternative method. It is applied in patients with severe perianal complications of Crohn's disease. The therapy's mechanism of action is growth suppression of anaerobic microorganisms which in addition to inducing local inflammation leads to ARA with development of ARF. It is carried out in series of hyperbaric pressure of 2.5 atm for 20 to 90 min, accompanied by local administration of 100% oxygen.[38]

The interest in investigating the cytokine imbalance in the intestinal mucosa in inflammatory intestinal diseases does not subside due to the appealing possibility that pro-inflammatory mediators are neutralized through proper therapy. Therapeutical approaches are focused on application of anti-inflammatory molecules. The need for such an approach is determined by the fact that complicated ARA and ARF in Crohn's disease may not be always successfully treated with surgical interventions because of risk of anal incontinence.

Candidates in the modern strategy for modulation of the inadequate immune response in Crohn's disease are two biological drugs: 1. humanized chymeric antibody against TNF-α (infliximab) and 2. human anti-TNF-α antibody (adalimumab).[39,40] The application of TNF-antagonists from the mid-90's of the last century is a novelty in the treatment of Crohn's disease and ulcerative colitis, especially in patients who are resistant to therapy with conventional medications like corticosteroids and immunomodulators.

Infliximab has local mechanism of action on the intestinal mucosa, related with neutralization of TNF-α.[41] The antibody suppresses also the quantity of T-cells, producing TNF-α cytokine in lamina propria by stimulation of their apoptosis and reduction of IFN-γ production by intestinal T-cells.[14] The reduced cytokine production after the treatment correlates with clinical improvement in about half of the patients - remission of the disease or even healing of the fistulas.

Treatment with Infliximab in Crohn's disease is conducted either by intravenous infusions or by local injections. Venous infusions are three times daily in a dose of 5 mg/kg for 2 hours, at certain intervals - most often at 0, 2 and 6 weeks.[14,42,43] Application of prolonged infusions (for more than 40 weeks) is possible. In case of systemic therapy with infliximab the following side effects may be observed[44-47]:

- autoimmune reactions - appearance of antinuclear antibodies or antibodies against infliximab in 7-10% of patients;
- anaphylactic and delayed type hypersensitivity reactions in repeated application in 20% of patients;
- infectious complications - reactivation of tuberculosis and pulmonary infections;
- appearance of carcinomas or lymphomas.

Local injections are applied in the treatment of non-complicated ARF in Crohn's disease.[28,48] Infliximab in a dose of 20 mg dissolved in 10 ml saline is injected in the primary fistula tract and around the external fistulous opening. The procedure is preceded from application of anesthetic drug (5 ml lidocain in 3 ml 8.4% bicarbonate solution). Three to five injections of infliximab are applied each week until the secretion entirely discontinues.[28] The effect is considered positive if reduction or total cease of fistula secretion for at least 4 weeks is achieved. External perineal fistulas are better treated by topical application in comparison to the internal (recto-vaginal and entero-vesical) ones.[28,49] The local treatment with infliximab solution is believed to prevent the side-effects observed in systemic infusions.

Adalimumab is applied in dosage of 80 or 160 mg weekly at the beginning of the therapy and 40 or 80 mg after the second week. It is followed by supportive treatment with 40 mg every second week.[37] Studies of patients with fistulizing Crohn's disease show good effect of the medication in achieving remission.[50,51] Generally, TNF antagonists stimulate ARF healing, increase the effectiveness of surgical intervention and reduce the risks associated with it.

CONCLUSIONS

Anorectal abscesses and fistulas are frequent complications of cryptoglandular disease and Crohn's disease or chronic inflammatory bowel disease. The etiology and pathogenesis of their occurrence and development, as well as of the diseases associated with them, are not quite clear. The prolonged inflammation of the colon wall causes local hyperproduction of pro-inflammatory and regulatory cytokines. The intestinal inflammation is controlled locally by a special population of regulatory T-cells. The histological alterations in the intestinal mucosa are represented by macrophages, lymphocytes and plasma cells. By presenting the main immuno-pathogenic mechanisms playing important role in the development of the inflammatory process in these diseases, we would like to underline the necessity of complex therapeutic approach in ARF, especially for those associated with Crohn's disease. This approach is concurrent with the modern trends for combined therapy - surgical treatment, antimicrobial control of infection and immunomodulation. Most of the research show rather encouraging results in using anti-TNF-α antibodies.

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ИММУНОПАТОЛОГИЧЕСКИЕ ОСОБЕННОСТИ АНОРЕКТАЛЬНЫХ ФИСТУЛ КРИПТОГЛАНДУЛЯРНОГО ПРОИСХОЖДЕНИЯ И ФИСТУЛ ПРИ КРОНА БОЛЕЗНИ

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РЕЗЮМЕ

Аноректальные фистулы представляют собой одни из самых часто встречаемых проктологических заболеваний. Это фистулы криптогландулярного происхождения или фистулы, связанные с болезнью Crohn-а и с хроническим илеоколитом. Ведущим механизмом в их развитии являются механическая обструкция и локальная инфекция. При Крона болезни наследственная предрасположенность и появление неадекватного иммунного ответа со сверхпродукцией проинфляматорных цитокинов являются основными моментами в ходе заболевания. В кишечной слизистой оболочке раньше всего продуцируется интерферон-гамма Th1 тип цитокин, маркирующий ангажирование клеточных иммунных механизмов. Постепенное повышение локальных уровней опухоль-некрозис фактора-альфа и регуляторных цитокинов (интерлейкин 10, трансформирующий ростковый фактор-бета) приводит к поддержанию и хронифицированию воспалительного процесса в слизистой толстой кишки, индуцированного богатой микробной флорой. Значительно увеличивается относительная часть локальных активированных Т клеток памяти CD4+CD45RB^{lo}. Повышается и число регуляторных CD4+CD25+ Т лимфоцитов, продуцирующих интерлейкин 10, пытаясь контролировать дисбаланс в цитокиновой реакции. Хронические воспалительные инфильтраты в слизистой оболочке толстого кишечника представлены лимфоцитами, плазматическими клетками, макрофагами. Продолжительное активирование макрофагов отделенным интерфероном-гамма приводит к повреждению ткани и потенцированию ангиогенеза (фактор риска для развития карцином). Лечение аноректальных абсцессов и фистул комплексно и ставит себе целью облегчить симптомы, предотвратить рецидивы и улучшить качество жизни. Ведущий подход - хирургический - желательно комбинировать с антимикробным контролем за инфекцией и иммуномодуляцией, где основное место занимает венозное или локальное применение анти-TNF-антител.

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ORIGINAL ARTICLES

Clinical Investigations

NON-INVASIVE VESSEL EXAMINATIONS IN CARRIERS OF LDL-RECEPTOR DEFECTIVE GENE VERSUS NON-CARRIERS WITH NEWLY DETECTED ASYMPTOMATIC SEVERE HYPERCHOLESTEROLEMIA

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ABSTRACT

The results of the research of early vascular alterations in LDL-R carriers in comparison with those in non-carriers with severe hypercholesterolemia are controversial.

Aim: To investigate the difference between severe hypercholesterolemia patients that carry LDL-R defective gene and those that do not have it, in their functional (flow-mediated vasodilation) and structural (intima-media thickness of carotid artery and ankle-brachial index) characteristics of arterial wall.

Pateints and methods: The study included 120 hypercholesterolemic patients. Biochemistry parameters were studied by routine methods. The flow-mediated vasodilation (%FMD), ankle-brachial index (ABI) and intima-media thickness (IMT) of common carotid artery were determined using Hewlett Packard Sonos 5 500; MedicaSoft. IMT.lab was the software programme used in the study.

Results: There was no significant difference between the groups with respect to total cholesterol, LDL, HDL, Apo-B, Apo-A_1, cellular adhesion molecules (sICAM-1, sVCAM-1, sP- and sE-selectine). The Apo-B/Apo A_1 index differed significantly ($t = 11.23$, $p < 0.001$) between the two groups; there was difference even after adjustment for age. There was no significant difference in the endothelial dependent and independent vasodilatation between the examined groups ($p > 0.05$). We found a significantly greater carotid IMT and lower ABI in the carriers than the respective parameters in the non-carriers. This significant difference was confirmed after adjustment for age.

Conclusion: Our data show that LDL-R carriers have a higher carotid IMT and lower ABI than non-carriers, whereas no difference between the groups was found with respect to the level of lipid parameters and %FMD.

Key words: *LDL-receptor, flow-mediated vasodilation, intima-media thickness, ankle-brachial index*

INTRODUCTION

International recommendations for treatment of hypercholesterolemia stress the importance of diagnosis and treatment of asymptomatic individuals with high absolute cardiovascular risk. Individuals with marked hypercholesterolemia fall into this group as well. Familial hypercholesterolemia (FH) can be due to different genetic defects in the LDL-R, which lead to impaired LDL clearance.[1,2] Lipoprotein levels are known to be of multifactorial genesis and are the result of the interaction of about 100 genes with various environmental factors.[1] Mutations of the LDL-R-gene are characterized by a high genetic heterogeneity.[2] Nearly 80% of the population specific heterogeneity is due to point mutations of the LDL-R gene. This fact requires genetic scanning of all exons. Monogenetic hypercholesterolemia is more frequent than it was thought before and it remains undiagnosed. On the other hand, the early diagnosis and the identification of the underlying genetic mutation are very important for risk assessment and therapy. The different expression of the

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defect gene in LDL-R mutation carriers as well as the presence of elevated LDL levels in non-mutation carriers makes diagnosis difficult. So far, there has been no optimal diagnostic algorithm in the clinical diagnosis of FH. Research efforts are focused on finding solutions that would support everyday clinical practice. It is known that routine lipid parameters are little informative with respect to identifying carriers or non-carriers of LDL-R defects.[3-6] Severe hypercholesterolemia and family history of early vascular diseases are important determinants of the development of the intima-media complex of the common carotid artery (IMT CCA), ankle-brachial index and flow-mediated vasodilation (%FMD) in FH.[7-18] The data reported in the literature from the investigation of early vascular alterations (functional - using %FMD, and structural - by measurement of the IMT CCA and ankle-brachial index) in carriers of molecular defects of the LDL-R in comparison to non-carriers with severe hypercholesterolemia are controversial.[12-25] Evidence on this issue could be used in developing the treatment algorithm of newly detected severe hypercholesterolemia. Most of the studies on severe FH are carried out in patients with manifested coronary artery disease[3-6]; data about patients with asymptomatic FH are scarce.[19-25]

AIM

To examine the difference between patients with newly detected severe hypercholesterolemia, who are carriers and non-carriers of LDL-R defective gene, with respect to their functional (%FMD) and structural (IMT CCA and ankle-brachial index) characteristics of arterial wall.

PATIENTS AND METHODS

Between June 2005 and September 2009 a total of 550 patients with primary hypercholesterolemia were examined in the Preventive Cardiology Surgery of the Clinic of Cardiology, St George University Hospital, Plovdiv. Of these, only 120 (age over 16 years) met the Simon-Broome register criteria (Table 1).[26] The patients were selected on total cholesterol criteria, family history of hypercholesterolemia or premature myocardial infarction and whether the subjects were examined for the presence of tendon xanthomas.

The exclusion criteria's are presented in Table 1. The table includes all known conditions besides hypercholesterolemia which are associated with development of endothelial dysfunction (Table 1). According to whether there were or were not molecular defects, patients were assigned to two groups: carriers (22 patients, 18%) and non-carriers (98 patients, 82%).

Prior to the study a written informed consent was obtained from the hypercholesterolemic patients. The procedures used in this study were approved

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
1. Total cholesterol level > 7.5 mmol/l in people over 16 years of age. 2. Tendon xanthomata in first or second degree relatives. 3. A myocardial infarction before age 60 in first degree relatives and before age 50 in second degree relatives. 4. Total cholesterol > 7.5 mmol/l in first or second degree relatives. A diagnosis of definitive FH requires meeting criteria 1 and 2. A diagnosis of possible FH requires meeting criteria 1 and 3 or 1 and 4.	1. Diabetes mellitus, impaired glucose tolerance or fasting blood glucose > 5.6 mmol/l. 2. Cigarette smoking. 3. Clinical and laboratory evidence of: 3.1. Coronary artery disease (CAD) in all forms 3.2. Cerebrovascular disease 3.3. Arterial hypertension 3.4. COPD, bronchial asthma 3.5. Chronic arterial insufficiency of the extremities (peripheral arteries) – ABI < 0.9 3.6. Chronic renal and hepatic dysfunction 3.7. Systemic disorders of connective tissues – collagenosis, rheumatoid arthritis, SLE 3.8. Neoplasms 3.9. Acute inflammation or chronic inflammatory process requiring active treatment. 4. Long-term use of NSAID (over the last six months and during the period of investigation), corticosteroids, hormonal medications, psychotropic drugs, lipid modifying drugs (fibrates, statins, antioxidants). 4.1. Chronic use of alcohol and drug abuse.

Folia Medica 2010; 52(4): 13-22

by the Ethics Committee at Medical University of Plovdiv.

All study subjects were evaluated with respect to any family history of early-onset coronary artery disease, clinical history, medication use, anthropometric characteristics, cardiovascular risk factors and tendon xanthomas. All patients were asymptomatic and had not taken statins before the beginning of medical examination (Table 2).

Laboratory tests were performed at the Central Clinical Laboratory of St George University Hospital, Plovdiv. The biochemical parameters of blood glucose, total cholesterol, triglycerides, high density lipoprotein cholesterol, urea, creatinine, and uric acid were measured using a biochemical analyzer Konelab 60i (Thermo Electron Co, USA). Determination of LDL-cholesterol was performed using a direct analysis and reagents from Thermo Electron Co KonelabTM (Finland).

MOLECULAR BIOLOGICAL ANALYSIS

The present study included 120 DNA samples of FH patients of both genders. The generic analysis used samples of high-molecular DNA isolated from nuclear blood cells. The blood samples were withdrawn 30 min to 1 hour after meals in plastic tubes with EDTA anticoagulant and stored at +4°C for 48 hours. The technique involved several stages: 1. Isolation of DNA; 2. Amplification of a specific target of DNA fragment using polymerase chain reaction; 3. A single strand conformation polymorphism analysis; 4. Direct sequencing. The R3500Q-mutation in the Apo-B gene was sought first. LDL-R gene mutation and polymorphism (and the promoter region), as large rearrangements, were then identified using denaturing gradient gel electrophoresis and DNA sequencing of the abnormal exon. When these analyses were negative, DNA was subjected to long-range polymerase chain reaction (PCR).

Determination of %FMD of brachial artery was performed based on Celermajer's recommendation (1992) and on %FMD manual book (2002).[27-28] The diameter of the brachial artery was measured by a 7.5 MHz transducer of Hewlett Packard 5 500, using automated computer software MedicaSoft. IMT.lab. A marker was placed at the starting point (the leading margin of the intima-lumen surface of proximal wall) and at the end point (10 mm away from the starting point). The diameter was measured automatically to the distal intima-lumen wall at the same distance. The percentage variation in the diameter of brachial artery was determined following a 5-min compression with the cuff of the blood pressure monitor up to 50 mm Hg

Table 2. Baseline characteristics of the examined hypercholesterolemic patients

Variables		Carriers (n = 22) Mean ± SEM	Non-carriers (n = 98) Mean ± SEM
Sex Male		58 (48.3%)	58 (48.3%)
Sex Female		62 (51.7%)	62 (51.7%)
Age (years)		46.68 ± 0.38	46.24 ± 0.43
Body mass index	(m/kg^2)	24.60 ± 0.55	24.36 ± 0.39
Waist circumference	(cm)	86.40 ± 2.05	84.05 ± 0.90
Arterial pressure	(mmHg)	115 ± 12	115 ± 12
Glucose	(mmol/l)	4.5 ± 0.4	4.6 ± 0.3
Alanin aminotransferase	(U/L)	33 ± 2	30 ± 3
Creatinine	(μmol/l)	105 ± 12	107 ± 15
C-reactive protein		4 ± 2	4 ± 1
Gamma glutamil transpeptidase	(U/L)	34 ± 10	33 ± 11
Total cholesterol	(mmol/l)	9.54 ± 0.47	8.55 ± 0.14
Triglycerides	(mmol/l)	1.50 ± 0.49	1.44 ± 0.34
HDL-cholesterol	(mmol/l)	0.95 ± 0.02	1.01 ± 0.24
LDL-cholesterol	(mmol/l)	7.42 ± 0.04	6.77 ± 1.90
Cholesterol × years score	(mmol-y/L)	445.32 ± 0.20	395.35 ± 0.06
Apolipoprotein-B	(g/l)	2.74 ± 0.11	2.08 ± 0.39
Apolipoprotein-A_1	(g/l)	1.21 ± 0.22	1.19 ± 0.15
Apolipoprotein-B/A_1		3.32 ± 0.61	2.48 ± 0.59

above the measured systolic blood pressure. The non-dominant arm was used. The examination was performed at a room temperature of 22-24°C. The subjects had fasted for 8-12 hours, and were advised to abstain from coffee, vitamin C and vasoactive drugs. Ten minutes after determination of %FMD, nitroglycerin (NTG) mediated vasodilation was achieved by administration of 0.4 mg NTG. The non-endothelium dependent vasodilation was determined at 4 minutes.

IMT was measure by the method of Pignoli et al.[29] MedicaSoft.IMT.Lab automated computer software was used to measure IMT CCA. The upper US demarcation line was defined as leading margin (intima-media border), the lower line was defined as a distant margin (media-adventitia border). The leading line is important in the automatic measurement. Using the left mouse button, a line is drawn parallel to the distant arterial wall. The leading and end measurement points are fixed at 10 mm apart. The IMT measurement is performed automatically - maximum, medium and minimal size, quality index and standard deviation.

ABI was measured by 7.5 MHz Hewlett Packard SONOS 2500 transducer and sphygmomanometer. Systolic arterial pressure was taken of both hands by blood stream in fossa decubitalis. Measurement of systolic arterial pressure was performed in the same way for lower limbs - by Doppler effect of blood stream in a. tibialis posterior and a. dorsalis pedis. The higher values of systolic arterial pressure of both brachial arteries were taken, respectively these of the arteries of lower limbs. ABI was determined for each lower limb as correlation between the higher SAP of lower to upper limbs. The lower index value of left and right side was taken. We made six measurements for each patient. Intima media complex of carotid artery was measured by the same transducer. The dimensions of both carotid arteries (right and left) were taken in the distal 10 mm of a. carotis communis. Automatic measurement on the carotid echograms was performed by MedicaSoft.IMT.Lab automatic programme. The higher demarcation echographic line was defined as a “leading fringe” (border intima-media) while the underlying line was named “far fringe”. The leading line is independent from gain tuning therefore it is the main line of automatic gauging. ABI was separated as: border: 0.9-0.99, low-normal: 1.0-1.09, normal: 1.1-1.29.[13]

Statistical analysis was carried out using the SPSS v.14.0 statistical software (SPSS Inc. Chicago, III). Descriptive statistics, the Student’s t-test, Pearson’s correlation coefficient, and analysis of covariance (ANCOVA) were used in the statistical analysis. We studied the distribution of all continuous data by applying a normality test on the distribution (one-sample Kolmogorov-Smirnov test). Results were expressed as mean ± standard error (SEM). $P < 0.05$ was used as a level of significance of the null hypothesis.

RESULTS

1. Characteristics of the study population - age, sex and anthropometric parameters

There was a statistically significant difference ($p < 0.001$) in the age distribution of patients, carriers and non-carriers of the molecular defects. This conclusion was confirmed by the calculated mean age in both patient groups. The mean age of non-carriers was 46.24 ± 0.43 years, the mean age of carriers - 46.68 ± 0.38 years. There were no statistical differences in the sex distribution in the study sample ($\chi^2 = 0.05$; $p > 0.05$). We found no statistically significant difference between non-carriers and carriers with respect to body mass index (24.36 ± 0.39 vs 24.6 ± 0.55, respectively, $t = 0.60$; $p > 0.05$). When waist circumference was compared in the groups, no statistically significant difference was found - waist circumference was 84.05 ± 0.90 cm in non-carriers, and 86.40 ± 2.05 cm in carriers (values within reference limits).

2. Molecular biological analysis

There were no objective and echocardiography evidence of xanthoma of the Achilles tendon in the patients. The most common family defect was in Apo-B caused by a substitution mutation at nucleotide position 10 780, which results in replacement of Arg in the defected polypeptide chain by Gln at position 3500. There was no mutation of this type in the study population in patients with total cholesterol above 7.0 mmol/l. The second stage included testing for spot mutations in the LDL-R gene. We screened all 18 exons of the LDL-R gene (apart from the recommended analysis of exons 6, 4 and 9).[3,5]

The results of the molecular analysis showed that spot mutations were found mainly in exons 5, 9 and 11 of patients with the clinical diagnosis of FH. Forty-five different mutations were documented in all 18 exons of the LDL-R gene - 4 in exon 4b, 5 in exon 5, 6 in exon 6, 2 in exon 8, 11 in exon 9, 7 in exon 11. The mutation 1073G > A was detected for the first time; it is not listed even in the most extensive mutation databases.

Folia Medica 2010; 52(4): 13-22

Nine polymorphisms were described (Table 1). In one patient, both polymorphism and 1646G > A mutation were detected in exon 11. The G > A mutation at position 1646 (FH Palermo-1 missence mutation, class 2A) leads to amino acid substitution G528D in EGF precursor domain of the LDL-R gene. LDL-R activity is reduced by 2% as a result. In exon 11 the polymorphism C > T at 1617 (StuI) causes no amino acid substitutions or splice mutations and therefore is not associated with elevated serum cholesterol levels. The C > A mutation at 858 (FH Greece-2) in exon 6 is a missence mutation; it induces the amino acid substitution S265R in LB7 region of the LDL-R gene. It is a class 2 mutation and is associated with a reduced activity of LDL-R by 10-15%. The T > C mutation at 1061-8 in intron 7 is a splice mutation in the precursor domain. The G > A mutation at position 1073 in exon 8 is a novel mutation detected first in this study. The G > A mutation at 1195 in exon 9 is a missence mutation. In exon 4b, the mutation G > A at 590 (FH El-Salvador) is a class 2 mutation; it is associated with amino acid substitution C176Y. It results in destruction of the SS bonds in LB5 leading to altered folding of the LDL-R. This molecular defect has also been associated with 2% reduction of LDLR activity. The mutation A > C at 761 in exon 5 is a frequent mutation associated with amino acid substitution in the ligand binding domain. The binding capacity of LDL-R is reduced by 5-30%.

3. Atherogenic biomarkers in the examined population

The patients were assigned to two groups based on the presence or absence of molecular defects: group I carriers (22 patients, 18%) and group II non-carriers (98 patients, 82%).

3.1. Lipid profile in the examined groups

Total cholesterol and LDL levels were higher in carriers (total cholesterol: 9.54 ± 0.47 mmol/l; LDL-C: 7.42 ± 0.04 mmol/l) compared to non-carriers (total cholesterol: 8.55 ± 0.14 mmol/l; LDL-C: 6.77 ± 1.90 mmol/l), but the difference between them did not reach statistical significance. Lower HDL-cholesterol levels were found in carriers (HDL-0.95 ± 0.02 mmol/l) compared with non-carriers (HDL: 1.01 ± 0.24 mmol/l), the difference being also not significant. There was not a statistically significant difference in the levels of triglycerides between carriers (1.50 ± 0.49 mmol/l) and non-carriers (1.44 ± 0.34 mmol/l) ($p > 0.05$). The cholesterol-years score was significantly higher in the carriers (445.32 ± 0.20 mmol-y/L) than in the non-carriers (395.35 ± 0.06 mmol-y/L).

4. Non-invasive vessel examinations

4.1. Endothelium-dependent and endothelium-independent vasodilation.

There was no significant difference in the endothelial dependent and independent vasodilatation between the examined groups: in the carrier group it was 4.47 ± 0.58%, and in the non-carrier group - 4.24 ± 0.55% (Table 3).

4.2. IMT and atherosclerotic plaques of the common carotid artery in carriers and non-carriers

4.2.1. We found a significantly higher carotid IMT in the carriers vs. non-carriers ($p < 0.05$) (Table 4). This significant difference was confirmed after adjustment for age and gender ($p < 0.05$).

4.2.2. There was no significant difference in the

Table 3. Endothelial dependent and independent vasodilatation in the study groups

%FMD parameters			n	Mean ± SEM	SD	t	p
Basal diameter of brachial artery	(mm)	Non-carriers	98	3.34 ± 0.04	0.44	- 0.47	NS
		Carriers	22	3.37 ± 0.05	0.52		
FMD	(%)	Non-carriers	98	4.47 ± 0.58	1.23	0.7	NS
		Carriers	22	4.24 ± 0.55	0.98		
Basal blood flow	(ml/min)	Non-carriers	98	125.23 ± 0.76	23.33	- 0.55	NS
		Carriers	22	124.23 ± 0.83	22.43		
Hyperemia	(% increased flow)	Non-carriers	98	536.43 ± 0.66	189.43	- 0.44	NS
		Carriers	22	538.55 ± 0.66	188.56		
NGL-mediated vasodilation	%	Non-carriers	98	16.41 ± 0.67	0.06	- 1.83	NS
		Carriers	22	16.13 ± 1.05	0.10		

atherosclerotic plagues (%) in the carotid artery between the two groups of patients ($\chi 2 = 0.05$; $p > 0.05$) (Table 4).

4.3. Ankle-brachial index in carriers and non-carriers

We found a significantly lower ABI in the carriers than in non-carriers ($p < 0.001$) (Table 5). This difference remained significant even after adjusting for age and gender ($p < 0.05$). All 22 carriers were in the group of the borderline and low-normal ABI.

5. Correlation between mean IMT of the common carotid artery and ankle-brachial index in carriers

We found a strong negative correlation between IMT mean and ABI ($r_{xy} = -0.457$; $p < 0.0001$) in carriers (Fig. 1).

DISCUSSION

The major findings of the present study are that carotid IMT is significantly higher and ankle-brachial index is lower in LDL-R defective gene carriers than in non-carriers, whereas the endothelial dependent FMD of the brachial artery does not differ between

Table 4. Intima-media thickness and atherosclerosis plaques of the common carotid artery

			n	No age and gender adjustment	p	Age and gender adjustment	p
				Mean ± SEM		Mean ± SEM	
IMT dex max	(mm)	Carriers	98	1.09 ± 0.16	< 0.05	1.06 ± 0.13	< 0.05
		Non-carriers	22	1.11 ± 0.13		1.28 ± 0.15	
IMT dex min	(mm)	Carriers	98	0.94 ± 0.14	< 0.05	0.98 ± 0.13	< 0.05
		Non-carriers	22	1.02 ± 0.12		1.13 ± 0.10	
IMT dex mean	(mm)	Carriers	98	0.98 ± 0.15	< 0.05	0.96 ± 0.14	< 0.05
		Non-carriers	22	1.06 ± 0.02		1.03 ± 0.04	
IMT sin max	(mm)	Carriers	98	0.01 ± 0.17	< 0.05	1.01 ± 0.11	< 0.05
		Non-carriers	22	1.19 ± 0.16		1.10 ± 0.10	
IMT sin min	(mm)	Carriers	98	0.93 ± 0.15	< 0.05	0.93 ± 0.12	< 0.05
		Non-carriers	22	0.94 ± 0.15		0.95 ± 0.10	
IMT sin mean	(mm)	Carriers	98	0.92 ± 0.16	< 0.05	0.97 ± 0.19	< 0.05
		Non-carriers	22	1.08 ± 0.05		1.09 ± 0.03	
IMT mean $\frac{\text{IMT dex mean} + \text{IMT sin mean}}{2}$	(mm)	Carriers	98	0.93 ± 0.15	< 0.05	0.96 ± 0.15	< 0.05
		Non-carriers	22	1.07 ± 0.03		1.06 ± 0.03	
Mean lumen diam.	(mm)	Carriers	98	6.10 ± 0.70	< 0.05	5.98 ± 0.67	< 0.05
		Non-carriers	22	5.58 ± 0.65		5.55 ± 0.60	
Wall thickness (mean) to lumen diameter (mean) ratio		Carriers	98	0.13 ± 0.12	< 0.05	0.12 ± .11	< 0.05
		Non-carriers	22	0.10 ± 0.15		0.10 ± 0.12	
Presents of plagues	n, (%)	Carriers	98	40 (40%)	> 0.05		
		Non-carriers	22	9 (45%)			

Table 5. Ankle-brachial index in the examined groups

		n	No age and gender adjustment	p	Age and gender adjustment	p
			Mean ± SEM		Mean ± SEM	
ABI	Carriers	98	1.16 ± 0.13	< 0.001	1.17 ± 0.02	< 0.05
	Non-carriers	22	0.98 ± 0.08		1.06 ± 0.02	

Folia Medica 2010; 52(4): 13-22

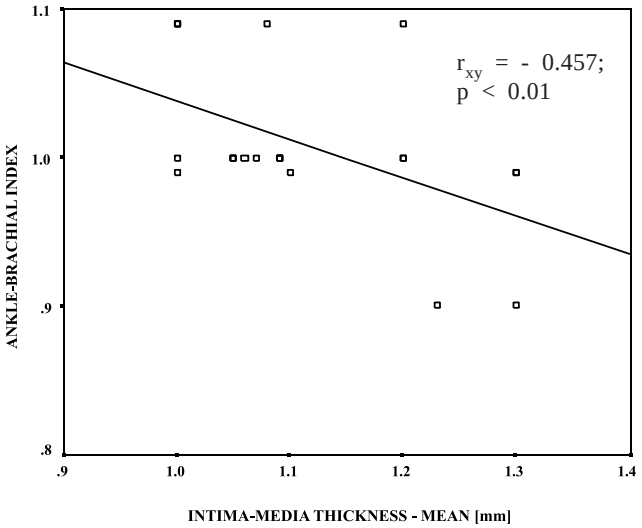

Figure 1. Correlation between IMT mean and ABI in carriers.

these groups of patients with newly detected severe hypercholesterolemia.

The serum cholesterol (total cholesterol and LDL-C) levels of individual carriers of molecular defects (spot mutations and polymorphisms of LDL-R) vary within large ranges and are similar to those in severe hypercholesterolemic patients with no defects. This shows that it is impossible to determine which patients should be referred to molecular-biological analysis for spot mutations and polymorphisms of LDL-R solely on the basis of the lipid parameters and the derivative atherogenic indexes. These results are consistent with the results of other studies that refute the thesis that lipid profiles can be informative for screening of patients.[4,6-7]

It has been shown that FH increases the response to vasoconstrictive agonists and leads to impairment of the endothelial-dependent relaxation. In all hypercholesterolemia patients (with or without point mutations or polymorphisms of the LDL-R) %FMD was impaired (< 7%); this could be explained by the fact that hypercholesterolemia decreases the endothelial NO, and thus decreases the endothelial-dependent vasodilation.[7-8] In our study the %FMD did not differ between the groups with and without the LDL-R defect, which is most probably due to the influence of some environmental factors.

The measurement of carotid IMT provides information on the structure of the vessel wall and it is an established indicator of early atherosclerosis.[9,24-25] In our study we examined the IMT in carriers and non-carriers of spot mutations and polymorphisms in LDL-R gene in patients without other cardiovascular risk factors. In the patients we examined, the patients with severe hypercholesterolemia and familial history of premature cardiovascular disease, we found that the patients with spot mutations and polymorphisms in LDL-R had a significantly higher carotid IMT in comparison with the non-carriers (Table 4). The existence of hypercholesterolemia and family history of cardiovascular disease at early age in patients with the molecular defect are associated with a greater increase of IMT. These data are consistent with reports of other authors. Multiple genes in pathways of production or metabolism of lipids and lipoproteins have been examined in relation to carotid atherosclerosis.[13] In the literature IMT is consistently higher and plaques more frequent in FH patients, but specific mutations have not consistently been implicated.[19-22] Most of the studies have examined IMT in FH and non-FH patients with additional cardiovascular risk factors.[12,20,23,30] It seems likely that carriers of the spot mutations and polymorphisms in LDL-R experience an earlier and longer exposure to higher Apo-B/A_1 atherogenic index (as indirect marker for small and dense LDL-particles than non-carriers. As a matter of fact, the cholesterol-years score, a measure of the lifetime cholesterol levels evaluated by the product of age with cholesterol level is considered as the parameter that is best correlated to IMT and cardiovascular disease occurrence in FH subjects.[31]

Most of the studies compare IMT in FH patients with a normolipidemic control group.[31] For the first time in a Japanese study 97 patients with FH were compared with 132 type IIa hyperlipidemic patients without the condition (non-familial hypercholesterolemia).[30] In this study a higher IMT was reported in FH patients suggesting that the IMT measurement is useful for identifying patients with a higher cardiovascular risk. At the beginning of 2010, Junyent et al.[32] considered more severe clinical phenotype and worse advanced carotid atherosclerosis in receptor negative mutation, compared to the receptor-defective, independently of age, gender, lipid and nonlipid risk factors and the cholesterol-years score. The increased number of atherosclerotic lesions in familial hypercholesterolemia patients versus the control group in comparison with the present study reported by Junyent et al could be explained by the existence of additional risk factors (diabetes mellitus, arterial hypertension) in these patients whereas we tried to get close to the pure familial hypercholesterolemia.

This result was confirmed in our study in a larger pool of patients with a severe hypercho-

lesterolemia and familial history of premature cardiovascular disease.

Another study aiming at comparing FH patients with other hypercholesterolemic patient included 273 Caucasian patients characterized by a severe HH and a family history of early cardiovascular disease and classified as having or not having FH on the basis of molecular screening for LDL-R and Apo-B mutations. The patients with genetic diagnosis of FH had a greater carotid IMT.[32] The difference was more pronounced in men suggesting a gender-specificity in receptor effect in contrast of the results of our research. In the study of Descamps et al. non-FH individuals had a higher prevalence of obesity, hypertension, diabetes and hypertriglyceridemia which is very common in the clinical practice but does not reflect adequately the effect of the genetic defect on IMT.[12] The atherosclerotic lesion is another phenotypic expression of the atherosclerotic process which is quite different from the IMT. The absence of statistically significant difference between non-carriers and carriers in the present study in comparison to previous reports could be due to the lack of concomitant risk factors among the patients studied.

The ankle brachial pressure index is a good predictor of subsequent cardiovascular events, and improves on predictions by conventional risk factors alone.[13-18] It is simple and accurate and could be included in routine screening of cardiovascular status. The reference values were presented in the Trans-Atlantic Inter-Society Consensus Working Group in 2005: normal is 0.9, mild PVD; 0.5-0.75, moderate PVD; less than 0.5 severe PVD. More than 1.3 - falsely elevated (non-compensated vessels). There have been conducted small studies on ankle-brachial index in patients with severe hypercholesterolemia in Bulgaria.[4-6,33-39] There is a dearth of evidence for this method of screening patients with this molecular defect-point mutations of LDL-R gene.

The major findings of the present study are that ABI is significantly lower in carriers of the LDL-R defective gene than in non-carriers. This is confirmed after sex and age standardization - this difference is associated with the presence of point mutations or polymorphism of LDL-receptor. Despite this fact, the lower levels of ABI are in borderline for this index about cardiovascular risk profile. Therefore, the reference values for ABI of patients with manifest family hypercholesterolemia do not exclude molecular defect of the LDL-R gene. Hence, ABI should be a factor in the diagnostic risk algorithm for patients with suspected family hypercholesterolemia as the lower it is the higher the likelihood is of a molecular defect of the LDL-R gene. ABI should be used as an additional marker in assessing the cardiovascular risk in asymptomatic patients with genetic predisposition of family hypercholesterolemia.

The strong correlation between ABI and mean IMT suggests that if ABI is borderline and low-normal levels must investigate IMT CCA and comment for molecular biological analysis in newly detected severe hypercholesterolemia. The results of the present study also suggest that only non-invasive structural measurements (ABI, IMT) can be used in the diagnostic algorithm of newly detected severe hypercholesterolemia. The functional vessel examinations in the context of the %FMD can't be used for screening purposes in patients with LDL-R molecular defects. According to the results of our previous study %FMD can be used for monitoring the statin therapy in the same patients.[40]

CONCLUSIONS

Our data show that carriers of the LDL-R defective gene have a higher carotid IMT; ABI is significantly lower than that in non-carriers with newly detected severe hypercholesterolemia. Lower levels of ABI are within the reference range for this index concerning cardiovascular risk profile. This fact shows that the reference values of ABI for patients with family hypercholesterolemia do not discount the possibility of molecular defect in the LDL-R gene. This result enables us to include ABI (within the reference range) and IMT in the diagnostic algorithm of severe hypercholesterolemia.

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НЕИНВАЗИВНЫЕ СОСУДИСТЫЕ ИССЛЕДОВАНИЯ ОТНОСИТЕЛЬНО НАЛИЧИЯ ДЕФЕКТОВ МОЛЕКУЛ LDL-РЕЦЕПТОРНОГО ГЕНА ПРИ НОВООБНАРУЖЕННОЙ ВЫРАЖЕННОЙ АСИМПТОМАТИЧЕСКОЙ ГИПЕРХОЛЕСТЕРОЛЕМИИ

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РЕЗЮМЕ

Данные исследований ранних сосудистых изменений носителей молекулярных дефектов LDL-рецепторного гена относительно неносителей при тяжелой форме гиперхолестеролемии противоречивы.

Цель: *Работа ставит себе целью исследовать разницу между пациентами с тяжелой формой гиперхолестеролемии, являющимися носителями или неносителями LDL-рецепторного дефектного гена, в контексте их функциональных (поток-индуцированная вазидилатация) и структурных (интима-медия комплекс каротидной артерии и лодыжка–рука индекс) характеристик артериальной стенки. Обследовано 120 пациентов с асимптоматической гиперхолестеролемией. Биохимические параметры определены с помощью рутинных методик. Определение поток-индуцированной вазодилатации, лодыжка-рука индекса и интима-медия комплекса каротидной артерии осуществлено эхокардиографом Hewlett Packard sonos 5 500 с применением автоматической компьютерной программы Medica Soft IMT. Lab.*

Результаты: *Не установлена сигнификантная разница между группами по отношению к общему холестеролу, LDL-холестеролу, HDL-холестеролу, Аполипопротеину B, Аполипопротеину A1, клеточным адгезионным молекулам (sICAM-1, sVCAM-1, sP and sE-selectine). Индекс Apo-B/Apo A1 различается сигнификантно между обеими группами ($t = 11.23$, $p < 0.001$) и эта разница подтверждается и после стандартизирования возраста. Не устанавливается разница в контексте эндотелий-зависимой и эндотелий-независимой вазодилатаций среди обследованных групп ($p > 0.05$). Устанавливается сигнификантно бóльший размер интима-медия комплекса и более низкий лодыжка-рука индекса среди носителей относительно неносителей. Эта разница подтверждается после стандартизирования возраста.*

Заключение: *Полученные данные показывают, что носители LDL-R дефектного гена имеют бóльший размер интима-медия комплекса каротидной артерии и более низкий лодыжка-рука индекса, чем неносители, при чем не устанавливается разница между группами по отношению к уровням липидных параметров и к эндотелий-зависимой вазодилатации.*

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THORACOSCOPIC RESECTION OF SOLITARY PULMONARY NODULES IN PATIENTS WITH PREVIOUS MALIGNANT TUMORS

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ABSTRACT

Round opacities in the lungs found in the course of a neoplastic disorder or during the initial tumor staging are most often regarded as metastases without histological studies to prove their nature. These presumed metastases are, however, very often diagnosed later as benign lesions or primary malignant pulmonary tumors.

Aim: To investigate the histological substrate of solitary pulmonary nodules in patients with a history of neoplastic condition and study the role of video-assisted thoracoscopy in their diagnosing and treatment.

Method: The study included 22 patients with solitary pulmonary nodules and history of previous malignant tumors who underwent video-assisted thoracoscopic surgery at the Clinic of Vascular and Thoracic Surgery, University Hospital Aschersleben, Germany between 01.01.2006 and 31.12.2009. Pulmonary wedge resection was performed and it was followed by histological verification.

Results: A diagnosis of pulmonary metastasis was confirmed in only 8 of the patients (36.4%). In another 8 of them (36.4%) the solitary pulmonary nodule proved to be a primary lung cancer, i.e. a second malignant tumor. The bronchial carcinoma was synchronous with the primary tumor in four of these patients, and metachronous in the rest. In the other 6 patients (27.2%) the lesions proved to be benign.

Conclusions: Not all solitary pulmonary nodules in patients with preceding malignant formations are metastases. In order to define their nature more precisely they should be resected by video-assisted thoracoscopy, if possible. In benign lesions video-assisted thoracoscopic resection is the definitive medical procedure too.

Key words: *solitary pulmonary nodule, malignant tumor history, video-assisted thoracoscopic surgery (VATS)*

INTRODUCTION

A solitary pulmonary nodule is a formation which is seen on a standard chest x-ray as a discrete, well-marginated opacity less than or equal to 3 cm in diameter surrounded by normal lung parenchyma and without associated atelectasis or hilar lymphadenopathy.[1]

As much as 50% of the solitary pulmonary tumours are considered malignant in nature, the majority of them being primary bronchial carcinomas at an early stage of development. Many solitary nodules, however, are pulmonary metastases from other malignant formations.[2]

A solitary pulmonary nodule in a patient with a history of previous malignant formations is initially, and most often, accepted to be a metastasis from the primary tumor without histological examination. However, it often happens that the presumed metastases are later diagnosed as benign lesions or primary malignant pulmonary tumors and depending on that their further medical treatment is differentiated.[3]

In spite of the modern diagnostic methods it is not always easy to diagnose such nodules precisely and to verify them histologically.

Video-assisted thoracoscopic surgery (VATS) recently has offered new diagnostic and therapeutic capacities for evaluation of poorly defined pulmonary lesions.[4,5]

The aim of the present study was to analyze the frequency of different solitary pulmonary nodules in patients with preceding malignant disorders, as

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well as the role of VATS for their diagnosing and treatment.

PATIENTS AND METHODS

Between January 2006 and December 2009, 22 patient (age range 27 - 80 years, mean age 67.5 years) underwent video-assisted thoracoscopic surgery. The patients had history of solitary pulmonary tumors and other malignant formations. The distribution of the primary malignant disorders was as follows: colorectal carcinoma - 6 patients, bladder carcinoma - 3, renal cell carcinoma - 2, breast carcinoma - 2, laryngeal carcinoma - 2, pulmonary carcinoma - 2, sarcoma - 2, pharyngeal carcinoma - 1, melanoma - 1, and prostate carcinoma - 1 patient.

As indications for VATS we accepted the following criteria:

- maximal size of tumor no larger than 3 cm in diameter;
- tumor located in the lateral third of the lung or in the inter-lobar region with the purpose of easier localisation of the nodule during surgery;
- absence of histological verification.

Patients with two or more than two nodules were excluded from our study.

In all patients we applied the following preoperation diagnostic procedures: standard radiography and computed tomography scanning (CT) of the chest, bronchoscopy, abdominal ultrasound examination, specific tests to exclude local recurrence of the different primary tumors and metastases in other characteristic organs. Fine-needle transthoracic aspiration biopsy (TTAB) was not used.

TECHNIQUE

We use standard equipment and instruments for video-assisted thoracoscopic surgery. The patient is placed on the operation table in a full lateral position. We use double lumen endotracheal tube for anesthesia. Surgery is usually performed using 3 trocars of 10 mm each which are placed depending on the tumor location. After the trocars are fixed we inspect the pleural cavity and remove any adhesions between the parietal and visceral pleura. With a special “palpation stick” we palpate indirectly the pulmonary parenchyma in order to localize the tumor. If the nodule could not be identified we perform direct digital palpation of the pulmonary parenchyma with the help of the index finger through the site of one trocar. If this method also fails to identify the tumor we move on to thoracotomy. We resect pulmonary parenchyma by “endo-Gia Stapler” of Ethicon Endo Sugery, Inc. which gives hermetic suture of the lung. The specimen is put in a special plastic surgical bag with the help of which it is removed through the trocar incision. After that we perform thorough hemostasis and lave the pleural cavity. Then the specimen is sent for frozen section biopsy histologic examination. If the tumor is benign or if it proved to be a metastasis we finalized surgery. At the site of the lowest trocar we fix 1 drainage tube and expand the lung under the control of the camera. If the nodule proves to be a primary pulmonary carcinoma we proceed with thoracotomy and radical oncologic resection.

RESULTS

In 21 of the patients we performed video-assisted thoracoscopic resection of the nodules and in one patient we proceeded with thoracotomy in order to localize the tumor. In only 8 of the patients (36.4%) the suspected diagnosis of pulmonary metastasis was confirmed. Table 1 presents the distribution of primary malignant tumors that metastasized to the lung.

In 8 of the patients (36.4%) the solitary pulmonary nodule was found to be primary pulmonary carcinoma, i.e. a second malignant tumor. In 4 patients the bronchial carcinoma was synchronous with the primary tumor and in 4 patients it was metachronous. In 5 of the patients we went on to conventional thoracotomy in order to perform oncologic pulmonary resection and lymphadenectomy. In the other three patients the rapid frozen biopsy examination could not realize differentiate between metastasis and primary pulmonary carcinoma. That is why we performed radical oncologic resection at a second stage, after the final histological results were obtained.

Table 2 presents the distribution of patients with a second primary tumor.

In the remaining 6 patients (27.2%) the lesions were found to be benign. In 14 patients (63.6%), of whom 8 with metastases and 6 with benign lesions, video-assisted thoracoscopic atypical resection of the lung with radical removal of the solitary nodule turned to be the definite medical treatment.

The postoperative period was uneventful with minimal pain in the first hours after surgery. The aspiration drainage tubes were removed between days 2 and 3 (an average of 2.4 days) only if there was not air leakage for a minimum of 12 hours and if the drainage liquid was less than 200 ml per 24 hours.

Folia Medica 2010; 52(4): 23-26

Table 1. Primary tumors, metastasized to the lung

Primary tumor	Number of patients	Metastases	
		synchronous	metachronous
Colorectal carcinoma	5	1	4
Pulmonary carcinoma	2	1	1
Breast carcinoma	2	–	2
Sarcoma	2	–	2
Renal cell carcinoma	1	–	1
Bladder carcinoma	1	–	1
Prostate carcinoma	1	–	1

Table 2. Distribution of the patients with a second primary tumor

Primary tumor	Number of patients	Bronchial carcinoma as a second primary tumor	
		synchronous	metachronous
Bladder carcinoma	2	1	1
Laryngeal carcinoma	2	1	1
Pharyngeal carcinoma	1	–	1
Colorectal carcinoma	1	1	–
Renal cell carcinoma	1	–	1
Melanoma	1	1	–

The patients were discharged on the following day, i.e. the postoperative hospital stay was no longer than 4 days. We had not cases with post operative complications.

DISCUSSION

When patients who underwent surgical treatment for malignant formation are followed up remote metastases are frequently found and they may appear months or even years after the first surgery. Metastases may also be present at the initial tumor staging. When one solitary pulmonary nodule is found in such patients the most frequent presumptive diagnosis is “metastasis” and corresponding treatment is started along these lines.[3]

The undetermined solitary nodule however may be radiological manifestation of quite a broad variety of different lung pathologies – bronchial carcinoma, benign tumors, infectious granulomas, hematomas, pulmonary infarctions, etc.[6] According to literature data 30–40% of all resected pulmonary nodules are malignant.[2] The nature of the nodule usually remains unidentified when common diagnostic procedures are applied and it presents a diagnostic challenge to the specialists.[7]

In the recent years VATS opened new possibilities of diagnosing and treatment of patients with solitary pulmonary nodules.[8]

The results of this study showed that only 36.4% of the solitary pulmonary nodules in patients with previous malignant disorders were metastases and the metachronous ones were significantly more frequent (12 patients) compared to the synchronous ones (2 patients).

In 8 of the patients (36.4%) with solitary pulmonary lesions bronchial carcinoma was diagnosed as a second primary tumor and there was no difference between the number of patients with synchronous (4) and metachronous (4) tumors. In the remaining 6 patients (27.2%) the lesions were benign.

Our results were closely consistent with the data of several previous studies which analyze

pulmonary nodules in patients with preceding cancer disorders.[9-11]

There are also reports according to which no primary bronchial carcinoma was found among the resected solitary nodules in patients with tumor history. Laisar et al. analyzed 34 solitary pulmonary lesions in patients with malignant diseases and they reported about 65% of pulmonary metastases and 35% of benign nodules. They explained the absence of pulmonary carcinoma in their study with the strict selection of patients done by them.[12]

CONCLUSIONS

Not all solitary pulmonary nodules in patients with malignant disorders are metastases. That is why they need to be resected by VATS to verify them morphologically and administer the proper treatment. In a large percentage of the cases VATS is the definitive therapeutic procedure and its application helps to avoid traumatic thoracotomy.

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ТОРАКОСКОПИЧЕСКАЯ РЕЗЕКЦИЯ СОЛИТАРНЫХ (ЕДИНИЧНЫХ) ЛЕГОЧНЫХ УЗЛОВ У ПАЦИЕНТОВ С ПРЕДШЕСТВУЮЩИМИ ЗЛОКАЧЕСТВЕННЫМИ ОПУХОЛЯМИ

Г. Присадов, Т. Ландес, Г. Крюгер

РЕЗЮМЕ

Если в течение опухолевого заболевания появятся легочные круглые тени или таковы устанавливаются при первоначальном обнаружении опухоли, то их чаще всего воспринимают как метастазы, не доказывая их гистологического происхождения. Однако, очень часто, эти предполагаемые метастазы позже диагностицируют как доброкачественные патологические изменения (лезии) или как первичные злокачественные легочные опухоли.

***Цель:** Настоящая работа ставит себе целью изучить гистологию единичных (солитарных) легочных узлов у пациентов с малигненной опухолью в анамнезе.*

***Методы:** В исследование авторы включили 22 пациентов с солитарными легочными узлами и с анамнезом «предшествующие злокачественные опухоли», оперированных видео-торакоскопически в Клинике сосудистой и грудной хирургии (Университетская больница Ашерслебен, Германия) в период 01.01.2006 - 31.12.2009. В целях постановки морфологического диагноза проведена клиновидная резекция легкого.*

***Результаты:** Только у 8 (36.4%) пациентов предполагаемый диагноз «легочный метастаз» подтвержден. У 8 (36.4%) больных единичный легочный узел оказался первичной легочной карциномой, т.е. второй злокачественной опухолью. У 4 пациентов бронхиальная карцинома синхронна с первичной опухолью, а у 4 - метасинхронна. У остальных 6 (27.2%) пациентов патологические изменения оказались доброкачественными.*

***Заключение:** Не все единичные легочные узлы у пациентов с предшествующими злокачественными образованиями являются метастазами. В целях гистологического исследования их следует резецировать - по возможности видео-торакоскопически.*

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SPECIFIC FEATURES OF VIBRATION-INDUCED DISORDERS

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ABSTRACT

Aim: Assessment of the specific clinical manifestations of hand-arm vibration syndrome (HAVS) or whole-body vibration syndrome (WBVS).

Patients and methods: Seventy - six patients (34 with HAVS and 33 with WBVS) were examined analysing the data from their medical history, clinical examinations and autonomic nervous system study, capillaroscopy, distal Doppler ultrasound study, vibrotactile sense, roentgenography, and electromyography.

Results: HAVS manifests mainly in the upper limbs as microcirculatory disturbances: RR 2.59; 95% CI (1.64 - 4.10), Raynaud's syndrome: RR 16.50; 95% CI (2.33 - 117.04), increased vascular resistance in the digital arteries of the hands: RR 9.71; 95% CI (3.28 - 28.75); distal autonomic neuropathy of the upper limbs: RR 15.04; 95% CI (3.91 - 57.88); sensory polyneuropathy predominantly of the upper limbs: RR 21.00; 95% CI (3.01 - 146.57); median neuropathy: RR 14.56; 95% CI (2.04 - 104.06); cervical spondylosis with/ without osteochondrosis: RR 2.09; 95% CI (1.33 - 3.28). In patients with WBVS we observed predominantly degenerative changes of the lumbar spine segment: RR 2.49; 95% CI (1.55 - 3.99); lumbosacral radicular symptoms: RR 8.53; 95% CI (3.73 - 19.52).

Conclusion: Dose-dependant, microcirculatory, peripheral vascular, peripheral nerve and musculoskeletal disorders of the upper limbs were found in HAVS and musculoskeletal and peripheral nerve injuries of the spine and the lower limbs were found in WBVS.

Key words: *hand-arm vibration syndrome, whole-body vibration syndrome, clinical characteristics*

INTRODUCTION

Occupational exposure to vibrations induces a wide spectrum of pathological vascular, neural and musculoskeletal alterations described as vibration disease caused by local and/or whole-body vibration impact. The internationally recognised terms are hand-arm vibration syndrome and whole-body vibration syndrome.[1-4] Currently, the universally accepted "gold standard" for diagnosing and determining the disease stage is the Stockholm scale[5], which is a subjective measure and registers only the anamnestic data from the patient[6]. Assessment based only on this scale does not take into account possible dissimulation and aggravation, and therefore there is a great potential for mistakes concerning the precise defining and staging of the disease.

The largest number of cases of occupational diseases by the end of 2007 in Bulgaria was registered in Plovdiv region - 5848, with vibration-induced and related to physical overload pathology being among the leading elements in the structure of occupational diseases.[7]

The aim of he present study was to perform comparative analysis and assessment of the clinical manifestations of hand-arm vibration syndrome (HAVS) or whole-body vibration syndrome (WBVS) in order to define its specific characteristics.

PATIENTS AND METHODS

Seventy-six patients, treated in the Department of Occupational Diseases between 2004 and 2009, were investigated. They were allocated to 2 groups: group I was of 34 patients with hand-arm vibration syndrome, and group II - 33 patients with whole-body vibration syndrome. The analyzed data included disease history, physical examination, data from the clinical studies of the autonomic nervous system and the neurovascular reactivity, including cold provocation test, thermistor thermometry, capillaroscopy, distal Doppler ultrasound study, roentgenography and electroneurography. The results were analyzed with the software package EPIINFO using Student-Fisher *t*-test and assessment of relative risk (RR) with a level of statistical significance $p < 0.05$.

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RESULTS

The first group of patients with hand-arm vibration syndrome included 34 men with mean length of service 13 ± 7 yrs and mean age 51 ± 13 yrs. The second group included 33 patients (11 females and 22 males) with whole-body vibration syndrome (mean age 53 ± 5 yrs, length of service 17 ± 7 yrs). The patients exposed to local vibrations were miners, chain-saw woodcutters, rotary hammer workers, and turners. The group of patients exposed to whole-body vibrations included truck drivers, dumper drivers, tractor drivers, excavator operators, skid steer operators, crane operators, and bulldozer operators.

Complaints of patients with hand-arm vibration syndrome were predominantly localized in the upper limbs. Symptoms related to peripheral nerve damage like tingling, formication, burning sensation in the hands, sweating or dryness of the palms and the fingers, pain in the hands, forearms and arms, the neck and between the scapulae, trophic changes of the skin and the fingernails and/or muscle weakness in the hands with dropping objects, were observed in 85% (n = 29) of the patients. The peripheral vascular and microcirculatory manifestations were palmar marbelization, paleness and/or cyanosis, redness of the fingers, especially following exposure to cold, tingling of the fingers - 94% (32 patients). The musculoskeletal disturbances presented with pain and weakness in the fingers, wrists, forearms, elbows, shoulders, humero-cervical regions in 70.59% (24 patients).

The characteristic clinical alterations in hand-arm vibration syndrome and whole-body vibration syndrome are presented on Table 1.

There was a statistically significant increase in the frequency of finger paleness, paresthesias and pain syndrome in the upper limbs with the increase of length of service, and respectively, with increased exposure to vibrations ($p < 0.05$.)

Concomitant low back pain syndromes were found in 15% of vibration disease patients.

Statistically significant increase of relative risk of the following alterations was found in the patients with hand-arm vibration syndrome: microcirculatory disturbances in the upper limbs: RR 2.59; 95% CI (1.64 - 4.10), Raynaud's syndrome with finger paleness and cyanosis: RR 16.50; 95% CI (2.33 - 117.04), peripheral vascular disturbances with increased vascular resistance, predominantly in the digital arteries of the hands found on Doppler sonography: RR 9.71; 95% CI (3.28 - 28.75), distal autonomic neuropathy of the upper limbs: RR 15.04; 95% CI (3.91 - 57.88), predominantly sensory polyneuropathy of the upper limbs: RR 21.00; 95% CI (3.01 - 146.57), neuropathy of the median nerve, resulting mainly from carpal tunnel syndrome, confirmed by electromyography: RR 14.56; 95% CI (2.04 - 104.06), cervical spondylosis with or without osteochondrosis established on roentgenography: RR 2.09; 95% CI (1.33 - 3.28).

In group II, the predominant alterations included low back pain syndromes (97%) with secondary peripheral nerves symptoms of damage of lumbosacral spinal cord roots, presented predominantly as excitatory and deficit sensory and autonomic, and more rarely as motor and reflex disturbances in the lower limbs. These facts determine also the more pronounced subjective data of low back pain and leg pain with tingling. In many patients there were pain syndromes in the upper section of the spine (64%) with cervical radicular syndromes presenting with pain, tingling of the upper limbs, changes in palmar and finger skin colour and sweating.

A statistically significant increase of relative risk of the following alterations was found in the WBVS patients: degenerative alterations of the lumbar spine: RR 2.49; 95% CI (1.55 - 3.99), lumbosacral radicular syndromes: RR 8.53; 95% CI (3.73 - 19.52).

Palesthesiometry data show severe or moderate decrease in vibrotactile perception in the fingers in 100% (n = 34) of the patients with HAVS, and predominantly moderate and mild in vibrotactile perception in 96.97% (n = 32) of WBVS patients. The vibrotactile sense is significantly associated with the levels of cumulative exposure to vibration impact in work environment.

DISCUSSION

Local vibration impact is related to paresthesias, stiffness, decreased hand muscle strength, disturbed manipulative dexterity.[8] T. Kákosy et al. describe the following disturbances in patients with HAVS: vascular in 90%, affecting peripheral nerves in 51%, Raynaud's syndrome in 33%, X-ray alterations in 37%, carpal tunnel syndrome in 85%, ulnar neuropathy in 4%.[9] R. Sauni et al. describe Raynaud's syndrome in 49%, sensory disturbances in 66%, carpal tunnel syndrome with median neuropathy in 56%, musculoskeletal injuries in 75% of the patients with hand-arm vibration syndrome and statistically significant dose-dependence on vibration exposure.[10] Our data confirm the findings of other authors of leading microcirculatory and peripheral nerve and musculoskeletal clinical manifestations in the upper

Folia Medica 2010; 52(4): 27-31

Table 1. Syndromes and pathologic manifestations in vibration disease

Syndromes / Disease	Hand-arm vibration syndrome n = 34	Whole-body vibration syndrome n = 33	p
	n (%)	n (%)	
Microcirculatory	32 (94.12)	12 (36.37)	0.0001
Raynaud's	17 (50)	1 (3.03)	0.0001
Peripheral vascular	30 (88.23)	3 (9.09)	0.0001
Distal autonomic neuropathy of:			
- Upper limbs	31 (91.18)	2 (6.06)	0.0001
- Lower limbs	5 (14.71)	13 (39.39)	0.02
Polyneural sensory syndrome in:			
- Upper limbs	28 (84.85)	3.03 (1)	0.0001
- Lower limbs	1 (2.94)	9.09 (3)	N.S.
Neuropathy of:			
- n. medianus	15 (44.12)	1 (3.03)	0.0001
- n. ulnaris	2 (5.88)	1 (3.03)	N.S.
- n. peroneus/tibialis	4 (2.94)	6 (18.18)	N.S.
Radicular syndrome:			
- cervical	27 (79.41)	21 (63.64)	N.S.
- lumbosacral	5 (14.71)	32 (96.97)	0.0001
Musculoskeletal:			
Humeral epicondylitis	24 (70.59)	30 (90.91)	N.S.
Humero-scapular periarthritis	9 (26.47)	3 (9.09)	N.S.
Cubital joint arthrosis	6 (17.65)	1 (3.03)	N.S.
Spondylosis and/or osteochondrosis:	3 (8.82)	-	N.S.
- cervical	28 (82.35)	13 (39.39)	0.001
- lumbosacral or spinal disc herniation	12 (35.29)	29 (87.88)	N.S.
Interphalangial joints arthrosis	1 (2.94)	-	N.S.
Focal osteoporosis	4 (2.94)	-	N.S.

N.S. – P > 0.05.

limbs significantly associated with the exposure to local vibrations.

M. Hirata and H. Sakakibara describe significantly slowed sensory nerve conductivity in the distal parts of the upper limbs due to focal neuropathy of the fingers, the carpal tunnel, Guyon's channel in 53% of the patients.[11] Our studies found electromyographic data for upper limb neuropathy in 50% of the patients. These data determine the necessity of investigation of upper limb peripheral nerve conductivity in cases of hand-arm vibration syndrome. The cold provocation test is a valuable objective tool for monitoring of vibration induced neurovascular disturbances with dose-effect relation between the cold-provoked vasomotor response and the cumulative vibration exposure.[12] Thermistor thermometry of the limbs, combined with distal Doppler sonography and cold provocation test correlate significantly with each other and with the stage of vibration disease[13], making their application for objective assessment of autonomic neurovascular injuries in vibration disease appropriate.[14]

C. Aström et al. determine significant correlation between whole-body vibrations exposure and the increased risk for musculoskeletal alterations in the neck, the shoulders and the wrists similar to those caused by local vibration impact as well as dependence on the cumulative time of exposure.[15] Whole-body vibration syndrome determines increased risk of low back pain syndromes with

secondary peripheral nerve disturbances in the lower limbs.[16] The pathologic findings in drivers included predominantly alterations in the spine, but also in the hands and wrists.[17] Our investigation found increased risk of musculoskeletal abnormalities in the lumbosacral part of the spine with secondary sensory nerve alterations in the lower limbs, significantly associated with the whole-body vibration impact, and possible synergic effect of static physical load and unfavourable microclimate.

Palesthesiometry is a valuable method for objective assessment of alterations in deep sensitivity in vibration disease. Vibrotactile sense is associated with the levels of cumulative exposure to local and whole-body vibration impact.

The risk from manifestation of the described typical clinical signs increases with the increase in the cumulative vibration dose.

Integrated assessment with a complex of laboratory methods is needed for the diagnosis and determination of the stage of vibration disease.[18]

Vibrations have a significant impact on subjective capability for performance of daily and work duties.[19] Vibration disease lowers the quality of life of the patients.

CONCLUSIONS

We found a dose-dependent correlation between the cumulative duration of the vibration impact and the sensory neural disturbances, peripheral neuropathies in the limbs and musculoskeletal injuries of the upper limbs and the neck has been found in the investigated patients. Local vibrations cause peripheral vascular, microcirculatory, peripheral nerve and musculoskeletal injuries in the upper limbs, and whole-body vibrations - primarily musculoskeletal and peripheral nerve injuries of the spine and the lower limbs. Longer service and higher daily vibration exposure are associated with more pronounced clinical manifestations and pathological alterations of the nervous, vascular and musculoskeletal systems. The applied complex of diagnostic methods significantly contributes to the objective assessment of the pathological alterations. The application of a complex of laboratory methods to diagnose and make an integral evaluation, in order to stage the vibration disease, is appropriate.

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ХАРАКТЕРНЫЕ ОСОБЕННОСТИ ВИБРАЦИОННО ОБУСЛОВЛЕННОЙ ПАТОЛОГИИ

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РЕЗЮМЕ

Цель: *Оценить характерные клинические проявления при вибрационной болезни вследствие локального воздействия (ВБЛВ) или общего вибрационного воздействия (ВБОВ).*

Материал и методика: *Анализированы анамнестические данные, объективный клинический статус, исследования автономной нервной системы, капилляроскопия, дистальная доплеровая сонография, виброощущение, рентгенография, электроневрография у 76 больных (34 с ВБЛВ и 33 с ВБОВ).*

Результаты: *Проявления при ВБЛВ наблюдаются преимущественно в верхних конечностях: микроциркуляторные нарушения RR 2.59; 95% CI (1.64 - 4.10); синдром Raynaud - RR 16.50; 95% CI (2.33 - 117.04); повышенное сосудистое сопротивление в дигитальных артериях рук - RR 9.71; 95% CI (3.28 - 28.75); дистальная автономная невропатия в верхних конечностях - RR 15.04; 95% CI (3.91 - 57.88); преимущественно сенсорная полиневропатия верхних конечностей - RR 21.00; 95% CI (3.01 - 146.57); невропатия n. medianus - RR 14.56; 95% CI (2.04 - 104.06); цервикальный спондилез с/без остеохондроза - RR 2.09; 95% CI (1.33 - 3.28). У больных с ВБОВ преобладают дегенеративные изменения поясничного отдела позвоночника - RR 2.49; 95% CI (1.55 - 3.99); люмбосакральные радикулярные синдромы - RR 8.53; 95% CI (3.73 - 19.52).*

Заключение: *Устанавливаются дозозависимые микроциркуляторные, перифернососудистые, периферонервные и мышечноскелетные нарушения в верхних конечностях у больных с ВБЛВ и мышечноскелетные и периферонервные повреждения в позвоночнике и в нижних конечностях у больных с ВБОВ.*

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AGE DYNAMICS AND SECULAR CHANGES OF INDICES CHARACTERIZING THE NEUROCRANIUM AND FACIAL CRANIUM IN ETHNIC BULGARIAN 7-17-YEAR-OLD CHILDREN FROM THE REGION OF THE EASTERN RHODOPES

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ABSTRACT

Background: It is impossible to give an objective anthropologic assessment of the overall physical development of a child's body during the time of intensive growth (7-17 years) without taking into account the age and gender changes in the absolute and relative head and face measurements. Head growth has specific characteristics that makes it different from the growth of other parts of the body. The head of a child differs from the head of an adult not only by metric characteristics but also by the proportions between the different head measurements. Growth and proportionality of the head comply with the general growth pattern, but there are also certain regional, populational and temporal differences. That was the reason why we conducted a study targeted at children and adolescents from the region of the Eastern Rhodopes.

The aim of the present study was to determine the growth dynamics in between-gender and between-age aspects of the variables characterizing the neurocranium and facial cranium, and establish the tendency and direction of the secular changes.

Material and methods: The study included 1481 children and adolescents (699 boys and 782 girls) aged 7 to 17 years that were examined using the classical methodology of Martin-Saller (1957). Head length, width, height and circumference, as well as face width, mandible width, morphological and physiognomic height of the face were measured. Head index, morphological face index and jugulormandibular index were calculated using standard formulas. The secular changes were analyzed comparing data from 1907 and 1960 with the data of the present study.

Results: The head index classified the children from both genders and all age groups as mesocephals. The girls from the study region had a relatively greater mandible width and boys - relatively greater face width. In the beginning of the growth period wider face forms prevailed especially in the girls, while narrower face forms were more characteristic for the adolescence and postadolescence and better manifested in the boys.

Conclusions: Throughout the entire study period the boys presented with greater measurements of the neurocranium and facial cranium than the girls.

For both genders the increase in the neurocranium measurements anticipates that in the facial cranium measurements.

In the examined children and adolescents the width cephalometric variables complete their growth earlier than the height variables.

The head circumference and head width decrease, while the differences in the head length and facial height increase in both genders and all age groups in the end of the 20th and beginning of the 21st century.

Key words: *head length, head width, face height, head growth*

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INTRODUCTION

Head growth is closely associated with brain growth. From the earliest stages of embryonic development brain is fairly close to its ultimate weight. The brain of a newborn weighs 25% of the adult brain, and the body weight is 5% of that in adults. By the age of 10 years the brain is about 95% of the adult weight, while body mass is 50% of that of adults.[1,2] Hence, the skull that encloses the brain attains its ultimate dimensions earlier than the rest of the skeleton. Accordingly, the head growth is most intensive in the early childhood period. The neurocranium of a 9-year-old child amounts to 92-95% of the size of a grown up subject. It is found that between 7 and 9 years of age the facial cranium shows a clear tendency to faster growth of the width compared with the height measurements. The mandible width is most rapidly increasing, followed by the face (bizygomatic) width.[3,4]

Head growth concerns not only absolute increase in the particular measurements, but also changes in the proportions. If head measurements of adult are accepted as 100%, at birth the head width represents 55-60%, head height 40-45% and head length 30-35% of the values of adult individual. That indicates different duration of growth in the specific variables. It has been found that width measurements reach their ultimate dimensions faster, while height measurements have longer growth period.[5-7]

The aim of the present study was to determine the growth dynamics in age and gender aspect of the variables characterizing the neurocranium and facial cranium and establish the tendency and direction of the secular changes.

MATERIAL AND METHODS

For the purpose of the study 1481 children and adolescents (699 boys and 782 girls) aged between 7 and 17 years were examined. The children were divided into 11 age groups and examined using accepted classical methodology[8] and standard anthropometric set. Head length, width, height and circumference, as well as face width, mandible width, morphological and physiognomic height of the face were measured. Proportionality was analyzed using certain cephalometric indices obtained by standard formulas:

$$\text{Head index} = \frac{\text{Head width}}{\text{Head length}} \times 100$$

$$\text{Morphological face index} =$$

$$= \frac{\text{Morphological face height}}{\text{Face width}} \times 100$$

$$\text{Jugomandibular index} = \frac{\text{Mandible width}}{\text{Face width}} \times 100$$

The secular changes were evaluated by comparing the present study data with data available for 1907[9] and 1960[10]. The data were analyzed using statistical package "STATISTICA 6.0".

Between-gender and between-group differences were tested with Student t-test with level of significance for the null hypothesis set at $P \leq 0.05$. The results are presented as mean ±SEM.

RESULTS

The results of the cephalometric characteristic of the studied children and adolescents aged 7 to 17 years determined the specificity of the size, shape and proportions of the head during this period of intense growth and development and the strength of expression of the between-gender differences. Analysis of these variables gives essential morphotypological information for peculiarities of the growth periods in children from the East Rhodopes region.

The head index classified the children from both genders and all age groups as mesocephals. The girls from the study region had a relatively greater mandible width and boys - relatively greater face width. At the beginning of the growth period wider face forms prevailed especially in the girls, while narrower face forms were more characteristic for the adolescence and postadolescence and better manifested in the boys. Head widths showed marked rise during adolescence in the boys, and head heights markedly increased in the girls. In both genders head height undergoes most intensive growth between 12 and 13 years of age. Although the cephalofacial variables present with higher mean values in the boys during the whole period of growing, their growth continues after the age of 16, while in the girls the face variables reach their ultimate values by that age.

DISCUSSION

A characteristic feature of the postnatal development is that the growing lines of the cephalometric variables do not cross. The head length (Fig 1) presents with higher values in the boys than in the girls throughout the examined period. The difference in 7-year-old children is 0.52 ± 0.10 cm and at 17-year-old children 0.77 ± 0.08 cm. In all age groups the difference reaches statistical significance ($p \leq 0.05$). At insignificant annual growth the highest growth (0.29 cm) in the boys is from 10 to 11 years of age and in the girls from 12 to 13 and from 15 to 16 years of age (0.27 and 0.25, respectively). For the whole growing period (7 - 17 years) the measure of the examined cephalometric variable increases by 1.08 cm in the boys and by 0.83 cm in the girls. Our findings reveal that at the age of 7 the head length in the boys is 94.29% of the measure in the 17-year-old boys, and in the girls - 95.43%, respectively.

In all age groups the boys present again with significantly higher mean values of the **head width** ($p \leq 0.05$) with differences at 7-year-old children are 0.45 ± 0.10 cm and at 17-year-old children 0.51 ± 0.07 cm (Fig. 2). The highest annual growth for the boys (0.22 cm) is between 13 and 14 years and for the girls between 9 and 10 and between 12 and 13 years (0.16 cm). For the 11-year period the head width increases by 0.77 cm in the boys, which is 94.87% of the basal values. In the girls the head width increases by 0.71 cm or 95.10%.

Analysis of the mean values of **head height** (Fig. 3) reveals priority of the boys and statistically significant between-gender differences ($p \leq 0.05$). At the age of 7 the difference between the boys and the girls is 0.62 ± 0.09 cm and at 17 - 0.75 ± 0.06 cm. In the boys intensive growth is observed in three age intervals: 7 - 8 years of age (0.22 cm), 12-13 years of age (0.26 cm) and 16-17 years of age (0.28 cm). In girls the maximum annual growth was established in the age intervals between 8 and 9 years of age (0.26 cm) and between 12 and 13 years (0.27 cm) During the examined age period the head height increases by 1.28 cm in the boys, which is 92.09% of the baseline values for 7-year-old boys. In the girls the head height increases by 1.14 cm or 92.61%, respectively.

The growth dynamics of the **head circumference** (Fig. 4) provides important information about the postnatal development of neurocranium. The mean values of this variable are higher in the boys than in the girls for the whole age period but the differences reach statistical significance only in the 10-year-old children ($p \leq 0.05$). The differences between genders are 1.03 ± 0.11 cm for 7-year-old children and reach 1.14 ± 0.10 cm for 17-year-old children. Two growth peaks are found in both genders. The first one is between 7 and 8 years of age for both genders (0.68 cm for boys and 0.84 cm for girls). The second one is between 10 and 11 years of age for the boys (0.69 cm) and one year later – between 12 and 13 years of age – for the girls (1.12 cm). For the period from 7 to 17 years the head circumference increases by 3.70 cm for boys and by 3.59 cm for girls, which is 93.33% and 93.39%, respectively of the initial measurements and those in 7-year-aged children.

The **facial cranium growth** differs from that of the neurocranium due to differences in their formation. If the size of the neurocranium of 7-year-old children is about 95% of the size of 17-year-old adolescents, the width measurements of facial cranium are about 86% and the height measurements - 87%.

Ours results show similar pattern of the growing curves of the cephalofacial variables, which present again with significantly higher values in the boys than in the girls (Figs 5, 6, 7, 8). In the 7-year-old girls the face **widths and heights** constitute 90% of those measurements in the 17-year-old girls, while in the 7-year-old boys these are about 86%. The results definitively show that face dimensions increase more rapidly in girls, ceasing to increase after 16 years of age, while the growth rate for boys is relatively high even after turning that age. An interesting finding is that in both genders, but more evident in the boys, the width variables cease growing earlier than the height variables.

The comparative analysis of growth intensity shows that in boys the most intensive growth of head length and head circumference and mandible width occurs between 10 and 11 years of age, and that of head and face height measurements - between 12 and 13 years of age. In girls the growth intensity of many of the head and face variables is the greatest in the age between 12-13 years.

Besides the complete values of the measured variables, anthropology also uses very much the proportionality indices, which indicate what the shape of the head, as well as certain head sections, can be. These findings are of particular importance in anthropological studies of children, for the head of children differs from the head of adults not only in the measured characteristics, but in the form and proportionality as well.

A special role in morphology is played by the

Folia Medica 2010; 52(4): 32-38

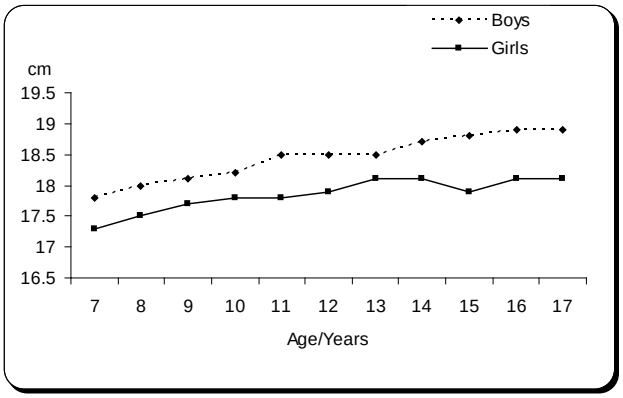

Figure 1. Head lengt.

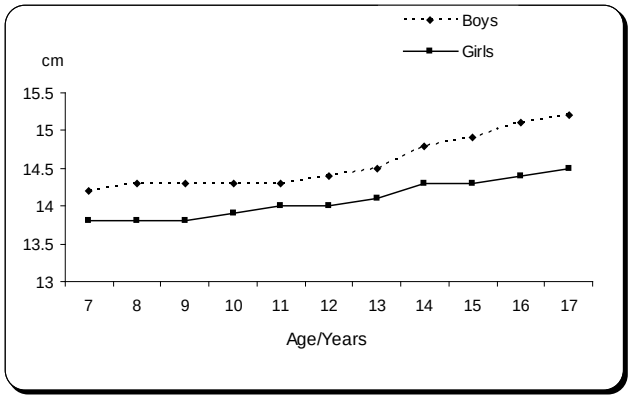

Figure 2. Head width.

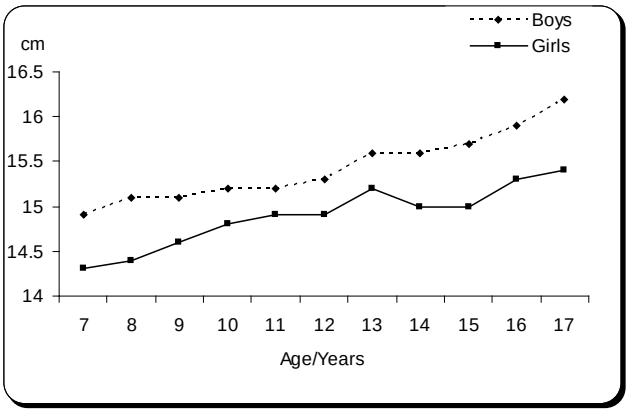

Figure 3. Head height.

Figure 4. Head circumference.

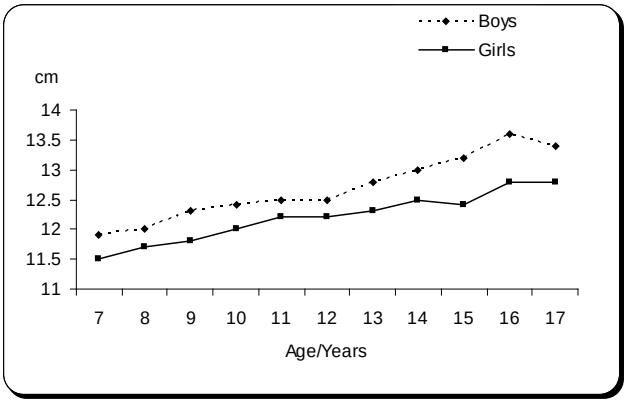

Figure 5. Face width.

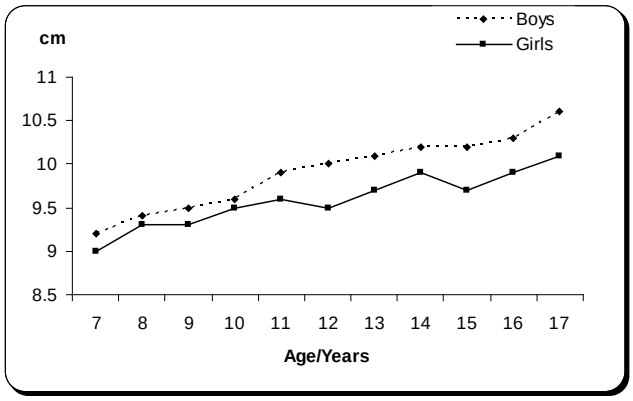

Figure 6. Mandible width.

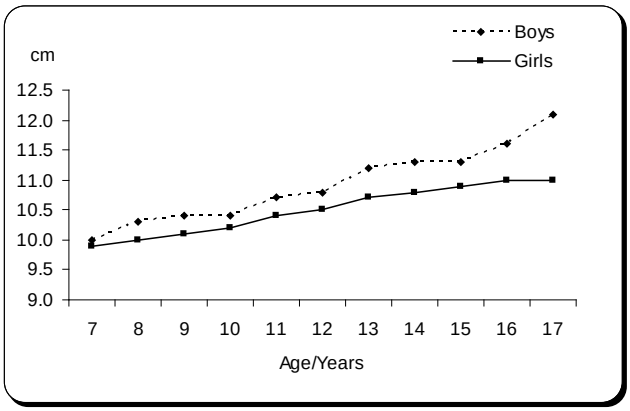

Figure 7. Morphological face height.

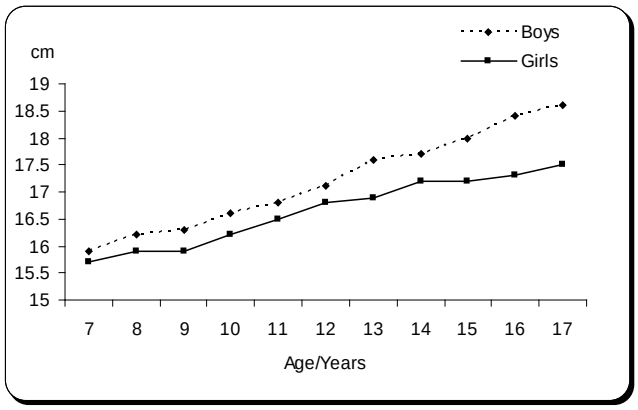

Figure 8. Physiognomic face height.

data of the **head index**, also called head length-width index, that represents the percentage of correlation between the length and width of the head, and gives a general idea of the head form.

Throughout the study period the boys and the girls present with comparable values of the index as only in the 15-year-old adolescents the differences reach statistical significance ($p \leq 0.05$). According to the head form categorization of Martin–Saller (1957) it appears that in all age groups in both genders the mean values of the head index fall within the limits of mesocephals, which represents the more accelerated growth of the head in height than in width.

The analysis of the **morphological face index** shows that at the beginning of the growth period wider face forms prevail in both genders yet more expressed in the boys, while during adolescent and post-adolescent period narrower face forms are more common and better manifested in the girls. The between-gender differences are statistically significant at 15 and 17 years of age ($p \leq 0.05$).

The index of **width proportionality** between middle and lower face shows that the girls have relatively greater mandible width and boys relatively greater face width. Statistically significant between-gender differences are found at 12, 15 and 16 years of age ($p \leq 0.05$). According to Lundborg-Linders-Saller categorization the boys from all age groups fall into the category middle face and the girls into the category wide face.

On the basis of our findings we can say that there are temporal and gender differences in the growth processes.

In terms of head circumference (Figs 9, 10) acceleration changes were present from the beginning of the century until the 60's of the previous century, which is characteristic for both boys and

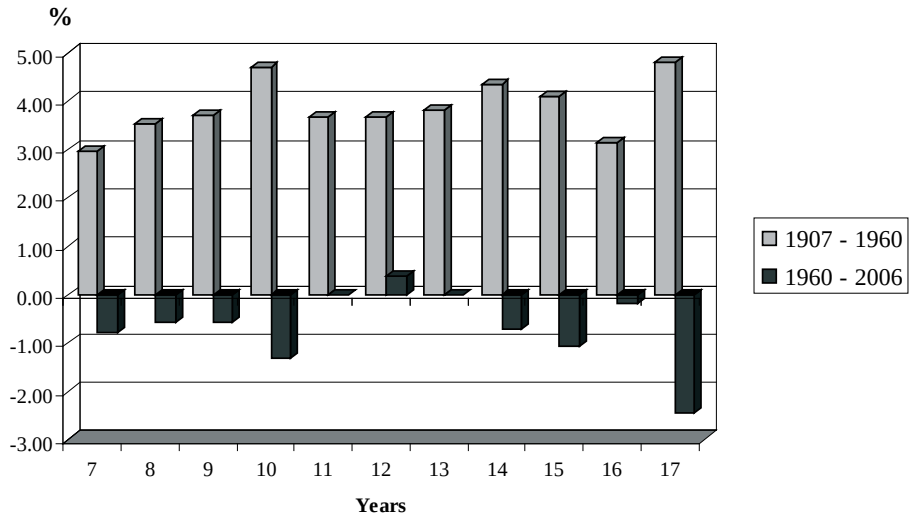

Figure 9. Changes in the head circumference in boys.

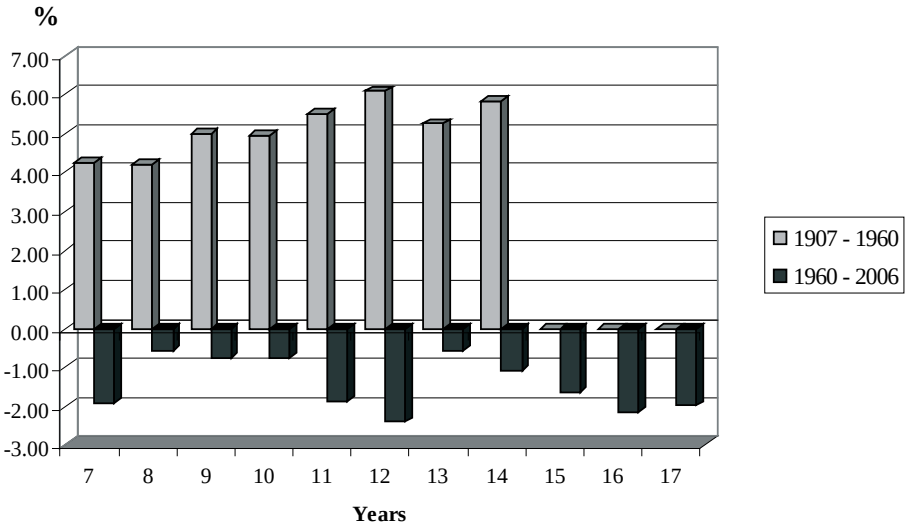

Figure 10. Changes in the head circumference in girls.

Folia Medica 2010; 52(4): 32-38

girls. After the 60's of the 20th century the acceleration processes slowed down and in the first decade of the 21st century deceleration processes took place characterizing both genders and all age groups. This can be explained with the tendency to debrachicephalisation in present-day children and adolescents reflected in the decrease in the head width and head index values and increase in the head and face height in the recent years.

CONCLUSIONS

Throughout the entire study period the boys present with greater measurements of the neurocranium and facial cranium than the girls.

For both genders the increase in the neurocranium measurements anticipates that in the facial cranium measurements.

In the study children and adolescents the width cephalometric variables complete their growth earlier than the height variables.

The head circumference and head width decrease, while the differences in the head length and facial height increase in both genders and all age groups at the end of the 20th and beginning of the 21st century.

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ВОЗРАСТНАЯ ДИНАМИКА И СЕКУЛЯРНЫЕ ИЗМЕНЕНИЯ ПРИЗНАКОВ, ХАРАКТЕРИЗУЮЩИХ МОЗГОВУЮ И ЛИЦЕВУЮ ЧАСТИ ГОЛОВЫ, У ДЕТЕЙ БОЛГАРСКОГО ЭТНОСА (ВОСТОЧНЫЕ РОДОПЫ) В ВОЗРАСТЕ ОТ 7 ДО 17 ЛЕТ

С. Тинешев

РЕЗЮМЕ

Введение: *Антропологическая оценка целостного физического развития детского организма во время активного роста (7 – 17 лет) не может быть объективной, если не прослежены возрастные и половые изменения в абсолютных и относительных стоимостях признаков головы и лица. Рост головы имеет специфику, отличающую его от роста остальных частей тела. Детская голова отличается от головы взрослых не только своей метрической характеристикой, но и соотношением между отдельными размерами головы. Рост и пропорциональность головы подчиняются общим закономерностям роста, но наблюдается и ряд территориально-популяционные и времевые различия. Именно этот факт мотивирует авторов предпринять целенаправленное исследование детей и подростков (район - Восточные Родопы).*

Цель: *Характеризовать динамику роста в межвозрастном и межполовом аспектах признаков,*

которые характеризуют мозговую и лицевую части головы, как и установить тенденцию и направление секулярных изменений.

Материал и методы: *Трансверсально, по классической методике Martin R. и K. Saller (1957 г.) проведены антропометрические измерения детей и подростков (1481 – 699 мальчиков и 782 девочки) в возрасте от 7 до 17 лет. Измерены длина, ширина, высота и горизонтальная окружность головы, как и ширина скул, ширина нижней челюсти, морфологическая и физиогномическая высота лица. По стандартным формулам вычисляют морфологический лицевой, югомандибулярный индексы и индекс головы. В целях оценки секулярных изменений использованы данные от 1907 и 1960 г., которые сравниваются с данными настоящего исследования.*

Результаты: *Во всех возрастных группах и при обоих полах форма головы определяется категорией "мезокефал". Лица женского пола исследуемого региона имеют относительно бóльшую нижнечелюстную ширину, а лица мужского пола относительно бóльшую ширину скулы. В начале периода роста преобладают более широкие лицевые формы, что лучше выражено у мальчиков, а в пубертатном и постпубертатном периодах преобладают более узкие лицевые формы, особенно у лиц женского пола.*

Выводы: *В течение всего исследуемого возрастного периода у мальчиков наблюдаются бóльшие размеры признаков, характеризующих мозговую и лицевую части головы. И при обоих полах размеры мозговой части опережают в своем развитии размеры лицевой части головы.*

Во всех случаях кефалометрические признаки ширины заканчивают свой рост раньше признаков высоты.

И при обоих полах в конце XX и в начале XXI века во всех возрастных группах окружность и ширина головы уменьшаются, в то время как различия в длине головы и в высоте лица увеличиваются.

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POSTERIOR TRANSPEDICULAR DECOMPRESSION FOR THORACOLUMBAR BURST FRACTURES

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ABSTRACT

Objectives: To demonstrate the feasibility of spinal canal decompression through the posterior transpedicular approach in patients with thoracolumbar burst fractures.

Methods: We present 25 consecutive patients (19 men and 6 women; mean age 36 years; age range, 24-48 years) with incomplete neurological deficits (ASIA B and C) resulting from thoracolumbar burst fractures treated by posterior transpedicular spinal canal decompression and posterior segmental instrumented fusion. Canal compromise at presentation was 51.7 ± 11.2%.

Results: The mean surgical time was 122 minutes (range, 108-122 minutes), and the mean blood loss was 528 ± 123 ml. Canal compromise improved to 15.3 ± 7.8%. At a mean follow-up of 14 months (range, 6-18 months), fourteen patients improved to ASIA D and were able to walk with an orthosis; seven improved to ASIA C, and four had no improvement (ASIA B). Seven ASIA B and all ASIA C patients had immediate postoperative neurological improvement to ASIA C and ASIA D; two ASIA B patients improved to ASIA C within six weeks after the operation. Anterior decompression was necessary in two (8%) ASIA B patients who did not improve after the initial operation; these patients, subsequently improved to ASIA C. There were no intraoperative complications. Superficial wound infections occurred in two patients and were treated with wound care and antibiotics; deep infection occurred in one patient and was treated with debridement and antibiotics.

Conclusion: Posterior transpedicular spinal canal decompression and instrumentation is a reasonable alternative technique to anterior decompression procedures and circumferential fusion, providing for satisfactory canal decompression and neurological improvement.

Key words: *thoracolumbar fractures, burst fractures, transpedicular decompression, spinal instrumentation*

INTRODUCTION

Management of thoracolumbar burst fractures, particularly those associated with neurological deficit is challenging. Many classification systems have been described to provide a comprehensive understanding of these fractures and offer guidelines for their management and timing of surgery.[1-3] Most authors agree that early stabilization and decompression is a generally accepted treatment option for unstable fractures associated with neurological deficits.[4] Early decompression intuitively will maximize the possibility of neurological recovery of the patients, and allow for early rehabilitation, which in turn will minimize complications related to prolonged best rest.[5]

The choice of surgical approach depends on associated injuries and patients' general health status. The anterior approach permits clear visualization of the thecal sac, thereby providin g the most reliable results for achieving a thorough decompression of the neural elements from displaced bony fragments.[6,7] In addition, the anterior approach addresses directly the reconstruction of the anterior spinal column and the sagittal alignment of the spine.[8] However, multiple injured patients cannot tolerate the anterior procedure well. Moreover, many acute (less than 5 days) thoracolumbar fractures can be indirectly reduced through the posterior approach and ligamentotaxis.[8,9]

The purpose of this study was to present our

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experience on the surgical treatment of patients with unstable thoracolumbar burst fractures and incomplete neurological deficits treated by posterior decompression through a transpedicular approach and posterior segmental instrumentation and fusion, and to discuss what is really the most suitable approach in cases like these.

PATIENTS AND METHODS

The medical files of 25 consecutive patients admitted to the authors' institution with incomplete neurological deficits resulting from unstable thoracolumbar burst fractures during a 12-month time period from March 2007 to February 2008 were retrospectively reviewed. There were 19 men and 6 women with a mean age of 36 years (range, 24 to 38 years). All patients gave written informed consent to be included in this study and were included in the postoperative evaluation. The study has been approved by the institutional review board of the authors' institution.

The mechanism of injury was motor vehicle accident in 14 patients, fall from height in 9 patients, and suicide attempts in 2 patients. The fractures were classified according to Denis classification (Tables 1 and 2). Neurological evaluation was performed according to the American Spinal Injury Association (ASIA) (Tables 2 and 3). Canal compromise and local kyphosis were evaluated preoperatively and postoperatively with plain radiographs and computed tomography scans (Table 4).

Table 1. Denis classification of thoracolumbar burst fractures

Type A	Fracture of both end plates
Type B	Fracture of superior end plate
Type C	Fracture of the inferior end plate
Type D	Burst fracture with rotation
Type E	Lateral burst fracture

All patients were operated within 72 hours from injury, by posterior decompression through a transpedicular approach and posterior segmental instrumentation and fusion. In the prone position, a midline skin incision was made at least two to three levels above and below the level of interest. The incision was carried down to the soft tissues and the ligamentous attachments; the muscles were taken off the spinous processes and laminae to the tips of the transverse processes. Pedicle screws were inserted two to three levels above and two levels below the fractured vertebra. Decompression of the spinal canal was done through a central laminectomy that was performed laterally with thinning of the pars interarticularis; this was done very carefully to preserve the continuity of the posterior elements, in order to preserve a bed to lay bone graft to facilitate fusion. Portion of the inferior part of the superior facet was removed to gain access to the top of the pedicle. With a high speed burr the pedicle was drilled down to its base (Fig. 1A). This was performed bilaterally, providing adequate visualization of the neural elements. The bony fragments were then removed with bone curettes and disc rongeurs (Fig. 1BC). The fracture fragments were impacted into the vertebral body using bone tamps to fill the void and provide structural support to the anterior column, with care to avoid retraction of the thecal sac (Fig. 2AB). Once decompression was achieved, rods were placed on the pedicle screws and reduc-

Table 2. The American Spinal Injury Association (ASIA) classification of spinal cord injuries

ASIA IMPAIRMENT SCALE	
A = Complete:	No motor or sensory function is observed in the sacral segments S4-S5.
B = Incomplete:	Sensory but not motor function is observed below the neurological level and includes the sacral segments S4-S5.
C = Incomplete:	Motor function is preserved below the neurological level, and more than half of key muscles below the neurological level have a muscle grade less than 3.
D = Incomplete:	Motor function is preserved below the neurological level, and at least half of key muscles below the neurological level have a muscle grade of 3 or more.
E = Normal:	Motor and sensory function is normal.

Folia Medica 2010; 52(4): 39-47

Table 3. Preoperative neurological deficits (ASIA classification), vertebral level and type of fractures (Denis classification) of the patients involved in this study

Level	Type A fracture (n = 16)	Type B fracture (n = 3)	Type C fracture (n = 5)	Type D fracture (n = 1)
Above T11	-	-	2 (ASIA B)	-
T11	-	1 (ASIA C)	1 (ASIA B)	-
T12	6 (5 ASIA B; 1 ASIA C)	0	2 (ASIA B)	1 (ASIA B)
L1	8 (6 ASIA B; 2 ASIA C)	2 (ASIA C)	-	-
L2	2 (ASIA C)	-	-	-
Below L2	-	-	-	-

Table 4. Preoperative and latest imaging evaluation.

	Canal compromise	Loss of vertebral height	Local kyphosis (Cobb angle*)	Loss of sagittal alignment
Preoperative	51.7 ± 11.2%	48.3 ± 7.4%	+7.8° ± 2.4**	
Postoperative	15.3 ± 7.8%	12.7 ± 3.1%	+1.2° ± 0.8**	2.3° ± 0.4

* The Cobb angles were measured from the inferior endplate of the vertebra above to the superior endplate of the vertebra below the fractured vertebra.
** (+) sign denotes kyphosis.

tion maneuver was performed to restore vertebral body height and sagittal alignment of the spine. Decortication was done with osteotomes and high speed burr. Locally harvested bone autograft and bone allograft was laid along the lateral gutters to facilitate fusion.

Postoperatively, physical therapy and rehabilitation was initiated as soon as associated injuries permitted. A thoracolumbar spinal orthosis (TLSO) was applied for three months. Postoperative neurological and imaging evaluation was done at 3, 6 and 12 months, and at the latest examination for the purpose of this study. Continues variables were expressed as mean ± SD.

RESULTS

The mean surgical time was 122 minutes (range, 108-122 minutes), and the mean blood loss was 528 ± 123 ml. Canal compromise improved to 15.3 ± 7.8% (Table 4). At a mean follow-up of 14 months (range, 6-18 months), fourteen patients improved to ASIA D and were able to walk with an orthosis; seven improved to ASIA C, and four had no improvement (ASIA B). Seven ASIA B and all ASIA C patients had immediate postoperative neurological improvement to ASIA C and ASIA D (Figs. 3 to 6); two ASIA B patients improved to ASIA C within six weeks after the operation. Anterior decompression was necessary in two ASIA B patients who did not improve after the initial operation; these patients, subsequently improved to ASIA C (Table 5).

Intraoperative complications were not observed. Superficial wound infections were diagnosed in two patients and were treated with local wound care and antibiotics; deep infection occurred in one patient and was treated with surgical debridement and antibiotics.

At the latest examination, radiographic evaluation showed local kyphosis of 1.2 ± 0.8 degrees, as measured by the Cobb angle, and an average of 2.3 ± 0.4 degrees loss of correction of the sagittal alignment (Table 4). Implant related complications such as screw cut-out or loosening, or rod fracture were not observed.

DISCUSSION

The treatment of thoracolumbar burst fractures requires a clear understanding of the mechanism of the injury in relation to the biomechanics of the

Table 5. Postoperative neurological deficits (ASIA classification), vertebral level and type of fractures (Denis classification) of the patients involved in this study.

Level	Type A fracture (n = 16)	Type B fracture (n = 3)	Type C fracture (n = 5)	Type D fracture (n = 1)
Above T11	-	-	2 (ASIA D)	
T11	-	1 (ASIA D)	1 (ASIA C)	
T12	6 (2 ASIA B; 2 ASIA C; 2 ASIA D)	0	2 (ASIA D)	1 (ASIA C)
L1	8 (2 ASIA B; 3 ASIA C; 3 ASIA D)	2 (ASIA D)	-	
L2	2 (ASIA D)	-	-	
Below L2	-	-	-	

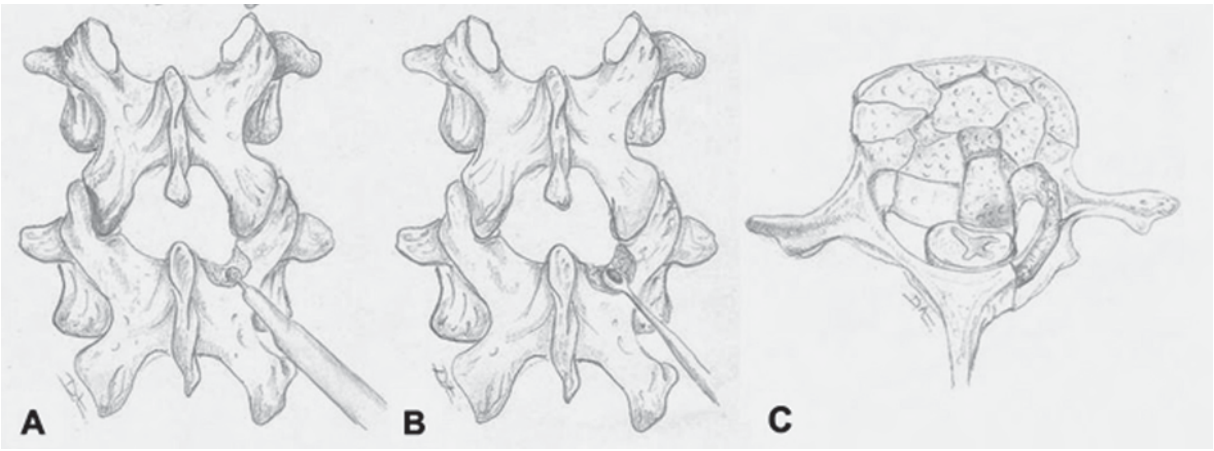

Figure 1. **(A)** Through the posterior approach, decompression of the spinal canal was done through a central laminectomy that was performed laterally with thinning of the pars interarticularis; using a high speed burr the pedicle was drilled down to its base. **(B)** The bony fragments were then removed with bone curettes. **(C)** for adequate visualization of the neural elements.

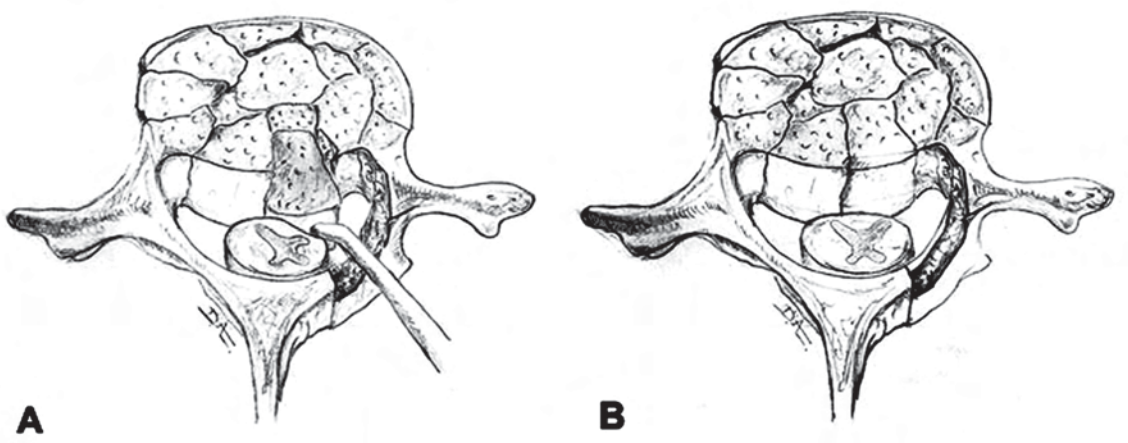

Figure 2. **(A)** Using bone tamps the fracture fragments were impacted into the vertebral body for **(B)** decompression of the spinal canal and void filling for structural support to the anterior column.

Folia Medica 2010; 52(4): 39-47

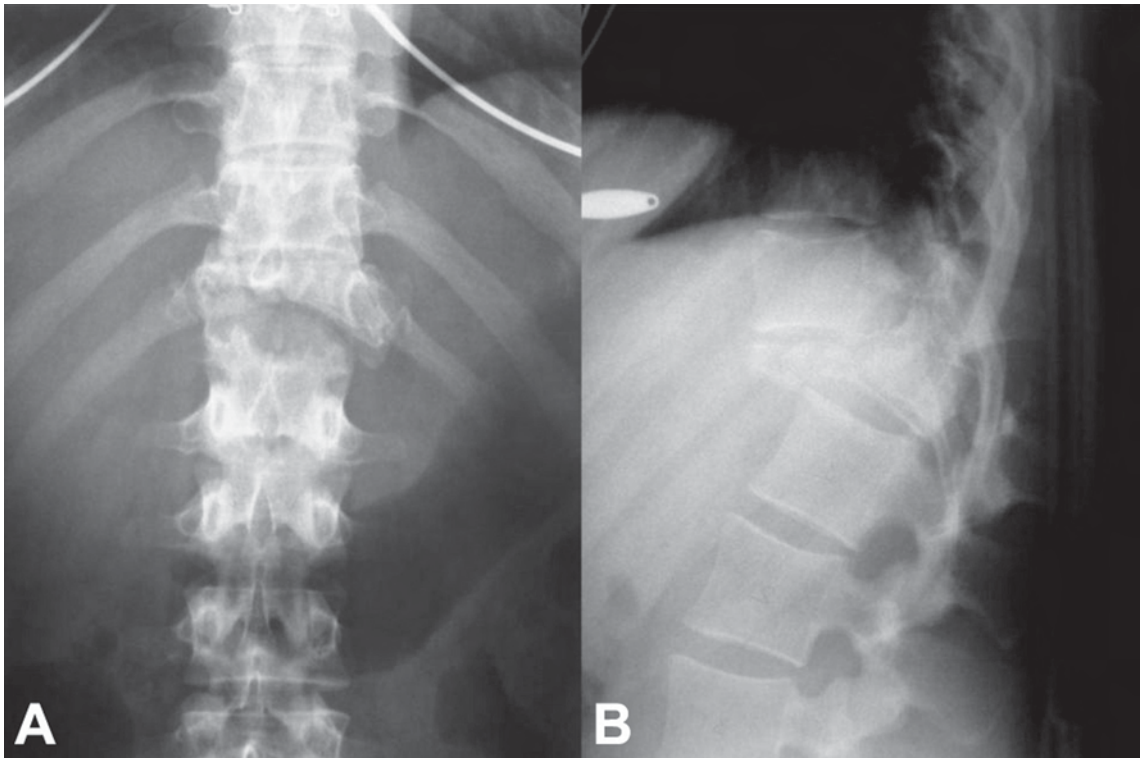

Figure. 3. **(A)** Anteroposterior and **(B)** lateral radiographs of the thoracolumbar spine of a 20-year-old woman with a type D burst fracture of the T-12 vertebra and incomplete neurological deficits (ASIA B).

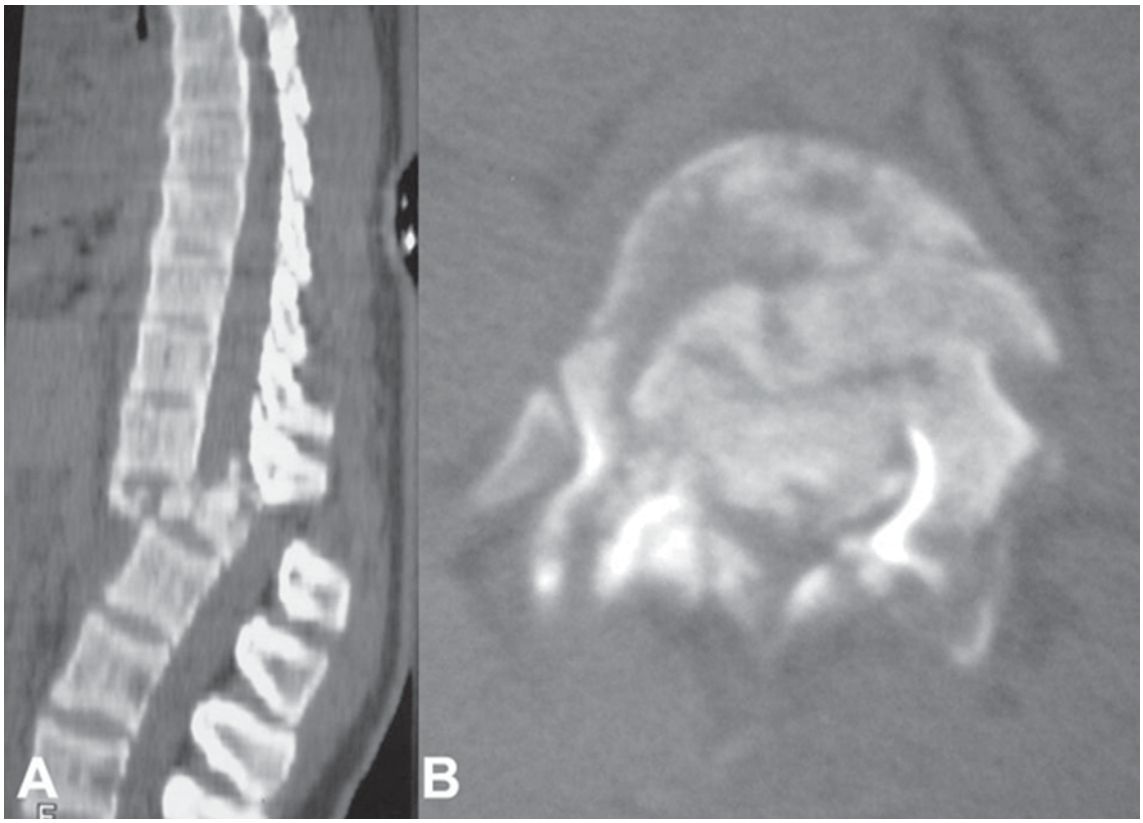

Figure 4. Preoperative **(A)** sagittal and **(B)** axial CT scan of the thoracolumbar spine show significant canal compromise.

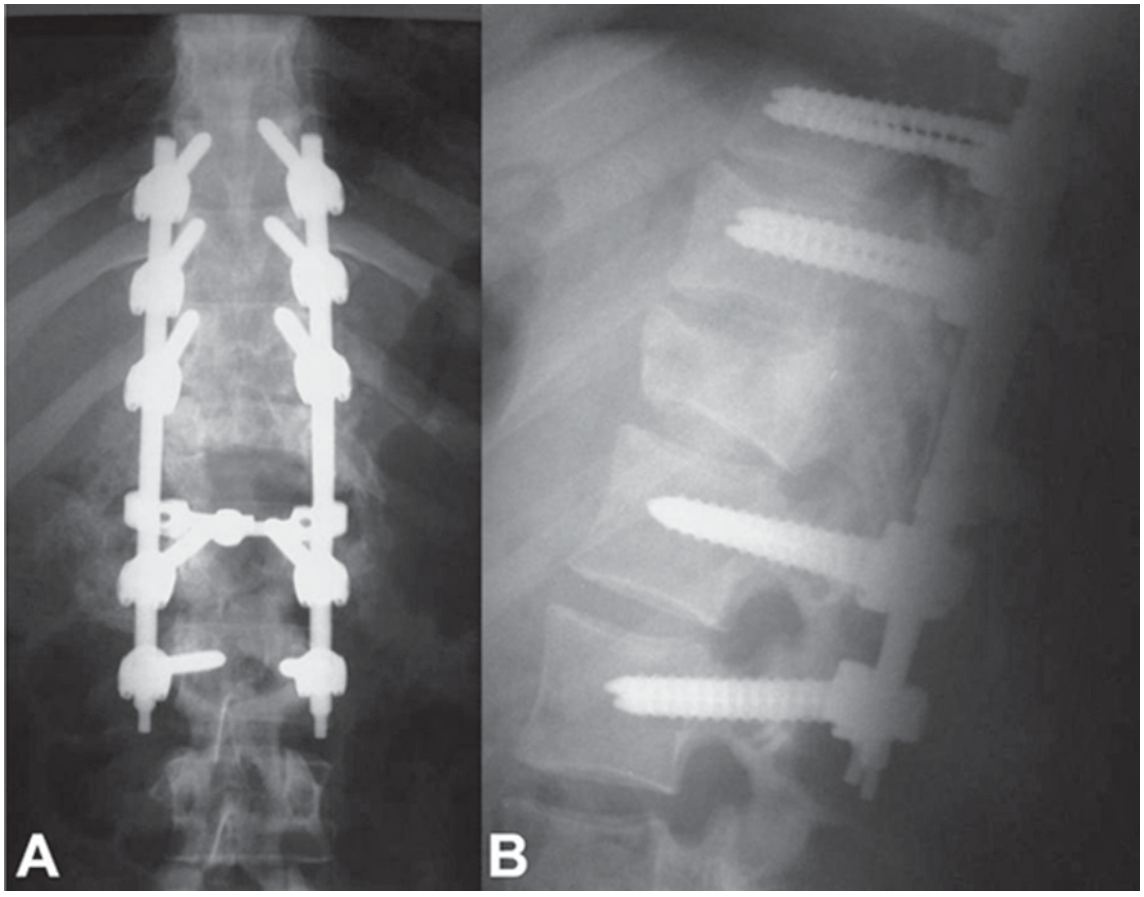

Figure 5. Postoperative **(A)** anteroposterior and **(B)** lateral radiographs of the thoracolumbar spine after posterior transpedicular decompression and spinal instrumentation. The patient had neurological improvement to ASIA C.

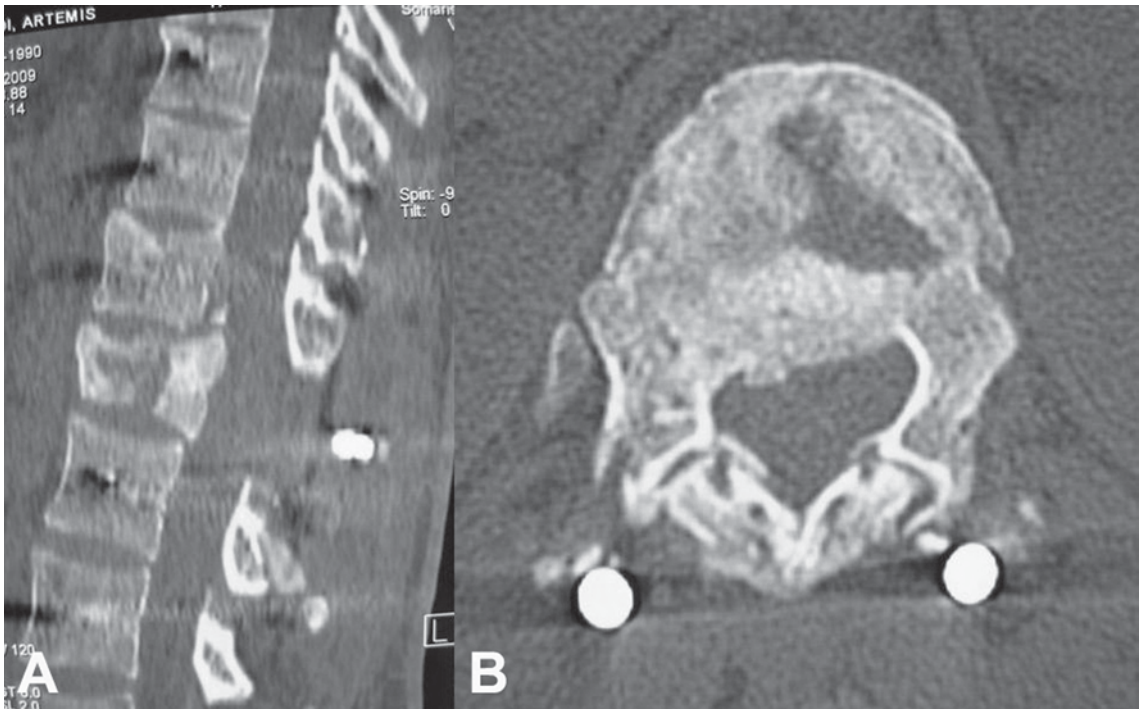

Figure 6. Postoperative **(A)** sagittal and **(B)** axial CT scan of the thoracolumbar spine show decompression of the spinal canal.

Folia Medica 2010; 52(4): 39-47

spine. Previous studies have reported different spinal models and criteria to evaluate spinal instability.[10-14] However, most surgeons agree that a select group of injuries that are clearly unstable, with or without neurological deficits warrant surgical intervention. In these cases, surgical treatment provides for a stable and aligned spine, and whenever indicated for decompression of the neural elements and the spinal cord.[15] The purpose of the present study was to evaluate the feasibility of decompression of neural elements through the posterior transpedicular approach in patients with thoracolumbar burst fractures and incomplete neurological deficits.

In the present study, the small number of the patients and the lack of a control group may be considered a limitation. In addition, we did not control for the use of different implant manufacturers and implants design, and neurological improvement of the patients was not related to the specific fracture type; patients' age, body mass, and comorbidities also may affect the validity and reproducibility of our results. Nonetheless, the surgery was conducted primarily by one surgeon, surgical technique was strictly controlled and there was no potential selection bias in regard to fracture type and level of decompression. Our incidence of neurological improvement, implant failure and postoperative kyphotic deformity is consistent with other published series.[9,16-18], and represents a valid finding. Furthermore, the purpose of this study was not to evaluate the outcome of patients with a specific thoracolumbar fracture type, but to evaluate the effect of posterior transpedicular decompression on neurological improvement of these patients with unstable thoracolumbar spinal fractures.

Spinal cord decompression following spinal trauma is of great importance in order to maximize the chances for neurological recovery of the patient. It is well-known that the methods of choice in different hospital units around the world differ and that methods and approaches are not easily changed. Decompression can be direct anterior, posterior, posterolateral or transpedicular, and indirect.[9,19] The proponents of anterior approach argue that direct anterior visualization of the canal will provide the best access to the spinal cord for removing bone fragments; in addition, anterior decompression and fusion will counter the compressive forces that are applied to the anterior column.[9,16,17] In 1992, Lemons et al.[20] claimed that compared with spinal instrumentation alone, transpedicular decompression showed no benefit in terms of postoperative canal dimensions or neurological outcome. On the basis of this experience, transpedicular decompression offers no advantage over spinal instrumentation alone.[20] The missing stabilization of the anterior spinal column may lead to a loss of sagittal alignment of the thoracolumbar spine with time and increased kyphosis.[21,22] This has also been observed in the present series; however, the degree of postoperative kyphosis was limited, without any significant effect on the sagittal alignment of the spine. Maybe the mean follow-up time of 14 months is too short to assess this. However, careful review of the literature does not demonstrate a clear advantage of the anterior versus the posterior decompression of the spinal canal in patients with incomplete neurological injury.[9,16,17] Triantafyllou and Gertzbein[18] evaluated the outcome of thoracolumbar spine fractures associated with incomplete neurological deficits. In their series, statistical significant results were in favor of surgical treatment with expected recovery rate ranging between 60 to 70% compared with patients with similar injuries that were treated non-operatively. However, they found no significant difference regarding recovery between the anterior and the posterior approach.[18] In the present series, 21 of the 25 patients (84%) improved for at least one grade in the ASIA scale. In two patients (8%), following posterior decompression there was persistent compression on the sac. In these patients, anterior decompression was necessary to remove the bony fragments and improve the neurological status.

Increased complications rates have been reported with the anterior approach for spinal cord decompression. These include vascular injury of the great vessels and compromise of the blood supply to the spinal cord.[4,14] By contrast, as the present study has shown, complications associated with the posterior approach are minimal. With the technological advance of the pedicle screws, the post-traumatic deformity can be corrected through indirect reduction by ligamentotaxis, provided that the procedure is performed early (less than 5 days) after the injury and the posterior longitudinal ligament is intact.[23,24]

CONCLUSIONS

After decades of treating spinal fractures with different approaches, the issue raised herein is still challenging. Posterior approach offers obvious advantages, and arguments like that are missing. Based on the findings of this study, adequate spinal decompression can be obtained through the posterior transpedicular approach in patients with

thoracolumbar burst fractures and incomplete neurological deficits. Although the posterior approach does not allow direct visualization of the neural elements, as is the fact with the anterior approach, adequate decompression can be achieved in most cases, leading to an acceptable recovery rate with minor complications and early mobilization and rehabilitation for these patients.

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ЗАДНЯЯ ТРАНСПЕДИКУЛЯРНАЯ СПИНАЛЬНАЯ ДЕКОМПРЕССИЯ ПРИ ТОРАКОЛЮМБАЛЬНЫХ ВЗРЫВНЫХ ФРАКТУРАХ

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РЕЗЮМЕ

Цель: *Представить применимость заднего транспедикулярного доступа в целях декомпрессии спинального канальца у пациентов с взрывными тораколюмбальными фрактурами.*

Методы: *Представлено 25 пациентов (19 мужчин и 6 женщин; средний возраст 36 лет - возрастная группа 24 - 48 лет) с неполным неврологическим дефицитом по шкале ASIA в B и C стадиях (дефицит спинального канальца - 51.7 ± 11.2%) в результате взрывных тораколюмбальных фрактур. Пациенты лечены по методу задней спинальной декомпрессии и заднего сегментарного инструментального спондилодеза.*

Результаты: *Среднее оперативное время 122 минуты (диапазон 108 - 123 мин.), а средняя кровопотеря между 528 ± 123 мл. Дефицит спинального канальца улучшился - 15.3 ± 7.8%. Среднее время прослеживания пациентов - 14 мес. (6 - 18 мес.). 14 пациентов показали улучшение до ASIA D и были в состоянии ходить с ортезом; 7 пациентов показали улучшение до ASIA C, а в 4 случаях не регистрировано улучшение (ASIA B).*

У 7 ASIA B и у всех ASIA C пациентов регистрировано непосредственное постоперативное неврологическое улучшение, а у двух ASIA C пациентов состояние улучшилось до ASIA B в сроке 6 нед. после операции. По необходимости передняя декомпрессия применена у двух ASIA B (8%) пациентов, у которых после первоначальной операции не наблюдалось улучшение. Впоследствии состояние этих пациентов улучшилось до ASIA C. Интраоперативные осложнения не регистрированы. Поверхностные раневые инфекции появились у двух пациентов, третированных антибиотиками. Глубокая инфекция констатирована только у одного пациента, и в этом случае пришлось произвести хирургическое очищение раны и лечение антибиотиками.

Заключение: *Задняя транспедикулярная спинальная аппаратурная декомпрессия представляет собой альтернативную технику в случае передней декомпрессии и 360º спондилодеза, обеспечивающая удовлетворительные результаты по отношению к декомпрессии спинального канальца, а также и неврологическое улучшение.*

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ORIGINAL ARTICLES

Dental Investigations

GINGIVAL DISEASE AND SECRETORY IMMUNOGLOBULIN A IN NON-STIMULATED SALIVA IN CHILDREN

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ABSTRACT

Aim: To find the relationship of secretory immunoglobulin A (SIgA) to gingival diseases in childhood and adolescence by quantitative study of these antibodies in non-stimulated saliva.

Patients and methods: The survey included 30 somatically healthy children (mean age 15.37 ± 1.06 yrs) with clinically healthy gingiva and another 30 children (somatically healthy) (mean age 15.07 ± 0.69 yrs) with manifested plaque-induced gingivitis. The diagnosis of periodontal status was made on the basis of clinical criteria, the oral-hygiene index of Silness & Loe, the papilla bleeding index (PBI) of Saxer & Mulheman and the periodontal screening index for evaluation - Periodontal Screening and Registration (PSR, after ADA - American Dental Association).

SIgA in saliva was quantified by ELISA with salivary secretory IgA kit of Salimetrics$_{LLC}$ – USA.

Results: In children with gingivitis the mean SIgA was 41.07 ± 32.14 µg/ml; it was higher in healthy children – 48.3 ± 32.41 µg/ml. A correlation was found between SIgA and the oral-hygiene index of Silness & Loe, ($P < 0.05$) and lack of dependence on the degree of gingival bleeding.

Conclusions: SIgA is a factor characterizing the local specific immunity which depends on local antigenic stimuli (plaque biofilm), but it does not affects the gingival pathology directly. SIgA can be considered an important part of an integrated assessment of oral risk environments.

Key words: *secretory immunoglobulin A (SIgA), plaque-induced gingivitis, ELISA, biofilm*

INTRODUCTION

Secretory immunoglobulin A (SIgA) is the main immunoglobulin isotype found in saliva and other body secretions.[1-4]

Biologically, SIgA provides the first line of immune defense in the oral environment. It is responsible for inhibiting the bacterial adhesion on the enamel and epithelial cells, acting in synergy with other defense mechanisms, making inactive bacterial enzymes and toxins and activating the complement. It is partially involved in cell-mediated immune responses. Thus, SIgA limits the invasion of various antigens in the mucosal epithelium and is involved in the maintenance of bacterial environment in the mouth and in the formation of biofilms on the enamel surface.[5,6]

In multi-factor periodontal pathology it is rather difficult to make a precise assessment of the importance of each risk factor in the oral environment, including the SIgA. However it has been found that these antibodies are in inverse relation with the salivary current, and the salivary current is essential for the accumulation of plaque on tooth enamel.[7,8]

Having in mind that SIgA do not enter the gingival sulcus, it is suggested that these antibodies, control the formation and composition of subgingival biofilm through modeling the accumulation of supragingival biofilm. They can affect the bacterial

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depot of microorganisms, including the subgingival area and to prevent bacterial transmission from one gingival niche to another. Studies on dependencies of periodontal pathology and the concentration of SIgA in saliva are contradictory.[9-12]

Evaluation of oral risk environments is an important part of modern approach to diagnosis and preventive treatment of oral diseases. Determining the risk of periodontal disease includes detection of the factors that cause disturbance in the balance between local subgingival biofilm and local protective processes in the periodontium.

The AIM of the study was to find if SIgA can be correlated with the gingival diseases in childhood and adolescence by studying these antibodies quantitatively.

PATIENTS AND METHODS

Patients

The study was conducted among 60 schoolchildren from Ruse. The children were allocated to two clinical groups: **group I** included 30 children aged 15.37 ± 1.06 (13 boys and 17 girls), in somatic health and with clinically healthy gingiva; **group II** consisted of another 30 children (mean age 15.07 ± 0.69 yrs) (11 boys and 19 girls), somatically healthy but with clinically manifested plaque-induced gingivitis.

To form these two groups we examined 180 randomly selected children aged 15-16 with stabilized periodontium; the children were given a thorough examination of their mouths focusing on their periodontal health; they were clinically healthy and without systemic diseases. Sixty children were selected - 30 with absolutely healthy periodontium and 30 with plaque gingival diseases.

The study was conducted with the permission of the Ethics Committee of Scientific Research at Medical University-Sofia obtaining informed consent from each probant over 16 years and from their parents if the children were younger than 16 years of age.

Evaluation of biofilm in the studied children

Oral hygiene status of the children was determined by recording the oral hygiene index (OHI of Silness & Loe) using the classical methodology of the index determination.[13]

Diagnosing the periodontal status

Diagnosis of children with healthy periodontium and children with chronic plaque-induced gingivitis was conducted using the following criteria to distinguish between the two groups:

1. Diagnosed due to clinical examination using the clinical criteria for healthy and inflamed gingiva (Table 1).

2. Provoked bleeding on probing. We used the Papilla Bleeding Index (PBI) of Saxer & Mulheman.[14] The obtained values were recorded on a pre-made card for evaluation and registration of oral status with periodontal orientation. To register the location of the gingival inflammation we grouped the children with gingivitis in 4 subgroups according to the proportion of bleeding papillae to the total number tested papillae for each child. This is an important indicator in children given that localized gingivitis in children is common and it must be registered as such. The idea was suggested by GBI of Aynamo & Bay.[15]

3. Determination of the depth of the gingival sulcus through probing (probing pocket depth). We used the index for screening periodontal assessment and registration - PSR (after ADA-American Dental Association).[16] In this index, as in the CPITN, dentition is divided into six sextants, and probing is done within 6 points of every tooth representative for each sextant. For each sextant the highest measured value was considered, and for the tested child - the value of the worst affected sextant. We used the following coding: 0 - no specifics, 1 - up to 3 mm and bleeding; 2 - up to 3 mm, bleeding + tartar; 3 - 3 - 5 mm; code 4 - > 6 mm; code (*) - detection of furcations, mobility, recession and other parodontal lesions.

Table 1. Clinical criteria for identifying healthy gingiva and gingival inflammation

Clinical criteria	For gingival inflammation	For healthy gingiva and parodontium
Gingival papilla	edematous, shiny, red, detached, and with possible ulceration	pale pink, tight-fitting
Gingival edge (free gingiva)	swollen, thickened, hyperemic	pale pink, tight, adhering to the tooth
Attached gingiva	shiny, red, smooth contour	pale pink, orange peel

In our study we found no children with scores 3 and 4 suggesting initial periodontal destruction and confirming the clinical diagnosis of gingivitis.

EXAMINATION OF SECRETORY IMMUNITY (SIgA) IN SALIVA

Methods for collecting saliva. A sample of non-stimulated saliva was taken in the morning before breakfast, after washing the teeth at least half an hour before taking the sample. The saliva was collected in a 5 ml plastic container, from which a certain amount (1 ml) was taken by a pipette and placed in another container for freezing. The samples were frozen in refrigerator (-20°C).

ELISA method for examination of IgA-S in saliva. For quantification of SIgA in saliva we used the ELISA assay with salivary secretory IgA kit of Salimetrics$_{LLC}$ – USA.[8] This is an indirect method. Conjugated goat anti-human SIgA is used. The induced enzyme reaction was read by an ELISA-reader by measuring the optical density at wavelength of 450 nm and 650 nm. Using an equation drawn from a standard curve, the concentrations of SIgA in μg / ml were calculated (Fig. 1).

Statistical analysis was performed using SPSS version 16 (SPSS Inc.Chicago USA). Analysis of variance (descriptive statistics) was used, as quantifiable results were presented as arithmetic mean ± standard error and standard deviation. We applied the *t*-test to compare the mean and the correlation coefficient of Pearson to find the relationship between various parameters. $P < 0.05$ was considered as level of significance for the null hypothesis

RESULTS

CHARACTERIZATION OF THE BIOFILM IN THE STUDIED CHILDREN

Comparative analysis of accumulation of plaque between the two groups of children was performed using the OHI of Silness & Loe (Table 2).

Table 2. Oral-hygiene index of the examined children after Silness & Loe

Children	Mean ± SD	SEM
Group I - with gingivitis	2.61 ± 0.54	0.09
Group II - without gingivitis	1.32 ± 0.87	0.16
Significance	t = 6.877, P = 0.0001	

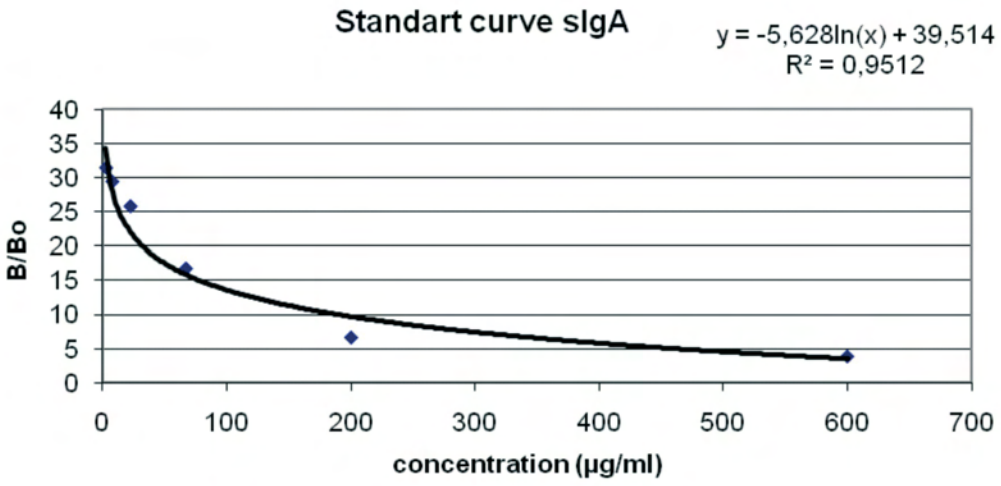


Figure 1. Standard curve obtained in the study.

There was a significant difference in OHI between the two groups of children ($P < 0.05$). The children with gingival disease had more biofilm and poorer oral hygiene which increases the risk of periodontal pathology and in the absence of other risk factors for such pathology proves the clinical diagnosis in children from the second group - plaque-induced gingivitis.

CLINICAL CHARACTERISTICS OF PERIODONTAL STATUS OF CHILDREN WITH GINGIVAL DISEASES

Clinical evaluation of periodontal health according to existing clinical criteria. In the group of children with periodontal pathology we observed only plaque-induced gingival diseases, and only one child was diagnosed with ulcerative necrotic gingivitis.

Folia Medica 2010; 52(4): 48-55

Clinical assessment of gingival bleeding using PBI. Gingival bleeding is the most characteristic symptom of gingival inflammation; its assessment using indices is the right way to measure it. The results showing the proportion of bleeding papillae compared with the total number of tested papillae are indicative of the degree of involvement of individual teeth in gingival inflammation (Table 3).

Almost half of children (43.33%) with gingival diseases presented with generalized gingivitis, affecting on an average more than 75% of the gingiva of each dentition. They are followed by the children with localized gingival diseases affecting from one fourth to half of the gingival papillae. They represent slightly more than one third of the whole group of children. The remaining children are equally distributed in children with bleeding in one fourth of the papillae and children with bleeding in more than half of the papillae. These are local gingival inflammations around individual teeth and inflammations involving more than half but no less than two thirds of the dentition. It is noteworthy that in all cases of localized plaque-induced gingivitis the localization of inflammation is related to available orthodontic anomalies and teeth, ectopically located to varying degrees (teeth with deviation from the dental arch).

Depending on the degree of bleeding of gingival papillae children with gingivitis are distributed as follows (Table 4):

The children with a third degree of bleeding were the most numerous - 13 (21.66%) which is indicative of significant inflammation of the gingiva. The second most numerous children were 10 children with PBI to first-degree provoked bleeding (16.66%), i.e. with mild gingival inflammation. 10% of all children had a mild inflammation, 6 of whom had moderate inflammation of the gingiva. One child had the most severe bleeding (up to code 4). This is the child with ulcerative gingivitis.

Assessment of the periodontal status with the help of the PSR-index

The children with gingivitis were in the age group of 15 to 16 years - an age in which the periodontium is stable. This is an important fact for the comparative study of the gingival sulcus and the attachment. Using a periodontal screening index for assessment (PSR) we registered the periodontal status of the surveyed children (Fig. 2).

The highest score for each child in probing the 6 sextants of the child was taken into consideration. The distribution of children with clinically detectable gingival diseases shows that 28 of the

Table 3. Distribution of children with gingivitis, according to the relative portion of bleeding papillae (after PBI)

Grouping according to the percentage of bleeding papillae (after PBI)		n	%	± Sp
Group I:	1 - 25%	3	10	5.47
Group II:	25 - 50%	11	36.66	8.95
Group III:	50 - 75%	3	10	5.47
Group IV:	75 - 100%	13	43.33	9.20
Statistical significance		$t_{1,2} = 2.79$ $P > 0.05$	$t_{2,3} = 2.79$ $P > 0.05$	$t_{3,4} = 3.16$ $P < 0.05$

Table 4. Comparison of children, grouped according to PBI

	n	Mean ± SD	SEM	Statistical significance
Children with code up to 1	10	1.007 ± 0.022	0.007	$t_{1,4} = -27.398$ $P = 0.0001$
Children with code up to 2	6	1.95 ± 0.260	0.106	
Children with code up to 3	13	2.867 ± 0.212	0.590	$t_{1,2} = -11.688$ $P = 0.0001$
Children with code up to 4	1	3.56		

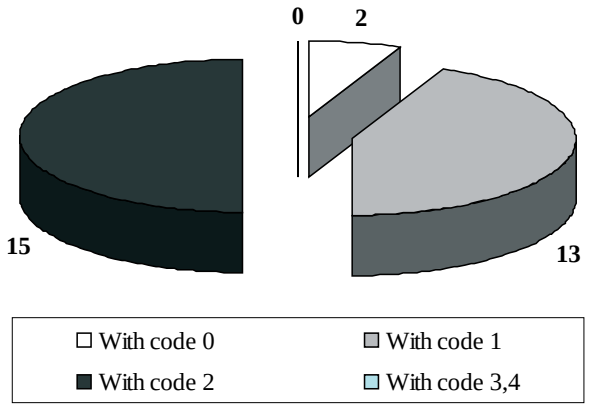
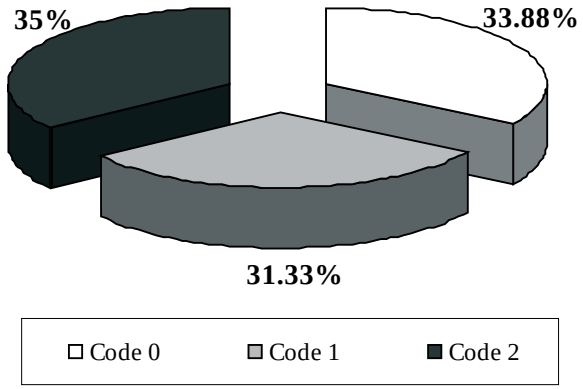


Figure 2. Distribution of children with gingival diseases according to the PSR-index.

children according to the PSR-index have at least one sextant with score 1 or 2 confirming the clinically detected gingival inflammation. If there are two children with score 0 it shows a discrepancy between the established clinical diagnosis (gingivitis)



$t_{0,1}$= 1.43 $t_{1,2}$ = 2.25 (P > 0.05)

Figure 3. Distribution of affected sextants according to PSR.

and the outcome of the PSR index. This is due to the localization of the gingival inflammation which is outside the representative teeth for examination. There were no children with PSR 3 and 4 which shows lack of destructive periodontal processes in the examined subjects.

The distribution of affected sextants is shown in Fig. 3

The distribution of the sextants with codes 0, 1 and 2 is almost even. There are no sextants coded 3 and 4. The greatest number of sextants is with code 2, but significant differences with sextants coded 0 and 1 were not found. This result proves the absence of periodontal destruction in the studied children and manifests even distribution between healthy sextants and those with mild and more severe gingival inflammation. Thus the clinical diagnosis of plaque induced gingivitis is confirmed. Some of the children have generalized gingivitis, others have localized gingivitis.

The distribution of the sextants by severity of the pathology observed in the group of children with gingivitis is shown in Fig. 4.

Of all examined 30 children the biggest percentage of healthy children are in the fourth sextant - 15 children (50% of all cases). The least healthy are the fifth sextants - in 4 children (13.3%) and these refer to the lower front teeth. The difference between them is significant (P < 0.05). Similar difference, although smaller, is found between healthy second and fourth sextants. In this comparison as well the second sextants are more affected than the fourth ones. i.e. the upper front teeth are more severely affected than the lower front teeth. There is no significant difference in the number of healthy first, third, fourth and sixth sextants.

Gingival inflammation in the study children is mainly in the front - significantly more affected are second and fifth sextants, as inflammation is predominant in the upper frontal sections.

Quantifying SIgA in saliva of healthy children and children with plaque-induced gingivitis

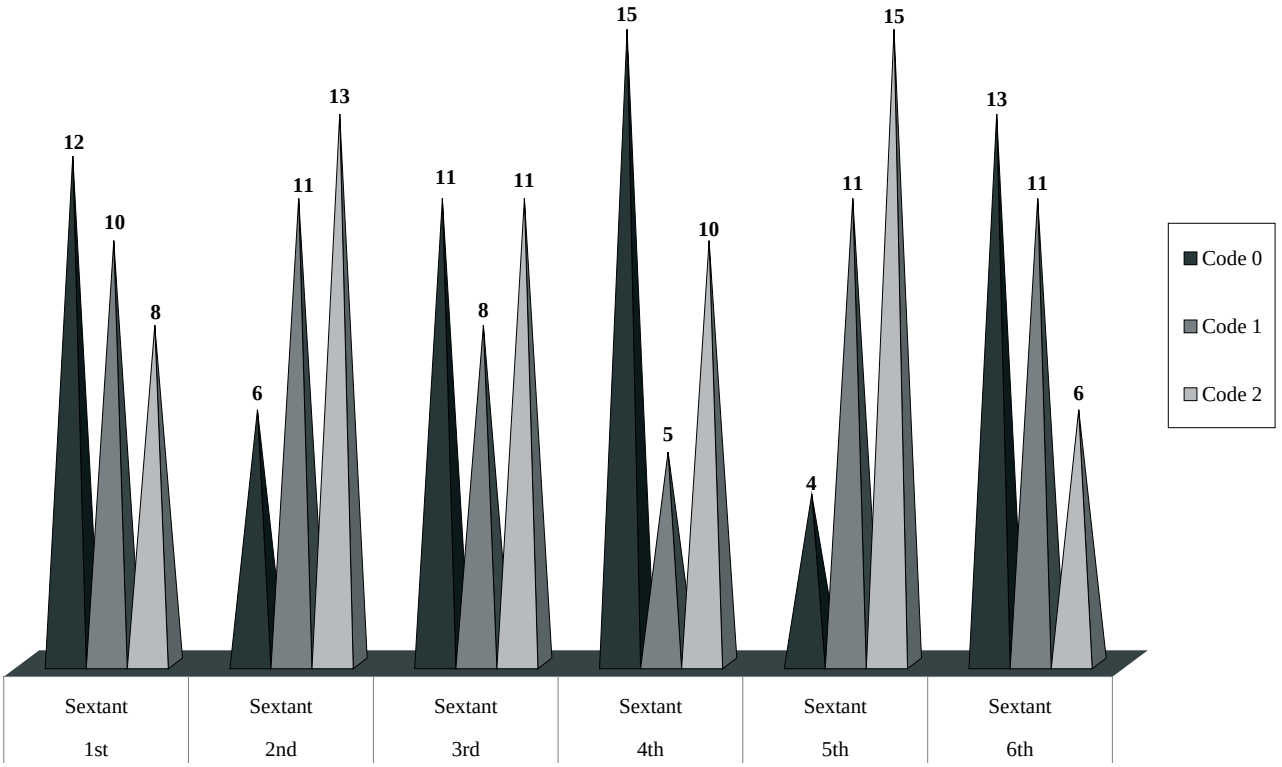
Oral secretory immunity is characterized by antibodies of the SIgA type which are secreted in saliva and are the result of local antigenic stimuli. The most important among these are the bacterial plaque biofilm antigens. As a protective factor of the macroorganism, SIgA provides the most important specific immune protective factor in the mouth and plays an important role in the homeostasis of the oral microbial environment.

SIgA was studied by using samples of non-stimulated saliva. Mean values of SIgA in mixed saliva of the examined children were 44.93 ± 32.24 μg/ml. The obtained values vary between 1.2 μg/ml and 175.5 μg/ml. The distribution of mean values of SIgA in both groups of children is shown in Table 5.

In children with gingivitis the mean SIgA is 41.07 ± 32.14 μg/ml, and in healthy children it is higher - 48.3 ± 32.41 μg/ml. Although the difference between them does not reach statistical significance (P > 0.05), there is a slight tendency to reduction of SIgA in children with plaque-induced gingivitis.

Given that gingivitis is plaque-dependent, using the correlation coefficient of Pearson we looked

Folia Medica 2010; 52(4): 48-55



Code 0	$t_{4,5} = 3.33$	$P < 0.05$
	$t_{2,4} = 3.00$	$P < 0.05$
Code 1	$t_{2,4} = 2.46$	$P > 0.05$
Code 2	$t_{5,6} = 1.84$	$P > 0.05$

Figure 4. Distribution of the sextants by severity of the pathology observed in the group of children with gingivitis.

Table 5. Mean SIgA values in saliva of children with and without plaque-induced gingivitis (μg/ml)

Children	n	$\bar{x} \pm S_{\bar{x}}$	SEM
With gingivitis	30	41.07 ± 32.14	5.86
Without gingivitis	30	48.3 ± 32.41	5. 91
t / P		t = -0.927, P = 0.358	

Table 6. Correlation between SIgA, OHI and PBI

	n	Pearson Correlation coefficient	P
OHI - SIgA	30	- 0.291	P < 0.05
PBI - SIgA	30	- 0.212	P > 0.05

into the possibility of a correlation between SIgA and plaque biofilm (OHI S & L) and correlation with bleeding gingival papillae (PBI) – an indicator of gingival inflammation. Results are presented in Table 6.

A correlation was found between SIgA and the plaque biofilm with a level of significance P < 0.05 and lack of dependence on the degree of gingival bleeding.

DISCUSSION

Dynamically changing saliva as material for analysis determines the difficulty of standardizing the testing for SIgA, as well as comparative analysis with other similar studies. In the present study we have used samples of non-stimulated saliva to measure the concentration of SIgA. In previous research and research reported in the literature have used for the same kind of research stimulated mixed saliva and clean parotid saliva.[5,17-19] According to some authors the use of mixed non-stimulated saliva is best for the study of SIgA because this is the secretion that washes the mouth cavity.[11,20] Bacteria in mixed saliva can adsorb antibodies which affects

the measurement of their quantity.[21]

Our research shows significant differences in the concentration of SIgA using the same test, but applied to samples of stimulated and non-stimulated saliva. In the present study SIgA in somatically healthy children (15-16 yrs) was 44.93 ± 32.24 μg/ml and also in previous studies in healthy children (7-15 yrs), whose saliva is stimulated, it is 121.[22] Although non-stimulated saliva shows lower levels of SIgA, their distribution is more even compared to SIgA from stimulated saliva (from a previous study[23,24], which is why we recommend non-stimulated saliva for the study of SIgA.

In the literature there is scanty information about the role of SIgA from saliva in the development of periodontal diseases. According to some authors, low levels of SIgA in saliva can be considered a potential risk factor for the development of periodontal disease and caries.[12,22] It is very difficult to ascertain how SIgA can control the subgingival biofilm, because secretory antibodies do not enter the gingival sulcus or pocket. It is possible SIgA to control the formation and composition of subgingival plaque and its potential to cause disease by modulating the accumulation of supragingival plaque.[11] The results of our study confirm this hypothesis because they prove the correlation between SIgA and the amount of biofilm. SIgA-antibodies could protect the proliferation of parodontopathogenes in the tongue which is a reservoir for these organisms and a source for their colonization in the gingival sulcus.[11]

The presence of correlation between SIgA and OHI shows that the biofilm is an important immunogenic factor for secretory antibodies in saliva, although their quantity can not be regarded as a risk marker of gingival inflammation.

Local antigens in the mouth are the most important stimulus for oral secretory immunity. This conclusion is supported by another study of ours, which demonstrated that the plastic orthodontic appliances are also local stimuli for secretory antibodies in the mouth in children.[24]

CONCLUSIONS

SIgA is a factor characterizing the local specific immunity which depends on local antigenic stimuli, but does not affect directly the gingival oral pathology. This conclusion is supported by the described mechanisms of action of SIgA in pathological processes in the mouth. Its quantitative evaluation is highly dependent on salivary current and the method of analysis. SIgA is a factor that can be used in comparative studies on the effects of different antigenic stimuli on oral risk environments.

As an indicator of specific local immunity in the mouth, SIgA can be considered an important part of an integrated assessment of oral risk environments.

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ГИНГИВАЛЬНЫЕ ЗАБОЛЕВАНИЯ И СЕКРЕТОРНЫЙ ИММУНОГЛОБУЛИН А (SIgA) В НЕСТИМУЛИРОВАННОЙ СЛЮНЕ У ДЕТЕЙ

М. Рашкова, А. Тошева

РЕЗЮМЕ

Цель: *Работа ставит себе целью провести количественное исследование SIgA в нестимулированной слюне, установить связь этих антител с гингивальными заболеваниями в детско-юношеском возрасте.*

Пациеты и методы: *В исследование включено 30 детей (средний возраст 15.37 ± 1.06), соматически здоровых и с клинически здоровой гингивой и 30 детей (средний возраст 15.07 ± 0.69) соматически здоровых с клинически проявленным бляшко-индуцированным гингивитом. Диагностицирование пародонтального статуса проведено с применением клинических критериев, орально-гигиенического индекса Silness & Loe, Papilla Bleeding Index (PBI) на Saxer & Mulheman и индекса относительно скрининговой периодонтальной оценки и регистрации - Periodontal Screening and Registration (PSR, по ADA - Американская дентальная ассоциация).*

В целях количественного определения SIgA в слюне использован метод ELISA с „Salivary secretory IgA KIT" на Salimetrics LLC - USA.

Результаты: *У детей с гингивитами средняя стоимость SIgA в границах 41.07 ± 32.14 μg/ml, а у здоровых детей она выше - 48.3 ± 32.41 μg/ml. Установлены корреляция между SIgA и орально-гигиеническим индексом Silness и отсутствие зависимости со степенью гингивальной кровоточивости.*

Заключение: *SIgA представляет фактор, характеризующий локальный специфический иммунитет, зависящий от локальных антигенных стимулов (бляшковый биофильм), но не оказывающий непосредственное влияние на гингивальную патологию. SIgA можно считать важной частью комплексной оценки оральной рисковой среды.*

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AGE AT AND REASONS FOR THE FIRST DENTAL VISIT

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ABSTRACT

Introduction: The ideal time for the first dental visit of a child as recommended by many professional dental organizations across the world is by the age of one year.

The aim of this study was to survey the age and the most common causes for the first visit to the dental office, as well as the recommendations of dentists regarding this visit.

Patients and methods: The present study included 289 children visiting a dentist for the first time. The children were divided into 5 age groups and the reasons for the visit were categorized into 9 groups. The variables we assessed were the percentages of children in each age group and the reasons for the first visit to the dentist.

Respondents to a questionnaire were 145 dentists. The survey included questions on gender, years of professional experience, specialty, ability to work with children and recommendations concerning the age of the first visit to the dentist.

Results: The greatest number of children making their first dental visit were in the 3-6 year-olds (51.90 percent) and the smallest number were the children younger than 1 year (1.73%). The most common reason for making this visit was caries and its complications (59.86%). The second most common reason was parents' decision to have a prophylactic examination of their child (26.99%).

The number of dentists in this country that recommended that the first visit should be before the age of 1 year is small (17.93%). 39.31% of the respondents recommended that the first dental visit should take place between 1 and 2 years of age, and 31.03 percent - between 2 and 3 years. It was only pediatric dentists that recommended a dentist appointment in the first year of life - 47.37%. This study found that it was the female dentists that predominantly admit and treat children in their dental practices.

Conclusions: The results of the study show that in this country there is no established practice for children to make their first dental visit before they turn one year of age. The predominant cause to make the first visit is caries and its complications. It is necessary to work out recommendations on the age for the first visit to the pediatric dentist and related prophylactic measures.

Key words: *first dental visit, pediatric age, tooth caries, prophylactic check-up*

INTRODUCTION

Worldwide it is recommended that the first visit to the pediatric dentist should take place within the first age of life.[1-7]

A timely first visit of a child to the dental office is an essential part of the children's health care. These early visits recommendations aim at detecting and controlling the various dental pathology and in particular the caries - a disease widespread among children and sometimes occurring immediately after teeth eruption.[7]

The main objective of the early first dental visit is to lay the foundations of preventive education and dental care in order to ensure optimal oral health during childhood.[1] This visit should allow a dentist to detect early lesions, to evaluate craniofacial and dental development, to advise parents on hygiene, nutrition and behaviour in possible traumas and to motivate them for preventive-oriented events.[8] This strategy establishes the practice of prevention leading to long-term benefits for the child and warrants that children will be free from caries[9] because age is a significant determinant for early childhood caries[10].

The age of the first preventive dental visit has a significant positive effect on costs related to dental health. [11]

Prevention of caries in the youngest children is

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possible if they have their visit to the dentist before or soon after the eruption of the first teeth.[4] For this reason, in the UK parents are offered to make an appointment for their children with the dentist immediately after the breaking out of the first deciduous teeth, i.e. about 6 months of age.[4]

The importance of an early visit to the dental office is highlighted by reports of oral colonization of *Streptococcus mutans* in six-month-old predentate infants with their mothers as a source of the infection.[12] Some authors even advise that the first visit concerning the dental health of the child should take place during 4th month of the pregnancy.[7] As a major source of *Streptococcus mutans*, the mother's oral health is a risk factor for the oral health of the newborn. A visit before birth is essential for the establishment of the first contact between the dentist and the parents and to acquire information and trust between both parties. The time is adequate to teach the pregnant mother the principles of health education and to explain to her that the first teeth of her baby begin to form during this stage of pregnancy. Visits to the dental office can provide the mother with information what care must be taken to ensure the normal eruption of the teeth of her child, and how to maintain dental health after teeth eruption. It also makes it possible for hazardous situations to be identified and to prescribe caries-preventive agents such as fluoride. This visit is also suitable to plan the first visit of the future child at about the age of 6 months.[7] This health strategy is easily realisable, economically viable and efficient. Thus a positive, conscious and responsible behaviour is achieved providing for the oral health of the child.

Regarding the recommendations of professional organizations for the first appointment of children with the dentist before the age of 1 year, some universities have develop public health programs associated with the oral health of the youngest population.[13]

Studies on the awareness of dentists on the importance of early first dental visit show different data on the number of those familiar with the recommendations that children visit dental offices before the age of 1 year.[14-16]

There has been no study of the first dental visit and the reason for it in Bulgaria which motivated the conduction of the present study.

AIM

1. To survey the age and the most common reasons for the first visit to the dentist;

2. To study the recommendations of dentists for the first visit to the dental office.

PATIENTS AND METHODS

This study was planned and conducted at the Department of Pediatric Dentistry at the Faculty of Dentistry, Medical University - Plovdiv. It included 289 children who visited the department between 2008 – 2010 for their first dental visit. The children were divided into 5 age groups: up to 1 year, 1-2 years, 2-3 years, 3-6 years and over 6 years. The reasons for the first visit were categorized as follows: prophylactic examination; dental caries/complications; pain, discoloration of teeth/unaesthetic depositions/ bad breath; trauma; malocclusion; missing/ supernumerary tooth: bad habits/other. The proportions of children in each age group and the reasons for the first dental visit have been assessed.

145 dentists answered the anonymous direct questionnaire. The survey included questions on gender, work experience, specialty, ability to work with children and recommendations concerning the age for the first visit to the dentist. The percentages of respondents giving different responses to the questions were calculated.

The results were analysed using the alternative analysis, χ^2 criterion and the Student's t-test. The level of significance for the null hypothesis was $p \leq 0.05$.

RESULTS

Only 1.73% of the children had their first dental visit before the age of one year, 15.92% - between 1 and 2 years, and 22.84% - between 2 and 3 years (Fig. 1). The largest number of children made their visit to the dentist at age 3 - 6 years (51.90%). The first appointment with a dentist for 7.61% of the study contingent was after the age of 6 years.

The distribution of children by reason for the first visit to the dental office is presented in Table 1. Statistically significant is the highest proportion of children who have visited a dental surgery because of caries and its complications (59.86%; $p < 0.001$, $t = 5.99$). The next most common reason is the desire of parents to have prophylactic examination of their child (26.99%). In 16.26% of the children the predominant cause for the visit was pain. Relatively small is the number of patients with trauma (2.77%), unaesthetic staining (2.42%), malocclusion (1.04%), missing tooth or supernumerary teeth (0.69%). Other reasons for the first dental visit (7.61 percent) were aphthous stomatitis, physiologic replacement of teeth and

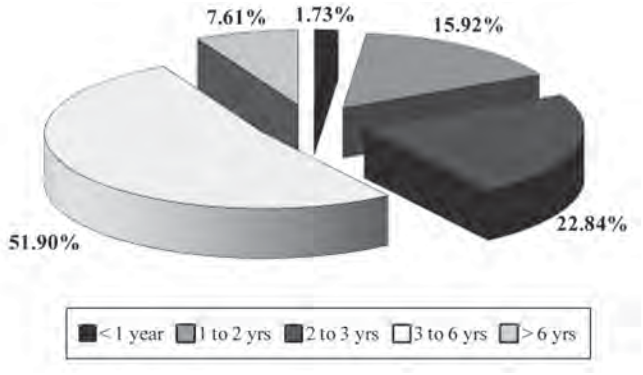

Figure 1. Distribution of children by age at their first visit to the dentist.

for the first dental examination. The largest is the proportion of dentists with another specialty that are of the opinion that the first visit should be between 2 and 3 years of age (44.26%) (Fig. 3).

DISCUSSION

A change in the prospective from seeing only the healing aspect of dental care to the evaluation of its preventive and informative value will lead to improvement of the oral status of the child.[8]

Dental treatment is stressful for all ages but especially it is so for children. To minimize the unpleasant moments during treatment it is important

Table 1. Reasons for the first visit of children (n = 289) to a dentist

Reasons	n	%	Sp
Prophylactic examination	78	26.99	2.61
Tooth caries/complications	173	59.86	2.88
Pain	47	16.26	2.17
Trauma	8	2.77	0.97
Stains/unaesthetic deposits/bad breath	7	2.42	0.90
Malocclusion	3	1.04	0.60
Missing/above-the-number tooth	2	0.69	0.49
Bad habits	0	0	0
Other	22	7.61	1.56

complications during teeth eruption.

Characteristic features of the surveyed dentists are presented in Table 2. The proportion of female dentists who treat children is significantly higher (90.22%), while for males the percentage of those who do not work with children is slightly higher. As a whole, the number of dentists who receive children in their practices remains roughly the same regardless of their work experience.

Only 17.93 percent of the dentists are familiar with the recommendation the first visit to be before the age of 1 year (Fig. 2). 39.31% of the respondents recommend the first dental visit to take place between the age of 1 and 2 years, and 31.03% - at the age of 2 to 3 years. There are doctors who prefer to examine children after the age of 3 years (11.72%) (Fig. 2).

The majority of pediatric dentists recommend the first visit to take place before the age of one (47.37%) or two years (42.11%) (Fig. 3). There is not a significant percentage of dentists without recognised specialty who recommend a specific age

Table 2. Characteristics of the surveyed dentists

Characteristics	n = 145 (%)
Sex	
Male	53 (36.55)
Female	92 (63.45)
Work experience	
Up to 10 years	67 (46.21)
Above 10 years	78 (53.79)
Specialty	
Without any specialty	65 (44.83)
Pediatric dentistry	19 (13.10)
Other specialty	61 (42.07)
Treatment of children	
Yes	110 (75.86)
No	35 (24.14)
Consultation of pregnant women on the oral health of their children	
Yes	100 (68.97)
No	45 (31.03)

Folia Medica 2010; 52(4): 56-61

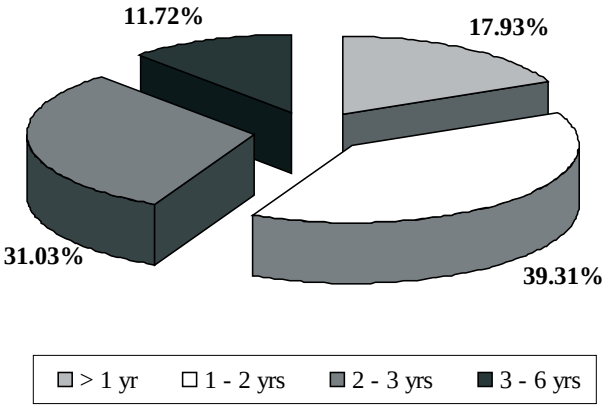

Figure 2. Distribution of respondents according to their recommendations on age for first dental visit.

the USA the children who have visited the dental surgery at the age of 2-4 years is 32%.[3] Slayton et al.[17] report that only 2% of children under the age of 3 visit the dentist during their first year of life. Similar figures are available for Bulgarian children with a first dental visit by the age of 1 year (1.73%). These data suggest that there is still no established practice for parents to take their children to an early visit to the dentist.

In this country, as shown by literature data[8,18,19], the most common reason that prompts the first dental visit is caries. Despite the high percentage of caries and its complications, only in 16.26% of the children the leading cause was pain. This can be explained by decreased sensitivity of deciduous

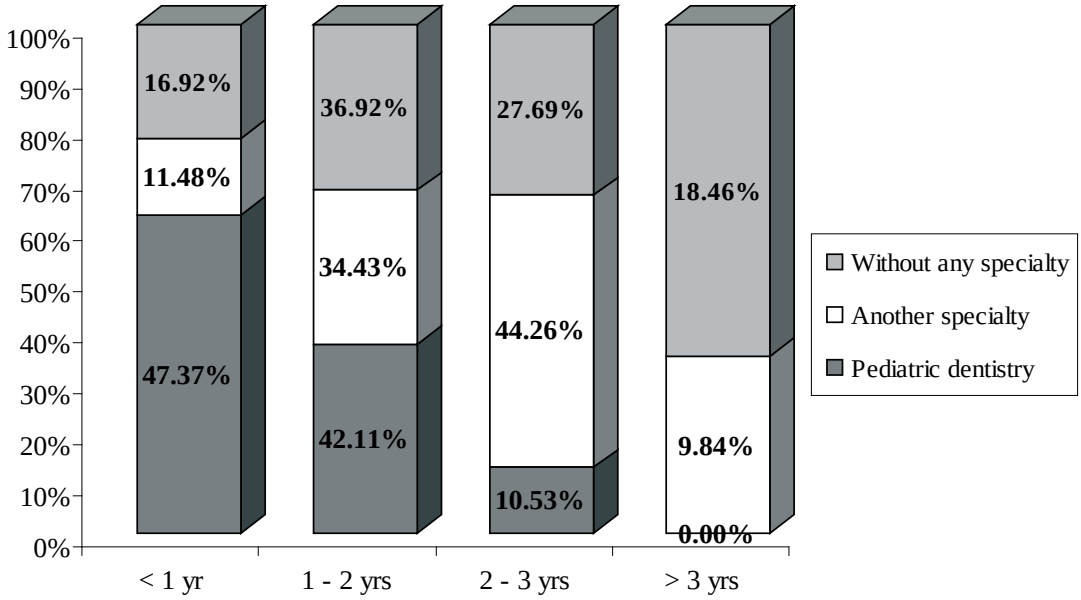

Figure 3. Distribution of respondents by specialty and recommendations for the first visit to the dentist.

that there is trust between the child and the dentist. Trust-building is a tough and gradual process. A timely first visit to the dental office without the need for treatment, and regular contacts between child and dentist, would facilitate the conduct of treatment, if necessary in future.

The results of this study indicate that in this country most parents take their children for the first time to the dental office between 3 and 6 years of age. These data do not match those reported for India. The average age at which children there see the dentist for the first time is over 6 years.[8] Between 6% and 8% of Indian children had their first visit before the age of 3 years. Data for Australia show that most children do not see the dentist before starting pre-school, i.e. about 5 years of age.[5] In

teeth due to changes that have occurred in the pulp. This means that the pain should not be regarded as an objective signal for the severity of the lesions. Age should be the criterion, which requires the preliminary examination and the dental care.

However, there are authors who report results different from ours. Study of the population in Brazil shows that in most cases the reason that parents seek dental care for children is prophylactic examination and prevention.[20] This encouraging result may be due to the fact that this study followed an educational program conducted at a clinic for newborn in the dental school in Aracatuba.

The analysis of the questionnaire results shows that in our country the number of dentists who recommend the first visit to be before the age of

1 year is low (17.93%). The percentage of their counterparts in Canada is higher (58%)[14] and also in Iowa, the USA (76%)[15], who are well aware of the recommendations of the professional organisations for first dental appointment before the age of 1 year. Although informed, only 11% of dentists in Iowa[15] and 12% of general practitioners in Virginia[16] assume that the first visit to the dentist should be between the age of 0 and 11 months. The majority of them (66%)[15] as well as predominant number of Canadian dentists[14] think that children must visit a dentist before the age of 2 years. In this country also the highest is the percentage (39.31 percent) of respondents recommending the first dental visit between the age of 1 and 2 years, followed by those (31.03%) who considered age 2-3 years as more appropriate. Only pediatric dentists most often recommend a dentist appointment in the first year of life - 47.37% in Bulgaria and 100% of respondents in Virginia, USA.[16]

The proportion of respondents who answered positively to the question whether they advise pregnant women on the oral health of their children is high. This result is inconsistent with the rest of the results. The question arises whether such consultations are meaningful and purposeful. There is the routine practice pregnant women to attend the dentist in relation to their own oral status. These visits should be directed to inform expectant mothers on the preventive oral health care of their children.

Questionnaires conducted among pediatricians, including questions on the oral health of their patients, show that they believe that the first dental visit should be about the age of 3 years[21] and only 5% of them recommend it to be done before 1 year of age.[16] The authors of these surveys conclude it is necessary to increase the knowledge of pediatricians about oral health, risk factors and oral pathology of early childhood.[16,21,22] The need for collaboration between all specialists in the field of health (pediatricians, obstetrician, gynecologists, general dental practitioners) is emphasized in order to promote timely first visit to the dental office and prevention aimed at the oral health of children.[7,22]

The present study found that the number of female dentists who see and treat children in their dental practices is predominant. Wolfe et al.[15] reached a similar conclusion. Their analysis shows that dentists who see young children in their offices are younger, recent graduates and very often female. However, we found no correlation between experience of dentists and their willingness to treat children.

Examination and treatment of young children poses a challenge for any dentist, even for professionals. Hardwick describes a detailed technique for the first examination.[23] The author focuses on the conversation between the dentist or their assistant with parents and clarification of the procedures that lie ahead. Another point is the examination itself that is easiest to perform in position knee-to-knee.[23]

CONCLUSIONS

Respondent dentists are not likely to recommend to parents early visit to the dental office, which is confirmed by the high percentage of children who have visited the dentist for the first time after the age of 3 years. Leading cause for the first visit is caries and its complications, which shows that parents do not seek prevention of dental diseases, and respond only if there is a visible problem.

Many dentists see and treat children in their practices, which requires them to be informed and in turn acquaint parents with the importance of the early first visit to the dental office.

Unfortunately, there is no professional debate on the age of the first visit of children to the dental surgery. Professional organizations in Bulgaria do not recommend any age for the first dental visit.

Given the lack of sufficient information to general dental practitioners, pregnant women and parents, it is desirable to have recommendations for the right age of the first visit to the pediatric dentist, techniques for carrying out the first screening and the preventive measures to safeguard the dental health of adolescents.

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К ВОПРОСУ О ВОЗРАСТЕ И ПРИЧИНАХ ПЕРВОГО ВИЗИТА ДЕТЕЙ В КАБИНЕТ ДЕНТАЛЬНОГО ВРАЧА

С. Милева, В. Кондева

РЕЗЮМЕ

Введение: *Ряд профессиональных дентальных организаций в мире рекомендуют осуществить первое посещение детей в кабинет дентального врача до исполнения первого года жизни.*

Цель: *Работа ставит себе целью установить возраст и самые частые причины первого посещения детей в кабинет стоматолога, как и рекомендации врачей относительно времени первого визита.*

Материал и методы: *Исследование охватывает 289 детей, впервые посетивших зубного врача. Дети разделены на 5 возрастных групп, а по причинам визита - на 9 групп (отмечен процент детей по отношению к возрасту и к причинам визита).*

Анкетировано 145 врачей. Анкета содержит вопросы относительно пола, трудового стажа, специальности, возможности работать с детьми, как и относительно рекомендаций, относящихся к возрасту первого визита к зубному врачу.

Результаты: *Самый высокий процент детей, посетивших впервые врача, наблюдается среди возрастной группы 3 - 6 лет (51.90%), а самый низкий - среди детей до первого года жизни (1.73%). Самая часто встречаемая причина визита - это зубной кариес и его осложнения (59.86%), за ней следует желание родителей провести профилактический осмотр их ребенка (26.99%).*

У нас процент дентальных врачей, рекомендующих осуществить визит к стоматологу до исполнения первого года жизни, незначителен (17.93%). Большинство анкетированных врачей (39.31%) рекомендуют осуществить визит в возрасте 1 - 2 года, а 31.03% - в возрасте 2 - 3 года. Единственно детские дентальные врачи рекомендуют осуществить первую встречу детей с врачом в течение первого года жизни (47.37%).

Настоящее исследование установило, что преимущественно дентальные врачи женского пола занимаются лечением детей.

Заключение: *Полученные результаты показывают, что у нас не наблюдается практика раннего первого визита (до первого года жизни) в кабинет стоматолога. Зубной кариес и его осложнения являются основной причиной посетить врача. Вот почему необходимы рекомендации со стороны дентальных врачей относительно подходящего возраста для первого визита в кабинет врача, как и рекомендации относительно профилактических мероприятий.*

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CASE REPORTS

ADULT BOCHDALEK HERNIA SIMULATING LEFT PLEURAL EFFUSION: A REVIEW AND A CASE REPORT

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ABSTRACT

The authors present a rare case of congenital diaphragmatic Bochdalek hernia in an adult stimulating left pleural effusion. The diagnosis of left pleural effusion was made on the basis of conventional chest X-ray and ultrasonography. The definitive diagnosis of Bochdalek hernia was made by left video-assisted thoracoscopy. The patient was successfully treated operatively by conventional surgery - a combination of left thoracotomy and median laparotomy.

The reported case supported the view that Bochdalek hernia in adults presents usually with atypical chronic abdominal and respiratory symptoms. Surgical treatment should best be performed, according to the authors, by competent surgeons with good command of both the thoracic and abdominal approaches to the diaphragm.

Key words: *congenital diaphragmatic hernia, Bochdalek hernia, pleural effusion*

INTRODUCTION

In 1848, Bochdalek described congenital diaphragmatic hernia with intrusion of abdominal organs into the thoracic cavity through a posterolateral diaphragmatic defect. Congenital diaphragmatic Bochdalek hernia results from failure of pleuroperitoneal canal closure typically occurring by the eight week of gestation.[1] The prevalence of posterolateral diaphragmatic hernia has been estimated to be 1 in 2,200 to 12,500 live births. Most cases of Bochdalek hernias are diagnosed in the neonatal period with 5 to 20% occurring after the first month till the end of the childhood.[1-4] In children, Bochdalek hernias most often present with acute respiratory failure.[1] The condition is rarely seen in adults and in contrast to children is usually manifested with atypical chronic abdominal and respiratory symptoms: abdominal pain, loss of appetite, chest pain, cough, and dyspnea. In some adults the hernia is even asymptomatic and is detected as an incidental finding in an image study which is the reason why the exact prevalence of adult Bochdalek hernia is impossible to determine.[1-5] There have been overall fewer than 200 cases of this congenial disorder described in the relevant literature.[5]

The aim of the present case report was to present a rare case of Bochdalek hernia in an adult patient which imitates clinically left pleural effusion. Diagnosis and treatment of this rare disorder in adults are discussed.

CASE STUDY

A 36-year old man was hospitalized in the pulmonology department for cough, poor expectoration and mild pain in the left half of the chest with the diagnosis of bronchopneumonia with left pleural effusion. The pleural effusion was detected by a conventional chest X-ray. The administered antibiotic therapy failed to achieve resorption of the effusion. Left pleural puncture was performed, but no pleural exudate was evacuated. Because of the persisting pleural effusion and suspicion for pleural tuberculosis, the patient was admitted to a thoracic surgery clinic. Video-assisted thoracoscopy with a view to pleural biopsy and pleural effusion drainage was performed on request from the pulmonologists treating the pulmonary infection.

After the patient was admitted to the thoracic surgery clinic, conventional chest X-ray was performed which revealed left pleural effusion (Fig. 1). Transthoracic sonography was performed showing heterogeneous echogenicity of the left pleural cavity content. Septated pleural effusion was diagnosed on the basis of the ultrasound study.

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Video-assisted thoracoscopy was performed suggested by the image study findings of left septated pleural effusion. It revealed herniated viscera in the left half of the thoracic cavity: spleen, colon, great omentum and small intestine. After diaphragmatic hernia was diagnosed conventional surgical intervention (left lateral thoracotomy) was performed. It was found that the viscera protruded through a posterolateral diaphragmatic defect, about 5 cm in size. There was no hernial sack. The herniated segments of the colon were the transverse colon, lienal flexure of the colon and the descending colon (Fig. 2). The herniated segment of the small intestine was about 100 cm long. Adhesions between the omentum and the pericardium were found.

Because it was impossible to reposition the herniated organs into the peritoneal cavity only by thoracic approach, the diaphragm was approached abdominally – median laparotomy was performed which allowed repositioning of the organs from the thoracic to the abdominal cavity. The diaphragmatic defect was repaired with single interrupted sutures, applied through the thoracic approach.

The patient was kept in hospital for 16 days postoperatively developing no complications. The control chest X-ray 30 days after the surgery showed normal position of the left diaphragmatic cupola (Fig. 3).

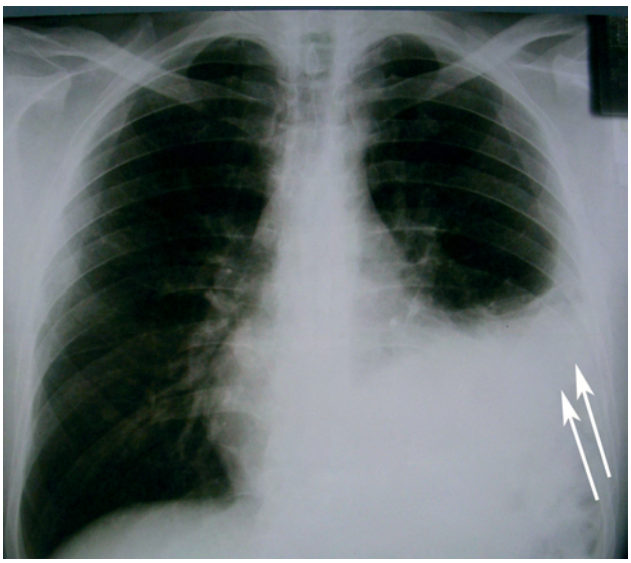

Figure 1. Conventional chest X-ray of patient with left Bochdalek hernia. The changes in the left half of the chest are interpreted as left pleural effusion (white arrows).

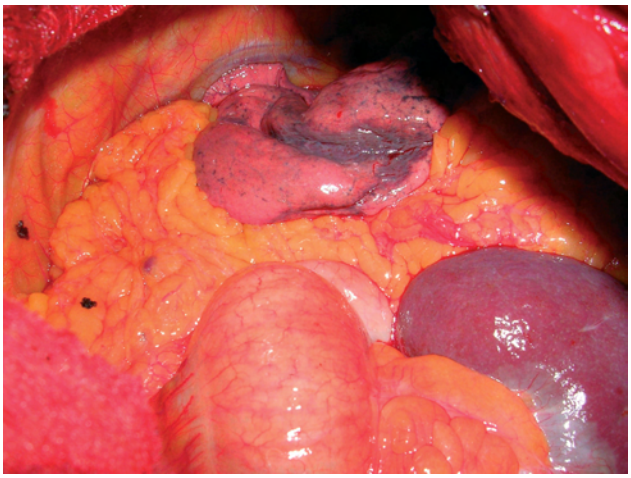

Figure 2. Digital photography of left pleural cavity (lateral thoracotomy approach) with presence of spleen, omentum, colon and small intestine in it.

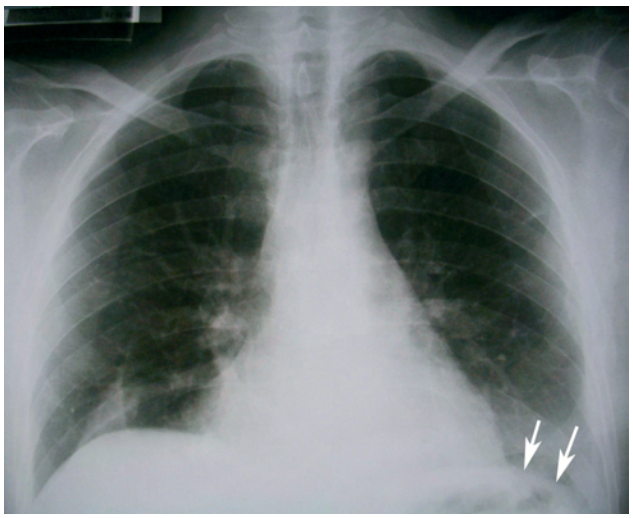

Figure 3. Conventional chest X-ray 30 days after the repair of diaphragmatic defect due to left Bochdalek hernia (arrows – normal position of the left diaphragmatic cupola).

DISCUSSION

The congenital diaphragmatic Bochdalek hernia is a posterolateral diaphragmatic defect through which abdominal organs herniate into the thoracic cavity. It is the most common congenital diaphragmatic hernia (78% to 90% of the cases).[1-3] It has been found that the right hemidiaphragm develops earlier than the left one during the embryonic period. This fact, and the protective role of the liver against herniation of abdominal organs into the thoracic cavity accounts for the greater incidence of left-sided localization of Bochdalek hernia (over 80% of all cases).[6-8] About 80 – 95% of the hernias are diagnosed in the neonatal period on the basis of the triad of symptoms: respiratory distress syndrome, scaphoid abdomen, and contralateral disposition of the mediastinum. More than 20% of congenital dia-

phragmatic hernias are associated with other inborn anomalies (cardiac defect, Down syndrome, median colostomias, mental retardation). Acute manifestation in childhood, as well as presence of associated severe anomalies explains why so few of Bochdalek hernias are diagnosed in adults.[1,4]

One of the reasons for late manifestations and diagnosing of Bochdalek hernia in adults is the late occurrence of herniation of abdominal organs through the diaphragmatic defect. It is believed that in prolonged or extreme increase of intra-abdominal pressure (pregnancy, delivery, abdominal trauma), abdominal organs pass through the diaphragmatic defect into the thoracic cavity. Once positioned in the thoracic cavity, the abdominal organs remain there due to the gradient between the intra-abdominal and intra-thoracic pressures.[1-3,8,9]

The presented case is interesting for several reasons. First, it is the late manifestation of the disease – in the 4th decade of patient's life, in the absence of triggering factors causing increase of the intra-abdominal pressure. In contrast to cases in children, Bochdalek hernia in adults usually presents with atypical chronic abdominal symptoms, accompanied or not by respiratory symptoms.[2,3,6,7,9,10] In our case, Bochdalek hernia was manifested only with respiratory symptoms.

Conventional X-ray study in most of the patients with Bochdalek hernia reveals hollow abdominal organs (stomach, small intestine and colon) in the thoracic cavity, which allows diagnosing of the disease. Cases of misinterpretation of X-rays of adult Bochdalek hernias with pneumatocele, lung cysts, pneumotorax, have been reported.[2,4-9,11] The presented case raises some interest because of the incorrect interpretation of the changes observed in the left pleural cavity: two radiologists independently diagnosed this case as left pleural effusion. There have been several similar case reports in the literature of misdiagnosing adult patients with Bochdalek hernia as having pleural effusion.[2,7] The false radiologic diagnosis can be accounted for by the presence of solid viscera and abdominal structures (omentum, spleen) in the thoracic cavity. Administration of nasogastral probe in addition to conventional X-ray study is considered complementary in diagnosing diaphragmatic hernias, but in our case it had no diagnostic value as there was no herniation of the stomach. A barium X-ray study would have diagnosed the presence of intestinal sections in the thoracic cavity.

The chest ultrasonography that was performed in this patient provided additional information about the changes in the left pleural cavity. The presence of solid abdominal organs in the pleural cavity explains why there was a wrong interpretation of the data from the investigation – the ultrasonographic image was interpreted as septated pleural effusion in an organisational stage.

Computer tomography of the chest is considered to be the definitive imaging method for diagnosing diaphragmatic hernias, in particular Bochdalek hernia.[1-3] The findings in this study can determine the characteristics of the pathologic content of the thoracic cavity and visualize the diaphragmatic defect. However, we should point out that a normal CT image does not rule out a diagnosis of diaphragmatic hernia. The method was found to have 78% sensitivity and 100% specificity in cases of left diaphragmatic defects.[1-3] The unconditional interpretation of the results in the conventional X-ray and ultrasonography as left pleural effusion explains why computed tomography was not applied in the presented case.

In the present case, misdiagnosing Bochdalek hernia as left pleural effusion had lead to performing procedures carrying very high risk for the patient such as pleural puncture and subsequently thoracoscopy. A case was reported in the literature in which due to misdiagnosis of Bochdalek hernia the patient was fixed with tube drain.[5] In this patient, the evacuation of gastro-intestinal content through the drainage tube had resulted in emergency thoracotomy during which Bochdalek hernia was finally detected. Fortunately, in the presented case, the two highly risky procedures we performed did not impair any of the herniated abdominal organs in the thoracic cavity. The video-assisted thoracoscopy we performed helped to make the final diagnosis. During the inspection of the left pleural cavity, very large Bochdalek hernia was found (with herniated spleen, colon, ileum, and omentum), surprisingly inconsistent with the scarce symptoms reported by the patient.

Because of the risk of intestinal obstruction and strangulation, surgery must be performed immediately after diagnosing Bochdalek hernia.[1,-4,11-18] The traditional surgical treatment of Bochdalek hernias is conventional intervention (laparotomy; thoracotomy). The treatment may also be accomplished by a minimally invasive approach (laparoscopy, thoracoscopy). The thoracic approach to the diaphragm is preferred because of the better visualization of the hernial content and the diaphragmatic defect, and also because of the possibility that possible pleural and pericardial adhesions with the herniated

Folia Medica 2010; 52(4): 62-66

organs may be safely resolved. For these reasons we opted for left thoracotomy. Attempts to reposition the hernial content into the peritoneal cavity of the patient through thoracic approach were unsuccessful. This can be accounted for by the abnormal increase of intra-abdominal pressure when trying to reposition the very large hernial content into the peritoneal cavity. Because of this we had also to perform surgery with abdominal approach (median laparotomy). The additional abdominal approach is further justified by the requirement that in large Bochdalek hernias it is obligatory to verify the presence of eventual intestinal malrotation as associated anomaly.[1,12,13,17,18]

After repositioning the abdominal organs into the peritoneal cavity, the diaphragmatic defect must be closed. If hernial ring edges can be drawn near each other without tension, the method of choice for repairing the defect is single interrupted sutures using non-resorbable material. Large diaphragmatic defects are closed with synthetic mesh[12,13,15,16] In our case, the size of the diaphragmatic defect (~ 5 cm) allowed successful closing without using any synthetic mesh.

CONCLUSIONS

The clinical manifestation of Bochdalek hernia in adults varies from asymptomatic cases (found accidentally) to development of serious complications, resulting from intestinal obstruction and strangulation. We present a rare case of Bochdalek hernia misdiagnosed as left pleural effusion and successfully treated by conventional surgical intervention combining thoracotomy and laparotomy. The diaphragm is an anatomical barrier, but it is hardly a surgical one. It is in this respect that the presented case gives support to the view that surgical treatment of Bochdalek hernia must be performed by competent surgeons that have a very good command of both the thoracic and abdominal approaches to the diaphragm.

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BOCHDALEK ГРЫЖА У ПОЖИЛОГО ПАЦИЕНТА, СИМУЛИРУЮЩАЯ ЛЕВОСТОРОННИЙ ПЛЕВРАЛЬНЫЙ ВЫПОТ (ЛИТЕРАТУРНЫЙ ОБЗОР И ВКЛАД – ОБСУЖДЕНИЕ ОДНОГО СЛУЧАЯ)

И. Новаков, Г. Паскалев

РЕЗЮМЕ

Авторы представляют редко встречаемый случай врожденной диафрагмальной грыжи у пожилого пациента, симулирующей левосторонний плевральный выпот. Диагноз "левосторонний плевральный выпот" поставлен на основании результатов конвенциональной рентгенографии и ультрасонографии грудной полости. Дефинитивный диагноз "Bochdalek грыжа" поставлен на основании видео-ассистированной торакоскопии.

Оперативное лечение пациента успешно. Оно осуществлено конвенциональной интервенцией (комбинированы левосторонняя торакотомия и срединная лапаротомия).

Вышеописанный случай подтверждает мнение, что у пожилых пациентов Bochdalek грыжа чаще всего проявляется нетипичными хроническими абдоминальными или респираторными симптомами.

Проведенное оперативное лечение подтверждает точку зрения, что только компетентный хирург, владеющий в одинаковой степени хорошо как торакальным, так и абдоминальным доступами к диафрагме, сможет осуществить хирургическое вмешательство.

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CASE REPORTS

A 20-YEAR-OLD MAN WITH LARGE GASTRIC LIPOMA - IMAGING, CLINICAL SYMPTOMS, PATHOLOGICAL FINDINGS AND SURGICAL TREATMENT

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ABSTRACT

A broad search of the available literature yielded no other report of gastric lipoma of that size (13.5×6.5×4.5 cm) at this early age. The patient (a 20-year-old man with giant lipoma in the anterior gastric wall) presented with haematemesis and melena after excessive alcohol consumption. Gastric resection was performed. At 5-year follow up the patient is healthy and doing well. Epidemiology of gastric lipoma, the differential diagnosis, means of diagnosis and treatment are discussed.

Key words: *gastric lipomas, lipomas, hematemesis*

INTRODUCTION

Lipomas of the gastrointestinal tract occur very rarely (1:600 necropsies[1]); the commonest site they are found is the colon, followed by the small intestine. Gastric lipomas are quite rare accounting for 5% of all the gastrointestinal lipomas and 3% of all benign tumours of the stomach. The peak incidence is in the seventh decade.[2]

Since resection was proposed by Ackerman and Chughtai in 1975, therapy of gastric lipomas has been changing. A CT scan preoperatively can change the treatment modality from mere observation in the asymptomatic to gastrectomy in symptomatic cases. Endoscopic removal is an alternative in pedunculated tumours.

To date, according to the literature available to us, only about 250 such tumors have been described and none of them involves a 20-year-old patient with lipoma of such size.[3]

CASE REPORT

A 20-year-old man was admitted to the University Hospital of Alexandroupolis because of haematemesis after consuming alcohol. Gastric endoscopic examination revealed a large smooth bilobed mass at the anterior wall of the gastric body with a central crater and elastic texture. The histologic analysis of endoscopically removed tissue showed the superficially ulcerated mesenchymatous neoplasm of the stomach obviously a lipoma. The patient undertook surgery during which a large tumor was found in the anterior wall and towards the minor curvature of the stomach with smooth margins and dimensions 13.5×6.5×4.5 cm causing a stricture of the lumen. The liver and the adjacent organs were found normal and the lymph nodes clean. A 4/5 Roux-en-Y gastrectomy was performed with CEEA 31. Post-surgically, the patient showed a six-day high fever attributed to pleural fluid (left) which was cured with proper drugs and physiotherapy. The ultrasound examination of the abdomen was normal. He received 2 blood units because of low Hct (22%). After the fifth day he began to receive fluids per os and then solid food without problems. The histology report of the surgically removed tumor showed a very large intramural gastric lipoma with intact surgical boundaries. The patient is currently alive and well without recurrence 5 years after surgery.

DISCUSSION

Lipomas of the gastrointestinal tract are uncommon, slow growing, fatty tumors that can occur anywhere along the gut. Although generally single, they may be multiple. Peak occurrence is in the fifth to seventh decade of life, with a slight female predominance. This case report concerns a 20-year-old man with a sizeable lipoma in the anterior gastric wall.

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The clinical symptoms were hematemesis and pain. The surgical excision was performed with Roux en Y gastrectomy.

Part of stomach and part of large omentum with total dimensions 22 cm in the major curvature and 11.5 cm in the minor curvature were examined. An intramural cylindrical mass 13.5×6.5×4.5 cm was found along the minor curvature and at the anterior gastric wall causing a degree of lumen stricture. The surface of this tumor presents an ulceration of 1.8 cm in diameter. The limits of the tumor and muscle layers were clearly outlined. The part of large omentum with dimensions 22×18 cm appears with no macroscopical lesions. Grossly, the tumor itself consists of bright yellow fat separated by fine fibrous trabeculae (Fig. 1).

Microscopically, lipomas are composed of mature adipose tissue with no cellular atypia. Areas of necrosis, infarct and calcification may be present. It is important not to confuse the histiocytes associated with fat necrosis with lipoblasts. The appearance of lipoblasts is the morphologic denominator of liposarcoma.[4] Liposarcoma, the malignant counterpart of lipoma, is a well circumscribed but not encapsulated tumor which when appearing yellowish can mimic lipoma. Approximately 90% - 95% of lipomas are located in the submucosa; the remaining 5% - 10% are subserosal.[5-7] In our case very large intramural lipoma of the stomach with areas of reactive fibrosis and/or fibroblastic activity in the ulcerated area of the tumor with mild inflammation and small hyperplasia of the gastric mucosa near the ulcer were recognised. The remaining gastric mucosa appears with no substantial lesions. Microcystic dilatation of the gastric glands is recognized near the tumor. Surgical limits of excision are clear. Moderate alterations of acute inflammation on the serosa are apparently due to the surgical procedure. Malignant neoplasmatic alteration in the examined material does not exist (Figs. 2, 3).

Morphologic variations of lipomas are: fibrolipoma, myxolipoma, chondroid lipoma, myolipoma, spindle cell lipoma, pleiomorphic lipoma, angiolipoma.[6,7] Lipomas may develop in the pharynx or in the esophagus, but the most common are these in the small bowel ranks (20-25% of lipomas) and in the colon (65-75%).[7] Lipomas may develop at other rare locations such as adrenal gland, parotid gland, parapharyngeal space, mediastinum, the pleura as reported by numerous case reports.

Gastric lipomas are a rare lesion accounting for only 5% of alimentary tract lipomas and for only 3% of all benign gastric masses. Most gastric lipomas are located in the antrum; the remainder are spread throughout the body and fundus. The usual antral location accounts for a high frequency of prolapse into the pylorus. Because of the lipoma's supple nature, however, complete obstruction of the gastric outlet seldom occurs. As in other segments of the gut, lipomas are usually single but may be multiple.[8]

Endoscopy and radiology play a major role in the diagnosis of lipomas. Endoscopy relies on the gross appearance of the mass to suggest the correct diagnosis. CT, in the properly prepared patient, is able to take advantage of the fat content, thereby

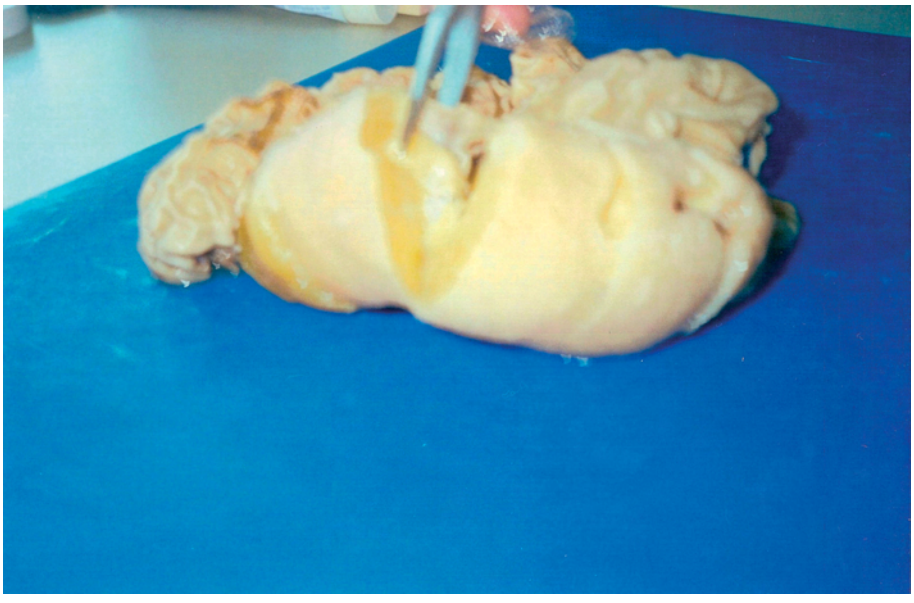

Figure 1. Macroscopic view of stomach lipoma 13.5×6.5×4.5 cm.

Folia Medica 2010; 52(4): 67-70

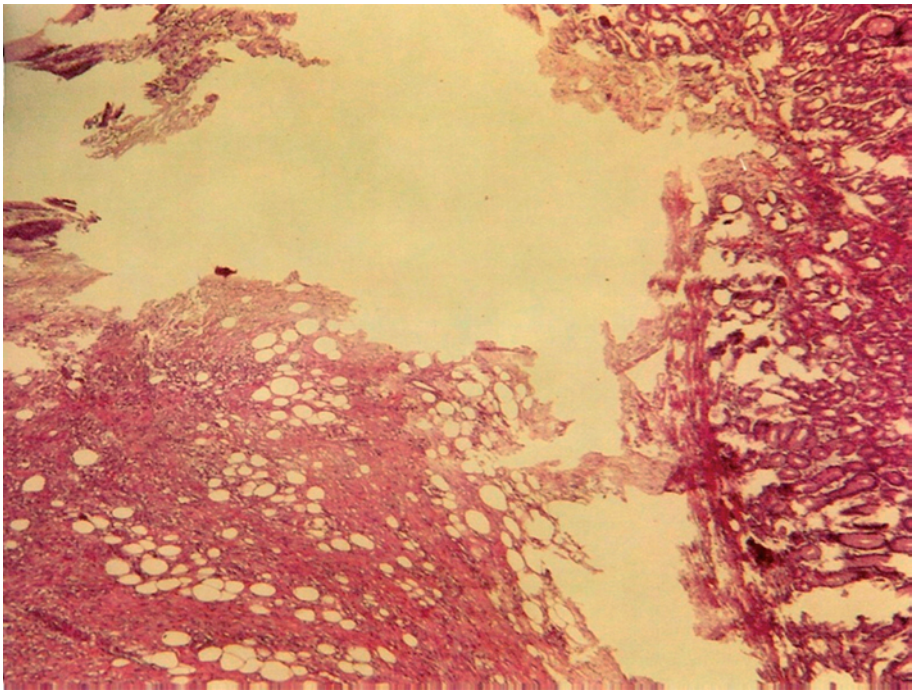

Figure 2. Pathological findings of ulcerated lipoma. H&E ×40.

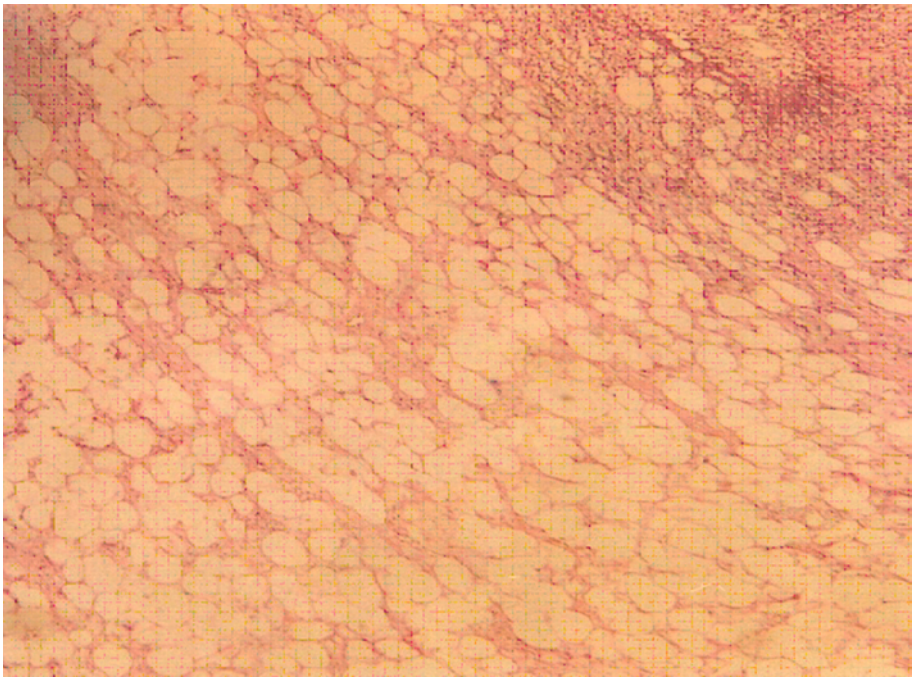

Figure 3. Pathological findings. Fatty tissue of the tumor with fibrosis near the ulceration. Absence of lipoblasts. H&E ×100.

identifying a mass as a lipoma. The finding of a homogeneous mass with Hounsfield units between -80 and -120 is nearly pathognomonic for a lipoma.[2]

No more than 250 cases of gastric lipomas have been published until now. The dimensions that have been described in these cases are smaller than this in our case. In the largest series of patients with stomach lipomas the average size of the lipomas was 6.5 cm (range 3.5 - 9.0) measured at the greatest dimension when in our case the lipoma was sizeable with the greatest dimension to be at 13.5 cm one of the greatest in English literature.[9-12]

Today, after 7 years, the patient is free of symptoms with no radiological or other findings. In conclusion, gastric lipomas are a rare entity that can

mimic symptoms of peptic ulcer at first appearance. Histologically, the absence of atypia and lipoblasts set the diagnosis and surgical resection remain the treatment of choice for symptomatic lipoma.

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СЛУЧАЙ ДВАДЦАТИЛЕТНЕГО МУЖЧИНЫ С БОЛЬШОЙ ЛИПОМОЙ ЖЕЛУДКА. ВИЗУАЛИЗАЦИЯ, КЛИНИЧЕСКИЕ СИМПТОМЫ, ПАТОЛОГИЯ И ХИРУРГИЧЕСКОЕ ЛЕЧЕНИЕ

С. Капетанакис, Я. Папатанасиу, А. Фиска, А. Ververидис, Т. Димитроу, Ж. Христов, Г. Паскалев

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One of the most beautiful reliefs of the healing cults in the Greco-Roman pantheon is the unique "Frieze of the healing family" exhibited in the Archeology Museum in Plovdiv.
It was excavated in the foundations of an old ruined Turkish mosque in 1921. The correct identification of all figures was performed by Professor Zapryanov in 1964 - Department of Social Medicine. The frieze, according to him, used to adorn a Roman valetudinaria - a military hospital - off the walls of the east entrance of the ancient city which was called Trimontium by the Romans in the late III century. It weighs about 3000 kg and is 2.80 m long and 1.08 m high. The figures on it are framed in a wide rim; it bears the personified images of the Moon (on the left) and the Sun (on the right).*
Presented on the frieze are (from left to right): Jaso and Panacea - Asclepios' daughters, Telesphor - the fortunate genius of the healing process, Asclepios - the god of healing art, Hygeia - his daughter, Epione - Asclepios' wife, Machaon and Podaleirios - his sons worshipped as military physicians.
All figures, except Panacea, are entirely in full face which is very rare in a general composition picture. The frieze's sculptor depicted in great detail the figures' anatomic features, clothes and peculiar attributes. All deities in the composition are on a par with the only association seen between Panacea and Asclepios (Panacea touches a bundle of herbs next to Telesphor's cowl with her left hand, while pouring the cure all (panacea) in Asclepios' bowl).

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* Folia Medica 1964; 6(3): 152 - 156