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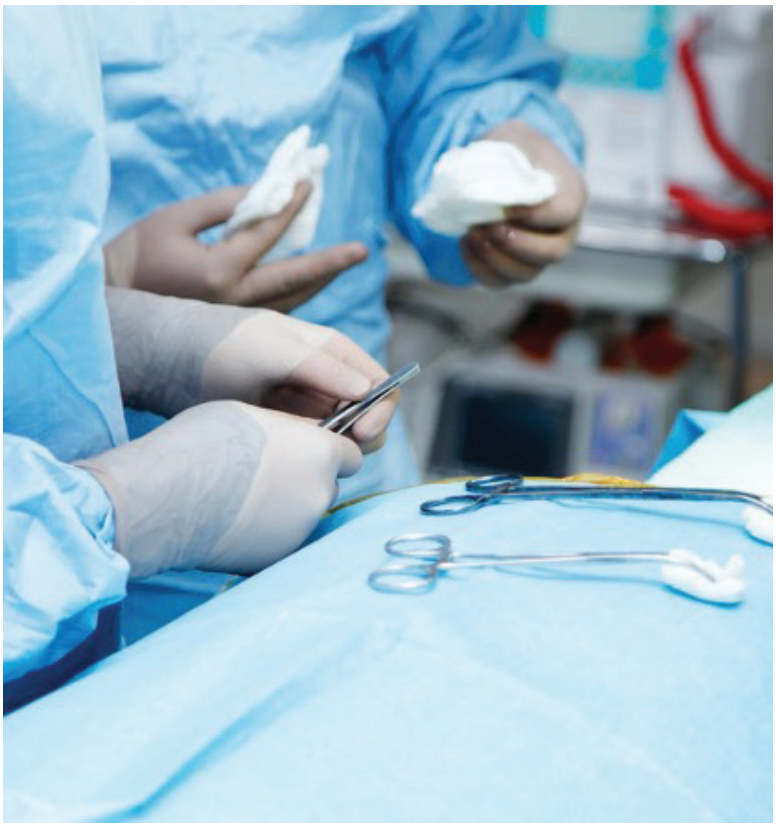

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Cell technologies in retinitis pigmentosa treatment

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Abstract

The growing crucial problem in practical ophthalmology relates to growth of hereditary degenerative diseases of the retina, in particular retinitis, causing progressive loss of visual functions. According to international estimates, the incidence rate of hereditary dystrophy contains 1 case per 3000 population. With the development of biomedical cell technologies, transplantation of stem (autologous and allogeneic) cells is at the stage of active research.

The article reviewed literature sources on prevalence, risk factors, etiopathogenesis, diagnosis, clinical picture and treatment of retinitis pigmentosa.

Key words: retinitis pigmentosa, etiology, pathogenesis, diagnosis, clinic, treatment

Introduction

The growing crucial problem in practical ophthalmology relates to growth of hereditary degenerative diseases of the retina, in particular retinitis, causing progressive loss of visual functions [1]. According to international estimates, the incidence rate of hereditary dystrophy contains 1 case per 3000 population [2]. With the development of biomedical cell technologies, transplantation of stem (autologous and allogeneic) cells is at the stage of active research [3].

Recent publications have been devoted to issues revealed the perspectives of applied regenerative medicine, in particular, cell therapy in the treatment of a whole spectrum of ophthalmic pathologies of various etiology, especially in hereditary degenerative diseases of the retina [3].

Retinitis pigmentosa represents the heterogeneous group of hereditary retinal dystrophies resulted by variation of mutations in more than 150 identified causative genes and various types of inheritance (autosomal dominant, autosomal recessive, X-linked), with syndromic or non-syndromic manifestaion [4].

Loss of vision caused by retinitis pigmentosa occurs with initial impaired night vision corresponding with a gradual narrowing of peripheral vision up to blindness in patients of different age groups: infancy, adolescence and maturity [5].

However, pathogenetic mechanism of the development of retinal cell death still remains unexplored, that creates a barrier in the development of an algorithm for therapeutic measures to prevent the progression of visual impairment [6].

As a result, patients face the number of problems: low efficiency of existing treatment methods, disappointing outcome of the disease, forced financial costs for medical services and lifelong rehabilitation measures, issues in professional activity, as well as in psychosocial adaptation in conditions of low vision, which significantly affects the quality of life [7-8]. The issues of significant economic costs rise simultaneously with quality of life fall [5].

The aim is to conduct a literature review of the available international and local scientific sources on the use of cell technologies in the treatment of retinitis pigmentosa.

Genetic research

Significant advances have been made in identifying new genes responsible for retinitis pigmentosa, and further research work in screening patients for genetic mutations continues to this day [9].

Heterogeneity in mutation genetics in more than 60 and 30 genes can lead to the emergence of about 3000 of non-syndromic and 1200 syndromic variations of retinitis pigmentosa, respectively [10].

Despite the variety of genetic prerequisites, retinitis pigmentosa unites a group of diseases occurring with progressive loss of peripheral and subsequent loss of central vision, which is initially caused by dysfunction and loss of rod photoreceptors and secondary death of cone photoreceptors. The difference between syndromic (systemic) and non-syndromic forms of retinitis pigmentosa comes in combination with other neurosensory disorders, ontogenesis abnormalities and phenotypic manifestations: congenital deafness (Asher's syndrome), kidney pathology, obesity, physical retardation and polydactyly (Bardet-Biedl syndrome). Authors cannot reject the appearance of retinitis pigmentosa as a secondary disease against the background of systemic disorders: degenerative changes in the cerebellum, mitochondrial disorders [11]. That makes difficulties in diagnostics due to heterogeneity, both genetic and phenotypic, since a mutation of the same gene can have different clinical manifestations due to allelic mutations and the type of inheritance [1]. Clinically, the general picture manifests the heterogeneity of such mutations: the appearance of "bone bodies" in the peripheral retina, narrowing of blood vessels, and blanching of the optic nerve head [4].

Authors consider progressive loss of photoreceptors as a result of several possible ways of cell death. One is the sufficiently studied mechanisms of programmed cell death is apoptosis [12]. The initiation of apoptosis can be triggered both by internal (activation of the complex: cytoplasmic cytochrome C, activation factor for pesticide protease 1, caspase-9) and external (immune cells, tumor necrosis factor, caspase-8) factors. This mechanism leads to the isolation and elimination of damaged cellular elements with maximum minimization of damage to the surrounding tissue [13,14]. However, there is convincing evidence for the existence of an alternative molecular pathway of cell death without the involvement of caspases [14-16].

One more way referred to as regulated necrosis, involves the destruction of the cytoplasmic membrane. Activation can be due to either stimulation of cell death receptors (necroptosis) or an excess of intracellular iron (ferroptosis) or the activity of poly-ADP-ribose-polymerase (partanatos) [17-22].

Many authors also do not exclude the activation of autophagy against the background of oxidative and metabolic stress. Normally, the lysosome-mediated autophagy mechanism is designed to maintain intracellular homeostasis, thereby performing a protective function [23-25]. In other cases, with serious damage, excessive autophagy can lead to a decrease in cell survival [26].

Authors supported arguments in the development of cell apoptosis in favor of oxidative damage, which may serve as evidence of the effectiveness of further development of antioxidant therapy [24]. Oxidative stress implies state of dysregulation of the balance between reactive oxygen species and the antioxidant defense complex [27-30]. Normally, such oxygen saturation is associated with an active blood supply to the retina, which requires high metabolic and energy costs to perform visual functions. However, with degenerative changes in the retinal cells, oxygen consumption decreases with a constant level of blood supply, which in the future can serve as a supersaturation of oxygen concentration in the interstitial space and cause an increase in oxidative stress in the tissue [31,32].

There are also other assumptions about the impact of other biological dysfunctions that affect the progressive death of photoreceptors, such as metabolic stress, inflammation [33,34].

Thus, the study of possible biological mechanisms of retinal cell degeneration remains unexplored, that requires further researches to develop effective methods of therapy for retinitis pigmentosa [35].

Diagnostic measures carried out to identify genomic abnormalities and the nature of the mutation, are the following: molecular methods, linkage mapping and DNA sequencing [33]. In recent years, the linkage mapping method based on the identification of more than 10 thousand informative markers and related genes linked became worldwide popular. This method allows you to test more than 1 million genetic markers and identify chromosomal mutations. Additional retesting required in case of variants of random "coincidences" of linkage possible due to large number of independent tests [36]. Sanger sequencing and ultra-high throughput (next generation sequencing) are considered the gold standard in detecting mutations at the DNA level [37,38].

Ophthalmology examination of retinitis pigmentosa is based on detecting the causes of genetic mutation, the nature of inheritance of phenotypic traits, with potential detailed study of the patient's family history. Diagnostics examination is performed for registration and subsequent monitoring of progressive visual disturbances: the presence of nyctalopia, pigment "bone cells" along the periphery of the fundus, a decrease in the amplitude of the electroretinogram. In order to record changes in visual functions, instrumental research methods are used: fundus photography, Goldman visual field testing, fundus autofluorescence, optical coherence tomography in the spectral region, electroretinogram [39].

Currently, ophthalmological care for these groups of patients is represented by a limited list of treatment methods with minimal effect, and is mainly aimed at slowing the deterioration of the disease and saving vision, since they do not eliminate the main molecular defect [39].

Significant advances have been made in the study of gene therapy carried out at the stage of preclinical trials: models of induced pluripotent stem cells, experiments on restoration of vision on a natural model of retinal dystrophy in dogs, which have a promising scientific future for further scientific developments [40-42]

At the same time, this direction meets certain complications in the process of implementation of gene therapy for patients. The process registration and approval by the European Commission requires long time together with significant financial costs for both researchers and patients, since the expected cost of treatment can exceed more than 1.5 million US dollars [5].

From one hand, some researchers stated the model of induced pluripotent stem cells has a number of advantages, due to the relatively low cost and potential in detecting retinitis pigmentosa gene mutations in vitro, as well as providing experiments in search of effective drugs. From the other hand, the recreation of the real conditions of biochemical interaction of drugs as on animal or a human model remains uncertain [42-45].

Another promising direction is the use of the model of induced pluripotent stem cells as a source of an unlimited number of cells for the treatment of damaged retina through their transplantation, since the use of embryonic stem cells, photoreceptor precursors, has limitations in matters of ethics and dietology [46,47]. Experimental models of transplantation of induced pluripotent stem cells carried out in animals have demonstrated the presence of similar characteristics with embryonic stem cells in the case of therapeutic use [48].

Overall, gene therapy for retinitis pigmentosa comes as promising innovative area for further study.

Current medical interventions are aimed at slowing the progression of vision loss leading to legal and functional blindness [49]. In general, medical care is implemented by prescribing vitamins with a trophic and antioxidant effect (vitamins A, E), food additives (omega-3 fatty acid, lutein, docosahexanic acid, gangliosides), drugs with a neuroprotective effect (0.2% bromonidine tartrate, ciliary neurotrophic factor), which have a protective effect on photoreceptors [50,51]. Systematic review analized traditional symptomatic treatment and revealed a certain efficacy of therapeutic interventions on indicators of visual function in patients with retinitis pigmentosa [52]. However, the presented methods of treatment, despite the proven safety of their use, have limitations due to their weak effect on a significant improvement in visual functions.

Thus, the search for effective pathogenetically grounded therapeutic interventions remains relevant and requires further consideration the existing genetic heterogeneity of retinitis pigmentosa.

Conclusion

Hereditary degenerative diseases of the retina, including retinitis pigmentosa, lead to progressive visual function. The search for effective methods of treatment remains relevant in view of the insufficient knowledge of the pathogenesis of retinal cell death, and the low effectiveness of treatment methods. The use of cellular and technologies for the treatment of groups of eye diseases is promising and promising.

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The use of autologous mesenchymal stem cells in complications of diabetes mellitus, in particular diabetic retinopathy: inputs and insights

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Abstract

The review provided reveals the analysis of the available scientific literature on the feasibility of using cellular technologies, such as, autologous mesenchymal stem cells (MSCs) for diabetic retinopathy treatment. The results indicated the viability of cellular technologies in clinical ophthalmology with respect to anatomical features and immune privilege of the organ of vision. Additionally, feasibility, safety, and optimization of pathogenetic therapy for MSC transplantation expands the prospect of their use in late complications of type 1 and type 2 diabetes mellitus, in particular, diabetic retinopathy.

Key words: mesenchymal stem cells, diabetic retinopathy

Introduction

Autologous mesenchymal stem cells (MSCs) reported as a considerable advance in biomedical cell therapy provide for the feasibility and safety of using cell technologies, in particular, in various fields of medicine, including ophthalmology [1,2]. The evidence represented in experimental studies in the direction of cellular technologies, repeatedly point to the advantages of the eye over other organs. It mostly relates to the presence of certain amount of non-invasive methods for diagnosing the organ of vision, the minimum risk of systemic complications (formation of tumors) due to hemato-ophthalmological barrier. Additionally, various mechanisms of immunological tolerance, the presence of a low immune response in the anterior segment of the eye, which is carried out according to a specific type of "immune deviation associated with the anterior chamber", which allows the use of not only allogeneic, but even more autologous MSCs without the threat of rejection in the recipient's body [2,3].

Mesenchymal stem/stromal cells include a group of multipotent cells extracted from various tissues of the body, in particular from bone marrow, adipose tissue, dental pulp, neonatal umbilical cord, amniotic membrane, amniotic fluid, placenta [4,5]. The unique fenomena of MSCs origined from the above biological tissues. When transplanted, MSCs demonstrate the ability to inhibit inflammation in case of tissue damage, secrete growth factors leading to tissue repair, and differentiate into any types of cells (nerve, bone, fat, etc.) depending on recipient tissue [6].

When transplanted, the nonspecific immunosuppressive therapy and the risk of transplant material rejection are excluded, the minimal susceptibility to malignant tissue degeneration and easy collection of biological material, and compliance with ethical standards are presented [7-10].

The clinical application of this line of cells in ophthalmology today is mainly experimental, especially in situations of late irreversible stages of degenerative processes in the photoreceptor layer of the posterior segment of the eye [11,17]. The reason for this is the insufficient in vivo potential of progenitor cells (ciliary body epithelium, iris pigment epithelium, Müller cells) capable of differentiating into retinal cells to replace a defect in such pathologies as age-related macular degeneration (AMD), retinitis pigmentosa (RP), diseases Stargardt and Leber's congenital amaurosis (AL), which lead to a significant decrease in visual function and, as a result, disability of the working population worldwide [12]. As known, the pathologies like ganglion-photoreceptor-pigment interface is first of all damaged, followed by activation of a cascade of irreversible complications: neuroinflammation, microglia, gliosis [13-15].

The failure of various traditional therapeutic methods for retinal diseases dictates the development of various methods of regenerative therapy. Recent achievements in the field of gene therapy, neuroprotection, anti-VEGF (vascular endothelial growth factors) and stem cell therapy make it possible to slow down the process of progressive vision loss [16]. The benefit of gene therapy is the recovery of certain types of degenerative retinal diseases associated with certain recessive gene defects. The therapeutic efficacy of neuroprotection is aimed at preventing the cascade of reactions leading to damage to retinal and optic nerve neurons [17-18]. Finally, anti-VEGF therapy is the gold standard for the treatment of neovascular age-related macular degeneration (AMD), diabetic retinopathy (DR), in particular in diabetic macular edema (DME), and neovascular retinal diseases [19]. The targeted role of in vitro cell therapy in these diseases of the retina lies in the possibility of their use in late incurable stages of apoptosis of the neuroepithelium and the inner layer of the retina, i.e. triggering the mechanisms of reversibility of the death of neuronal cells in the retina and optic nerve [20]. Pathological complications of the retina occured in diabetes type 1 and type 2 affect neurons in the light-receiving and light-transmitting layer of the posterior segment of the eye, which combines DR with degenerative diseases of the retina [21].

The global prevalence of DR impressed by almost 200 million people suffering from eye complications due to diabetes mellitus. The proliferative stage of DR and DME is a particular threat to visual functions [22].

At the basis of pathogenetic processes, the main role belongs to the long-term state of hyperglycemia of the body and, resulted in prolonged hypoxia of the retina, sequented to gross violation of the homeostasis of retinal vessels. The outcome of such transformations of the microcirculation of the retina, mostly the vascular wall, is the development of microaneurysms, exudations, activation of neovascularization causing secondary ischemia of the retina, which exacerbates the processes of neurodegeneration, gliosis, and neuroinflammation [23,24].

Routine pathogenetic methods of treatment for the above conditions, such as retinal photocoagulation (panretinal and focal) in the proliferative stage of DR and macular edema, and intravital administration of anti-VEGF (bevacizumab, ranibizumab and aflibercept) significantly reduce retinal edema and cause regression of neovascularization [17,22]. However, the implementation of these techniques is accompanied by the risk of possible complications and adverse reactions: recurrence of a bleeding episode, subcapsular cataract, steroid-induced glaucoma, endophthalmitis, intraocular inflammation, rhegmatogenous retinal detachment, traction retinal detachment, which entails additional economic costs [25,26].

Drawbacks of existing methods motivate the search for the best ways to treat DR, taking into account modern scientific achievements, including in the field of stem cell therapy, has great promise and relevance, especially in terms of restoring damaged retinal architectonics [27].

Thus, the aim of the study is to analyze the available scientific literature reported about using cell technology, in particular MSCs, to optimize the treatment of DR.

Material and methods

The search conducted based on medical and biological publications in "PubMed", the "Biotechnology" section, created by the National Center for Biotechnology Information (National Center for Biotechnology Information). The search allowed identifying the studies on the issue of potential use of stem cells / progenitor cells in DR. It addressed the therapeutic efficacy by having four areas of application such as precursors of photoreceptors and other retinal neurons, also, Muller / glia stem cells - the ancestors of glia and retinal neurons, pigment epithelial stem cells, endothelial, myeloid cells - precursors, adult stem cells, induced pluripotent stem cells (iPSCs). The cells mentioned can contribute to the microvasculature of the retina, thereby exerting vasculo- and neurotrophic effects [22].

Results

As the therapeutic efficacy of stem cells depends on many factors: donor-recipient matching, tissue of origin, MSC cultivation protocol we underlined the following. When chose a donor tissue, it is mandatory to consider the characteristics of the obtained stem cells, affecting the level of therapeutic efficacy: the ability to proliferate and get aged, paracrine activity, the simplicity of collection method, and its option effective, as well as the route of transmission of the cell material. Accounting these aspects, MSCs of the bone marrow, adipose tissue and umbilical cord meet pointed conditions. Therefore, the issue of MSC integration into the retinal tissue has a number of unresolved issues that require further study and research [5].

As additional problem we defined the favorable mode for the delivery of MSCs to the retina. Previously mentioned the results obtained in preclinical trials, which found low efficiency of MSCs on ganglion cells and photoreceptors when administered systemically. Regarding the method of local injection of MSCs, a review showed the performed preclinical trials with preference of intravitreal transplantation of MSCs, given their ability to synthesize neurotrophic factors that prolong the survival of ganglion cells, regenerate axons, thereby slowing down the rate of loss of the latter [22]. With the background of prolonged ischemia of the retinal tissue and a decrease in the secretion of growth factors, MSCs can replenish their volume by producing two categories of neurotrophic factors. Some stimulate cell proliferation (transforming growth factor-alpha (TGF-α), TGF-β, hepatocyte growth factor (HGF), epidermal growth factor (EGF) and fibroblast growth factor-4 (FGF-4), others are responsible for maintaining angiogenesis, (VEGF, interleukin-8 (IL-8) and insulin-like growth factor-1 (IGF-1) [10]. The rat models demonstrated the trophic effect of the retinal neuroepithelium by experiment on laser-induced glaucoma and damage to the visual nerve induced. The damage caused by intravitreal transplants of dental pulp MSCs (DPSC), simultaneous cultivation of porcine

retinal cells and human MSCs differentiated in the Transwell system (brain-derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) in the eyes of rats with chronic hypertension with intravitreal transplanted MSCs (brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF) [12].

Thus, these studies have shown the possibility of successful use of autologous MSCs to improve retinal gliosis through their differentiation, which significantly increases the thickness of the macula and improves visual functions [11].

In modulating angiogenesis, MSCs play an important role in diabetic retinopathy (DR), which is characterized by the triggering of the formation of disordered and physiologically defective blood vessels. This leads to a violation of the histology of neurons in functionally significant areas of the retina and irreversible loss of vision. Experimental intraperitoneal administration of MSCs from the human amniotic membrane to mice inhibited neovascularization due to the expression of transforming growth factor beta (TGFβ), subconjunctival administration of bone marrow MSCs resulted in corneal wound healing and suppression of the formation of new vessels due to VEGF inhibition [28-29]. In an induced model of mice with DR, when adipose tissue MSCs were injected into the vitreous body, the researchers observed a decrease in the activity of the VEGF receptor 2 due to an increased expression of thrombospondin-1 (TSP1), which led to the suppression of angiogenesis [20].

With the background of early stages of DR, the use of MSCs makes interest because of the ability to replace lost pericytes of the retinal microvascular bed due to the existing morphological similarity of mesenchymal cells. This assumption appeared during the experimental intravitreal administration of human MSCs in diabetic mouse models, where an improvement in the physical properties of the retina was noted [22, 30].

Regarding the preservation of the survival of photoreceptors after the introduction of MSCs, positive experimental results were obtained in rat models with induced retinitis pigmentosa, retinal degeneration, and diabetic retinopathy, which demonstrate the neuroprotective effect of stem cells administered by subretinal or intravitreal injections [31].

Naturally, each mode of injection is associated with certain risks and side effects; therefore, optimization of procedures is required to minimize the consequences, such as secondary glaucoma, epiretinal membrane, differentiation into myofibroblast-like cells with subsequent development of fibrosis, proliferative retinopathy, traction retinal detachment, neovascularization [27].

Another advantage of the use of MSCs is associated with the production of extracellular vesicles, secreted bilipid-layered nano-microvesicles containing functional molecules (lipids, proteins, RNA, etc.). With subsequent endocytosis, retinal cells provide the content of the necessary biochemical subunits, which suppresses cell apoptosis, promotes inhibition of pro-inflammatory mediators and stimulates retinal regeneration. Experimentally, in DR, there is a positive effect of neuroprotection and regeneration of the retina from the movement of miRNA-222 in exosomes secreted by MSCs from adipose tissue into different layers of the retina in three ways (intravenous, subconjunctival, intraocular), damaged and deficient miRNA-222 expression resulted in persistent hyperglycemia [32]. This direction has the prospect of development due to the low risk of a number of complications: the quality of transplantation, immunogenic and oncogenic risks [27].

Studies reviewed evidenced the additional mechanism that provides the protective function of cells; we are talking about the transfer of functional mitochondria into damaged target cells via tunneling nanotubes [33]. Such structures have active intercellular communication and are able to transfer cytosolic material to ganglion cells. These data were obtained in rat models with induced inflammation and glaucoma [17]. As is known, mitochondrial dysfunction is observed in many diseases of the retina, including DR, and treatment with this method will help to suspend and restore the function of ganglion cells [34].

With regard to the immunological reactivity of the organ of vision, there is scientific information on an interesting mechanism of the immune response that is triggered by the introduction of MSCs: suppression of the proliferation of T-cells, B-cells and natural killer cells, inhibition of differentiation and maturation of monocyte-derived dendritic cells, and promotion of the formation of regulatory T cells. It is based on the ability of MSCs to release mediators, including cytokines, chemokines, and some metabolites (IDO, IL-6, PGE2 and TGF-β1), which provide an immunomodulatory effect, thereby causing protection of the hemato-retinal barrier, infiltration of immune cells and subsequent tissue inflammation. (edema, violation of the MCC, growth of microglia). The significance of the immunosuppressive role lies in the phagocytic absorption of the retinal tissue of apoptotic MSCs, which results in the production of indoleamine-2,3-dioxygenase (IDO), which provides an anti-inflammatory function [21].

In international practice, the therapy of ocular pathology remains in process of testing at the stage of clinical trials, in particular DR, is being tested with retinal stem cell transplantation. Clinical trials in humans are carried out with retinal stem cell transplantation using pluripotential stem cells (PSCs) and iPSCs, which are morphologically similar to photoreceptors and retinal pigment epithelial cells (RPE) [35]. Following this, in 2017, Japan researchers presented two cases of transplantation of MSCs into the retinal pigment epithelium obtained from iPSCs, the cell layer was previously monitored for the absence of genetic breakdowns and anomalies, in patients with wet macular degeneration after removal of the neovascular membrane. At an intermediate stage of this clinical study, the authors proved the survival of induced retinal pigment epithelial cells during the first year after transplantation, improvement in visual acuity and the absence of complications. This study and follow-up of patients is in progress [35].

Since 2012, the biotechnology company SmartCell, based on the Institute of Advanced Medicine VIRTUS, has been developing and implementing unique cellular technologies. They reported AMK (platelet automesoconcentrate), which is a cellular preparation containing growth factors, in particular, bioactive substances that stimulate the process of regeneration of damaged tissues, the growth of new vessels, and as a result, improvement of local blood circulation. That were isolated in the laboratory from the patient's blood platelets used in regenerative medicine, in particular in diabetic retinopathy, to start the natural process of tissue and organ repair in diabetes mellitus. [32].

Bhattacharya, 2017, prospective clinical study provided an evaluation of changes in visual functions, the degree of DME and the state of microcirculation of retinal vessels after transplantation of MSCs in patients with non-proliferative and proliferative stages of DR by intravenous administration. The study revealed the ability of MSCs to control the processes of phagocytosis and reduce inflammation of the retinal tissue. The indicators of the two groups were experimentally compared, without the participation of the placebo group, which is the main drawback of this study [35]. Summarizing the results

of the studies, it was concluded that ABMSCs have an anti-inflammatory effect, reducing the thickness of the macula that controls inflammation. With respect to improvement in BCVA and electrophysiological function (data not shown), MSC-derived neurotrophic factor [36] and control of inflammation may be indicative results. Although several studies have obtained damaged stem cells from people with diabetes [36,37], enough cells have been successfully extracted for transplantation to demonstrate their efficacy. Thus, ABMSCs extracted from diabetic patients may be suitable seed cells for DR.

Baek, 2011, long-term clinical study (NCT01736059) reported the intravitreal injections of bone marrow CD34+ MSCs are administered to subjects with irreversible visual loss due to retinal degenerative disease or retinal vascular disease, including DR. Nirwan, 2019, (NCT03403699) examined the ability of iPSCs to generate endothelial cells and pericytes in areas with capillary degeneration seen in DR [38].

In support of this assumption, recently obtained autologous MSCs from bone marrow were found to be beneficial in patients with NPDR, with significant improvement in macular thickness and improved best-corrected visual acuity (BCVA) from baseline [39].

Overall, the calculation of integral hematological parameters found as no less important before the start of the study and in dynamics, which makes it possible to predict the results after transplantation of autologous MSCs and the state of the body's immunological reactivity [40].

Conclusion

Overall, the review in the field of application of biomedical cellular technologies, in particular, autologous MSCs for the treatment of late complications of diabetes type 1 and type 2 , such as diabetic retinopathy, which is currently one of the leading causes of blindness worldwide, found the technology as perspective.

The presented experimental and clinical cases of achieving an optimal result in transplantation of autologous MSCs explain sufficient potential in improving the homeostasis of the retinal microvasculature and the pathomorphological picture of the neuroretinal interface at all stages of DR. That does not exclude the stage of proliferation, as well as an increase in the state of the body's immunological reactivity, including including the organ of vision, provided that international ethical standards are observed when using cellular technologies.

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Local transport of antibiotics in the treatment of tubular bones chronic osteomyelitis: Literary review

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Abstract

Local bacterial infection after surgery is a formidable complication in traumatology and orthopedics. Local use of antibiotics as an independent type of therapy and in combination with systemic use of antibiotics can lead to the cure of wound infection. Currently, local transport of antibiotics directly into infection focus is increasingly used, in addition to the local use of antiseptics, which has become a traditional method of purulent wounds treating. This method has undeniable advantages in the treatment of osteomyelitis, since due to the variety of forms of local antibiotic transport available today; it allows effective treatment of intraosseous infection without resorting to daily opening and wound trauma. The purpose of this literature review is to analyze all the available data on the treatment of local bacterial infection after surgery in traumatology and orthopedics, as well as on the most promising methods of treating osteomyelitis today.

Key words: antibiotic impregnated allograft, cement with antibiotic, local application of antibiotics

Introduction

Local bacterial infection after surgery is a formidable complication in traumatology and orthopedics [1]. Wound infection, which is quite common in surgery, is also common in traumatology. The main operations in traumatology are metal osteosynthesis of long bones and endoprosthetics of large joints. At the same time endoprosthetics of large joints is complicated by an infectious process in 0.3-3.0% of cases at primary endoprosthetics and in 2.6-4.8% at revision endoprosthetics [2-4]. The course of a purulent infection in orthopedic patients is aggravated by the need for repeated operations, removal of the metal fixator (endoprosthesis components) and resynthesis (revision arthroplasty).

The purpose of reoperations is to stabilize the fracture, promote of bone defects restoration and destroy the bacterial flora [5].

The causes of infectious complications are:

1. Failure to comply with asepsis and antiseptics rules during an operation.

2. The formation of hematomas in the subcutaneous fat and soft tissues in violation of the surgical technique, which subsequently fester.

3. The fact of contamination of metal fixators with microbial flora. Microbial biofilm formation on the metal clamps, which can cause a purulent complication due to immunity decrease [6].

Local antibiotics use as an independent therapy, as well as in combination with the systemic antibiotics use, can help to heal wound infection [7]. Treatment of an orthopedic patient is a difficult task, regardless of the cause of the purulent process development, as it has been proven that blood circulation is impaired in the zone of the pathological process. Therefore, it is impossible to achieve antibiotics concentration in the pathological zone by parenteral drugs injection [8]. Moreover, massive systemic antibiotic therapy will inevitably lead to the development of toxic effects [9]. Currently, local transport of antibiotics directly into infection focus is increasingly used, in addition to the local use of antiseptics, which has become a traditional method of purulent wounds treating. The method has clear benefits in osteomyelitis treatment,

since the variability of methods of antibiotics local transport allows effective treatment of intraosseous infection without daily opening and traumatizing the wound [10]. The given literary review aims to analyze the development of the philosophy of antibiotics use.

History

The history of topical antibiotic use dates back to the 19th century. Scientific discoveries in this matter have always changed public opinion. Alexander Fleming, a British bacteriologist, noticed during the First World War that the local antiseptics application immediately after injury did not completely destroy pathogens, as it was previously thought. He proved that in the case of an open wound, bacteria penetrated very deeply into muscles and bone fragments, and it was impossible to completely wash them out with antiseptic solutions. When antiseptic solutions came into contact with the wound, lymphocytes contained in the wound discharge were washed out, which negatively affected wound healing [11]. In 1939, N. K Jensen et al. first informed of the local sulfonamides application [12]. Subsequently, interest in the local antibiotics use waned. In the 1960s, the method of «irrigation and aspiration» of wound with antibiotic solutions during osteomyelitis treatment was widely used in the West. The method was positioned as a method of high concentrations of antibiotics. But the high consumption of antibiotic solution and low efficiency did not contribute to the further development of the method [13].

A truly rapid dynamic development of topical antibiotic use began in Germany in connection with the widespread use of hip arthroplasty. Endoprosthetics of large joints has become a mass flow surgery. Naturally, the number of purulent complications began to grow with the increase in the number of operations. Buchholz and Engelbrecht reported in 1970, that cement-based hip arthroplasty with the addition of erythromycin, gentomycin, and penicillin to the cement caused the distribution of the antibiotic into soft tissues for several months [14]. In 1979, Clem used gentamicin-impregnated cement for osteomyelitis treatment of 128 patients, and he achieved the cure rate of 91.4% [15].

Surgeon practitioners have not always been optimistic despite the success with topical antibiotics. Thus, according to survey in 1992, in America only 90 (27%) of 336 hospitals used antibiotic impregnated cement in their practice, only in exceptional cases [16, 17]. In 1946, Prigge first proposed the treatment of osteomyelitis using bone grafts, impregnated by antibiotic. He used an autologous graft concurrently with local penicillin application to fill bone defects after affected bone tissue resection in 61 patients with chronic osteomyelitis [18]. De Groude used cancellous bone as an antibiotics bearer for the first time a year later [19]. After infected bone resection, he filled the bone defects with a cancellous bone graft impregnated with penicillin (2 cases). The results were very promising. Nevertheless the procedure was discontinued, after treatment failures, presented by Hogeman in 1949 [20], and Buchman and Blair in 1951 [21]. And the idea of cancellous bone graft using as a carrier for antibiotics delivery was announced by McLaren and Miniachi in 1986 – more than 30 years later [22].

Infections in traumatology devalue the benefits of modern joint arthroplasty and other surgical methods of treatment.

Treatment of an infection boils down to three principles:

1. Reoperation – removal of all foreign bodies (including an endoprosthesis with the installation of a spacer).

2. Long-term systemic antibiotic therapy.

3. The need for repeated endoprosthetics, as joint dysfunction remains after removal of the endoprosthesis.

Long-term antibiotic treatment may have complications [23] or not have the desired effect due to poor blood supply in this area. According to modern concepts, one of the important problems of biofilms presence is the reason for the lack of a therapeutic effect from antibiotics [24]. The emergence of new antibiotics and the improvement in the manual technique of surgeons do not lead to a significant decrease in surgical complications.

Until now, the following questions remain:

• how to transport the antibiotic to the outbreak;

• what antibiotic to use;

• is it possible to use an antibiotic locally for therapy and for infection prevention.

In this review, we will consider these and other issues according to current medical knowledge [25].

Sources searching

The present systematic sources searching were realized in the PubMed database. *Osteomyelitis bone graft substitutes and osteomyelitis antibiotic bone graft* search strings were used, and 285 works were found. Only articles on orthopedic pathology were considered. The full-text assessment was realized after the abstracts were checked and selected. Publications in Russian were also included in the review.

Cement with antibiotic

In 2017, Heinz Winkler and Peter Hayden found that most pathogens that cause bone infection are gram-positive and vancomycin sensitive. However the most gram-negative bacteria are tobramycin susceptive. Hoff et al. showed that antibiotic concentration in tissue is significantly higher when the antibiotic was delivered by cement beads than when systemic antibiotics using. The disadvantage of using antibiotic cement is the fact that the cement is not biodegradable. In this connection, a second operation is required for cement removal. For example, gentamicin beads must be removed in 7-14 days after the wound has healed and the infection has eradicated. The most common combinations of cement with antibiotics used according to the studies reviewed are shown in Table 1. Recently, the use of polymethyl methacrylate considered as a method for local antibiotics delivery [26, 27]. Klemm et al. used granules of gentamicin-polymethyl methacrylate for local antibacterial treatment after surgical removal of damaged tissue in chronic osteomyelitis. The success rate was 91.4% of 128 cases [28].

Polymethyl methacrylate has the following advantages: accessibility, sufficient elution and excellent structural support properties, is the gold standard of treatment [29]. Polymethyl methacrylate can be used as a bearer for vancomycin [30], tobramycin [31, 32], daptomycin [33, 34], and gentamicin [34, 35]. But, the use of polymethyl methacrylate has several imperfections. Thus, an increase to high temperatures (to 100° C) is observed during polymethyl methacrylate preparation and mixing, which can occur antibiotic denaturation and thermal necrosis at the implant site. Also, the implant must be removed as it is not biodegradable. The implant is a substrate for bacteria when the concentration of the antibiotic in it falls below the minimum inhibitory concentration [36, 37]. Thus, the use of cement with an anbiotic is limited to cases when long-term maintenance of the antibiotic in the focus of infection is necessary. In addition, when replacing large bone cavities, cement after a while can be recognized by the organism as a foreign body, and the course of the infectious process will worsen.

Treatment of osteomyelitis in orthopedics (current state)

Today, the gold standard therapy for chronic osteomyelitis is two-stage treatment. The Ist stage of treatment includes systematic therapy by antibiotics, surgical removal of damaged tissue and local antibiotic therapy by Polymethyl methacrylate. The IInd stage includes removal of the PMMA implant and surgery to restore the resulting bone defect. The global trend in healthcare organizations is expedient, aimed at reducing the time of stay and treatment in the hospital, the number of repeated surgical interventions and minimizing financial costs.

Therefore, at present, a promising direction in research all over the world is the search for ways to eliminate the disadvantages of PMMA, the use of biodegradable substitutes of bone grafts with an antibiotic for reoperations reducing [38].

Bone graft alternate materials

The shortage of autografts due to the limited donor sites in the patient for bone collection and the increasing demand for allografts due to the increase in the volume and complexity of surgical operations have contributed to the development of the industry of substitutes for bone grafts (Table 1).

Table 1 The most commonly used cement with antibiotic

Name	Antibiotic	Origin
Simplex P	Polymetil metacrilat 1,0 tobramycin	Stryker Howmedica Osteonics, New-Jercy
SmartSet GHV SmartSet MHV	1 g Gentamycin	Depuy Inc., Poland

Table 2 Comparison of local antibiotic transport systems

Resesarch	Publication year	Bone graft	Antibiotic	Term of antibiotic retained (days)
Mader et al.	1997	PLA (polylactic acid)	Clindamycin Tobramycin Vancomycin	30 days
	1997	PLGA (polymethylmethacrylate)	Clindamycin Tobramycin Vancomycin	30 days
	1997	PLA (polylactic acid)+ PLGA (polymethyl methacrylat)	Clindamycin Tobramycin Vancomycin	30 days
	1997	PMMA (polymethyl methacrylat)	Vancomycin	12 days
Liu et al.	2002	PLGA (polylactide-co-glycolide)	Vancomycin	55 days
Turner et al.	2005	Calcium sulphate	Tobramycin	14-28 days
Raushman et al.	2005	Nanocrystolic hydroxyapatite and magnesium sulfate	Vancomycin Gentamycin	10 days
Web et al.	2008	Calcium sulphate	Daptomycin	28 days
Yeng et al.	2011	PLGA+(polylactide-co-glycolide) collagen	Vancomycin	56 days
Wand et al.	2011	Calcium sulphate and BMP-2	Vancomycin	21 days
Mayer et al.	2013	Betatricalcium Phosphate	Vancomycin Gentamycin	4-6 days

The biodegradable characteristics of substitutes for bone grafts exclude the implant removal necessity [38]. And the decomposition of the biodegradable substitute of bone graft ensures that the remedy is released into the outer tissues. Table 2 shows the results of studies of biodegradable bone graft substitutes.

As can be seen from Table 1, the antibiotic elution time varies from 4 to 55 days. It should be borne in mind that prolonged release is accompanied by a drop in concentration. Therefore, the effectiveness can be much shorter than the release time. Research has been carried out since 1997 and is ongoing.

Calcium sulphate

Calcium sulfate has clinical potency and dependability as an antibiotic carrier [39, 40]. In 2002, the problems encountered with the destruction of the antibiotic during the manufacturing and sterilizing of calcium sulfate were solved by Gitelis and Brebach [41]. The calcium sulfate structure causes its mechanical peculiarities. When stretched, its stability is slightly below, and resistance to compression is higher than in cancellous bone [42]. Calcium sulfate has osteoconductive properties. Calcium sulfate demonstrated the good resorption and good biocompatibility in various studies [43, 44]. Nevertheless, calcium sulfate solution stipulates to inflammatory processes at the implantation site [43, 45]. McKee et al. used bone infections with calcium sulfate impregnated by tobramycin for treatment of 25 patients (15 men and 10 women) in 2002 [46]. 92% eradication rate was noted after post-traumatic osteomyelitis. An urgent problem of clinical practice is the increasing prevalence of antibiotic-resistant bacterial infections. Richelsoph et al [47] and Webb et al [48] offered to use daptomycin as a potent lipopeptide agent to impregnate calcium sulfate to counteract antibiotic resistance. Webb has shown that daptomycin can reduce the growth of *S. aureus and Staphylococcus epidermidis* by up to 28 days [48].

Unfortunately, calcium sulfate also has its drawbacks. Such as the low rate of bioresorption in comparison with the rate of formation of new bone tissue and the impossibility of filling complex-shaped defects. The above factors restrict calcium sulfate application for traumatology and orthopedics.

According to Turner et al., local concentrations of tobramycin antibiotics are sufficient for 14-28 days after the ingrafting of calcium sulfate beads with 10% tobramycin [49]. In 2005, Thomas et al. reported affirmative facts when using calcium sulfate impregnated with tobramycin for the treatment of stable single crystal damages of proximal tibial metaphysis captured by *S. aureus* [50].

Requirements for ideal bone graft

According to the data, a perfect bone graft must show three peculiarities: osteoinduction, osteoconduction and osteogenesis. Also, the bone graft must be in position to desegregate into the recipient's body in order to avoid a graft rejection reaction [51]. Autologous bone grafts are still considered ideal for bone grafts. They contain bone matrix, growth factors, osteoblasts [51, 52]. However, the number of autologous grafts is limited, and complications associated with the site of graft collection remain high. The market of orthopedic allografts is increasing due to the growing demand for bone grafts in the United States [52]. Given the increasing use of bone graft substitutes and the growing number of multi-resistant organisms, antibacterial factor should be considered the fourth property for a perfect bone graft.

Materials of substitutes of bone grafts distinguish in their peculiarities of osteoinduction, osteoconduction, osteogenesis and stability. The biologically active properties of osteoinductive materials potentiate the undifferentiated and pluripotent cells separation into a bone-forming cell line. The surface of the osteoconductive bone graft promotes bone vascular growth. Osteogenesis is the process of new bone tissue forming from transplanted living cells. Bone substitutes do not possess osteogenetic properties by themselves in contrast to autologous bone. Osteogenetic peculiarities can only be added in composites – for example, using bone marrow aspirate. A perfect substitute of bone graft must be biocompatible, bioabsorbable, structurally similar to bone, easy to use, and inexpensive in addition to the four main properties.

Synthetic polymers

Synthetic polymers include polylactide co-glycolide (PLGA), polylactidic acid (PLA), polyglycolide, polycaprolactone, polyhydroxybutyrate co-hydroxyvalerate, polydimethylsiloxanes and polyhydroxyalkanoates [53-55]. All of these materials have antibiotic delivery properties [55].

Mader et al. [56] estimated the elution of clindamycin, tobramycin, and vancomycin from polylactidic acid, polylactide co-glycolide, a composite of polylactidic acid and polylactide co-glycolide, and polymethyl methacrylat in 1997. The polylactidic acid and polylactide co-glycolide composite releases clindamycin, tobramycin and vancomycin concentrations higher the sensitivity point for 30 days. Polymethyl methacrylat effectively eluted vancomycin for only 12 days. Liu et al. examined vancomycin-impregnated polylactide co-glycolide beads in rabbits in 2002. The trough concentration of vancomycin measured for 55 days [57]. The other authors has created a copolymer composite of polylactide co-glycolide as vancomycin bearer in complex with collagen impregnated by mesenchymal stem cells as a biodegradable substitute of bone graft for the treatment of modeled osteomyelitis in animals [57]. Polylactide co-glycolide is a lactic acid based organic substance. As an antibiotic bearer, it is non-toxic material with minimal inflammatory reaction during its biodegradation, whereas collagen is known as a good and low immunogenic bone graft [57]. Ueng et al. revealed low vancomycin concentration in rabbits and good bone regeneration for 56 days [58].

The disadvantages of using polymers include their irritating effect on body tissues due to residual monomer.

Bioactive glass: substitutes for bone grafts

Bioactive glasses (BAG) can serve as a biodegradable osteoconductive substitutes for bone graft, being a silicon-based material. The capacities of BAGs can vary from absorbable to non-absorbable, altering their structural composition [59, 60]. They cannot be useful as antibiotics bearers, but they have antibacterial and angiogenesis peculiarities [61] and bind to bone and muscles. There are data on 11 cases of osteomyelitis, in the treatment of which healing was achieved after surgery and implantation of BAG-S53P4. In 9 out of 11 patients, wound healing was primary, while 1 patient had an infection due to a hematoma, and 1 patient had a superficial wound infection associated with vascular problems [32]. A good treatment outcome was published [23], in which treatment of 27 patients with osteomyelitis using BAG-S53P4 [61] showed good results in 88.9% of cases (24 of 27 patients) over 18 months. One patient was to have plastic surgery, and two patients had recurrent infections. *MRSA* was inoculated in one case and polymicrobial infection was recorded in the other.

The disadvantages of using bioactic glasses include the difference in the mechanical properties of bioglass and bone. Mechanical glasses of bioactive glasses are significantly inferior to the properties of bone tissue. Therefore, bioactive glasses cannot be used for implants of the supporting bones of the body, for example, on the femur, shin bones.

Composites

Vancomycin-impregnated calcium hydroxyapatite was used in rabbits with modeled osteomyelitis after intramedullary MRSA injection by Shirtliff et al. [51]. The researchers compared the efficacy of calcium hydroxyapatite at 81.8% versus the 70% PMMA vancomycin group on *MRSA* strains.

PerOssal® in the form of hydroxyapatite and calcium sulfate composite was used by Rauschmann et al. [57] to reduce the calcium sulfate cytotoxic effects. Gentamicin was eluted from both bearers equally for 10 days when comparing calcium sulfate with a nanoparticle-based calcium sulfate/hydroxyapatite composite. But vancomycin release was initially higher in the composite, but it was higher in calcium sulfate after 5 days. The composition of nanoparticles of hydroxyapatite and calcium sulfate demonstrated better antibiotic release and better resorption and biocompatibility. Calcium sulfate showed cytotoxic reactions in 2 of 4 tests.

Calcium sulfate formulation with recombinant human BMP-2 and vancomycin was examined by Wang et al. [58]. At the same time, good elution of the antibiotic was observed within 21 days and an increase in osteogenesis in the experiment on rabbits. An in vitro study using a composite disc of calcium sulfate and hydroxyapatite (Cerament™) as an antibiotic bearer with different antibiotics against S. aureus and P. aeruginosa was undertaken by Karr et al. in 2011 [32]. In the same year, Karr [32] published a study on the resultative therapy of diabetic osteomyelitis of the foot with the clinical extracellular CeramentTM application impregnated with vancomycin.

A comparative analysis of beta-tricalcium phosphates Cerasorb® and Cerasorb® M as antibiotic carrier systems impregnated with gentamicin and vancomycin was undertaken by Mayer et al. [61]. Elution levels for both S. aureus materials were demonstrated. However, Cerasorb® showed a higher elution rate during 6 days and Cerasorb® M demonstrated lower rates during 4 days.

The disadvantages of composites are low strength and high modulus of elasticity. The presence of these properties does not allow the use of composites in load-bearing bones.

Discussion

Today, the search for the best bone allograft, ideal for the treatment of osteomyelitis, continues. And while there are many options already available, each one has its drawbacks. Thus, polymethyl methacrylate maintains a sufficient minimum concentration of antibiotics in the wound.

This helps to sanitize the wound, but due to the lack of biodegradation properties in the above graft, it becomes necessary to reoperate to remove non-absorbable materials in order to prevent recurrence and to create conditions for bone tissue resorption.

The imperfection of bioactic glasses includes the difference in the mechanical properties of bioglass and bone. Mechanical glasses of bioactive glasses are significantly inferior to the properties of bone tissue. Therefore, bioactive glasses cannot be used for implants of the supporting bones of the body. For example, on the femur, shin bones.

The disadvantages of polymers include their irritating effect on body tissues due to residual monomer.

The shortcomings of composites are low strength and high modulus of elasticity. The presence of these properties does not allow the use of composites in load-bearing bones.

Studies with the isolated use of calcium sulfate showed good efficacy, but later the cytotoxic effect of calcium sulfate was noted, which made it difficult for wound healing. At the same time, a composite of hydroxyapatite and calcium sulfate has shown itself well in a number of studies both as an allograft for good bone resorption and as a local antibiotic delivery system.

Autologous bone grafts are still the gold standard of bone transplantation. They contain bone matrix, growth factors, osteoblasts, and are well impregnated with antibiotic solutions. The disadvantages are the limited amount of autograft, high cost, and difficulties in the process of obtaining bone tissue allotranslantate.

It is necessary to continue the search of an «ideal» bone allograft and to integrate promising developments into clinical practice. The search for the best carrier of the antibiotic continues. Our scientific team continues research in this area.

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Ultrasonographic measurement of optic nerve sheath diameter as a bedside tool in critical care unit to identify raised intra cranial pressures

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Abstract

Aim: Raised intracranial pressure is a common problem in critical care unit and is associated with worse outcome. Several methods have been used to investigate for elevated intracranial pressure (ICP). Here usefulness of optic ultrasound as a bedside tool for assessment of raised ICP in critical care setting was studied.

Material and methods: A prospective study was carried out in Intensive Care Unit (ICU) of tertiary care hospital for a period of 12 months. Patients admitted in ICU during the period of study and who were at the risk of development of raised ICP and needed CT scan for diagnosis were part of the study. 115 patients were included. The optic nerve sheath was identified on ultrasound, and optic nerve sheath diameter (ONSD) was measured 3 mm posterior to the retina. A mean ONSD value of >0.5 cm was taken as positive. CT scan was done immediately afterwards to find any signs of raised ICP.

Results: The mean age of participants was 42.3±10 years with majority of them male (61.7%). The number of cases diagnosed as raised ICP with optic ultrasound was 36 (31.3%) and with CT scan 33 (28.7%) cases. The calculated sensitivity and specificity of optic ultrasound in detection of raised ICP is 100.0% (95% CI: 89.4-100.0) and 96.5% (95% CI: 90.0-99.3) respectively with accuracy of 97.5%.

Conclusion: The present study revealed that ultrasonographic measurement of ONSD as a bedside tool is accurate for screening of patients with probable elevation of ICP. It has advantage of being quick, is without any complication, and patient is not exposed to radiation, besides it does not expose patient to risk of transit during transport to imaging unit as in case of CT scan.

Key words: intracranial pressure, traumatic brain injury, critical care, optic nerve sheath, ultrasound

Introduction

Raised intracranial pressure (ICP) is a common problem in critical care units and in trauma units and is associated with worse outcome. Close ICP monitoring is vital for management of severe traumatic brain injury (TBI) patients to reduce mortality. Indications of intracranial pressure monitoring in TBI has been defined and revised in the 2016 guidelines [1]. Early recognition and management of raised intracranial pressure is essential to maintain cerebral perfusion and to minimize intracerebral damage.

Invasive intracranial monitoring is gold standard method in assessing ICP [2]. As an invasive procedure it has certain limitations, including need of a specialist, risk of infection and bleeding [3,4]. Its availability may also be an issue in resource poor settings.

Lumbar cerebrospinal fluid (CSF) opening pressure or counting CSF drops over time are often used as surrogate markers of ICP. However, it may not correctly depict ICP as pressure is not evenly distributed throughout the subarachnoid spaces [5] and it is an invasive procedure, it also maybe contraindicated.

Numerous methods have been described for non-invasive ICP monitoring, including magnetic resonance imaging (MRI), computed tomography (CT), optic nerve sheath diameter (ONSD) measurement by ultrasonography (USG) and fundoscopy [6,7].

CT scan and MRI is commonly used to diagnose increased ICP. Presence of CT and MR signs suggestive of raised ICP include effacement of basal cisterns, diffuse sulcal effacement and the presence of significant midline shift. These modalities require patient transfer to imaging units, additional manpower and equipment is needed and is time consuming. Critically ill patients requiring high inotropic support or ventilator support present with increased risk during transit and transfer to imaging unit.

Raised ICP can manifest as papilloedema, for which patients can be screened by fundoscopy, a low cost technique, but it has limitations of providing only qualitative assessment and has operator dependence [8]. Also papilloedema does not develop immediately after raised ICP, hence limiting its use in acute settings.

Ultrasonographic measurement of optic nerve sheath diameter (ONSD) is a quick, easy to learn method and can be used at patients bedside for measurement of raised intracranial pressure. It does not have any complication, also patient is not exposed to radiation.

The optic nerve is a part of the central nervous system (CNS), sheath surrounding optic nerve is continuation of dura. It is distensible in its retrobulbar segment. When ICP is elevated transmitted pressure results in increased diameter of optic nerve sheath. It eventually results in swelling of the optic disc and papilloedema [9]. Studies have shown that increased ICP results in development of papilloedema which can take hours to days, but distension of the optic nerve sheath occurs within seconds [10,11]. Hence size of optic nerve sheath measured by ultrasound gives an idea about ICP even in acute settings.

Ultrasonographic measurement of the ONSD a fixed distance from the retina has been studied as a non-invasive measure of ICP [12,8].

Aim: The aim of study was to determine usefulness of optic ultrasound as a bedside tool for assessment of raised ICP in critical care setting. Ultrasound was used to measure ONSD in patients who required CT imagining.

Material and methods

Study design

A double-blinded prospective observational study was carried out in Intensive Care Unit (ICU) of Government Medical College Srinagar, a tertiary care hospital, from November 2020 to October 2021. Institutional ethics committee approval was taken before conduct of this study. Informed consent was taken from the patient or his/her attendants/relatives.

Study setting and population

Patients admitted in ICU during the period of study and who were at the risk of development of raised ICP were part of the study. Patients with wide range of pathologies were enrolled, and included those with traumatic brain injury and those with non traumatic causes like tumor, infection, vascular malformation, or obstruction to CSF outflow. This is a single investigator study, patients were enrolled depending on availability of investigator. Patients less than 18 years of age or those having any ocular injury or pathology were excluded from the study.

Patients with clinical suspicion of raised ICP were to undergo CT scan imaging for confirmation as part of their clinical care and diagnosis. Occular ultrasound was done before CT scan imaging for patients included in the study.

Procedure and Measurements

Measurement of ONSD was performed by single investigator experienced in ocular ultrasound; he was blinded to the CT imagining study results.

Scans were conducted in supine position using point of care Ultrasound machine SonositeTM M-Turbo (SonoSite Inc., Bothell, WA, USA) using linear array probe of frequency 13-6 MHz. B-mode was used for sonography as it provides a high-resolution two dimensional evaluation of orbital and intraoccular tissue including optic nerve. A sterile transparent film was placed on closed eye and sterile ultrasound gel was used as a coupling medium. The probe was placed against the eyelids of closed eye (Figure 1) until the optic nerve was visualized as a hypoechoic linear structure with clearly defined margins posterior to the globe (Figure 2).

Figure 1

Figure 2

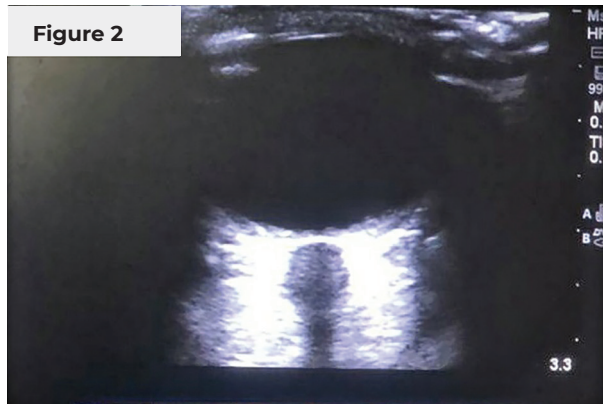
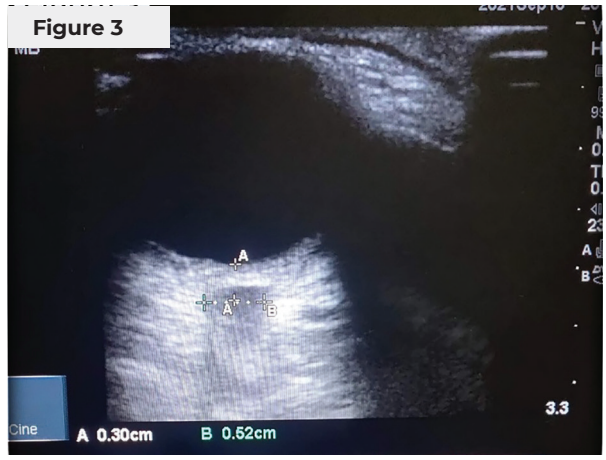


Figure 3



Precautions for use of ocular ultrasound included gentle placement of probe on the closed eye and never in direct contact of cornea or sclera. There has been no reported complication of using ocular ultrasound.

The optic nerve sheath was identified on ultrasound, and ONSD was measured using electronic calipers on a static image of the optic nerve from edge to edge of the nerve at 3 mm posterior to the retina (Figure 3). ONSD was measured in both the eyes, both transverse and sagittal plane, and mean of the measurements was recorded. A mean ONSD value of >0.55 cm was taken as positive. Time taken for the measurement was recorded.

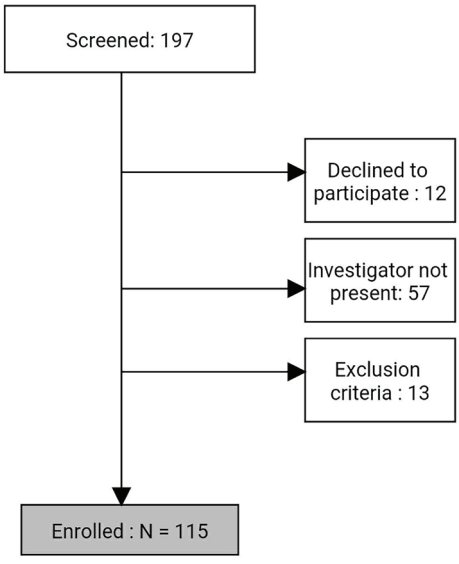
CT scans were done immediately after ONSD measurements and were reviewed by independent investigator for signs of raised ICP, the investigator was blinded to ONSD measurements.

Signs of raised ICP on CT imaging included: Effacement of sulci with evidence of significant oedema; Collapse of mesencephalic cisterns; Midline shift of 3 mm or greater; Collapse of third ventricle; Hydrocephalus or Evidence of herniation. Presence of raised ICP was based on presence of any of above findings on CT and absence determined by absence of all the findings noted.

Statistical analysis

Data were analyzed using STATA version 11.2 statistical software. Brain CT was defined as a gold standard. Using data obtained from the study, sensitivity, specificity, positive and negative predictive value, positive and negative likelihood ratio and accuracy of ultrasonographic measurement of ONSD in prediction of raised ICP was calculated.

Figure 4 - Patient enrollment flowchart



Results

A total of 115 patients were enrolled in the study (Figure 4). There were 71 (61.7%) male and 44 (38.3%) female patients with mean age of study population being 44.6 years.

Traumatic brain injury was the most common (54.8%) diagnosis followed by CNS Infection (15.7%) and Seizures (14.8%) (Table 1).

Out of total study population, 36 patients had raised ONSD measurements (>0.55 cm). Mean ONSD measurement in this group was 0.61cm. Average time taken for optic ultrasound for ONSD measurement in a patient was 3.2 minutes.

Table 1 Patient characteristics

Gender	
Male	71 (61.7%)
Female	44 (38.3%)
Diagnosis	
Traumatic Brain Injury	63 (54.8%)
CNS Infection	18 (15.7%)
Seizures	17 (14.8%)
Hydrocephalus	8 (7%)
Vascular Malformations	5 (4.3%)
Tumors	4 (3.5%)

CT scan imaging was positive for sign of raised intracranial pressure in 39 patients. It included all patients who had raised ONSD measurements (>0.55 cm).

Three patients had signs of raised intracranial pressures on CT scan but ONSD measurements on optic ultrasound were normal (<0.55 cm) (Table 2).

Table 2 Diagnostic accuracy of raised ONSD measurement in prediction of elevated ICP

STATISTIC	VALUE	95% CI
Sensitivity	100%	89.42-100.00
Specificity	96.47%	90.03-99.27
Positive predictive value	91.67%	78.35-97.10
Negative predictive value	100%	-
Positive likelihood value	28.33	9.32-86.1
Negative likelihood ratio	0.00	-
Accuracy	97.46%	92.75-99.47

On analysis of the data, ONSD has sensitivity of 100.0% (95% CI: 89.42-100.0) and specificity of 96.47% (95% CI: 90.03-99.27).

Discussion

Raised ICP is frequently encountered in critical care units in patients with different pathologies and it is life threatening. It is essential to identify patients with high ICP and intervene to maintain adequate cerebral perfusion. Hence, a rapid and reliable bedside tool for detection of raised ICP is much needed.

Use of ultrasound in critical care has increased in recent years. A portable ultrasound machine is ubiquitous in critical care units nowadays. Ultrasonographic ONSD measurement for raised ICP is based on anatomical fact that optic nerve is in communication with CNS with the intraorbital part of subarachnoid space being distensible.

In our study ultrasonographic ONSD measurement for raised ICP has sensitivity of 100% and specificity of 96.47%, with accuracy of 97.46%

Our results support use of ultrasonographic ONSD measurement for detection of raised ICP in critical care setting. It is quick, noninvasive, safe and inexpensive. Portable ultrasonography equipment is commonly available in critical care units and hence can be used as a bedside tool. It provides quantitative assessment of ICP and is repeatable. Also no complications were observed during the procedure.

These results are in conformation with other studies in which ONSD was studied in different clinical settings and showed good correlation with ICP measured with methods including invasive methods and CT imaging [13,8].

Recent studies suggest that daily monitoring of ONSD measurement by ultrasonography can be useful for determination of increased ICP, it also can be used for evaluating

the neurological prognosis of the patients [14,15,16]. With the development of technology the USG devices are getting more portable. Smaller handheld or pocket-sized USG devices are already being used. These devices can be used to screen high risk patients at their bedside.

Our study was aimed at detecting usefulness of OSND measurement for detection of raised intracranial pressures in a group of patients with different pathologies. CT scan imaging was used for comparison as it is the most common diagnostic tool being used in our set up.

CT scanning requires equipment to which access is variable in resource limited settings. It exposes patient to increased risk of transit as it needs transport of patient to specific imaging unit. There is also risk of radiation exposure.

We support use of invasive method to measure and continuously monitor absolute ICP values for high risk patients in accordance with guidelines for TBI management. However as shown in our study ultrasonographic measurement seems to be of value as it is accurate, reliable non invasive tool and can be used in situations where there is clinical suspicion for intracranial hypertension but invasive monitoring is unavailable, contraindicated or risky to perform. It does not interrupt treatment as can other modalities like CT scan, can be done on bedside of patient and is repeatable. It can also be used for screening and to select patients for invasive monitoring in resource limited settings.

Limitations

Sonographic experience is one of the limitations to use of ocular sonography. Many patients could not be included in the study as only one investigator was available. However due to single investigator, inter-observer variability was excluded. Continuous measurement is not possible with ocular ultrasound and it needs to be repeated in patients at higher risk of raised ICP.

The criterion standard used to compare ultrasound examination is not ideal because CT imaging is not a true measurement of ICP. Though CT scan has good reliability in acute settings

ONSD measurement may be raised without elevated intracranial pressure as seen in few rare conditions like optic neuronitis, optic nerve trauma or cavernous sinus mass leading to false positive finding, though we did not encounter these in our study.

Conclusion

ONSD measurement for detection of raised ICP is an effective diagnostic tool and has a strong correlation with CT scan imaging for raised ICP detection. It is quick, easy, inexpensive and without any complications.

Disclosures: There is no conflict of interest for all authors.

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Screening of specimens by Ziehl-Neelsen staining technique for the diagnosis of extra spinal musculoskeletal tuberculosis: A retrospective study

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Abstract

Introduction: Musculoskeletal tuberculosis is a significant form of extra pulmonary tuberculosis (EPTB) which has substantial consequences if not diagnosed and treated early.

Reliable and rapid confirmation of the diagnosis is now possible by advent of Polymerase Chain Reaction (PCR), Cartridge Based Nucleic Acid Amplification Test (CB-NAAT/Gene-Xpert) and radiometric method (BACTEC)

However, most of these sensitive technologies are not available at places with constrained resources. Hence, diagnosis of tuberculosis at these places, continues to rely on smear microscopy using Ziehl-Neelsen (ZN) staining. Specificity of ZN staining and microscopy is high, but its overall sensitivity is variable (20-53%). Despite of low sensitivity, it is still an easy, cheap, efficient and accessible tool due to its high specificity.

Aim: Aim of the present study was to assess the role of ZN staining in diagnosis of extra-spinal musculoskeletal tuberculosis in resource limited settings.

Material and methods: A retrospective study was done from 1st January 2016 to 31st December 2019 on specimens received in microbiology laboratory for ZN staining from clinico-radiologically suspected cases of extra-spinal musculoskeletal tuberculosis. The clinical information of the cases was noted from the Orthopaedics Department case files, while results of ZN microscopy were retrieved from the records maintained in Microbiology Department.

Results: Specimens from 95 patients with clinico-radiologically suspected extra-spinal musculoskeletal tuberculosis were examined for Acid Fast Bacillus (AFB) by ZN staining technique. Out of 95 patients; 11 patients (11.58 %) were found to be AFB positive.

Conclusion: Owing to low yield percentage of ZN staining in extra spinal musculoskeletal tuberculosis; ZN stain alone cannot be used as a tool for diagnosis of this form of tuberculosis.

Key words: extra-spinal musculoskeletal tuberculosis, ZN staining, diagnostic tool

Introduction

Tuberculosis is a disease known to mankind since ancient times. The disease is more common among the poor and marginalized sections of the community. According to the World Health Organization (WHO), there were 9.6 million cases of tuberculosis in the world in 2014 and 1.5 million died from the disease. In addition, most of the deaths (>90%) occurred in developing countries [1]. Untreated tuberculosis cases infect 10 to 15 people every year [2, 3]. In 2010, the incidence

of tuberculosis was estimated as 2.15 million in Central Asia, which is expected to be tripled by 2030 [4].

Extra pulmonary tuberculosis (EPTB) accounts for 10-15% of all the forms of tuberculosis.5 The most common site of EPTB are lymph nodes, central nervous system, abdomen, skeletal system, pleura, pericardium, genitourinary system, skin and others [5,6].

Musculoskeletal tuberculosis includes tuberculous myositis and osteoarticular tuberculosis. Osteoarticular (spinal and extra-spinal) tuberculosis represents 1-5% of all cases of tuberculosis and 10-18% of EPTB, with spine being the most common site accounting for 50% of osteoarticular tuberculosis [7-9].

Early diagnosis and prompt initiation of antitubercular treatment is imperative for successful management of musculoskeletal tuberculosis, thereby decreasing the morbidity/disability. A good clinic-radiological assessment along with proper investigations with highly sensitive/specific tools is key for early diagnosis.

Clinically extra- spinal musculoskeletal tuberculosis is suspected in patients who present with pain, swollen joints with/without constitutional symptoms like night sweats, loss of appetite, weight loss and mild grade fever.

Tubercle bacilli do not stain readily, but once stained, they resist decolorization by acid or alcohol and are hence called acid-fast bacilli (AFB). They can be identified early and easily with ZN staining method in resource-limited settings [10, 11]. ZN staining is highly specific, but its overall sensitivity is variable (20-53%) and poor in EPTB [10, 12]. Viable and dead bacilli cannot be distinguished by smear microscopy, but can be distinguished by culture methods. They are obligate aerobes and take about 15-30 days to grow on culture in an enriched media with a moderately acid-base media at a temperature of 37 degree C.

Despite the recent development of more sensitive technologies like PCR, CB-NAAT/Gene-Xpert and radiometric method (BACTEC); diagnosis of tuberculosis in most low socioeconomic countries, continue to rely on smear microscopy for diagnosis of tuberculosis [2]. Although sensitivity of ZN staining is low/variable, still it is an easy, cheap, efficient and accessible tool due to its high specificity, for early diagnosis and prompt start of antitubercular treatment of ZN positive cases in resource limited settings. Literature on ZN staining for screening of extra-spinal musculoskeletal tuberculosis is scarce. Hence, the aim of the present study was to study the role of ZN staining in early diagnosis of extra-spinal musculoskeletal tuberculosis in resource constrained settings.

Material and methods

Institutional research committee approval and ethical committee clearance were obtained for the study. A retrospective study was done between January, 2016 and December, 2019.

Inclusion criteria: Clinico-radiologically suspected cases of extra-spinal musculoskeletal tuberculosis; whose specimens were sent to the microbiology laboratory from Orthopaedics Department for ZN staining were included in the study.

Exclusion criteria: Cases with spine tuberculosis, multiple system involvement and/or disseminated miliary tuberculosis were excluded from the study

The demographic characteristics of the patients and clinical data were noted from the case files, while results of ZN microscopy were retrieved from the records maintained in Microbiology Department.

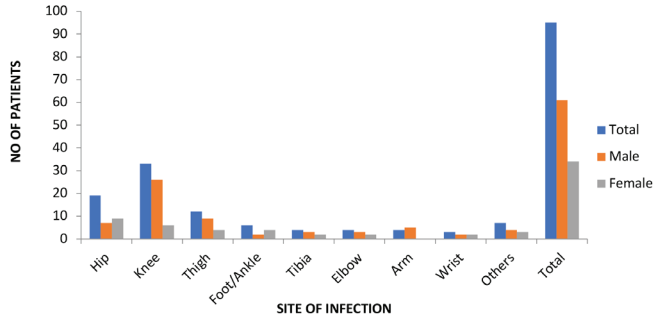
Results

Specimens from 95 patients with suspected extra-spinal musculoskeletal tuberculosis were screened for AFB.

The mean age of patients was 31.07 years (range 4-70 years). Most of the patients were less than 40 years old (68.42%). There were 61 (64.21%) males and 34 (35.79%) females (male: female ratio 1.79:1).

The commonest symptom was pain with/without swelling. One patient presented with multiple sinuses over ankle/foot. Two cases of suspected knee joint tuberculosis presented with cold abscess. All the cases were human immunodeficiency virus (HIV) negative. Number of cases based on involvement of site is depicted in Figure 1. Knee was the most common site of involvement followed by hip and thigh.

Figure 1 - Distribution of suspected cases according to site of involvement



Out of 95 patients, only 11 patients (11.58%) were found to be AFB positive, most being from pus specimens 9 (9.47%) while 2 (2.10%) from synovial fluid. Distribution of the 11 AFB positive cases based on age, gender and site of involvement is shown in Table 1. Maximum AFB positive cases were seen in specimens from knees 5 (45.45%) followed by 1 (9.09%) each from wrist, elbow, hip, thigh, tibia and ankle. Seven of the 11 AFB positive cases were males (63.63%) while 4 (36.36%) were females. Mean age of the AFB positive cases was 27.82 years (range 06-68 years).

Table 1 Distribution of AFB positive cases

S.No.	Age (years)	Sex	Infection site	Sample
1.	12	M	Knee	Pus
2.	45	M	Wrist	Pus
3.	68	F	Ankle	Pus
4.	45	F	Elbow	Pus
5.	13	M	Knee	Synovial fluid
6.	22	M	Knee	Pus
7.	10	F	Thigh	Pus
8.	15	M	Knee	Synovial fluid
9.	06	M	Hip	Pus
10.	18	F	Knee	Pus
11.	52	M	Tibia	Pus

Discussion

Tuberculosis is a worldwide public health problem, although 90% of cases being in the underdeveloped countries [13]. There has been a recent re-emergence of tuberculosis in the industrialized developed countries which has been attributed to an increase in life expectancy, immigration from endemic regions, emergence of multi-drug-resistant tubercular strains and HIV infection [14-16].

In a study by Patel et al., out of 18 AFB positive patients, maximum patients (5 each) were in age group 11-20 years and in 21-30 years (27.8%) [17]. In our study, out of 11 AFB positive patients, maximum patients (4) were in age group 11-20 years (36.36%). In another study by Prasad et al, mean age at presentation was 33.35 years (range 4-72) [18]. Mean age of patients in our study was 31.07 years (range 4-70 years).

In study by Patel et al, male to female ratio was 1.5:1 [17]. In another study by Prasad et al, it was 1.1:1 [18]. In our study, it was 1.79:1.

In a study by Patel et al, out of 793 EPTB samples screened for AFB, 18 (2.26%) samples were found to be AFB positive [17]. In another study by Prasad et al, 51 patients with extra-spinal musculoskeletal tuberculosis, 18 (35.3%) were AFB positive [18]. In our study, 11 (11.58%) patients were AFB positive out of 95 extra-spinal musculoskeletal tuberculosis patients.

In study by Prasad et al, commonest site (18 out of 51 patients) of articular involvement in musculoskeletal tuberculosis was knee joint (35.29%) [18]. Knee was most commonly (33 out of 95 patients) involved joint (34.74%) in our study too.

In study by Patel et al, 14 out of 18 (77.78%) AFB positive samples were received as pus [17]. In our study, 9 out of 11 (81.81%) samples were received as pus.

Anti-tubercular treatment was started immediately in the 11 AFB positive cases. The other 59 AFB negative cases were either advised more sensitive investigations like TB-PCR or histopathology while remaining were started on antitubercular treatment based on sufficient clinico-radiological evidence and blood investigations (erythrocyte sedimentation rate, C-reactive protein and liver function tests).

Extra-spinal musculoskeletal tuberculosis should be diagnosed at the earliest to prevent limb and life-threatening complications. AFB load is scanty in musculoskeletal specimens, hence difficult to diagnose. Although culture is considered as a gold standard method but it takes at least 4 weeks to come positive [19]. Newer and more sensitive techniques like PCR, fluorescence microscopy, CBNAAT/Gene-Xpert and histopathology should be used for rapid diagnosis.

Gene-Xpert and AFB smear microscopy share almost same specificity but sensitivity of Gene-Xpert is much higher than AFB smear microscopy. Although ZN staining method for AFB plays a key role in the diagnosis of tuberculosis, its major disadvantage is its low sensitivity [20].

Limitations:

There were certain limitations of the study: The study was performed retrospectively and results could not be correlated with radiological findings and histopathological reports. We could not comment on the disease status of the 59 patients who had AFB negative specimens as they were subjected to ZN staining only which has a low sensitivity.

Conclusion

Extra-spinal musculoskeletal TB is one of the important forms of EPTB and has significant consequences if not recognized and treated early on. Involvement of weight-bearing joints like hip and knee is common. High index of clinical suspicion, timely judicious use of invasive diagnostic methods and confirmation of the diagnosis by more sensitive methods like fluorescent stain, CBNAAT, TB-PCR, biopsy, Gene X-pert in comparison to ZN stain and early institution of specific antitubercular treatment with close clinical monitoring for adverse drug reactions are the key to the successful management of extra-spinal musculoskeletal TB.

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Research trends on Legionellosis

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Abstract

Aim: The aim of the study is to analyze the scientific studies about Legionellosis which published in all around the world.

Material and methods: The Elsevier's Scopus database (https://www.scopus.com/) was searched with the for bibliometric analysis method. Articles were selected only according to their valid scientific properties which accepted by scientific community with their methodologic design. The publications were analyzed according to publication years, countries, authors, institutions, funding institutions, publishing languages, themes, citations, keywords, methods, and samples. The Scopus database was filtered for document type which only the articles. The data containing the keywords "legionellosis" or "Legionella pneumophila" or "Legionella" or "Pontiac fever" in their title were retrieved till December 13, 2021.

Results: In total, 8778 documents relating to Legionellosis were identified in the Scopus database till December 13, 2021. 7073 of the documents were articles. The articles were published in mainly in the discipline of medicine (n=4766, 67.38%). The first articles were published in the year 1977 and was from United States of America (USA). There were two surges in the number of publications in the years 1983 and 2013. After 1980, the annual number of articles never dropped below 100 articles per year. The USA dominated the scientific production on Legionella with the number of 2214 (31.30%) publications. The top 5 leading scientifically productive countries on Legionella literature after USA were Germany (n=8.41%), United Kingdom (7.64%), France (7.36%) and Japan (n=512, 7.23%). The publications on Legionellosis were from more than 100 countries globally. The maximum number of the citation was in the year of 1978. 1126 (15.9%) of the articles were not cited yet.

Conclusion: The number of publications was high since the first reports were published, but the number of cites decreased since 2020.

Key words: bibliometric analysis, Legionnaires' disease, Legionellosis

Introduction

Legionnaires' disease or Legionellosis is a serious lung infection caused by *Legionella* species. The *Legionella* family currently consists of more than 60 species comprising 70 distinct serogroups [1,2]. Only a few of these are associated with disease in humans. The most common type of disease is *Legionella pneumophila* and *L. pneumophila serogroup* 1, with different invasion and virulence abilities than others, are responsible for 75-80% of cases. However, it can cause nosocomial pneumonia. The disease was first described in 1976 at a congress of American Legionnaires at a Philadelphia hotel (Bellevue Stratford Hotel), with an outbreak of pneumonia among attendees. Of the participants attending the congress, 221 were affected by *L. pneumophila* and 34 died. Since the infection first appeared in the meeting with the legionnaires and the causative microorganism was isolated from lung tissue samples, the disease was called "Legionnaires' disease" and the bacterium causing the infection was named *L. pneumophila*. Legionnaires' disease was first identified with a hotel-acquired epidemic, and it was soon realized that it could occur with hospital-acquired outbreaks or sporadic cases [2,3].

The term of Legionellosis is used to describe bacterial infections that can range from mild febrile

resolving illness (Pontiac fever) to pneumonia, which can be rapid and fatal (Legionnaires' disease) [1]. Legionnaires' disease is a systemic infection that can occur in a wide clinical spectrum, from mild lower respiratory tract involvement to forms which can progress to severe coma and death that affecting all organs [4]. The main pathological events occur in the lungs and the state of the defense mechanisms determines the course of the disease. Since it cannot be distinguished from other types of pneumonia clinically and radiologically, its definitive diagnosis is made by microbiological examination. Legionnaires' disease will not develop if there is no contaminated water source. It is considered that there are two ways for the bacterium to reach the lungs. The widely accepted route is the inhalation of water aerosols containing *Legionella*, which are emitted into the respiratory air from environmental sources (fans of cooling towers, whirlpools and showerheads, spray humidifiers, decorative fountains...). Another important transmission route is the passage of bacteria settled in the oropharynx into the respiratory tract as a result of aspiration of water containing *Legionella*. There is no human-to-human transmission [2,4]. This disease is seen globally and as it is known to be a nosocomial pathogen for hospital plumbing, and its importance is increasing [5,6].

In this study, we aimed to analyze the publications on Legionellosis and to guide researchers about Legionella.

Material and methods

The aim of the study was to analyze the articles on Legionellosis. The Elsevier's Scopus bibliometric database (https://www.scopus.com/) was searched for bibliometric analysis method. Articles were selected only according to their valid scientific properties which accepted by scientific community with their methodologic design. Congress book abstracts, reviews, letters, etc. were not included.

The articles on Legionellosis were analyzed according to publication years, countries, authors, institutions, funding institutions, publishing languages, themes, citations, keywords, methods, and samples. The Scopus database was searched for article document type. The data were retrieved until the date of December 13, 2021 which containing the keywords "legionellosis" or "Legionella pneumophila" or "Legionella" or "Pontiac fever" in their title.

The searches were performed only at the day of December 13, 2021, to avoid bias as the database is daily updating. Duplications were included in the review only once. The obtained data were analyzed in the Excel forms created by the researchers. The data was analyzed with both quantitative and qualitative methods and the top-rated publications were analyzed comprehensively.

Çanakkale On Sekiz March University's online library and digital resources were used to accessing information.

Ethical Approval

The study complied with the Helsinki Declaration, which was revised in 2013. Ethics committee approval is not required as there is no human or animal research.

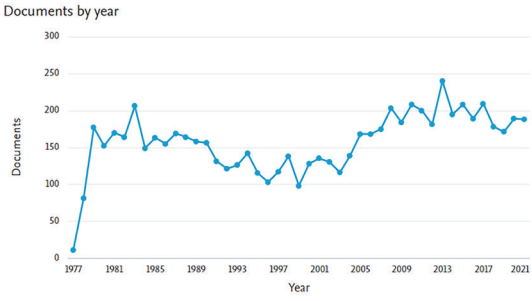
Statistical methods

The data in the tables were given as absolute values (frequency and percentage) by using Microsoft Excel 2010. No advanced statistical analyses tests were used. The visualization of the citing analyses was done with the Dimension program (Free Version) and WOS viewer (https://app.dimensions.ai/discover/publication)

Results

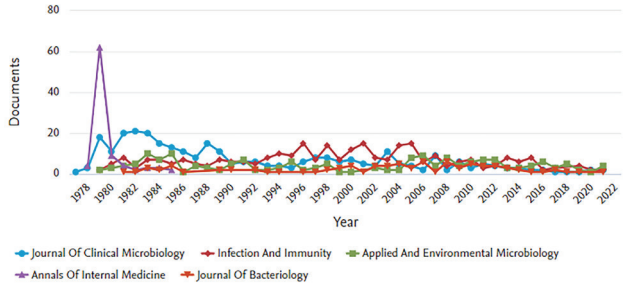
In total, 8778 documents relating to Legionellosis were identified in the Scopus database till December 13, 2021. 7073 of the documents were articles, and we only analyzed the articles which has a trustworthy scientific impact. 2859 (40.42%) of the articles were published as open access (OA). Articles were published in mainly in the categories of medicine (n=4766, 67.38%), Immunology and Microbiology (n=2067, 29.22%), Biochemistry, Genetics and Molecular Biology (n=1286, 18.18%) and Environmental Science (n=667, 9.43%). The first articles were published in the year 1977 and from United States of America (USA). There were two surges in the number of publications in the years 1983 and 2013. After 1980, the annual number of articles never dropped below 100 articles per year (Figure 1). The USA dominated scientific production on Legionella with the number of 2214 (31.30%) publications. The top 10 leading scientifically productive countries on Legionella literature were after than USA were Germany (n=595, 8.41%), United Kingdom (n=541, 7.64%), France (n=521, 7.36%), Japan (n=512, 7.23%), Italy (n=301, 4.23%), Canada (n=265, 3.74%), Spain (n=258, 3.64%), China (n=214, 3.02%) and Netherlands (n=193, 2.72%). Turkey was in the 22nd place. The publications on Legionellosis were from more than 100 countries globally.

Figure 1 - Number of publications by the years



The majority of the articles (n=5960, 84.26%) were written in English language. French (n=189, 2.67%) and German (n=187, 2.64%) languages were the other most preferred languages. The highest number of articles on Legionellosis were published in the journals; Journal Of Clinical Microbiology (n=307, 4.34%), Infection and Immunity (n=292, 4.12%), Applied and Environmental Microbiology (n=179, 2.53%), Annals of Internal Medicine (n=89, 1.25%) and Journal of Bacteriology (n=89, 1.25%). The published numbers of top 5 journals by years were given in Figure 2.

Figure 2 - Published numbers of top 5 journals by years



Most funding sponsors were National Institute of Allergy and Infectious Diseases (n=595, 8.41%), National Institutes of Health (n=290, 4.10%), U.S. Department of Health and Human Services (n=178, 2.51%) National Institute of General Medical Sciences (n=91, 1.28%), Deutsche Forschungsgemeinschaft (n=75, 1.06%) Japan Society for the Promotion of Science

(n=62, 0.87%) and National Natural Science Foundation of China (n=57, 0.8%). The most of the funding sponsors were from USA. 5559 (78.59%) of the articles were not funded.

Nicholas P. Cianciotto (Northwestern University Feinberg School of Medicine, USA) and Sophie Jarraud (Claude Bernard University, France) were the most productive authors on legionellosis literature with 91 articles. The Centers for Disease Control and Prevention was the leading affiliation on this topic with 302 articles (Table 1).

Table 1 Top 10 affiliations on Legionellosis literature (n=7073).

Affiliation, Country	Number of publications (%)
The Centers for Disease Control and Prevention, USA	302(4.26)
VA Medical Center, USA	142(2.00)
CNRS Centre National de la Recherche Scientifique, France	121(1.71)
National Center for Infectious Diseases, USA	111(1.56)
Université Claude Bernard Lyon 1, France	108(1.52)
Inserm, France	93(1.31)
Public Health Laboratory Service, United Kingdom	86(1.21)
Julius-Maximilians-Universität Würzburg, Germany	80(1.13)
Howard Hughes Medical Institute, USA	77(1.08)
University of Pittsburgh, USA	74(1.04)

Citing analysis

The maximum number of the citation was in the year 1978. 1126 (15.9%) of the articles were not cited yet. The top cited article was published by Fraser et al. (Figure 3) [7].

Citing analyses of the most productive authors on Legionella research was given in Figure 4.

Figure 3 - Publication citation number is the number of times that publications have been cited by other publications in the database.

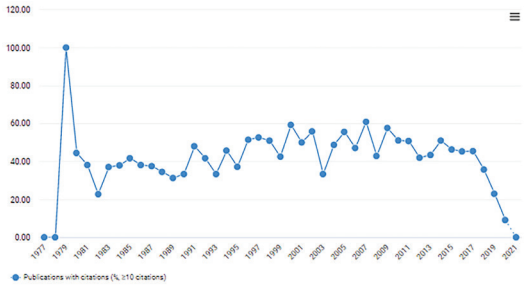
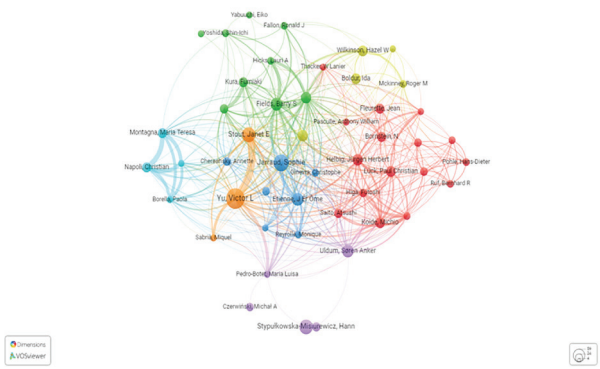


Figure 4 - Citing analyses of the most productive authors on *Legionella* research



Discussion

Many different bibliometric analysis methods have started to enter the medical literature in recent years, and analysis studies on this subject can be enriched by methods such as mapping and graphing. These studies can be done by using many methods such as content analysis, comparison of scientific productivity by years, countries, and citation numbers. Databases that are frequently used for bibliometric analysis are databases that provide easy and comprehensive data analysis such as Pubmed, EBSCO, Scopus, Pro-Quest, Web of Science. However, other sources such as any database, theses, journals, congresses, etc. can be analyzed with this method [8-13]. The Scopus database is a comprehensive, well-compiled database of abstracts and citations which combining academic literature. This subscription database provides access to metrics and analytical tools [10-12]. With the comprehensive analysis feature Scopus provides, this database also enables publication analysis, which is a different research method [10-14]. There are limited bibliometric studies on infectious diseases [8,15-19]. The bibliometric method refers us quantification about overall trends and highlighting connections or correlations hidden within large amounts of data.

We think that the scientific efficiency of infectious diseases as well as many other disciplines in health, should be evaluated with this method and a roadmap should be created for further studies. In this study, the Scopus database was used for the research. Before conducting this current study, we conducted a literature review on Legionellosis, and we could not reach any similar study. This disease is important as many countries have surveillance tracking systems available on Legionellosis [20]. But the publications were not analyzed yet in bibliometric vision. We aimed to add vision to Legionellosis researchers.

According to the findings of our study, the annual number of articles on the topic Legionellosis never dropped below 100 articles per year after the year 1980. But the number of citations decreased over the years. The majority of articles were published in the year 2013. The USA, Germany, United Kingdom, France, Japan, Italy, Canada, Spain, China and Netherlands produced most of the articles (nearly 75%) on Legionellosis. Although this disease is seen worldwide, the distribution of scientific productivity by country did not reflect disease prevalence. Although there were publications from more than a hundred countries globally, there were very limited publications from developing countries.

The most productive affiliations were also from the USA and France. The first articles were also from the USA. The highest number of USA publications may be due to the fact that the funder affiliations are often from the USA. Additionally, the Centers for Disease Control and Prevention was the leading affiliation on this topic is from the USA, too.

The examining the writing languages of the publications showed that more than 95% of the publications were written in English. This may be due to the predominant language of literature being English. Because greater visibility and a higher number of citations are expected for articles in English, most researchers tend to publish their work in English, even if their native language is not. In addition, English is accepted as the common language of the scientific world. However, non-native English speakers still publish studies in their native language, which explains the lower citation rates, perhaps due to local or regional interest and accessibility.

As a result of the study, the number of publications was high since the first reports were published, but the number of cites decreased since 2020. This situation may be attributed to the global pandemic.

Limitation

The current study has several limitations. Studies in other than Scopus database were not analyzed. Also keywords only in English, so publications written in other languages may be omitted. Content analysis was not performed in our study.

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Human HMGB1 does not induce eryptosis in vitro

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Abstract

Aim: To study the ability of human high mobility group box protein 1 (HMGB1) to induce eryptosis *in vitro*.

Material and methods: Blood collected from six healthy volunteers was incubated with HMGB1 (0-0.2-1-5 ng per ml). Eryptosis of red blood cells was assessed by Annexin V staining and 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) staining by flow cytometry. The forward scatter (FSC) fluorescence was used to evaluate the morphology of red blood cells.

Results: Exposure of erythrocytes to HMGB1 did not affect the morphology of erythrocytes, evidenced by no changes in the percentage of cells with small volume, i.e. shrunken cells, and erythrocytes with large volume, i.e. enlarged cells. HMGB1 had no impact on phosphatidylserine externalization, which is confirmed by the absence of statistically significant changes in the amount of phosphatidylserine-displaying cells and the mean fluorescence intensity (MFI) values of Annexin V-FITC in cells exposed to different concentrations of HMGB1. Furthermore, H2DCFDA staining revealed that the HMGB1 did not induce oxidative stress.

Conclusion: HMGB1 does not promote eryptosis of human erythrocytes at concentrations of up to 5 ng/ml.

Key words: high mobility group box protein 1, erythrocytes, inflammation, cell death

Introduction

Human high mobility group box protein 1 (HMGB1) is a highly abundant evolutionary conserved nuclear protein composed of 215 amino acid residues with a molecular weight of approximately 30 kD [1]. It has attracted a lot of attention, since it can be secreted by cells or released from dead cells and act extracellularly as an alarmin or damage-associated molecular pattern (DAMP) [2,3]. As for its intracellular functions, HMGB1 is a non-histone, chromatin-binding protein, which is involved in transcription regulation and DNA repair [1,4]. However, nowadays effects of extracellular HMGB1 are under extensive research due to its ability to regulate inflammation. In particular, HMGB1 secreted from viable cells or cells, which underwent apoptosis, necrosis, netosis, pyroptosis, and necroptosis, can induce the inflammatory response [5-9]. It has been reported that extracellular HMGB1 is a ligand for many receptors, including the innate immunity ones, but its pro-inflammatory effects are mainly mediated by binding to toll-like receptor-4 (TLR4) and receptor of advanced glycation end products (RAGE) [10]. Both TLR4 and RAGE signaling culminates in nuclear factor-kB (NF-kB) activation and generation of pro-inflammatory cytokines [11].

It is important to mention that HMGB1 can undergo some post-translational modifications, such as oxidation, acetylation, methylation, phosphorylation, ubiquitination, glycosylation and ADP-ribosylation, which can affect its effects on cells [1]. Inside the nucleus, HMGB1 is fully reduced and its oxidation is tightly regulated both under normal and pathological conditions [12]. Reduced and oxidized forms of HMGB1 differ in their effects on inflammation, which suggests the importance of HMGB1 redox state and its regulation [13,14].

In addition to inflammation, HMGB1 is involved in regulation of cell death. It is mainly considered to be an inhibitor of apoptosis and necrosis [15]. However, some evidence is provided that HMGB1 can induce apoptosis in tumor cells [16]. Moreover, this DAMP can promote autophagy, necroptosis, and pyroptosis [16-18]. Moreover, HMGB1 is reported to regulate the crosstalk between autophagy and apoptosis in inflammation [19]. Thus, HMGB1 is a multi-faceted protein involved in regulation of inflammation and cell death. Its effects on cells are pleiotropic and context-dependent. Despite the fact that erythrocytes don't have nucleus and unlikely release HMGB1, its role in red cell biology is of huge interest. No data are available on the ability of human HMGB1 to influence eryptosis, which is a suicidal cell death of red blood cells.

The study was designed to analyze the impact of human HMGB1 on eryptosis *in vitro*.

Material and methods

Subjects and incubation conditions

Blood samples were collected from six conditionally healthy male volunteers aged 24-29 years in K2EDTA Vacutainers (IMPROVACUTER Evacuated EDTA K2 Spray Dried PET Tubes, Guangzhou, China). All volunteers enrolled for the study signed an informed consent. Exclusion criteria included the presence of acute or chronic inflammatory diseases, hypertension, endocrine diseases, intake of steroid hormones for the last 3 months, obesity (BMI over 30), and fever. Blood aliquots of 5 µl were incubated with human HMGB1 (0-0.2-1-5 ng per ml, n = 6) purchased from Elabscience (Houston, TX, USA) in 500 µl RPMI-1640 medium with stable glutamine (Biowest, France) and 5% fetal bovine serum (BioWhittaker®, Lonza, Belgium) for 24h in sterile conditions. The sample size was calculated using G*Power software (Germany).

Following incubation, erythrocytes were obtained after washing the samples twice with phosphate buffer saline (PBS). The obtained erythrocyte suspensions were used for Annexin V staining and 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) staining.

The research was conducted in accordance with the Declaration of Helsinki and adhered to Good Clinical Practice guidelines. The design was approved by the Ethics and Bioethics Committee of Kharkiv National Medical University (Kharkiv, Ukraine; minutes No 5 dated September 17, 2019). The study was performed in December 2021.

Annexin V staining

Erythrocyte suspensions obtained from blood exposed to HMGB1 were stained with Annexin V-FITC purchased from Becton Dickinson (USA). Erythrocytes were resuspended in 100 µl 1x Annexin-binding buffer (Becton Dickinson, USA) after washing and then 5 µl Annexin V-FITC was added. Cells were incubated for 15 minutes. Then 400 µl 1x Annexin-binding buffer was added to each tube. The fluorescence of Annexin V-FITC was detected by BD FACSCanto™ II flow cytometer. Samples treated with hydrogen peroxide (0.1 mM) were used as positive controls. Negative controls included erythrocyte suspensions treated with no Annexin V-FITC [20, 21].

H2DCFDA staining

To analyze reactive oxygen species (ROS) generation, erythrocyte suspensions were stained with H2DCFDA. According to the staining protocol [20, 21], washed red blood cells were resuspended in 100 µl PBS. Thereafter, stock solution of H2DCFDA (5 mM) was added, so that the working solutions had 5µM H2DCFDA. Suspensions were incubated in the dark for 30 minutes, and then washed in PBS to remove the extracellularly located dye and resuspended in 500 µl PBS for acquiring the fluorescence of dichlorofluorescein (DCF), produced in cells from H2DCFDA when interacting with intracellular ROS. The fluorescence of DCF was registered by BD FACSCanto™ II flow cytometer. Excitation wavelength was 488 nm, while the emission wavelength was 525 nm for both Annexin V-FITC and DCF.

Eryptosis and cell morphology indices

FlowJo™ (v10, BD Biosciences, USA) and BD FACSDiva™ software (Becton Dickinson, USA) were used to process initial files of fluorescence acquisition. To analyze the impact of HMGB1 on eryptosis, five parameters were compared. The morphology of erythrocytes exposed to HMGB1 was assessed by comparing the percentage of cells with small volume (FSC-low) and large volume (FSC-high) [22]. To analyze the cell membrane scrambling, the percentage of Annexin V-positive cells and the mean fluorescence intensity (MFI) values of Annexin V-FITC were detected. The MFI values of DCF characterized ROS production in erythrocytes.

Statistical analysis

The Kruskal-Wallis and *post-hoc* Dunn's tests were used to statistically process the data obtained in this study. All numerical values are shown in figures as the median (Me) and interquartile range (IQR; 25%–75%). p values above 0.05 indicated no statistical significance. Graph Pad Prism 5.0 (USA) software was used to process the results.

Results

Analysis of forward scatter (FSC) histograms of erythrocyte suspensions exposed to various concentrations of HMGB1 allowed identifying three populations of cells, i.e. the cells with normal volume, small (FSC-low) and large (FSC-high) volumes, respectively (Figure 1a). The percentage of cells with small and large volumes, i.e. shrunken or enlarged erythrocytes,

Figure 1 - Forward scatter (FSC) intensity analysis for determination of red blood cell volume. Representative forward scatter (FSC) histograms of erythrocytes treated with HMGB1 (0-0.2-1-5 ng per ml) for 24 h. The populations of shrunken (FSC-low) and enlarged (FSC-high) erythrocytes are shown (panel a). The impact of HMGB1 (0-0.2-1-5 ng / ml) on the morphology of erythrocytes. The percentages of FSC-low (shrunken) and FSC-high (enlarged) cells were found to be comparable in four groups of samples (n=6).

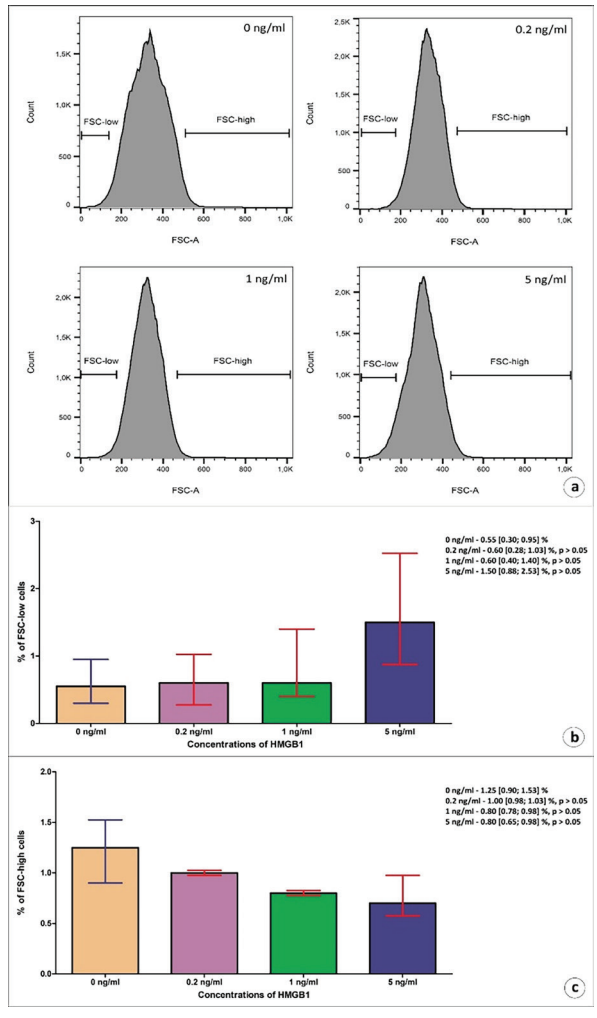
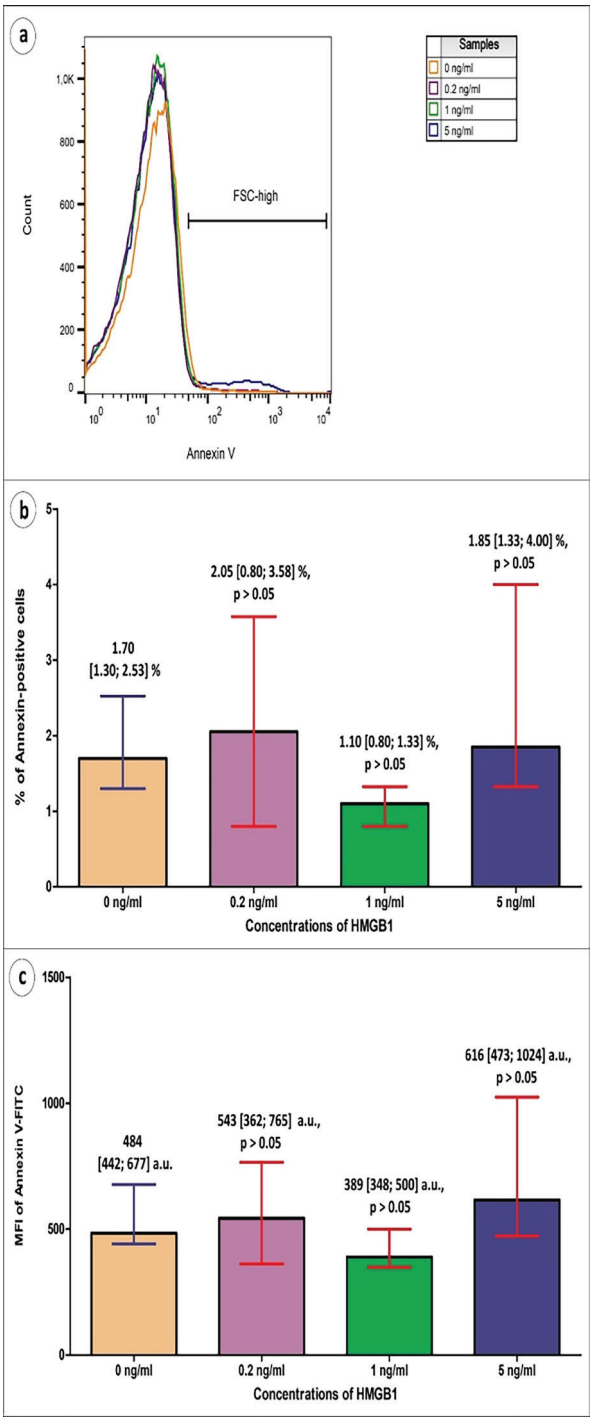


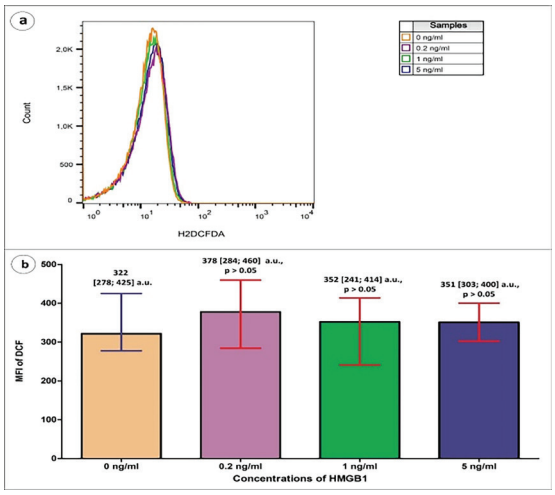
Figure 2 - Phosphatidylserine externalization in erythrocytes exposed to HMGB1 (0-0.2-1-5 ng per ml) for 24 h. Representative side scatter (SSC)/FL1 histograms are shown (panel a). Phosphatidylserine externalization was assessed quantitatively by comparing the percentage of Annexin V-positive cells (panel b) and the mean fluorescence intensity (MFI) values of Annexin V-FITC (panel c). Both eryptosis indices differed statistically insignificantly.



respectively, were compared between 4 groups of samples exposed to different concentrations of HMGB1. No statistically significant differences were found in these parameters (Figure 1b,c). Thus, incubation of blood with HMGB1 did not cause the changes in the morphology of red blood cells.

Annexin V staining was used to analyze the rate of phosphatidylserine externalization in cell membrane of cells, which is a hallmark of eryptosis. Two parameters that characterize the cell membrane scrambling were analyzed, namely the percentage of cells with externalized phosphatidylserine (Annexin V-positive cells) and the MFI values of Annexin V-FITC.

Figure 3 - HMGB1 did not promote ROS production in erythrocytes. H2DCFDA staining of red blood cells incubated with HMGB1 (0-0.2-1-5 ng per ml) for 24 h. Representative side scatter (SSC)/FL1 histograms are demonstrated (panel a). Generation of reactive oxygen species (ROS) was estimated by comparing the mean fluorescence intensity (MFI) values of dichlorofluorescein (panel b).



The latter reflects the degree of phosphatidylserine translocation to the outer leaflet of cell membranes. Both parameters were observed to be unaffected (Figure 2) indicating no changes in phosphatidylserine externalization in response to HMGB1.

Another marker of eryptosis is ROS overgeneration, which can be analyzed by H2DCFDA staining. No statistically significant changes in the MFI values of dichlorofluorescein, which is produced inside the cells from H2DCFDA upon the interaction with ROS, were revealed after incubation with different concentrations of HMGB1 (Figure 3).

Discussion

In this study, the impact of human HMGB1 on eryptosis of red blood cells was assessed in a complex way by analyzing the morphology of erythrocytes, the cell membrane scrambling and oxidative stress development. Eryptosis is a form of suicidal, programmed cell death of red blood cells characterized by certain morphological changes, including cell shrinkage [23]. This is a protective mechanism used to eliminate dysfunctional cells from the bloodstream. Multiple triggers of eryptosis have been described, including energy deficiency, osmolar damage to erythrocytes, action of xenobiotics and ROS [24]. It is important to note that the hallmarks of eryptosis are cell shrinkage, phosphatidylserine externalization, i.e. translocation of this phospholipid from the inner layer of cell membrane to the outer leaflet, oxidative stress and calcium ion entry [25]. Thus, these parameters can be used as reliable markers of eryptosis.

HMGB1 is known to regulate multiple cell death modes. However, its impact on eryptosis is not studied. The average HMGB1 levels in serum of healthy individuals vary from 0 to 1.3 ng/ml (on average 0.2-0.5 ng/ml) and can increase over 20-fold in case of various pathological conditions [26-28]. Thus, circulating erythrocytes can contact high concentrations of HMGB1.

There is accumulating evidence that the HMGB1–TLR4 and HMGB1–RAGE axes mediate apoptosis induced by HMGB1 [29, 30]. However, despite the reports on the ability of erythrocytes to modulate innate immunity and express some innate immunity receptors, including TLRs [31], the role of

innate immunity pathways in erythrocytes is extremely limited. We believe that this could be the reason behind the inability of HMGB1 to induce eryptosis. In addition, it has been reported that the impact of HMGB1 is context-sensitive and depends on crosstalks between many factors, which limits the results of *in vitro* studies [15]. Nevertheless, our findings indicate that HMGB1 does not induce eryptosis of human erythrocytes despite the widely recognized ability of this alarmin to regulate apoptosis of different cells.

The study has some limitations. In particular, this research was performed on a relatively small sample size. In addition, not all eryptotic indices that can elucidate underlying mechanisms, including intracellular calcium ion levels and ceramide abundance, were analyzed.

Conclusion

Human HMGB1 does not induce eryptosis of erythrocytes at concentrations below 5 ng/ml. Nor HMGB1 triggers ROS overproduction and oxidative stress. This study contributes to covering the knowledge gap in understanding the impact of HMGB1 on cell death and survival. Future studies are necessary to reveal the influence of higher levels of HMGB1 on eryptosis to figure out the role of HMGB1 in eryptosis regulation under pathological circumstances.

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The 16s ribosomal ribonucleic acid microorganisms' detection in mesenteric lymph nodes by a polymerase chain reaction in view of colorectal cancer

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Abstract

Objective: This study proposes a method to detect 16s rRNA microorganisms in mesenteric lymph nodes (MLN) using a polymerase chain reaction (PCR) in patients with colorectal cancer (CRC).

Material and methods: To quantify the presence of microorganisms in MLN, it is proposed to determine the dependence of the accumulated amplification products on the number of colony-forming units of bacteria (CFU/ml). The pure culture of Escherichia coli, GFP 6 serotype of biotype 1 (ATCC® 25922GFP™) with the CFU values from 102 to 108 (group 1) as well as the mixtures of E.coli with CFU/ml from 102 to 108 with the MLN tissues (group 2) were calibrated. The third group consisted of the MLN patients (60 people) with CRC without bowel obstruction. The 16s rRNA bacteria in MLN was detected by using real-time PCR by the BIO-RAD CFX96 amplifier.

Results: To assess the dependence of the bacteria's CFU/ml logarithm on the value of the threshold cycle amplification, a model was developed in the form of an equation. The amplification curves, threshold cycle values, and PCR efficiency differ from the first two groups. This can be due to the presence of DNA amplification-inhibiting compounds as well as the non-specific binding of MLN primers to DNA. Therefore, a mathematical model of the second group (suspension of E.Coli and MLN) was used to study the translocation of microorganisms in MLN. According to the developed mathematical model, depending on the values of the threshold amplification cycles, the positive PCR result in the study group (patients with CRC) was detected in 15 patients (25%). At the same time, the level of CFU/ml with bacterial translocation in MLN does not exceed 104.

Conclusion: The developed method allows to determine the microbial DNA in MLN quantitatively in a wide range of its concentrations (102 to 108 CFU).

Key words: bacterial translocation, gut microbiota, intestinal barrier, colorectal cancer, PCR, 16s rRNA, mesenteric lymph nodes

Introduction

Colon and rectal cancer (CRC) ranks third in prevalence among all diagnosed malignancies [1]. Over the past 20 years in Kazakhstan, CRC has moved up in the structure of cancer incidence from 6th to the 3rd place [2]. It is the fourth leading cause of death due to cancer in the world [3, 4]. As of today, the rates of postoperative infectious and inflammatory complications remain high. These are one of the main causes of death in patients with cancer [4,5]. In case of CRC, there are disturbances in the microbiota of the large intestine as well as the violations of the microcirculation of the intestinal wall and the

intestinal barrier. These changes lead to the so-called bacterial translocation through the damaged intestinal wall into the mesenteric lymph nodes (MLN) and further into the systemic circulation, as a result of which postoperative infectious and inflammatory complications may occur [6].

To identify bacterial translocation in MLN, microbiological methods are used. These include the isolation of a pure culture of bacteria, yet this technique is time-consuming (it takes an average of 3-7 days). Besides, not all bacteria can be cultured in the microbiological media [7]. Another detection method of bacterial translocation in MLN is the use of radioactively labeled microorganisms. From the point of view of ethical standards, this technique is applicable only in the experiment. Recently, the technique for determining bacterial DNA in MLN, namely 16S rRNA (ribosomal ribonucleic acid), by using a polymerase chain reaction (PCR) in real-time (Real-time PCR) has become relevant. The 16S rRNA gene contains some hypervariable regions unique to each microorganism and some "rigid" regions common to all microorganisms. Therefore, there are universal primers that bind to the known common gene sequences of most bacteria. This is a relatively recently proposed method in which most pathogenic bacteria can be detected using universal primers [8].

The team of the authors has proposed a method for detecting 16S rRNA microorganisms in mesenteric lymph nodes by using a polymerase chain reaction in the patients with colon and rectal cancer.

Material and methods

To quantify the presence of microorganisms in MLN, it is proposed to determine the dependence of the accumulation of amplification products on the number of colony-forming units (CFU) of bacteria per milliliter (CFU/mL). Escherichia coli (E. coli) GFP 6 serotype 1 (ATCC® 25922GFP™) with CFU/mL values from 102 to108 as well as the mixtures of E. coli with CFU/mL from 102 to 108 with MLN tissues were calibrated to the pure culture.

The first group consisted of the pure culture of E. coli samples with 102, 104, 106, 108 CFU/mL in saline (0.9% NaCl solution) – 5 samples of each CFU/mL value, totally comprising 20 samples.

The second group consisted of the MLN samples with a suspension of E. coli with 102, 104, 106, 108 CFU/mL – 5 samples of each CFU/mL value (subgroups), a total of 20 samples.

The third group consisted of the MLN patients (60 people) with colorectal cancer without bowel obstruction. The operating surgeon performed a MLN sampling in sterile conditions during surgery after resection of the intestine from the mesentery of the gross specimen. MLN was placed in a sterile tube without any fillers. The MLU specimen was stored in the refrigerator (+4°C to +8°C) up to the moment of transportation (for a maximum period of up to 12 hours). The collected material was transported strictly vertically in a special container with cooling elements at a temperature of +2°C to +8°C for 6 hours.

The DNA was extracted by the GeneJET Genomic DNA Purification Kit manufactured by Thermo Fisher Scientific, USA, in accordance with the manufacturer's instructions. The samples of MLN weighing up to 20 mg (pure, bacterial-free and with added 102 to 108 bacteria) were placed in the microcentrifuge tubes with the addition of 180 µL Digestion Solution and 20 µL of Proteinase K solution. The tubes were shaken thoroughly by Vortex and incubated at 56°C. The samples had been shaken periodically by Vortex until the tissues were completely lysed. Furthermore, 20 µL of RNAse A solution was added, followed by a 10 min incubation at room temperature. 200 µL of lysing solution and 400 µL of 50% ethanol were added afterwards. After each step, the sample was thoroughly mixed by using Vortex. The mixture was then transferred to special spin columns with a test tube for collection and centrifuged for 1 min at 6.000 x g. After each centrifugation, the spin column was placed in a new test tube for collection. The samples were then washed with 500 µL of wash buffer I and centrifuged at 8.000 x g for a minute. The second stage of washing was carried out by adding 500 µl of wash buffer II with centrifugation at maximum speed for 3 minutes. The spin column was then placed in a sterile microcentrifuge tube and 200 µL of elution buffer was added, followed by incubation for 2 min at room temperature and centrifugation for 1 min at 8,000 x g.

The obtained DNA was immediately used for PCR diagnostics. The 16s rRNA bacteria in MLN was detected by using real-time PCR and BIO-RAD CFX96 amplifier. For this purpose, a reaction mixture was prepared, which consisted of:

1) 4 µL of the DNA sample under test;

2) 1 µL of 16s rRNA forward and reverse primers (U16SRT-F FACTCCTACGGGAGGGAGGCAGGT and U16SRT-R TATTACCGCGGCTGCTGGGC);

3) 10 µL of the Master Mix Maxima SYBR Green reaction mixture;

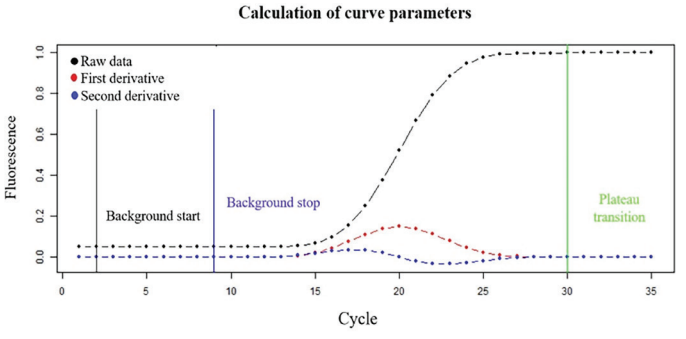
4) 4 µL of nuclease-free water.

Afterwards, the samples were loaded into the tablet of the BIO-RAD CFX96 amplifier (refer to the Figure 1), and amplified under the following parameters:

- denaturation at 95°C for 10 minutes;
- "annealing" and elongation – 40 cycles at 95°C for 15 seconds and 40 cycles at 62°C for 60 seconds.

After PCR amplification, the fluorescence values for the three groups were imported into R statistics (v.3.6.3) for further analysis. The qpcR and pcr packages were used to construct the sigmoid curves (Figure 1) [9]. To assess the dependence of the CFU/mL logarithm on the value of the threshold amplification cycle, a model was developed in the form of an equation.

Figure 1 - Amplification based on a sigmoid model with 4 parameters and calculated threshold cycles based on a derivative nonlinear model as well as the efficiency of amplification. First derivative – red line, second derivative – blue line, amplification graph – black line



Results and discussion

Figure 2 shows the curve of the PCR standards of the first calibration group (suspension of E. coli in saline), while Figure 3 demonstrates the second group of calibration (suspension of E. coli with tissues of MLN).

Using the average values of the threshold cycles (Ct) of the first and second groups (Tables 1 and 2), the graphical curves of the PCR standards were drawn up in the subgroups with different

Figure 2 - Amplification of E. coli samples with 102, 104, 106,108 CFU/mL in saline

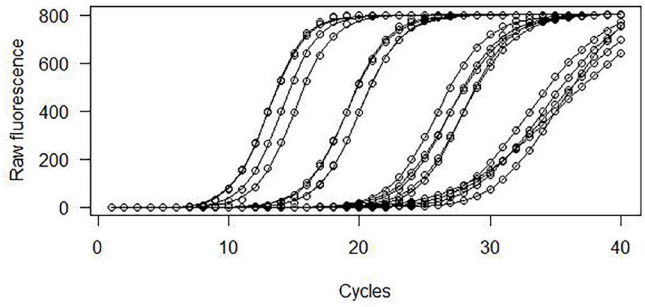


Figure 3 - Amplification of E. coli samples with 102, 104, 106,108 CFU/mL with mesenteric lymph nodes tissues

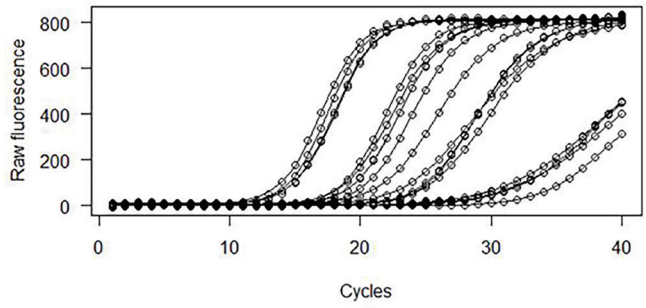


Table 1 Average values of threshold cycles in the E.coli group of 102, 104, 106,108 CFU/mL in saline

Subgroups, CFU/mL	n	Lg (CFU/mL)	Ct
102	5	2	34.885 ± 0.986
104	5	4	27.024 ± 1.086
106	5	6	19.694 ± 0.475
108	5	8	13.836 ± 0.639

The results are expressed as mean and standard deviation and number. CFU, colony-forming unit; Lg (CFU/mL) - logarithm of colony-forming units of bacteria per milliliter; Ct, cycle threshold.

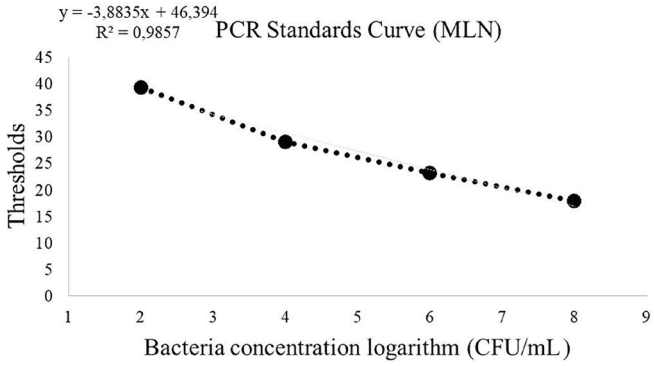
Table 2 Average values of threshold cycles in group E. coli of 102,104, 106, 108 CFU/mL with MLN tissue

Subgroups, CFU/mL	n	Lg (CFU/mL)	Ct
102	10	2	39.235 ± 0.87
104	10	4	29.002 ± 0.89
106	10	6	23.211 ± 1.11
108	10	8	17.899 ± 1.3

The results are expressed as mean and standard deviation and number. CFU - colony-forming unit; Lg (CFU/mL) - logarithm of colony-forming units of bacteria per milliliter; Ct - cycle threshold.

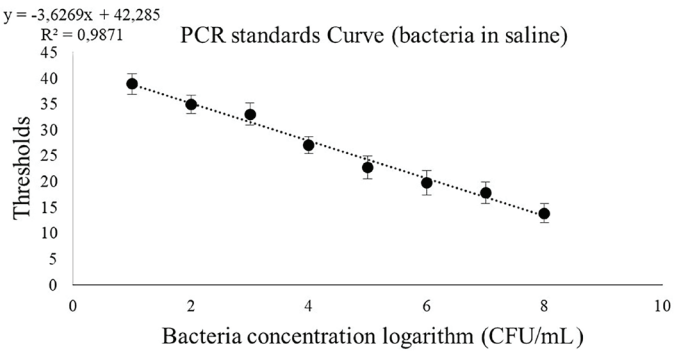
CFU/mL (from 108 to 102 CFU/mL). A model was developed in the form of an equation to assess the dependence of the CFU/mL logarithm on the value of the threshold amplification cycle (Figures 4 and 5).

Figure 4 - Polymerase chain reaction (PCR) standards Curve in group E. coli of 102,104, 106, 108 CFU/mL with mesenteric lymph nodes tissue (MLN)



The amplification curves, threshold cycle values and PCR efficiency differ between the two groups. This can be due to the presence of DNA amplification-inhibiting compounds as well as the non-specific binding of MLN primers to DNA. Some

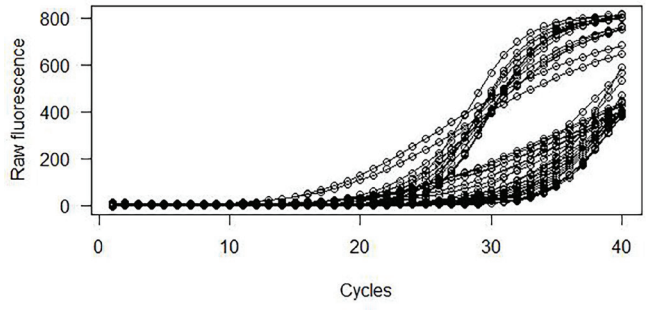
Figure 5 - Polymerase chain reaction (PCR) standards Curve in group E. coli of 102, 104, 106, 108 CFU/mL in saline



researchers reported that 16s rRNA primers align to a region within the human mitochondrial DNA and it can be amplified by this primers when human DNA is overwhelming [10, 11]. Therefore, to study the translocation of microorganisms in MLN, a mathematical model of the second calibration group (suspension E. coli and MLN) was used.

In accordance with the developed mathematical model, depending on the values of the threshold cycles amplification, the positive PCR result in the study group (patients with CRC) was found in 15 patients (25%). At the same time, the level of CFU/mL with bacterial translocation in MLN does not exceed 104 (Figure 6).

Figure 6 - Amplification of mesenteric lymph nodes tissue samples



Bacterial translocation (BT) to the lymph nodes occurs because a large area of intestinal mucosa is drained by lymphatic capillaries, each of which passes into larger lymphatic vessels and then to the lymph nodes [12]. Number of researches support the view that BT is associated with an increase in postoperative infectious complications and septic morbidity, as BT can induce an enhanced immune response and ultimately lead to systemic inflammatory responses [13, 14]. For example, Takashi Mizuno

et al. in patients after hepatectomy for biliary malignancies and Eiji Nishigaki et al. in patients after esophagectomy, reported that the detection of bacterial 16 s rRNA in MLN, was strongly associated with the occurrence of postoperative infectious complications [15, 16]. In this two studies bacterial 16s rRNA was detected in 37.3% MLN (19 of 51 cases) and in 56% MLN (10 of 18 cases), respectively. There are also several studies of 16s rRNA in MLN in patients with inflammatory bowel disease, where 16s rRNA was found in no more than 40% of MLN. But the sample size in these studies was small (20 patients each) and to determine the sensitivity of the PCR, researchers amplified ovine lymph nodes spiked with 103 CFU/mL, but not 102 CFU/mL, bacterial cells [12, 17]. Studies on the detection of bacteria in the MLN in colorectal cancer were carried out, but they used conventional culture-based methods, while in present study was used the novel method for determining bacterial 16s rRNA.

Because culture-based studies are limited and have only identified 20% of the bacteria present, detection of bacterial 16s rRNA is widely used. Today, the research on human microbiota has focused on material such as oral or vaginal swabs, feces that have a large number of bacterial populations and few human DNA [18]. Bacteria from MLN more difficult to assess by standard PCR due to small amount of bacteria and relatively large amount of human DNA.

Also, today optimal PCR methods for analysis of low biomass microbiota samples like MLN has not been developed. Remy Villette et al. tested the lower bacterial concentration required to perform 16S rRNA gene analysis: microbiota community standard and low biomass samples (108, 107, 106, 105 and 104 CFU/mL) from two healthy donor stools were employed to assess optimal sample processing for 16s rRNA gene analysis. Using the improved protocol they report a lower limit of 106 bacteria per sample for robust and reproducible microbiota analysis [19]. Studies to determine the lowest concentration of bacteria in the lymph nodes, necessary for the analysis of the 1s rRNA gene, have not been previously performed. In present study mathematical model depending on the values of the threshold cycle's amplification, was developed and the lower limit of bacteria in the lymph nodes was 104 CFU/mL.

Conclusion

The developed method allows to determine the microbial DNA in MLN quantitatively in a wide range of its concentrations (CFU/mL from 102 to 108). It was found that the level of CFU/mL in the event of CRC with the bacterial translocation in MLN does not exceed 104. Detection bacterial 16s rRNA gene in MLN, and other human tissues with low biomass microbiota samples are receiving increasing attention. Therefore, a more in-depth study of this problem and the development of an optimal standardization for this analysis are needed.

Ethical aspects: The study was conducted in accordance with the Ethical Guidelines for Medical Research with Human Subjects as established by the Ministry of Health of the Republic of Kazakhstan and with the guidelines outlined in the Helsinki Declaration and its amendments. This study was approved by the Ethics Committee of the NJSC "Karaganda Medical University" (Protocol No.6 with the assigned number No.30). An informed consent was obtained from all participants included in the study.

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The assessment of plasma asprosin levels in acute coronary artery disease and its correlation with HEART score

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Abstract

Objective: It was aimed to compare the serum asprosin levels in patients with ischemic heart disease with healthy subjects, and to evaluate the relationship between asprosin levels with HEART score and mortality in the patients with coronary heart disease.

Material and methods: This study was designed as a single-center, prospective study. Sixty-two patients who presented with acute chest pain and underwent digital subtraction coronary angiography and 31 healthy subjects were included in the study. Fasting serum asprosin levels were compared between patients and healthy individuals. The HEART score was calculated for each individual, and its relations with asprosin and one-month mortality were evaluated.

Results: The minimum age of 93 cases included in the study was 24, the maximum age was 85, and the median age was 64. HEART score was higher in cases who had mortality within one month ($p<0.0001$). Plasma asprosin values were higher in patients with one-month mortality ($p<0.0001$) and lower in the control group compared to the study group ($p=0.015$). There is a statistically significant weak positive correlation between HEART score and asprosin value ($p=0.006$, $r=0.285$).

Conclusion: Serum asprosin level can be used both diagnostically and as a biochemical marker in the evaluation of mortality and prognosis in patients with ischemic heart disease.

Key words: acute chest pain, asprosin, HEART score

Introduction

Asprosin is a newly defined centrally acting orexigenic adipokine secreted from white adipose tissue that regulates glucose metabolism [1]. Increased asprosin levels are shown in patients with clinical conditions associated with metabolic syndrome such as obesity, coronary artery disease, insulin resistance, polycystic ovarian disease, lipid metabolism, anaerobic and aerobic exercise, type 1 and type 2 diabetes mellitus, and non-alcoholic fatty liver disease [2-4]. It is known that blood asprosin level has a positive correlation with body mass index [5]. Besides, significant changes were found in plasma asprosin levels in patients with anorexia nervosa, anorexic cachexia in cancer patients and malignant mesothelioma [6].

There are very few studies in the literature evaluating heart diseases and serum asprosin levels, and current studies suggest that asprosin has a protective effect on myocardial cells and is associated with ischemic heart diseases [7]. The relationship between acute coronary syndrome and asprosin has not been clearly elucidated yet. It is also not defined the relationship between plasma asprosin level and the prognosis of the disease in these cases. The correlation of asprosin with the HEART

scoring, used in the prognosis of the disease in triage as an essential cornerstone in the diagnostic-therapeutic decision, is not investigated. It is thought that the asprosin level may be a diagnostic and prognostically significant marker in this sense.

In this study, it was aimed to compare the plasma asprosin levels in patients with the acute coronary syndrome with healthy individuals in similar age groups and examine the relationship between the HEART score and plasma asprosin level at the time of admission in patients with ischemic heart disease to aid physicians in making a timely appropriate management of patients.

Material and methods

Study design and setting

This study was designed as a single-center, prospective study. The results are reported in accordance with Standards for Reporting of Diagnostic Accuracy Studies (STARDS). Before the study, the local ethics committee approval (23.01.2020 date and 2020/1-3 number) was obtained, and the research was conducted between 01.02.2020-01.05.2020 afterward. Before the study, an informed consent form was obtained from all included cases.

Selection of participants

The study group consisted of 62 cases who a presented to the emergency department with acute chest pain and underwent digital subtraction coronary angiography between 01.02.2020-01.05.2020 and 31 cases without known heart or metabolic disease constituted the control group. Subjects under 18 years of age and those who refused to give informed consent were excluded from the study. Cases with obesity (body mass index >30), polycystic ovarian disease, omission - anoxia nervosa, heart disease, type 1 or type 2 diabetes, and patients who exercised heavily in the last six hours were not included in the control group.

Interventions

In order to determine the asprosin level, blood samples were obtained from venous blood between 7-9 am after overnight fast both study group and the control group. The samples were collected in EDTA tubes, and after 15 minutes of centrifugation at 1500 rpm, the plasma was obtained. The plasma samples obtained were stored at -860. ELISA kit (Sunred Biological Technology Cooperation, Shanghai, China) with high sensitivity to human asprosin level (<0.938 ng/ml) was used to determine plasma asprosin concentration. The manufacturer's instructions were followed in the measurement.

Measurements

Demographic data including age and gender were analyzed. One month mortality, treatment methods and involved coronary arteries in coronary artery disease were recorded. Artery stenosis were group as LAD and the other arteries.

HEART scoring was made according to the patients' evaluation in the study group at the time of admission to the emergency department in accordance with the scoring system specified in the literature, the patient's history, ECG, risk factors of the patients for coronary artery disease and the troponin level at the time of admission were recorded [3,4]. HEART scores between 0 and 10 were obtained by giving 0, 1, or 2 points for each item. Information showing the detailed evaluation of scoring is shown in Table 1.

Table 1 HEART scoring system applied in the study

History	• High suspicion • Moderate suspicion • No or slight suspicion	• 2 points • 1 point • 0 points
ECG	• Significant ST-Depression • Nonspecific Repolarization • Normal ECG findings	• 2 points • 1 point • 0 points
Age	• ≥ 65 years • Between 45-65 years • ≤ 45 years	• 2 points • 1 point • 0 points
Risk Factors	• ≥3 risk factors or history of coronary artery disease • 1 or 2 risk factors • No risk factors	• 2 points • 1 point • 0 points
Troponin • • • •	• ≥3 x Normal limit • Abnormal troponin but <3x upper limit • Normal troponin	• 2 points • 1 point • 0 points
Risk factors: Diabetes Mellitus, current or recent smoker, hypertension, hyperlipidemia, family history of coronary artery disease, obesity		

Outcomes

A comparison of asprosin levels between the study group and the control group constitutes the primary outcome. The correlation between the HEART scoring obtained from the patients in the study group and the asprosin levels represents the secondary outcome. Other secondary outcomes are the comparison of age and gender between the study group and the control group, the relationship of serum asprosin level with mortality, treatment methods presented at the time of admission, findings obtained after angiography in the patients in the study group.

Analysis

SPSS 21.0 (SPSS Inc., Chicago, IL) software was used for statistical analysis. Categorical data are expressed in numbers and percentages. Pearson's chi-square and Fisher's exact tests were used to compare categorical data with each other. Shapiro Wilk test was used to evaluate the distribution pattern of numerical data. Since numerical data do not show a normal distribution, Mann Whitney-U and Kruskal Wallis tests were used to compare numerical data with categorical data. In cases where a significant relationship was found in the group comparisons, the sensitivity and specificity values were calculated with the ROC analysis at the most appropriate cut-off values. Spearman correlation analysis was used to compare the numbers and data with each other. Statistical analyzes where the p-value is less than 0.05 was considered to be significant.

Results

Characteristics of study subjects

Of the 93 cases included in the study, 62 were the study group (66.7%), and 31 were the control group (33.3%). 41 (44.1%) of the cases were male, and 52 (55.9%) were female. The minimum age of 93 cases included in the study was 24, the maximum age was 85, and the median age was 64.

Mortality was observed within a month in 10 (16.1%) of the cases constituting the study group. In the classification made according to treatment methods, percutaneous coronary angiography was performed in 52 cases, and stent or balloon treatment was arranged. Coronary artery by-pass grafting was performed in 3 cases (4.8%) after coronary angiography. In 7 cases (11.3%), the interventional therapeutic procedure was not

presented during or after coronary angiography. In the evaluation of coronary angiographies of the patients in the study group, left descending artery stenosis was found in 11 cases (17.7%), while circumflex artery stenosis was found in two cases (3.2%). No stenosis was found in the coronary arteries in 44 cases (71%). In 5 cases (8.1%), stenosis was observed in more than one coronary artery.

According to the classification made according to the blood troponin value in the study group, troponin value at presentation was high (>0.4 ng / mL) in 18 cases (29%), while it was within normal limits in 44 cases (71%). In the evaluation made according to the HEART score, the minimum HEART score was 1, the maximum HEART score was 10, and the median HEART score was 4.5.

Main Results

There is no statistically significant relationship between sex and plasma asprosin level, between gender and HEART score, and sex and troponin level (p=0.951, 0.173, and 0.937, respectively). There was no statistically significant difference in age between the study group and the control group (p=0.18) (Table 2).

Table 2 Characteristics of study and control groups

	Study group	Control group	p-value
Age (median, min-max)	64 (24-85)	55 (35-77)	0.07
Male (n, %)	31 (50%)	10 (24.4%)	0.104
HEART score (median, min-max)	4.5 (1-10)	0	<0.0001*
Asprosin, ng/mL (median, min-max)	119.6 (77.8-388.6)	102.2 (78.9-280.1)	0.015*
Troponin ng/mL (median, min-max)	0 (0-13)	-	-

A statistically significant difference was found in the comparison of the HEART score between the study group and the control group, and the HEART score in the study group was statistically higher (p<0.0001). A statistically significant difference was also observed in the comparison of asprosin values between the study group and the control group (p=0.015). Parameters of the study group and their comparison between the survivor and non-survivor groups are given in Table 3.

Table 3 Parameters of the study group and their comparison between the survivor and non-survivor groups

	Non- survivor	Survivor	p-value
Age (median, min-max)	75 (33-84)	64 (24-85)	0.246
HEART score (median, min-max)	9.5 (8-10)	4 (1-9)	<0.0001*
Asprosin, ng/mL (median, min-max)	288.8 (92.7-388.6)	112.7 (77.8-196.4)	<0.0001*
Troponin, ng/mL (median, min-max)	0.053 (0-13)	0 (0-3.5)	0.058

The one-month mortality rate for the male of the study group was 22.6% and for female 9.7%. The difference in mortality rate in the working group by gender was not statistically significant (p=0.167). When mortality was compared with age, no statistically significant relationship was found between the mean age of the cases with one-month mortality (p=0.246).

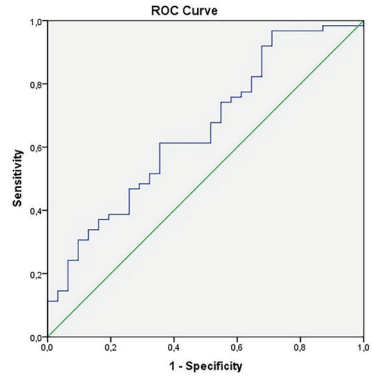
There was no statistically significant relationship between troponin and asprosin levels (p=0.828). When one-month mortality and troponin level was compared, one-month mortality was present in 5 (27.8%) of 18 cases with high troponin value, while one-month mortality was present in 5 (11.4%) of 44 cases with normal troponin value. The relationship between them is not statistically significant (p=0.137). While normal findings were obtained in 9 (50%) of 18 cases with high troponin value, normal angiography findings were found in 35 (79.5%) of 44 cases with normal troponin value (p=0.033).

In the comparison between mortality and HEART score, the HEART score was higher in cases with one-month mortality (p<0.0001). In the comparison of mortality and asprosin value, a statistically significant relationship was found, and the asprosin value was statistically higher in cases with mortality within one month (p<0.0001). When comparing the troponin value between the survivor and non-survivor groups the p-value was found to be 0.058, and a significant relationship was not obtained. Numerical data showing the relationship between mortality and numerical data are detailed in Table 3.

In the comparison between the HEART score and the coronary angiography findings of the patients in the study group, a statistically significant relationship was found (p<0.0001). In paired group comparisons, a statistically significant difference was found between cases with normal coronary angiography findings and cases with more than one coronary artery stenosis, and the HEART score was found to be lower in cases with normal findings on angiography (p=0.005). In addition, a statistically significant difference was found between the HEART score of patients with normal coronary angiography findings and patients with LAD stenosis, and the HEART score was higher in cases with LAD stenosis (p<0.0001). No statistically significant difference was found in the artery stenosis groups (p>0.05).

A statistically significant result was obtained in the ROC analysis to determine the relationship between asprosin and coronary artery disease (p=0.015). The area under the curve was calculated as 65.6%, sensitivity 61.3%, specificity 64.5% at a cut-off value of 109.5 ng/mL (Figure 1).

Figure 1 - ROC curve in the comparison of plasma asprosin level between study and control groups



A statistically significant (p<0.0001) relationship was found in the ROC analysis performed to evaluate the relationship between asprosin and mortality, and the area under curve was calculated as 92.1%. 90% sensitivity and 100% specificity values were obtained at the cut-off value of 236.55 ng/mL (Figure 2).

A statistically significant correlation was found in the ROC analysis between the HEART score and mortality (p<0.0001), and the area under curve was calculated as 98.3%. When the cut-off value was 7.5, the sensitivity was 100%, and the specificity was 92.3% (Figure 2).

Figure 2 - ROC curve in the relationship between plasma asprosin level (first), HEART score (second) and one-month mortality

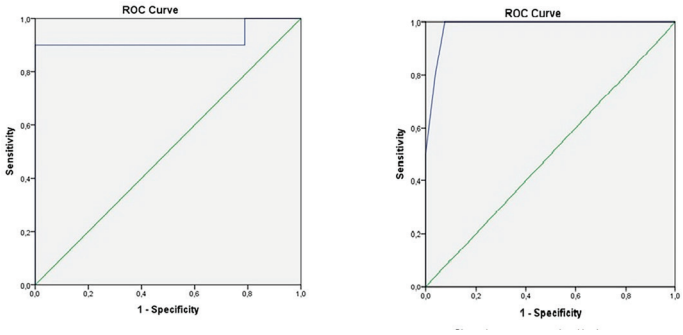
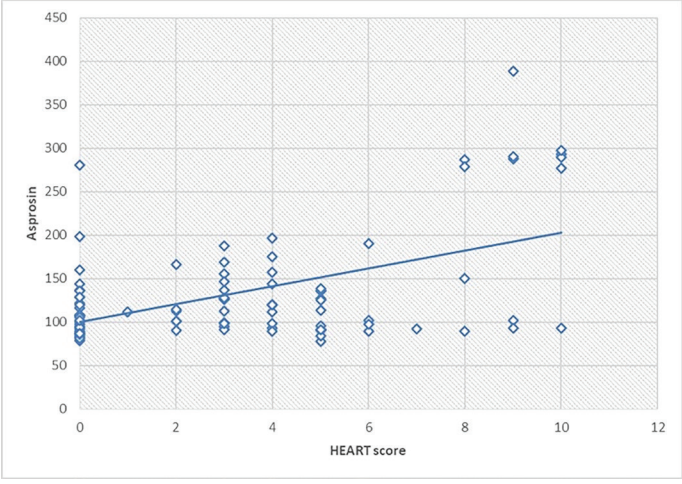


Figure 3 - Scattered plot graphic demonstration correlation between plasma asprosin level and HEART score



In correlation analysis, a statistically significant weak positive correlation was found between age and HEART score (p=0.03, r=0.309). There was no statistically significant correlation between age and asprosin and troponin values (p=0.182 and 0.128). There was a statistically significant weak positive correlation between the HEART score and the plasma asprosin level (p=0.006, r=0.285) (Figure 3). A statistically significant moderate positive correlation was found in the comparison of HEART score and troponin values (p<0.0001, r=0.583). There was no significant correlation when comparing asprosin and troponin values (p=0.966).

Discussion

Asprosin, a protein adipokine discovered in recent years, increases glucose release by activating the G protein-cAMP-PKA pathway in the liver [8]. Asprosin is the C-terminal product of the fibrillin protein encoded by the FBN1 gene, crosses the blood-brain barrier, and activates the hypothalamic circulation and affects adiposity by increasing appetite [9]. In this study, the relationship of this hormone, which is known to be associated with glucose metabolism and metabolic syndrome, with coronary artery disease was evaluated. Asprosin was found to be higher in patients with coronary artery disease than in healthy individuals. Moreover, it was concluded that patients with higher HEART score in coronary artery disease have higher asprosin levels. In addition, blood asprosin level was found to have high sensitivity (90%) and specificity (100%) in determining mortality within one month.

Although there are no studies in the literature that directly examine the relationship of serum asprosin level with HEART scoring and ischemic heart disease mortality, there are several studies evaluating asprosin associated with coronary heart disease. In a study, it has been shown that asprosin has a protective effect from apoptosis caused by the presentation of hydrogen peroxide in myocardial cells [10].

In another study, asprosin levels were compared with SYNTAX scoring used in ischemic heart disease. As a result, a significant positive correlation was obtained between serum asprosin level and SYNTAX score. This suggests that asprosin may show the severity of acute coronary syndrome in unstable angina pectoris cases and that asprosin may play a predictive role in cardiovascular diseases [11,12]. Unlike HEART scoring, SYNTAX scoring is based only on imaging findings in coronary angiography and does not include clinical and laboratory findings [13]. It is not a scoring system used in triage like HEART scoring. Nevertheless, it can be said that parallel results were obtained with this study since this study also showed the relationship between coronary artery disease findings in the coronary system and serum asprosin level. Besides, in this study, when the HEART score and coronary angiography findings were compared, it was found that the patients with stenosis in two or more coronary arteries also had higher HEART scores.

In a meta-analysis conducted in 2018, 25 studies between 2010 and 2017 were evaluated, and data on 25266 cases were collected. According to the results of the study, it has been shown that patients' HEART score can predict major cardiac events in the short term with high sensitivity, high negative predictive value and likelihood ratio [14]. Similarly, in this study, it was shown that one-month mortality was higher in cases with high HEART scores. Eight or more HEART scores were found to have high diagnostic value with 100% sensitivity and 92.3% specificity at one- month mortality prediction.

In the current literature, the number of scientific studies examining the relationship of asprosin with ischemic heart disease is limited. There are no studies in the current literature examining the HEART score and asprosin level and comparing the serum asprosin level with the mortality rate. This study provides preliminary and illuminating data that asprosin is higher in the non-survivors and study groups and shows that the value of asprosin can be used to predict short-term mortality in patients with ischemic heart disease. On the other hand, the lack of correlation between asprosin level and troponin level may indicate that asprosin provides additional information as a marker independent of the troponin level.

In retrospect, in addition to the asprosin level at presentation, evaluation of the asprosin level at follow-up in patients who responded to treatment, compared to other cases, could have yielded more impressive results for this study. In addition, it is remarkable that asprosin hormone, whose protective effect on the myocardium has been shown in the literature, is associated with mortality in metabolic syndrome and coronary artery disease, so the comparison of asprosin levels between coronary artery disease cases with and without metabolic syndrome and its relationship with mortality in these cases could provide additional valuable information.

Limitations

There are some limitations to this study. First, the fact that the study is single-centered and the number of cases in the study and control groups are limited are among the factors that may affect the result of the study.

Second, in this study, a single commercial kit was used to evaluate the asprosin level in plasma. In the publications mentioned in the literature, it is seen that various trademarks kits

are used in asprosin measurements. This may affect the study results.

Thirdly, one month was used as the limit in the evaluation of mortality, and statistical analysis was not performed in this sense, as specific mortality time was not recorded. The time of mortality could not be compared with the asprosin level.

Fourth, the total number of your patients is insufficient for proper ROC analysis (there must be 100 and higher in each sample). It appears to be extremely difficult to get statistically significant p-values in fewer cases. However, we presented the p values we found because they were statistically significant. So, ROC analysis results may need to be validated with large samples.

Fifth and last, only the patients who presented to the emergency department during the study and had coronary angiography constituted the study group. Coronary artery disease may result in sudden cardiac death, or there may be cases with coronary artery disease for which coronary angiography is not planned in the first line assessment. In this respect, the patients in the study group may not reflect the general profile of acute coronary artery disease.

In summary, according to the results of our study, serum asprosin level and HEART score have high sensitivity and specificity in predicting mortality in coronary artery disease. There is a significant correlation of asprosin level with HEART scoring. Asprosin levels are higher in patients with ischemic heart disease than healthy individuals. Serum asprosin level can be used as a biochemical marker in the evaluation of mortality and prognosis in patients with ischemic heart disease. Given the possible role of confounding factors, we recommend confirming our results with further larger cohorts, multicenter studies.

Ethical Considerations: Ethical approval for this study was obtained from Adıyaman Training and Research Hospital Ethics Committee. (23.01.2020 date and 2020/1-3 number) The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

Disclosures: There is no conflict of interest for all authors.

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Relationship between gamma-glutamyl transferase/albumin ratio and coronary slow flow phenomenon

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Abstract

Aim: The coronary slow flow phenomenon (CSFP) is a pathology characterized by decreased coronary flow without stenosis on angiographic imaging. It is known that gamma-glutamyl transferase (GGT) and albumin play a role in cardiovascular disease. Our aim was to investigate whether GGT-to-albumin ratio could predict CSFP.

Material and methods: Our cross-sectional study included 149 patients who had myocardial ischemia and underwent coronary angiography in our clinic. Our study consisted of two groups, with and without CSFP. The GGT-to-albumin ratio values were compared between the groups, and the presence of a risk factor for CSFP was evaluated with a regression analysis.

Results: A statistical significance was observed between the groups with and without CSFP in terms of GGT-to-albumin ratio values, (6.16 and 4.46, respectively; p<0.001). There was a moderate correlation between GGT-to-albumin ratio and the mean thrombolysis in myocardial infarction frame counts (r=0.423, p<0.001). In the univariate logistic regression analysis revealed that GGT-to-albumin ratio was predictive of CSFP [odds ratio: 0.460, 95% confidence interval (CI): 0.341–0.620, p<0.001]. In the receiver operating characteristic curve analysis performed on GAR to distinguish CSFP, the GGT-to-albumin ratio exhibited 84% sensitivity and 59% specificity for values ≥4.67 (area under the curve: 0.78, 95% CI: 0.708–0.859, p<0.001).

Conclusion: GGT-to-albumin ratio values were found to increase in the presence of CSFP. Our findings advise that GGT-to-albumin ratio might also play a function inside the pathogenesis of CSFP.

Key words: angiography, coronary slow flow, gamma-glutamyl transferase, gamma-glutamyl transferase to albumin ratio

Introduction

Coronary slow flow phenomenon (CSFP) is defined as timely filling of coronary arteries despite the absence of occluded coronary arteries on angiographic imaging [1]. CSFP has an incidence of approximately 7% in coronary angiographic series [2]. It has been shown that atherosclerosis, inflammation, vasomotor dysfunction and endothelial damage play a role in its pathogenesis [3,4] CSFP may cause chest pain in patients because of reduced coronary perfusion. 80–90% of patients with slow coronary flow have recurrent chest pain and that 33% require hospitalization for treatment. Although there is no definite therapeutic recommendation, antianginal treatments can be started according to the characteristics of the disease [5].

It is known that gamma-glutamyl transferase (GGT) and albumin may be associated with the development of cardiovascular disease [6]. GGT protects the cell against the harmful effects of oxidized molecules [7]. In addition, GGT is located on the cellular membrane and can trigger the atherosclerotic process [8]. On the other hand, albumin is an important protein that indicates the nutritional status of human metabolism. Albumin has anti-inflammatory and antioxidant. Decreased level of albumin is risk factors for cardiovascular diseases [9-11]. Therefore, the combination obtained by taking the GGT-to-albumin ratio may be a strong predictor for cardiovascular diseases.

According to our recent literature review, the relationship between GGT-to-albumin ratio and CSFP has not been investigated. As a result, we sought out to investigate the relationship between GGT-to-albumin ratio and CSFP, as well as whether GGT-to-albumin ratio may predict CSFP.

Material and methods

Study population

Our cross-sectional study conducted at a tertiary care center between January 2013 and September 2021. A total

of 149 patients who were admitted with suspected coronary artery ischemia (with evidence of coronary artery ischemia in myocardial perfusion scintigraphy and positive treadmill exercise test results) were included. According to the result of coronary angiography (CAG), our study consisted of two groups. 70 patients with CSFP, and age-and sex-matched 79 controls with normal CSFP.

CSFP was confirmed by the CAG records of the patients and thrombolysis in myocardial infarction frame count (TFC) was used.

Patients with a history of coronary artery bypass graft or percutaneous coronary intervention with stent placement, statin users, chronic kidney [estimated glomerular filtration rate <30 (mL/min/1.73m2)], liver failure (ALT and AST >3x the upper limit of normal), coronary artery ectasia and tortuosity, coronary artery myocardial bridge, newly diagnosed stroke, chronic inflammatory disease, malignancy, pre-CAG pathological Q wave on electrocardiography, reduced heart failure (left ventricular ejection fraction ≤40%), moderate or severe heart valve disease, abnormal heart structure (dilated or hypertrophic cardiomyopathies congenital heart disease), and <18 years of age, coronary artery stenosis of >50% were excluded from the study. Information of the patients were obtained from the recorded electronic data and patient file archives.

The study was performed in accordance with the Declaration of Helsinki, following local ethics of Clinical Research of Çanakkale Onsekiz Mart University (Decision no: 2011-KAEK-27/2021-2100169944).

Coronary angiography and TFC evaluation

The standard Judkins method with a femoral or radial approach was used to produce coronary angiographies (GE Healthcare Innova 2100, New Jersey, USA). Two expert cardiologists reviewed the angiographic images. Stenosis was defined as the observation of >50% stenosis in the coronary arteries.

In the evaluation of coronary flow, the and thrombolysis in myocardial infarction (TIMI) frame count method, which was previously defined by Gibson et al. [12] was used. The frame in which the coronary artery ostium is fully filled with contrast material was determined as the initial frame and the one in which the contrast agent reached the distal branch was determined as the last frame. When defining the distal segment of the artery, it was determined as the distal fork branch for the left anterior descending (LAD) artery, the distal fork of its longest segment for the circumflex artery (Cx) and the first lateral branch of the posterolateral artery for the right coronary artery. The corrected frame number [(LAD (corrected)] was determined by dividing the LAD TIMI frame number by 1.7 since the LAD is longer than other epicardial coronary arteries. Right anterior oblique angles were used to calculate LAD and Cx TIMI frame counts, whereas left anterior oblique angles were used to calculate RCA TIMI frame counts. The mean TFC was calculated as follows (LAD TFC+LCX TFC+RCA TFC/3). In this method, 36.2±2.5 frames for LAD, 21.1±1.5 frames for LAD (corrected), 22±4.1 frames for Cx, and 20.4±3.1 frames and above for RCA were evaluated as CSFP.

Statistical analysis

To examine the distribution of continuous variables, the Kolmogorov-Smirnov test was performed. The data that did not conform to normal distribution that was expressed as median and percentiles (25th and 75th percentiles). Continuous variables were expressed as mean ± standard deviation. Categorical data are expressed in percentages and numbers. The Chi-square test was used when comparing the probability ratios of categorical variables. Mann-Whitney U test and independent samples t-test had been used to compare variables between groups. Spearman correlation analysis was used for identify the relationship between mean TFC and variables. For independent predictors of CSFP, univariate logistic regression analysis was used. The percentage sensitivity and specificity of independent CSFP predictors were assessed using the receiver operating characteristic (ROC) curve. P<0.05 was considered statistically significant using SPSS 20.0 (SPSS Inc, Chicago, IL, USA).

Our study was evaluated by a priori using G*Power power analysis (software version 3.1.9.6) (effect size 0.50, alpha error: 0.05, power: 90%, and a minimum of 70 patients in group 1 and group 2) were calculated.

Results

Characteristics of the study patients

Our study consisted of 149 patients with CSFP (46 men, 24 women) and without CSFP (33 women, 46 men). The mean age in the CSFP group was 58.3±12.6, while in the non-CSFP group it was 57.7±12.5. When the biochemical parameters were compared between the two groups, statistically significant differences in GGT values were found in the CSFP group [(26 (22–33) and 20 (18–25), p 0.001), respectively], while no differences in albumin values were found [(4.39 (4.00–4.68) and 4.26 (4.19–4.53), p=0.492], respectively]. GGT-to-albumin ratio values were statistically different in patients with CSFP compared to those without [(6.16 (5.13–7.75) and 4.46 (4.11–5.73), respectively; p 0.001) (Table 1). The differences in the TFC values in the CSFP group were statistically and numerically significant when compared with the control group for LAD (corrected) (37.48 ± 1.60 and 20.92±1.49, respectively; p<0.001), LCX (25.61±1.31 and 20±0.94, respectively; p<0.001), and RCA (24.92±1.55 and 19.64) (Table 1).

CSFP was observed in 70 patients. CSFP was observed in one vessel in 21 (30%) patients, in two vessels in 30 (42.9%) patients, and in three vessels in 19 (27.1%) patients. When the GGT-to-albumin ratio and the number of involved vessels were examined, it was noted that the number of vessels with CSFP increased as GGT-to-albumin ratio increased (Figure 1).

Figure 1 - The average GAR and the number of involved vessels. * p<0.001

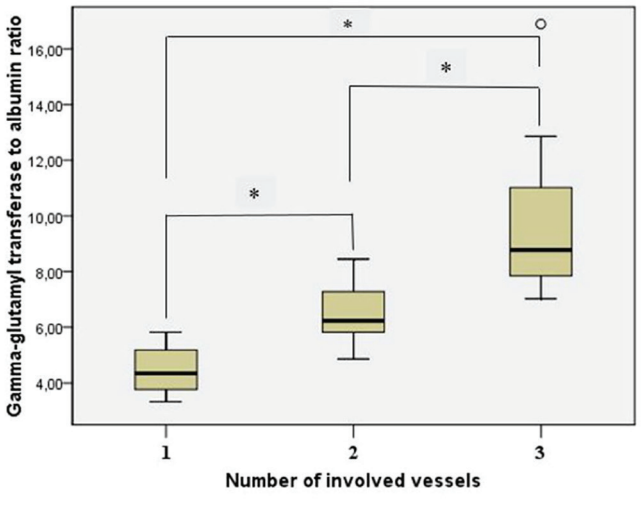


Table 1 Demographic and laboratory findings of patients

	Coronary slow flow phenomenon		
Clinical characteristics	Yes (n= 70)	No (n= 79)	P- value
Age (year) (Mean±SD)	58.3±12.6	57.7±12.5	0.788
Gender			0.348
Female, n (%)	24 (34.3)	33 (41.8)	
Male, n (%)	46 (65.7)	46 (58.2)	
DM, n (%)	10 (14.3)	6 (7.6)	0.293
HT, n (%)	7 (10)	10 (12.7)	0.802
COPD, n (%)	5 (7.1)	2 (2.5)	0.254
Smoking, n (%)	6 (8.6)	9 (11.4)	0.765
ACE/ARB, n (%)	10 (14.3)	8 (10.1)	0.599
Beta-blockers, n (%)	15 (21.4)	13 (16.5)	0.572
Biochemical variables			
Glucose (mg/dL)	106 (94-116)	105 (92-116)	0.504
Creatinine (mg/dL)	0.78 (0.63-0.94)	0.82 (0.63-0.89)	0.600
Hemoglobin (g/dL)	12.40 (11.5-13.5)	12.30 (11.3-12.3)	0.411
White blood cell count (x103 /mL)	5 (4-6.22)	5 (4-6.10)	0.793
Neutrophil count (x103 / mL)	3.5 (2.4-7)	3.5 (1.7-7)	0.361
Lymphocyte count (x103 / mL)	1.20 (0.8-1.7)	1.20 (0.8-1.8)	0.923
Triglyceride (mg/dL)	116 (94-150.25)	116 (86-139)	0.280
HDL-C (mg/dL)	46 (38-61)	55 (39-61)	0.302
LDL-C (mg/dL)	109 (78-130.25)	118 (101-136)	0.200
GGT (U/l)	26 (22-33)	20 (18-25)	<0.001
Albumin (g/dL)	4.39 (4-4.68)	4.26 (4.19-4.53)	0.492
GGT-to-albumin ratio	6.16 (5.13-7.75)	4.46 (4.11-5.73)	<0.001
TIMI frame count			
LAD (corrected)	37.48±1.60	20.92±1.49	<0.001
LCX	25.61±1.31	20±0.94	<0.001
RCA	24.92±1.55	19.64±0.86	<0.001
Mean TFC	29.34±0.56	20.18±0.69	<0.001
Vessel involved			
1-vessel, n (%)	21 (30)		
2-vessel, n (%)	30 (42.9)		
3-vessel, n (%)	19 (27.1)		

ACE- Angiotensin-converting enzyme; ARB- Angiotensin receptor blocker; COPD- Chronic obstructive pulmonary disease; DM- Diabetes mellitus; GGT- Gamma-glutamyl transferase; HDL-C- High-density lipoprotein cholesterol; HT- Hypertension; LAD- Left anterior descending coronary artery; LCX- Left circumflex artery; LDL-C- Low-density lipoprotein cholesterol; TIMI- Thrombolysis in myocardial infarction; TFC- Thrombolysis in myocardial infarction frame count; RCA- Right coronary artery

Table 2 Correlation of mean TFC with variables

		Age	Glucose	Cr	TG	LDL-C	HDL-C	GGT	ALB	GGT-to-ALB ratio
mTFC	r	0.018	0.128	0.019	0.069	-0.103	-0.173	0.400	0.079	0.423
	P value	0.825	0.120	0.819	0.405	0.211	0.035	<0.001	0.341	<0.001

ALB- Albumin ;Cr- Creatinine; GGT- Gamma-glutamyl transferase; HDL-C- high-density lipoprotein cholesterol; LDL-C- Low-density lipoprotein cholesterol; mTFC- Mean Thrombolysis in myocardial infarction (TIMI) frame count; TG- Triglyceride

Table 3 Univariate regression analysis to determine Coronary Slow Flow Phenomenon

Variables	OR	95% CI	p
Age	0.996	0.971-1.022	0.787
Sex	0.727	0.374-1.416	0.349
Smoking	0.729	0.246-2.163	0.569
Diabetes	2.028	0.697-5.901	0.195
Hypertension	0.767	0.275-2.136	0.611
HDL-C	1.013	0.988-1.038	0.300
LDL-C	1.004	0.996-1.013	0.346
Triglyceride	0.998	0.993-1.003	0.467
Albumin	1.138	0.546-2.372	0.730
GGT-to-albumin ratio	0.460	0.341-0.620	<0.001

CI- Confidence interval; GGT- Gamma-glutamyl transferase; HDL-C- High-density lipoprotein cholesterol; LDL-C- Low-density lipoprotein cholesterol; OR- Odds ratio

Correlation analysis

Age, glucose, creatinine, low-density lipoprotein (LDL)-C, triglyceride (TG) and albumin had no correlation on mean TFC levels. However, a moderate correlation was observed between the mean TFC, GGT, and GGT-to-albumin ratio (r=0.400, p<0.001, r=0.423, p<0.001, respectively) (Table 2).

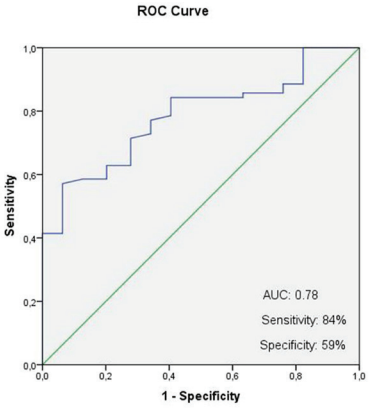
The effect of the variables on CSFP

In the univariate logistic regression analysis, GGT-to-albumin ratio (OR: 0.460, 95% CI: 0.341–0.620, p < 0.001) were found to be the independent predictors of CSFP (Table 3).

ROC analysis

In the ROC analysis performed on GGT-to-albumin ratio to distinguish CSFP, the GGT-to-albumin ratio had 84% sensitivity and 59% specificity for values ≥4.67 (area under the curve: 0.78, 95% CI: 0.708–0.859, p<0.001) (Figure 2).

Figure 2 - Receiver operator characteristic curve of gamma-glutamyl transferase/albumin ratio to predict coronary slow flow phenomenon



Discussion

The relationship between CSFP and GGT-to-albumin ratio in patients undergoing elective coronary angiography has never been studied. Our research yielded some important results. Firstly, GGT-to-albumin ratio was found to be significantly higher in patients with CSFP than in those without CSFP. Secondly, as the number of vessels with CSFP increased, higher GGT-to-albumin ratio values were observed. Thirdly, it was seen that GGT-to-albumin ratio can be used to distinguish between patients with and without CSFP.

Coronary angiographic imaging performed for angina shows an increase in the number of patients without obstructive coronary artery disease. In the angiographic imaging of these patients, no structural heart disease is observed apart from a delay in the distal vascular blood flow [13]. Although the exact pathogenesis of CSFP is not known, it is assumed that it develops in response to inflammation [14]. CSFP, also called delayed coronary artery filling is not a harmless clinical condition. Endothelial functions have been shown to be impaired in the brachial artery in patients with CSFP that CSFP may play a role in the pathogenesis of endothelial dysfunction is an important proof [15]. In addition, abnormally slow flow without coronary artery disease is also a pertinent indicator of diffuse atherosclerosis [16]. In the light of this information, the presence of CSFP appears to be a significant risk factor for atherosclerosis and heart disease although it is defined simply as a delayed blood flow on coronary angiographic imaging.

GGT has been known for many years as an indicator of hepatobiliary dysfunction [17]. GGT is responsible for the entry of amino acids and peptides in the form of gamma-glutamyl into the cell. Furthermore, there is an important relationship with glutathione which is a key antioxidant [18]. Indeed, glutathione is an important antioxidant produced from metabolic processes. As a result of oxidative stress, the production of GGT is induced to maintain the intracellular glutathione levels at the desired level. In cases where the induction of GGT production is insufficient, excessive oxidative stress will cause new endothelial damage in the cells or aggravate the existing damage [19]. Previous studies have shown a relationship between GGT levels and the severity of coronary artery disease [20]. In another study, it was shown that there is a relationship between GGT and cardiovascular mortality, independent of cardiovascular risk factors [21]. In our study, we found that GGT can be used in patients with CSFP in addition to the literature. Especially, in our study, a moderate correlation was observed between GGT and TFC. Moreover, statistically significant increased GGT levels were observed in patients with CSFP when compared with those without CSFP.

Albumin is an important protein in the human plasma and its plasma concentrations are associated with inflammation and hemostatic processes [22,23]. Low albumin levels were proven to be related to long-term cardiovascular events in sufferers with stable coronary artery disease [24]. Albumin was also found to be a predictor of delayed flow after percutaneous coronary intervention in individuals with acute coronary syndrome in another investigation [25]. Considering the literature examples, it is possible that both GGT and albumin trigger atherosclerotic processes in different ways. In our study, no difference was observed in the albumin values between patients with and without CSFP. We think that the main reason for this result may be that patients with severe coronary artery lesions were not included in our study. As a matter of fact, it has been shown that lower albumin levels may be observed with an increase in the synthesis of inflammatory proteins secondary to the elevated inflammatory response with the severity of the coronary artery disease. This finding may be an important reason for the variations in the results [26,27].

GGT-to-albumin ratio is a simple and noninvasive marker. In our study, the collective effects of GGT and albumin on CSFP were evaluated and it was shown that GGT-to-albumin ratio can be used to predict the presence of CSFP.

Our study had some limitations. First of all, a single center and relatively few patients were included in the study. Advanced imaging methods such as optical coherence tomography were not used in the evaluation of CSFP. However, mean TFC values were used in the evaluation of CSFP and it was found to be associated with GGT-to-albumin ratio. In order to interpret the results of our study more reliably, those with known chronic liver disease were not included in the study. GGT and albumin levels may be affected by factors such as laboratory testing techniques and one-time measurement values. Prospective studies are needed to better evaluate the relationship between CSFP and GGT-albumin ratio.

Conclusion

GGT and albumin are known to be associated with cardiovascular diseases. The use of laboratory tests in the pathogenesis of CSFP is limited. GGT-to-albumin ratio can be easily calculated, and the consequences of our study show that it may play a role in the pathogenesis of CSFP. To conclude, this study is the first to demonstrate that, our results support the hypothesis that GGT-to-albumin ratio plays a role in the etiology of CSFP.

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Digitalization of the clinical exam in Covid-19 pandemic: Karaganda Medical University's experience

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Abstract

Nowadays, Medical education in the Covid-19 pandemic poses a new challenge to explore approaches for delivering quality distance education, especially in the clinical competence assessments. Assessments of clinical competence approaches to conducting an exam, implementation of a new innovative strategy to the assessment procedure, minimize costs, and efficiency in using existing digital resources, have been revised.

The study aimed to compare paper and electronic checklists used in Objective Structured Clinical Exams and assessors' self-perception of electronic checklists and technologies.

Material and methods: The authors compared three types of checklists (paper, bubble, and electronic checklists) according to the following criteria: ease of use, registration, and authentication of examinees and examiners, processing of exam results, and cost benefits.

Results: In the course of analysis, three versions of checklists were compared, thus identifying the advantages and disadvantages of each one, including the assessors' attitude to new technologies.

Despite the high cost of the tablets compared to paper checklists, the use of tablets at OSCE had many advantages, and assessors mentioned that number of errors significantly dropped.

Conclusion: Thus, digitalization of the assessment procedure helped streamline the exam process, securing evaluation data, and speeding up information processing, which significantly minimized the cost of human resources.

Key words: medical education, checklist, OSCE, assessment, clinical competence, performance

Introduction

The Covid-19 epidemiological situation has led to a global change in the approaches to teaching medicine both around the world and in Kazakhstan [1]. It is time to be creative to explore approaches for developing and delivering quality distance education. Medical education in Kazakhstan is faced with the challenge of implementing distance learning, because clinical skills cannot be fully taught and assessed online. The pandemic necessitated a transition from traditional educational programs, to online education. All educators had to switch over to distance learning overnight while having different levels of experience in using educational technologies and devices. Assessment of clinical competence is a very important part of the evaluation of educational program outcomes and does not depend on any critical situation. Nowadays, one of the most important assessment tools is the Objective Structured Clinical Exam (OSCE) which is a comprehensive examination for testing students' clinical skills and competences [2-4]. During the ten-year period of using OSCE at Karaganda Medical University, various methods have been used to assess students' achievements ranging from simple checklists to "advanced" [5]. With the widespread use of available digital resources, the term "digitalization" emerged as gradual transformation of business processes using digital resources. During the pandemic, using digital resources in student assessment accelerated faster but examiners have had some difficulties in using digital technologies. Electronic resources can offer a reasonable solution for assessing students [6,7]. The experience of using paper and electronic assessment checklists and examiners' self-

perception had not been studied in our university, and therefore, this was the goal of our research.

The purpose of this study is to describe and compare student assessment approaches using paper and digital resources during an Objective Structured Clinical Exam and study examiners' perception of new electronic checklists and technologies

Material and methods

This is a descriptive and comparative study. Three types of OSCE checklists were used: a paper checklist (pCHKL, n=7 850), a bubble checklist (bCHKL, n=12 530), and an electronic checklist (eCHKL, n=687). The OSCE is the second part of the clinical skills final examination carried out among senior students. In this study, the investigators attempted to evaluate the effectiveness of tools in assessing clinical competence of students, to identify the advantages and disadvantages of paper and digital resources; and to assess the impact of integrating digital technologies on student assessment (87%) and perception of the examiners (n=70) in using them. To accomplish this goal, the costs for material resources and human resources were assessed, and an online survey of all participants in the assessment was conducted. To evaluate the effectiveness of the digital technologies, methods of relative values and efficiency at the microeconomic level were used.

Results

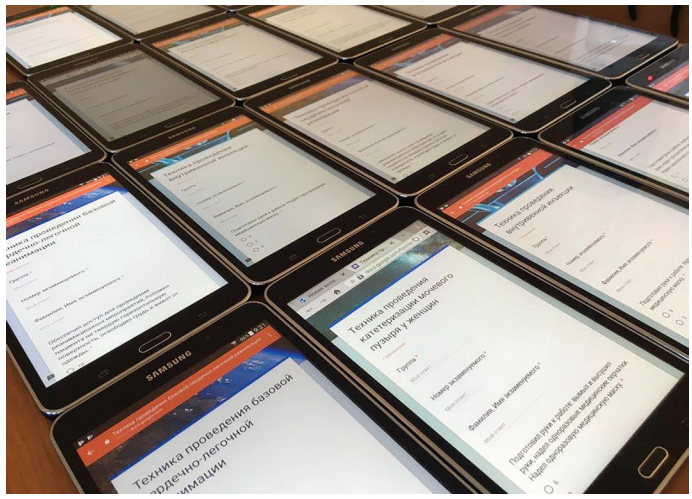
The three types of OSCE checklists, paper checklist (pCHKL), bubble checklist (bCHKL), and e-checklist (eCHKL) were used, analyzed, and compared for their effectiveness. A single checklist was used to assess the OSCE but the way in which the results were recorded and processed was different.

Paper checklists (pCHKL) is a simple paper version and included criteria of clinical skills algorithms and were fairly simple and easy to fill out by examiners. The OSCE results were transferred to a computer database manually, which required high concentration from the staff to minimize errors that may be related to human factors (inattention, heavy workload), which resulted in processing delays of up to 14 days. With a large number of students, the amount of time spent and the quantity of random errors increased, which delayed the processing and delivery of exam results. The disadvantages of pCHKL were the additional resources related to printing of up to 10,000 copies, staff time to review all materials to identify scoring errors, populate the Excel table for analyzing, and to process and distribute the results. The paper form was easy for examiners to fill, but it was more difficult for staff support personnel to transfer data from paper to Excel.

Bubble checklists (bCHKL) were implemented to further improve the assessment process and paper scanned versions of the checklists were used for automatic data analysis by FormReturn, which, as expected, could allow optimizing the processing of OSCE results. FormReturn allows users to develop their own form of an evaluation sheet template, and then use barcodes to track information across individual checklist categories. The development of a template of the checklist was personalized with the identification number of the examinee, which cannot be changed during OSCE. Preparing all the bCHKL required a long time and preliminary approval of all stages, points, and features of the FormReturn program. All bCHKL filled by examiners were scanned, and the results immediately were loaded into the server database. However, scanned bCHKL have disadvantages. The bCHKL had to be sorted before and after the OSCE day by student ID, cohort, and OSCE stations. It also took up extra time and staff resources. For example, 10 bCHKL were printed per student, and 1,253 students required at least 12,530 sheets. During one OSCE period, with 3,163 examinees at 10 stations, 64 reams of paper were used, which averages US $220, excluding printer ink and other print shop services. Additional costs during OSCE included office supplies, which also required financial costs associated with the acquisition of a high-resolution printer during scanning. Also, using pCHKL and bCHKL, required up to 10 staff members who were employed for uninterrupted exams and timely processing of OSCE results.

Electronic checklists (eCHKL) are an alternative form for faster and objective student assessment. With the advent of widespread use of available digital resources, the term “digitalization” emerged as the need to transform processes using digital resources [Hochlernet, 2015]. In connection with the development of digitalization strategy, improvement and automation of assessment processes, interest in digital resources arose in the center of practical skills, and an electronic evaluation form was introduced on tablets to streamline the OSCE evaluation process. For this, a tablet with the Android operating system was used. The interface was designed to fill out the fields of the evaluation form conveniently and quickly. For this, the tablets with the Android operating system and eCHKL developed in Google Form (Figure 1), and 687 electronic student results were analyzed.

Figure 1 - e-checklists for Objective Structured Clinical Exam. Electronic checklists for clinical competence assessment on Objective Structured Clinical Examination used



For comparison of three types of checklists, the utilization of resources in OSCE was analyzed. A total of 20 tablets were used in the examination, costing about US $1,700, thus, the cost of one examination period of the OSCE is US $340. The benefits of using tablets and electronic estimating are greater than evaluating using pCHKL and bCHKL. For example, the relative cost-effectiveness of using the tablets showed the profitability of the resource used and the relative cost savings, including labor costs for staff time. For the evaluation of students in OSCE, more than 75 available electronic programs were studied. According to the analysis of the capabilities and effectiveness of the program, Google Forms and Microsoft Forms are superior to other programs in functionality. Tablets can be used for 10 years, and the Google Forms cloud environment does not require financial expenditures, OSCE results are available at any time, and are stored for a long time. The advantages of using Google forms are the simplicity and convenience of creating new forms, the availability of information at any time of the day and on

Table 1 Comparative table of the evaluation sheet in pCHKL, bCHKL, and eCHKL in OSCE with the calculation of savings due to the increase in staff productivity. http://www.antegra.ru/news/experts/_det-experts/4/

Criteria	pCHKL	bCHKL	eCHKL	Savings, multiplicity	Productivity
Pi (%)					
Filling time per one checklist,min	10	10	5	2,0	100
Identification of ID students,					
min	30	60	1	0,03	2900
Time for data processing,					
Day/hour/min	7/42/2520	5/30/1800	0/0/60	0,02	3500
The cost of resources per OSCE, $USD	220	4220	340	1,5	120
Number of personnel (n=HR)	10	10	2	0,2	400
Number of OSCE days, days	14	14	7	0,3	500
Staff salary per OSCE period, $USD	1800	1800	370	4,8	400

any Internet-connected device. However, this online service has some drawbacks such as the lack of template variety, which limits the use of more complex evaluation forms. Also, some of the senior examiners were not familiar with tables and had difficulty using them.

Below, the comparative table of the evaluation sheet in paper and electronic versions of OSCE shows the calculation of savings due to the increase in staff productivity in one examination period as an example (Table 1).

The calculation of indirect savings of economy at the expense of increasing the user's labor performance was made using the formula: $P_i = \left(\frac{\Delta T_i}{F_j - \Delta T_i}\right) * 100$, and Pi (в %) - labor productivity, ΔTi - i-view savings with automation in hours, and Fj - the time that were planned for the j-type work before the introduction of automation in hours. The table shows that with the OSCE automation, labor productivity in hours and HR increased significantly. Despite the high cost of the tablets compared to the pCHKL/bCHKL, the use of the tablet and the N3 have more advantages.

At the end of OSCE, feedback is regularly collected through questionnaires among examiners and students. Seventy out of 114 examiners participated in the online survey, which amounted to 61.4%. When using pCHKL / bCHKL, there was a low satisfaction with filling out paper evaluation sheets (52 %) and there were many comments and recommendations to simplify the assessment procedure. When using an e-checklist, an online survey among examiners was used, obtained by simply clicking on the tablet screen. Thus, online survey shows that 92% of the respondents were satisfied with the innovation, and in the comments section indicated their interest in further use of e-checklists. The survey among 87% students, who took part in the OSCE, was also conducted. The feedback was accepted positively by students, as students are the cohort that is closer to digital technologies and the use of modern smart technologies in the educational process and the examination only give them support and approval. In the introduction of electronic checklists and the automation of the exam process, while piloting the OSCE, fear of technology arose among examiners over 70 years old. To reduce technophobia, examiners were trained, and they were questioned before and after the exam the self-perception exam during the assessment. The results showed that before the training, more than 80% of senior teachers had technophobia, after the training, the phobia decreased up to 5%. Therefore, automation of assessment processes requires planning the digitalization process to reduce stress for examiners and teachers.

Discussion

The COVID-19 emergency triggered a rapid transition from simple forms of assessment to digital electronic assessment formats. With the aim of comparing the effectiveness of the paper and electronic versions of the evaluation sheets used during the Objective Structured Clinical Exam, a descriptive and comparative study was conducted. To assess the effectiveness of the evaluation procedure in OSCE, the evaluation sheets were divided into three types and compared by the convenience of filling out checklists, registering and authenticating the examinees and examiners, processing the exam results, and studying the indirect economic benefits of using various types of evaluation sheet. The three types of evaluation sheets were used: (i) a simple paper evaluation form, (ii) a bubble version of the evaluation form, and (iii) an electronic evaluation form. The use of digital resources made it possible to optimize the assessment process, secure data and OSCE results, and ensure the efficiency of processing all received information. The use of electronic resources greatly facilitates the collection and processing of test data by automating the process. E-checklists record personal data by IP-addresses, which eliminates the entry into the system of unauthorized persons and allows you to trace the geography of users. To reduce technophobia and stress of examiners, automation of assessment processes should be planned the digitalization process. Feedback from the students, examiners, and administrative staff shows effectiveness of the eCHKL as compared to pCHKL/bCHKL, in generating exam results and minimizing scoring and data processing errors.

Conclusion

In conclusion, the results of the study allowed us to draw the following conclusion: the state of emergency caused by the Covid-19 pandemic accelerated digital implementation of assessment and using digital resources allows faster processing of exam data, to trace the personalization and track each examiner, allows controlling the points of contact of all forms, and increasing the target audience by expanding the geography of IP addresses, and are the main driver for improvement and modernization of OSCE. The effective planning of the digitalization process in education will reduce technophobia and stress of examiners, automation of assessment processes. The charges of resources for digitalization in medical education show appropriate cost-efficacy that necessity for making the decision of the budget of the university.

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Clinical outcome of percutaneous common extensor tenotomy for recalcitrant lateral humeral epicondylitis

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Abstract

Introduction: Tennis elbow is a commonly encountered problem in orthopaedic clinical practice. The patients are usually in their financially productive years and many a times, this is a work related disorder that becomes severe enough leading for lost work days. Conservative treatment is almost always the first choice; but once it fails, there is no clear consensus for the management of recalcitrant cases. We undertook this study to evaluate the clinical outcomes of percutaneous tenotomy in cases of resistant lateral epicondylitis.

Materials and methods: This was a prospective study done over a period of 5 years. A total of 108 patients with recalcitrant tennis elbow were recruited and underwent percutaneous tenotomy. They were followed up for an average period of 14 months. The outcomes measured were: tenderness, DASH score, Roles & Maudsley scale, Visual analogue scale (VAS), and Grip strength. Statistical analysis was done using Paired t-test.

Results: 95% patients were pain free after an average of 12 weeks. The 6 months postoperative improvement in VAS score was 6.84 and change in mean DASH score was 23.04. The average increase in mean grip strength was 13.30 kg. These results were statistically significant.

Conclusion: Percutaneous tenotomy can be considered as a useful treatment option for resistant cases of tennis elbow.

Key words: lateral epicondylitis, office procedure, percutaneous tenotomy, tennis elbow

Introduction

Lateral humeral epicondylitis (tennis elbow) is a frequently occurring musculoskeletal condition presenting to the orthopaedic clinic. The condition is usually a 'repetitive strain injury' and is seen in patients performing work related repeated and forceful supination and pronation of the forearm with the elbow in extension [1, 2]. The annual incidence is 1-3% of the U.S. population. Runge is credited to first describe this condition in 1873 [3]. Contrary to its name, it commonly affects non tennis players and workers.

Clinically the disease manifest itself as pain over the radial aspect of elbow exacerbated by resisted extension of the wrist and by making a fist. Symptoms are usually mild, but occasionally they flare up so severely as to prevent lifting or holding a book or even a cup. The significant pain and disability can be enough to cause a working man to leave his job [2, 4].

Clinically, tenderness is localized to outer epicondyle and to a centimeter distal to lateral epicondyle onto the common extensor muscles. Pain is aggravated by wrist extension against resistance in the pronated position, particularly with elbow in extension. Elbow and wrist range of motion are typically not affected [5]. Differential diagnosis typically includes other causes of pain on radial side of elbow e.g. radial tunnel syndrome, lateral collateral ligament injury, elbow arthritis, and osteochondritis of the capitellum. They must be excluded before reaching a diagnosis of lateral epicondylitis.

With time, pathoanatomy of lateral epicondylitis has become conclusive and has guided treatment methodologies. Histopathology of the affected Extensor

Carpi Radialis Brevis (ECRB) attachment has demonstrated non-inflammatory angiofibroblastic tendinosis with neovascularization, a disordered collagen scaffold, mucoid degeneration, and micro tears [6, 7].

Currently available non-operative treatment methods include rest, nonsteroidal anti-inflammatory drugs, ultrasonic therapy, steroid injections, counterforce brace, and stretching and deep friction massage. Off late, extracorporeal shock waves, laser light and non-coherent light therapy have also come into picture. Similarly, the recent fad of injecting platelet rich plasma has not left Lateral Epicondylitis untouched [8, 9].

Failure of the above non operative modalities, taken for at least 6 months is an indication for surgical management of the disease. By an estimate, 8% fail to respond to non-operative means, and this is the subgroup that might require surgery [10]. Several surgical procedures like open surgical debridement, common extensor origin release, endoscopic techniques and percutaneous tenotomy have been pronounced in texts [3, 11]. It is unclear which procedure is best.

A direct approach to the pathology i.e. open ECRB release has some downsides like protracted postoperative recovery time, a risk of posterolateral instability of the elbow due to lateral ligament injury, and the formation of cutaneous neuroma.

Another approach i.e. Endoscopic ECRB release has a long learning curve and has issues with re-suturing of the detached ECRB. Therefore we felt that a minimally invasive approach like percutaneous common extensor tenotomy shall be tried for recalcitrant lateral epicondylitis.

So far, literature is unclear on superiority of one procedure over the other and most studies have proved a success rate of more than 80 percent. We undertook this study to evaluate the clinical results and functional outcome of percutaneous tenotomy of common extensor origin performed in the out-patient setup in cases of recalcitrant lateral humeral epicondylitis.

Material and methods

A prospective study was conducted at our hospital in 5 years (from 2011 to 2016) on 108 patients. They go through percutaneous release of the common extensor origin. Average follow up period was 14 months.

Patients presenting to the outpatient department were enlisted for the study; provided they meet inclusion and exclusion criteria. An informed written consent was taken from all of the recruited patients. Proper approval was taken from the institutional ethics committee of the hospital.

Inclusion Criteria:

Patients of either sex with a proven clinical diagnosis of lateral epicondylitis of the elbow along with-

1. Failure of at least six months of non-operative treatment including minimum 4 of the following- nonsteroidal anti-inflammatory drugs, steroid injections, physical therapy, stretching exercises and tennis elbow brace, and unacceptable quality of life.

2. Pain induced by two or more of these diagnostic tests

a. Palpation of the lateral epicondyle

b. Resisted wrist extension (Thomsen test)

c. Chair test: With the shoulder flexed to 60° and the elbow extended, the patient attempts to lift a chair weighing 3.5 kg.

Exclusion Criteria (any one of the following):

Age less than 18 years, complain of generalized polyarthralgia, presence of local infection, history malignancy, and radiological evidence of elbow arthritis, ipsilateral shoulder disorder and radial tunnel syndrome.

Operative Technique

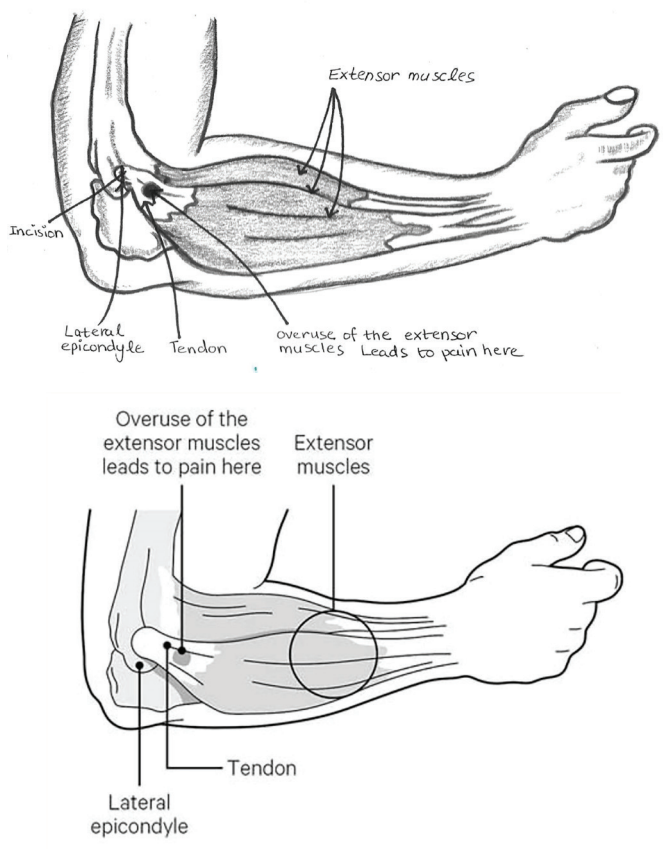
The procedure was performed under local anesthesia and as an OPD procedure under all aseptic precautions [3, 12]. Patient was kept supine with the elbow was kept flexed at 90 degrees. This advances the radial nerve volarly from the tenotomy incision. No tourniquet was used in the procedure. Steps that were followed were-

1) Palpated the posterior edge of the lateral epicondyle, and made a fingernail impression at a point 1 cm anterior to this edge and at the midpoint of the width of the epicondyle

2) Inserted the local anesthetic needle at this exact point and infiltrated the entire region

3) Inserted a number 11 blade at the needle entrance side and cut through the entire width of the common extensor origin from proximal to distal in a direction parallel to the axis of humerus. Skin pliability allows the superficial incision to be one fourth inch long while a three-fourth inch tenotomy is accomplished (Figure 1) (adapted from http://www.phyzio.biz/tennisElbow.php).

Figure 1 - Incision site for percutaneous common extensor tenotomy



4) While digital pressure was maintained to control bleeding, the wrist was flexed to complete the procedure. No suturing was required.

5) A small sterile gauge was then placed with a pressure dressing over the wound. The dressing was removed on the seventh day.

Assessment

Functional assessment was done at follow-up of 4-weekly intervals for a minimum of 6 months and then every 3 months for the next 1½ years. We assessed at every follow up-

1. Tenderness at the lateral epicondyle.

2. Disabilities of the Arm, Shoulder and Hand (DASH)

Table 1 On Visual Analogue Scale mean difference was 6.84 with p value of .0001 at 6 months follow up.

Time	Range	Minimum	Maximum	Mean	Std. Deviation
0 weeks	2	7	9	8.08	.60
4 weeks	4	3	7	4.69	1.03
8 weeks	3	2	5	3.42	.73
12 weeks	3	1	4	2.31	.74
16 weeks	3	1	4	1.58	.64
20 weeks	3	1	4	1.31	.62
24 weeks	4	0	4	1.14	.68

Table 2 Mean DASH score dramatically decreased from 71.35 to 48.31

Time	Range	Minimum	Maximum	Mean	Std. deviation
0 weeks	30	58	88	71.35	6.97
4 weeks	24	51	75	62.75	6.03
8 weeks	30	46	76	57.81	5.58
12 weeks	32	42	74	54.86	5.57
16 weeks	29	42	71	51.36	4.77
20 weeks	32	40	72	49.42	5.16
24 weeks	32	40	72	48.31	5.11

score: The DASH questionnaire was used to get data regarding capacity to recommence daily activities, occupational activities, and sports.

3. Roles & Maudsley scale: for clinical assessment

4. The Visual Analogue Scale (VAS): was used for evaluation of pain.

5. Grip strength of both hands: measurement of grip strength of both hands was done using a hand dynamometer. Quantifications were made with the elbow fully extended and then flexed to 90 degrees. The mean of the two measurements was recorded as the grip strength.

6. Any complication: if found, was reviewed and managed.

Statistical analysis

Paired student t-test was applied for statistical analysis. SPSS software was used for paired t test.

Results

The mean age of the patients we studied was 44.5 years. Dominant hand was involved in 78% of the patients. The post-operative improvement in mean VAS score (Table 1) was 6.84 (p= .0001) at 6 months and change in mean DASH score (Table 2) was 23.04 (p= .0001) (Table 3). The average increase in mean grip strength was 13.30 kg (Table 4) with a p value of .0001 (Table 5). The overall results according to Roles and Maudsley rating scale (Table 6) were excellent in 94 elbows (87.03%), good in 9 elbows (8.33%), fair in 3 elbows (2.77%) and poor in 2 elbows (1.85%) because of the recurrence. In 95% of the patients lateral epicondylar pain was relieved at the mean duration of 12 weeks after the surgery. All patients had a full range of elbow and wrist movement at follow up examination. One patient developed synovial fistula. Two patients had superficial infection which resolved after a short course of oral antibiotics. Recurrence of pain occurred in two patients (Table 7). All the patients were very satisfied with minimal post-operative scar (Figure 2, 3, 4, 5).

Table 3 DASH Score showed mean difference of 23.04 with p value of .0001

	Mean	Std. Deviation
Pre procedure	71.35	6.97
Post procedure	48.31	5.11

Table 4 Grip strength measurements in Kg- average increase in mean grip strength was 13.30 kg

Time	Involved side	Control side
0 weeks	22.81±8.68	41.44+10.90
4 weeks	24.83±8.63	41.47±10.93
8 weeks	26.97±9.2	41.42±10.9
12 weeks	29.81±9.6	41.47±11.02
16 weeks	33.53±9.7	41.17±10.7
20 weeks	35.28±9.44	41.64±10.74
24 weeks	36.11±10.01	41.47±10.94

Table 5 Grip Strength p value

Side	
p value*	
Involved side	.0001
Control side	.911

*Paired t test

Table 6 Roles and Maudsley Scale, 87% patients had excellent results at 6 months

		Number of patients	Percentage
Excellent	No pain Full movement Full activity	94	87.03%
Good	Occasional discomfort Full movement Full activity	9	8.33%
Fair	Some discomfort after prolonged activity	3	2.77%
Poor	Pain limiting activities	2	1.85%

Table 7 Complications

Complication	Number of patients	Percentage
Superficial Infection	2	1.85%
Synovial Fistula	1	0.92%
Recurrence	2	1.85%
No Complication	103	95.37%

Figure 2 - Eliciting tenderness at lateral epicondyle

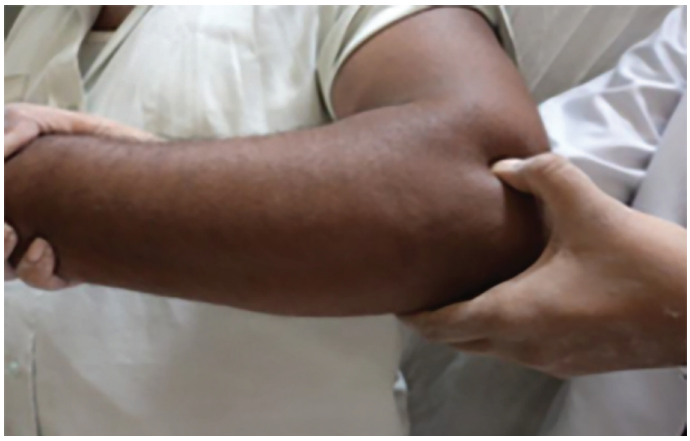


Figure 3 - Release of common extensor origin

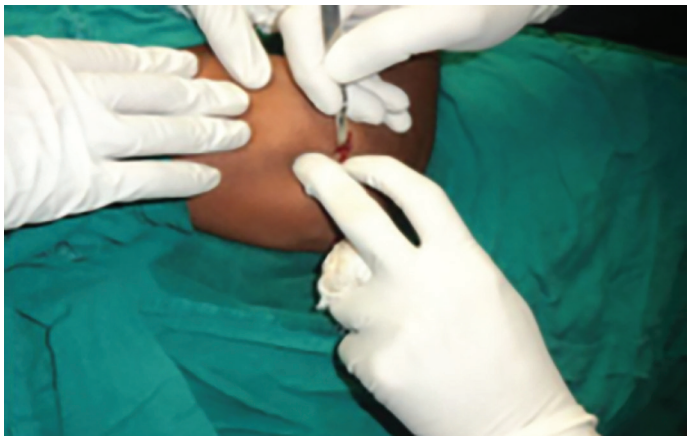


Figure 4 - Completion of the tenotomy

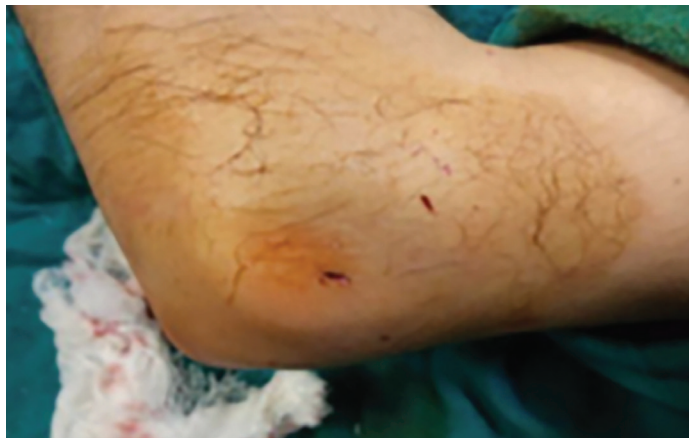
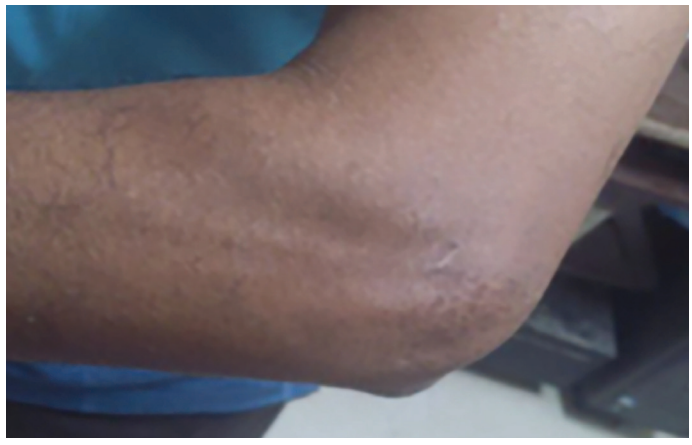


Figure 5 - Post-procedure scar of tenotomy at 8 weeks



Discussion

The notion that lateral humeral epicondylitis is an auto-limiting condition and does not need any intermediation is not fair for those patients in whom pain and limitation of activities have been upsetting their daily routine for nearly 1-2 years. Greenbaum et al. in their anatomical studies came to the conclusion that "pain of lateral epicondylitis appears to arise more from the 'common extensor' origin", the pathology cannot be isolated to a single structure [13]. Any surgical treatment for recalcitrant lateral epicondylitis should therefore focus on the common extensor origin.

The open debridement approach is the most direct of the available methods; but, there has been a recent increase in reports on per-cutaneous and endoscopic approaches due to a protracted rehabilitation course of open surgery. Insufficient data is available in literature to favor one procedure over the other.

We found very few published reports in the literature concretizing the results of percutaneous release of common extensor origin [14-16]. Keeping these background facts in mind, 108 patients with recalcitrant lateral humeral epicondylitis were treated with percutaneous release of common extensor origin.

The mean age of our patients was 44.5 years. The range was 30-70 years. 36.11% of the patients (n=39) were between 31-40 years and 50% (n=54) were in the age group 41-50 years. This shows that it commonly afflicts middle aged and productive working population [17]. In our study, the mean pre-operative VAS score was 8.08 (range 7-9) and post-operative (6 months) the mean VAS score was 1.14 (range 0-4). Hence, the VAS score changed from 8.08 to 1.14 in pre-operative and postoperative values respectively (Table 1). The results were similar to those of shown by Rayan et al. for extensor fasciotomy of common extensor origin in recalcitrant lateral epicondylitis [18].

The mean pre-operative DASH score (Table 2, 3) in our study was 71.35 (range 58-88) and post-operative (6 months) DASH score was 48.31 (range 40-72). The change in post-operative DASH score was 23.04 (p= .0001).

Average time for pain to disappear was 12 weeks with a range from 10-16 weeks in our study. Almost all patients were free from tenderness at 24 weeks after the procedure was performed. Whereas pain on resisted wrist extension and activity related pain disappeared in 95% (n==103) of patients after 24 weeks of procedure and only 5% had mild to moderate pain.

The grip strength (Table 4, 5) on the affected side and the normal side were measured before and after the release for each patient, and strength on the two sides were compared. Pre-operative mean grip strength on involved side was 22.81+8.68 kg and on control side pre-op mean grip strength was 41.44+10.9 kg. Post-operative mean grip strength on involved side was 36.11+10.01 kg and on control side mean grip strength was 41.47+10.94 kg. The post-operative increase in grip strength was 13.30 kg on involved side (p= .0001) and 0.03 kg on control side (p= .911). This result was significant on involved side.

According to Roles and Maudsley rating system, excellent results were obtained in 94 elbows (87.03%), good in 9 elbows (8.33%), fair in 3 (2.77%) and poor in 2 elbows (2.70%). Yerger [3] et al. has reported 93.5% excellent and good results. Baumgard et al. [14] reported 35 cases of percutaneous release of tennis elbow in which excellent result were achieved in 32 cases (91.4%) while in 3 cases results were unsatisfactory. Powell and Burke followed up 20 patients and showed 85% excellent results

[19]. Nirschl and Pettrone [2] achieved an excellent outcome in 66 of 88 and Verhaar [20] got 46 out of 57 excellent outcomes of tennis elbows using an open technique.

Post-operatively, the complications (Table 7) encountered in our study were synovial fistula in 1 patient and superficial infection in 2 patients. This could be a result of tenotomy being taken too distally, which resolved after 3 weeks of regular dressing and course of antibiotic therapy [21]. There were two cases of recurrence after three months of tenotomy, it might have been due to imperfect release of the common extensor origin. The revision percutaneous tenotomy was performed on the same patients with achievement of a pain free elbow after 8 weeks of procedure. All these three patients achieved excellent results without any loss of elbow and wrist movement.

Conclusion

Resistant lateral epicondylitis remains a difficult therapeutic problem. Various surgical procedures have been described in the literature for recalcitrant lateral epicondylitis. Of all these, percutaneous tenotomy is a modest, benign, minimally invasive, effective and straightforwardly reproducible method of treatment. Furthermore, this procedure can be done as an OPD procedure under local anesthesia. In addition, percutaneous extensor tenotomy very well tackle with the shortcomings of open ECRB & arthroscopic ECRB release i.e. protracted rehabilitation .We recommend that Percutaneous common extensor tenotomy is a beneficial management option for recalcitrant cases of Lateral humeral epicondylitis.

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The first experience of radical corrections of total anomalous pulmonary vein return by "sutureless technique" in the Republic of Kazakhstan

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Abstract

Total anomalous pulmonary vein return is a critical heart defect with 4 anatomic forms. It belongs to the severe category of complexity of the STAD scale with an incidence of about 0.013% among all newborns, most of them requiring urgent surgical treatment in the first days and hours of life. Currently, there are many surgical techniques for its correction. The present work presents a comparative assessment of two groups of patients with earlier classic and "sutureless" method of surgery with the results of reduction of myocardial ischemia by an average of 14 minutes, artificial circulation by 33 minutes. We proved that "sutureless technique" has a number of advantages in comparison with the previously performed methods, the main ones being reduction of myocardial ischemia time, artificial circulation and universalism in application to any form of malformation.

Key words: total anomalous pulmonary venous return, "sutureless technique", congenital heart defect (CHD), critical congenital heart disease (CCHD), heart surgery

Introduction

Total anomalous pulmonary vein return (TAPVR) is a congenital heart defect (CHD) presenting as a complete absence of pulmonary vein (PV) flow into the left atrium, with multivariate forms of atypical drainage. TAPVR can be isolated or combined with other heart defects. The incidence is as high as 13 per 100,000 newborns [1]. Almost always TAPVR occurs from the first days of life with high pulmonary hypertension, extremely aggravating the course of severe malformation. The main causes of surgical mortality after radical correction of TAPVR are pulmonary hypertension and postoperative PV obstruction [2]. "Sutureless technique" in TAPVR correction was initially developed for patients with postoperative PV stenosis [3]. Today, the technique has become a standard part of primary radical correction of TAPVR in many centers [4-6]. The plasty can be called universal as an application to any type of TAPVR, including its complex mixed forms [7]. The advantages of this method become obvious. It has been observed that in comparison with traditional surgery, the "sutureless technique" was associated with lower incidence of postoperative PV obstruction (4.6% vs 13.5%) and reoperation (3.4% vs 12.4%) [8], and the torsion of venous sinus system and reactive process of noncontact anastomosis zone are minimized [9]. TAPVR is a grade 4 of 5 critical heart defect according to the STAD (The Society of Thoracic Surgeons-European Association for Cardio-Thoracic Surgery) scale analysis of mortality risk associated with congenital heart disease surgery [10].

Most patients require emergency surgery during the newborn period and sometimes in the first hours of life. "Sutureless technique" significantly reduces the time of surgery and artificial circulation, with the consequent known positive aspects, which is especially important when several CHDs need to be corrected simultaneously [7]. The method is universal, since we have managed to perform this technique to correct almost all types of TAPVR.

Case-presentation

71 patients with isolated TAPVR without concomitant CHD were hospitalized on the bases of the National Scientific Medical Center Nur-Sultan, Center of Modern Medicine "Mediterra" and Center of Perinatology and Pediatric Cardiosurgery in Almaty during the period from 2004 to 2021. The patients were distributed between two groups. In the control group of the study, surgery was performed using the technique of anastomosis between the left atrium and PV collector through incision accesses: right atrium (RA) - interatrial septum - posterior wall of the left atrium (LA) - PV collector (64 patients); in the experimental group correction was performed using the "sutureless technique" (7 patients) who were operated on between July 2020 and July 2021 (Table 1). When carrying out operations by this technique, we did not use circulatory arrest, all operations were carried out in conditions of moderate hypothermia in 33^{0}C.

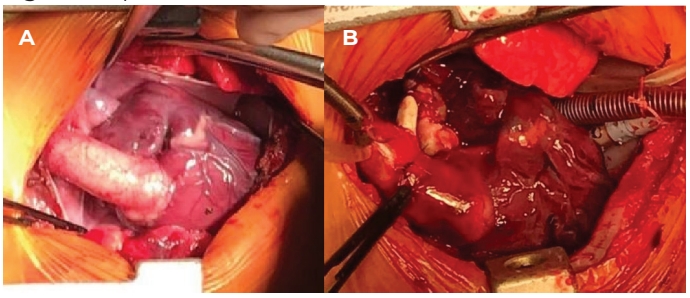
Table 1 Summary data

Minimum score	Group	
	Second (7)	Control (64)
Myocardial ischemia, min	53	67
Cardiopulmonary bypass, min	98	131
Operation, min	179	211

In the experimental group there was one fatal outcome: the child from birth was on artificial ventilation, on the 6th day of life was admitted in an emergency severe decompensated condition. Against the background of poly-segmental pneumonia, periventricular hemorrhage, surgery was performed, which ended with ECMO and lethal outcome after 2 weeks.

Our experience with the "sutureless technique" objectively demonstrates a number of advantages. The results of the study showed that the average time of myocardial ischemia using this technique was more than 20% shorter than that of the previously used method. As the experience accumulates, the time of myocardial ischemia will decrease even more. The need for deep hypothermia, which takes a significant part of the total duration of the operation, is eliminated. The operation technique itself is simple, without opening the right sections, which statistically reliably reduces the aortic occlusion time by at least one third. Thus, such complications of artificial circulation as: fatal arrhythmias correlated with electrolyte disturbances, vasoconstriction due to changes in viscosity properties, coagulation disorders, renal and metabolic disorders, as well as central nervous system lesions are minimized [11, 12]. "Sutureless technique" allowed us to perform correction of almost all types of TAPVR, including its most complex mixed forms, and one patient successfully performed the first and second stages of the most highly lethal CHD - combination of TAPVR with single ventricle and atresia of pulmonary artery heterotaxy syndrome (Figure 1A, B).

Figure 1 - Specific characteristics of case



A - intraoperative preoperative view, B - postoperative view: sutureless TAPVR plasty, dilating LA bifurcation plasty, central systemic pulmonary anastomosis

Conclusion

We have proved that the "sutureless technique" significantly reduces the time of myocardial ischemia, artificial circulation and surgery, avoids the negative effect of deep hypothermia, is universal in relation to correction of almost any type of TAPVR including its complex mixed forms. All advantages of this technique incline many surgeons to choose it as the primary correction of TAPVR.

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Ethical approval: All procedures performed in research involving human subjects met the ethical standards of the institutional and national research committee, as well as the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This case does not contain animal studies conducted by any of the authors.

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CONTENTS

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ДЛЯ ЗАМЕТОК