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Femoropopliteal bypass using vena saphena magna graft in patients with Diabetes Mellitus Type 2

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Abstract

The most common cause of long-term shortness of blood delivery in the lower extremities is atherosclerosis. This process is generalized in all blood vessels but is most commonly localized on the ramifications of large blood vessels where it results in a narrowing of the blood vessels. Dilemmas about transverse types in the treatment of critical stenoses at the lower extremities are not yet completely resolved, especially in the femoropopliteal abutment.

Aim of this study was to analyze complications, early mobilization rate, duration of hospitalization and comorbidity in patients with Diabetes Mellitus type 2 (DM2) who were underwent to femoropopliteal bypass with VSM prosthesis as a graft. The study included 30 patients with DM2 and control group was 30 patients without DM2, all of them were treated at Department of Surgery, University Clinical Center Tuzla.

The results of this study have shown there is statistically significant difference in duration of hospitalization, patients with DM2 stayed in hospital 9.7 days and those ones without DM2 8.3 days. The most common complications were infections, wounds, ischemia of the lower leg and ischemia of the feet. The frequency of complications has been statistically greater in patients with DM2. In patients with DM2 early mobilization was longer than in control group 5.7 vs. 3.7 days. Coexisting diseases in patients with DM2 were more frequent (96,7% vs 76,7%).

Femoropopliteal bypass using VSM should be used as the first choice of treatment of outpatients with and without DM2 due to clinical characteristics, as it gives priority to early mobilization of patients despite longer duration of operation and higher frequency of complications.

Key words: femoropopliteal bypass, Diabetes Mellitus Type 2, complications, vena saphena magna graft (VSM graft)

Introduction

World Health Organization estimates worldwide 2.1% of people have diabetes mellitus (DM). It is predicted that this figure will rise by more than 3% in the next decade (Amos et al., 1997), and that in 2025, around 5.4% of the world's population (about 300 million) will have this disease (Monteiro et al. 2005). It is well known that Diabetes Mellitus is a risk factor for atherosclerotic diseases of the blood vessels. Atherosclerotic disease in such patients is more extrinsic, rapidly progressive and affects the terminal branches of the blood vessels. The frequency of diabetics among patients undergoing surgical technique of femoral prolapse bypass (FP) is estimated at 12-38% (Herlitz et al., 1997; Szabo et al., 2002; Barsness et al., 1997). Traditionally, diabetic patients have a worse outcomes of femoral prolapse (Filsoufi et al., 2007), that is, having higher morbidity and mortality, although the influence of Diabetes mellitus on the femoropopliteal overlaps is rather vague and there are still many controversies, especially about the early results of femoral hyperplasia in these patients. Because of these controversies, there is a need for continuous monitoring of femoral hyperplasia in patients with Diabetes mellitus. It is even more pronounced if it is known that they represent approximately one quarter of the patients undergoing this operation and that their number is constantly increasing as well as that there are numerous studies that show superiority of femoral prolapse over the percutaneous intervention, the presence of diabetes in some practitioners still have an influence on the femoro-

popliteal premonition strategy, ie, it favors the use of percutaneous methods.

There are still not enough studies in the world to give a definitive answer to the question of how Diabetes mellitus affects the results of surgical techniques of femoral hyperplasia, which is also the main reason for carrying out this study. The long-term lack of arterial blood in the lower extremities is most commonly occurs as a result of occlusive disease of the artery and is termed as chronic extremity ischemia. The most common cause of atherosclerosis is atherosclerosis, and vasculitis (Morbus Burger), vasospastic disease (Morbus Raynaud), fibromuscular dysplasia, and other rare conditions may appear beside it. Although atherosclerosis is a generalized disease, however, it is a segmental distribution, and is most commonly found in the aortic and infrared end of the artery tree. Due to segmental distribution, this condition can be surgically treated. The aim of our study was treatment of occlusion and stenosis of the infrared endothelial segment through femoral hypoplastic overlapping with the use of autogenous graft vein saphena magna (VSM).

It is known that during the preparation of the VSM as a crossover there is a greater manipulation of the tissue, ligating the VSM branch, greater possibility of injury to the tissue and structure, longer wound length, postoperative pain level, ambulatory overflow, distance from the hospital where it is necessary to perform controls as well as accommodation (83.3%) compared to patients without Diabetes Mellitus (63.3%). Statistical analysis and comparison of coexisting diseases revealed statistically significant differences in the frequency of coexisting diseases in patients with Diabetes mellitus (83.3%). All co-diagnosis was divided into four groups: cardio-vascular, respiratory, renal and gastro-intestinal. Cardiovascular diseases include: arterial hypertension, atrial fibrillation, cardiomyopathy, post-pacemaker implantation, and post-myocardial infarction or cerebrovascular infarction. Respiratory diseases include chronic obstructive pulmonary disease and chronic bronchitis. Chronic renal insufficiency has been reported in kidney disease. Gastrointestinal diseases have been reported as Chron's disease.

The greater the number of complications in patients with Diabetes mellitus is explained by the longer duration of surgery, the longer stay in JIT, the longer the time of early mobilization, longer hospitalization times, higher rates of comorbidity (Kwolek et al., 2004). One of the most important rehabilitation procedures that improves the general condition of the patient, reduces complications in the sense of deep venous thrombosis, lung-induced pneumonia, and preparation for hospital discharge and return to normal life activities.

Patients and methods

This was retrospective study included 60 patients who have been underwent to femoropopliteal translocations with vena saphena (VSM) graft at Cardiovascular Surgery Clinic, University Clinical Center Tuzla. Follow-up period was six months. Patients were divided into two groups. First one consisted of 30 patients with DM2 and second one 30 patients without DM2, as a control group.

Including criteria

Patients with or without DM2 who were underwent to femoropopliteal bypass using VSM have been included in this study.

Excluded criteria

- Anastomoses made by other type of procedures (eg. termino-terminal)
- Anastomoses or vascular interventions performed above the level of femoral polyneus anastomosis (eg. aortic bifemoral peripheral artery)
- Femoropolital roundabouts of "de novo"
- Anastomoses made with alogenic vascular prostheses (PTFE, dacron and others).

All patients involved in the study were underwent to standard preoperative preparation, CT-angiography the lower extremities and colorectal-doppler arterial and vein system of the lower extremities. General endotracheal anesthesia and same surgical procedure using VSM graft were applied to all patients. Also, in the postoperative course patients had standard monitoring of vital parameters, basic laboratory parameters, wound healing and nursing care. The use of antibiotics in the early postoperative course was also unified (Cefazolin).

In this study it has been analyzed following parameters: age, gender, duration of hospitalizatio, degree of early mobilization, number and type of postoperative complications, Comorbidity (heart, lung, stroke, kidney disease).

Results

There was not statistically significant difference between experimental and control group acording to the gender. Male patients were 86,7% with DM2 and 83,3% without DM2, and 13,3% femeale patients were with DM2 and 16,7% without DM2. Comorbidity was statisticaly more frequent in patients with DM2 (96,7% vs 76,7%; figure 1).

Duration of the surgery was longer in patients with DM2 but not stisticaly significant (Figure 2). Postoperative complications, early and late (postoperative bleeding requiring audit, acute emboliation of athletic bypass grafts, deep infection of the wounds, ischemia, amputations and others) were statisticaly more frequent in patients with DM2 (figure 3 and 4).

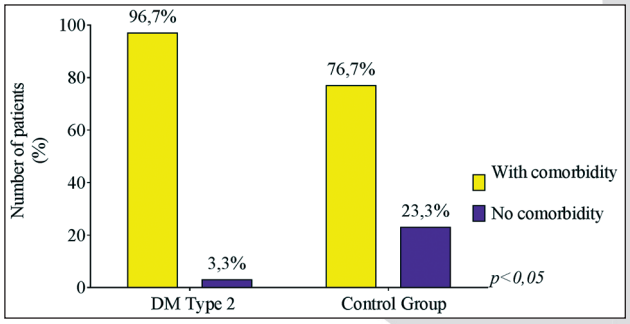

Figure 1. Comorbidity in pateints with and witout DM2 underwent to femoropopliteal bypass with VSMG

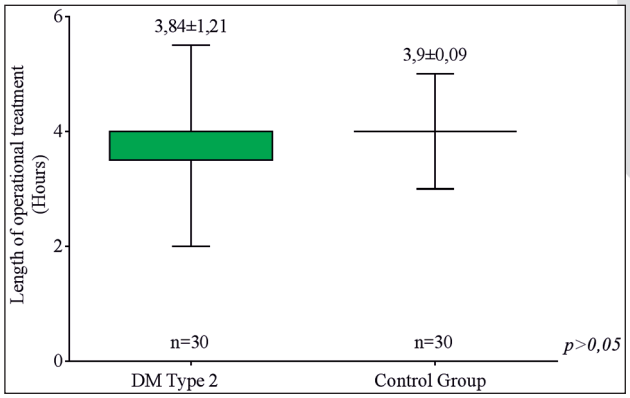

Figure 2. Duration of surgery in patients with and without DM2 underwent to femoropopliteal bypass with VSMG

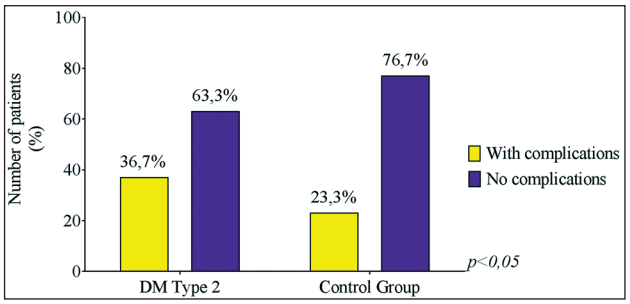

Figure 3. Postoperative complications in patients with and without DM2 underwent to femoropopliteal bypass with VSMG

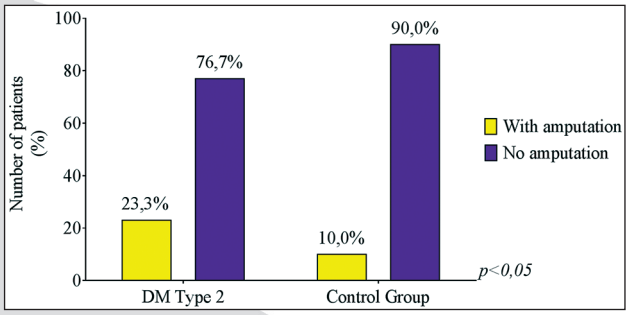

Figure 4. Frequency of amputation in patients with and witout DM2 underwent to femoropopliteal bypass with VSMG

Discussion

When performing femoral hyperplasia in the treatment of occlusion and stenosis of the femoropopliteal segment of the artery tree there are still dilemmas in the selection of graft types for performing femoro-popliteal abutment. VSM and PTFE grafts are most commonly used. VSM has certain advantages such as longevity, economic viability (due to the use of contralateral VSMs) but also the negative side because applying VSM as a graft has a higher operational risk due to longer operation, greater operative section (weaker cosmetic effect). (Klinkert et al., 2004). In this type of graft the preoperative patient should be made with color dopler to determine the size, passage of the veins, caliber veins, strings and injuries. Restrictive circumstances for using VSM as a graft are fibrous processes that are trying to prevent this search increases the costs and time needed for preoperative patient preparation, especially in case of VSM use reversed.

There is a description of the occurrence of safenous neuralgia in the use of VSM as a graft in arterial reconstructive surgical procedures (Roder et al., 1984). In patients with high morbidity and

mortality, PTFE is preferred for a shorter duration of operation and at good flow distal from the forearm. (Rutheford et al., 2000, Klinkert et al., 2003, Pereira et al., 2006). In this study wehave shown next: a) patients with DM2 had significantly longer postoperative mobilization; b) patients with DM2 had more postoperative complications and more frequent amputation; c) ischemic changes of the feet were equally frequent in subjects with and without DM2, but ischemic changes in the lower limbs were significantly more frequent in patients with DM2, d) amputation was significantly more frequent in patients with DM2, e) patients with DM2 who had postoperative complications or amputations were significantly longer hospitalized compared to those without complications or without amputation, but were equally long hospitalized in comparison to non-diabetic subjects with complications or amputation, f) patients with DM2 postoperative complications or amputation were significantly longer treated in intensive care units and had significantly longer mobilization time compared to subjects without complications or without amputation, regardless of whether they had diabetes mellitus or not.

The results obtained will suggest to all those concerned with this problem that the operative femoropopliteal overhang is still a safe method in treating a patient with Diabetes mellitus, which should dispel all doubts about the efficacy of this method as well as increase its share in the day-to-day treatment of atherosclerotic disease peripheral blood vessels in these patients.

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Total and Specific Immunnoglobulin E in Bronchial Asthma Diagnostics in Children

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Introduction

Asthma is a chronic airway inflammatory disease of multicausal genesis, with the increased irritability in its basis, and complex activity of inflammatory cells and mediators on airway cells and tissues, and that leads to their stenosis, variable airflow restriction with reversible cough attacks, hissing, chest tightness and tension, pain especially at night and/or early in the morning.[8]

Regardless of the type of asthma, most of the patients with this disorder have bronchospasm attacks provoked by different alergic, chemical, physical and other stimuli, which represents a bronchial irritability.[12]

The most commonly used test for the identification of allergens is a skin test, which is of subjective character and an anaphylactic reaction can occur during the test itself. The other method is enzime immunoassay test which gives the exact concentration of allergens in blood circulation.[9]

It has been described in literature that the skin test is more sensitive than the specific immunoglobulin E obtained by enzoimmuno test, and therefore the aim of the research paper was to examine is that the case in our sample in children and how often is the overall immunoglobulin E increased in such patients.

Key words: prick test, total IgE, specific IgE, stool test on intestinal parasites.

Subjects and working methods

There were 35 subjects included in the research study, children aged 0-14 years of age diagnosed with bronchial asthma by a medical specialist in pediatric diseases - pulmologist at the Pediatric Clinic of the University Clinical Centre in Tuzla, either through outpatient service or during hospitalisation.

The control group was selected randomly and consisted of 10 subjects from the population of children having no alergic disease simptomatology nor bronchial asthma in the anamnesis.

Total IgE in serum on DADE BEHRING II device quantitatively, with nefelometric method and skin tests with prick method were performed on patients diagnosed with bronchial bronchal asthma at Pediatric Clinic. Having conducted the prick test, we used the same serum from which we determined the total IgE, we used that method to test the serum on specific IgE on HYTEC 288 device quantitatively, with enzimoimunotest. We had the stool tested on intestinal parasites with kato method for all subjects who had the increased IgE.

The criterium of including the subject in this research study was the diagnosis of bronchial asthma in children while the criteria for excluding the subjects from the research study were the subjects diagnosed with bronchial asthma, an the stool findings were positive on intestinal parasites.

Results

Table 1.1 Subject age and sex

Age	Male	Female	Total	Percentage
3 – 6	4	0	**4**	**11,43**
6 – 10	8	10	**18**	**51,43**
10 – 14	8	5	**13**	**37,14**
Total	**20**	**15**	**35**	**100**
%	**57,14**	**42,86**	**100,00**	

Table 1.2 Prick test results review per subjects and ellergens

	House dust	Grass pollen	Dermatophagoides pteronyssinus	Roof pollen
P-1	3	3	4	
P-2				
P-3	3,5		5	
P-4	3		5	
P-5		5	4,5	
P-6		3,5	4	
P-7	2,5	3,5	9	
P-8	2,5	3	5,5	
P-9	3,5		4,5	
P-10	3		5	4
P-11	3		8	
P-12	3		6	
P-13		3,5	4	
P-14			3,5	
P-15	3	3,5		3
P-16			4	
P-17			5,5	
P-18			3	
P-19			6	
P-20			4	
P-21			4,5	3,5
P-22	2		4	3,5
P-23			3,5	3
P-24				
P-25	3		3	
P-26		3	6,5	
P-27			5	
P-28	3	3	5	
P-29	3,5	3	4	
P-30		3,5	3	
P-31			6	
P-32			4,5	
P-33	3		4	
P-34		7		
P-35		3,5		
Count	15 (42.85%)	14 (40%)	30 (85.71)	5 14,28

Table 1.3 Specific and total IgE per subject and allergens

	House dust	Grass pollen	Dermatophagoides pteronyssinus	Roof pollen	Total IgE
P-1	15,02	2,06	36,45		776
P-2					692
P-3	3,5		101		604
P-4	6,95		101		960
P-5		2,06	101		737
P-6		1,8	82,01		264
P-7	14,34	2,5	101		794
P-8	26,91	3,77	101		1340
P-9	1,26	3,86	101		181
P-10	36,65		101	15,01	3140
P-11	2,19		38,78		453
P-12	4,59		101		743
P-13		1,39	22,32		61,7
P-14			101		866
P-15	1,54	3		1,52	627
P-16			6,42		131
P-17			101		656
P-18			101		2690
P-19			101		419
P-20			45,35		111
P-21					62,6
P-22	3,98		68,48	16,26	592
P-23			61,41	1,23	162
P-24					236
P-25	**1,0**		6,5		225
P-26		16,65	101		2700
P-27			71,9		508
P-28	2,69	1,06	17,86		131
P-29	1	3	9,56		390
P-30		33,88	92,8		2110
P-31			101		503
P-32			47,35		566
P-33	15,21		35,83		566
P-34		101			1110
P-35		15,31			475
Count	15 (42.85%)	14 (40%)	29 (82.85)	4 (11.43)	97.14%

Table 1.4 Total IgE descriptive statistics

Mean	759,49429
Standard Deviation	764,91177
Sample Variance	585090,02
Range	3078,3
Minimum	61,7
Maximum	3140
Count	35
Trust Interval (95.0%)	262,75639

Table 1.5 Prick test frequency arrangement per age groups and allergens

Age	House dust	Grass pollen	Dermatophagoides pteronyssinus	Total
3 – 6	0	1	2	3
6 – 10	10	4	17	31
10 – 14	5	9	11	25
Total	**15**	**14**	**30**	58

Table 1.6 Prick test descriptive statistics

Prick test	House dust	Grass pollen	Dermatophagoides pteronyssinus
Mean	2.9643	3,7143	4,7833
Median	3,00	3,50	4,50
Mode	3,00	3,50	4,00
Standard Deviation	0,4144	1,0869	1,3752
Sample Variance	0,1717	1,1813	1,8911
Range	1,50	4,00	6,00
Minimum	2,00	3,00	3,00
Maximum	3,50	7,00	9,00
Count	15	14	30
Confidence Level(95,0%)	0,2393	0,6275	0,5135

Table 1.7 Specific IgE descriptive statistics by allergens

Specific IgE	House dust	Grass pollen	Dermatophagoides pteronyssinus
Mean	9,7021	13,6993	70,9317
Standard deviation	10,8364	25,8193	35,4969
Sample variance	117,4278	666,6388	1260,0272
Scope	35,6500	99,9400	94,5800
Minimum	1,00	1,06	6,42
Maximum	36,65	101,00	101,00
Number	15	14	29
Trust Interval (95.0%)	6,2568	14,2983	13,5023

Table 1.8 Specific IgE frequency arrangement by age groups and allergens

Age	House dust	Grass pollen	Derm. Pt.	Total	%
3 – 6		1	2	**3**	**5,17**
6 – 10	10	5	17	**32**	**53,45**
10 – 14	5	8	10	**23**	**41,38**
Total	**15**	**14**	**29**	**57**	**100,00**
%	**24,14**	**25,86**	**50,00**	**100,00**	

Table 1.9 Correlation between total IgE and age by age groups

	All	Male	Female
3 - 14	0.157721	0.330462	-0.22203
3 - 6		-0.84637	
6 - 10	-0.33149	-0.35757	-0.3559
10 - 14	-0.3203	-0.47018	0.549106

Table 1.10 Correlation between total IgE and prick test by sex

Male	Female	All	
-0,3626	0,0183	-0,1680	House dust
7	7	14	← Incidence
0,1882	-0,2731	0,0328	Dermatophagoides pteronyssinus
16	14	30	← Incidence
-0,0217	0,3977	0,0555	Grass pollen
9	5	14	← Incidence

Table 1.11 Correlation between total IgE and prick test by age groups

Age group	House dust	Grass pollen	Dermatoph. Pteron.
All	-0.16802	0.055533	0.032827
3 – 6			
6 – 10	-0.72233	-0.02896	-0.12068
10 – 14	-0.03377	-0.04198	0.397247

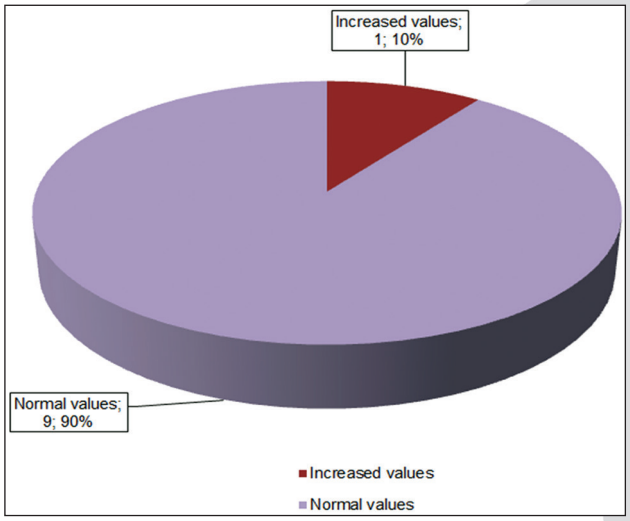

Chart 1. Value ration between the total and specific IgE for control group

Discussion

Asthma is one of the oldest known diseases. The word asthma is of the Greek origin and means gasping or heavy breathing. In the first half of XIX century Leannec discovered structural damages on airways and introduced the term "asthma bronchiale").[13] This is the disease of the whole world and it can be claimed with confidence that it is a disease of the past, present and future.[17]

Asthma is a common disease and it is spread widely in all areas of the world with constand tendency of growth in the last decades. About 3% of overall population in Great Britain and about 5% in the USA suffer from asthma. Compared to the developed Western countries the disease rate is lower in the countries of Central and Eastern Europe. In childhood, asthma affects boys more often than girls.[8]

There were 57.143% of boys and 42.857% of girls in our sample.

Similar results were obtained by Neda Aberle and Zeljka Rainer-Banovac in 1998 in their study on epidemiologic research on asthma in children where they had 377 children between 2 and 15

Table 1.12 The comparison between total and specific IgE

Number	Allergen	t-test	Correlation	Count
1.	House dust	0,000634	0.872	14
2.	Grass pollen	0,000621	0,274	15
3.	Dermatophagoides pteronyssinus – all patients	0,0000169	0,495	29
3a.	Dermatophagoides pteronyssinus - male	0,00138	0,450	15
3b.	Dermatophagoides pteronyssinus - female	0,002114	0,541	14

Table 1.13 The influence of sex on prick test per age groups

	House dust	Grass pollen	Dermatophagoides pteronyssinus		
			All	Male	Female
3 - 14	0,132797	0,143594	-0,08998		
3 - 6					
6 - 10	0,020642	-0,01733	0,129728	0,518614	-0,29484
10 - 14	0,334787	-0,30775	-0,46132		

Table 1.14 The relationship between the prick test and Specific IgE

Age group	House dust	Grass pollen	Dermat. Pt.		
			All	Male	Female
All	-0,28907	0,817449	0,306575	0,464326	0,125852
3 - 6					
6 - 10	-0,75225	0,169432	0,267282	0,510848	0,062919
10 - 14	-0,04777	0,806819	0,427021	0,371867	0,834888

years of age. There were considerably more boys (63.7%) than girls (36.3%) present among subjects. 65.8% or 248 children of the total number were diagnosed with allergic asthma, while in others it was about the influence of the environment, weather changes, infection etc.[1]

These figures confirm that boys suffer from asthma more often than girls. 34 children had high IgE, two of them neither had a positive skin test nor positive serum specific IgE, while one child had low IgE but it had a positive skin test and serum specific IgE.

The American Academy of Allergy, Asthma and Immunology (AAAAI) states that 50% of adults and 85% of children suffer from asthma with the allergic origin.[18]

In our results, 34 children had high IgE, two of them neither had a positive skin test nor positive serum specific IgE, while one child had low IgE but it had a positive skin test and serum specific IgE.

In 50% of cases, asthma starts before 10 years of age, but it is not rare that it starts after 60 years of age. Less than half of the children with asthma will have asthma when they grow up. If asthma occurs in adult age it rarely retreats. It is a general opinion that asthma and other allergic diseases prevalence is in constant increase, especially in industrially developed Western Countries. There are more and more reports from lots of countries about the increasing number of sick people hospitalized because of asthma, and also about the increased mortality rate, especially in young people.

Including the centres for disease control and prevention, the asthma prevalence in the USA increased from 3.6% to 5.8% in 2003.[16]

Lagre differences were noticed in asthma prevalence between the rich, moderately rich and poor people in Australia, but there is no reliable claim whether this difference occurs as a consequence of a response to different allergens or other environmental factors, or due to more common parasitic infections in poor patients, or due to the influence of social-economic status in a particular country on adequate healthcare protection benefits.[12]

It can be found in literature that IgE plays the main role in asthma immunopatogenesis, so the objective of this scientific study was, by using random selection, to try to check this statement on the sample of children, and to check if it is really important in routine diagnostics when suspecting asthma to immediately check total and then specific IgE.

Immunoglobulin E is one of 5 classes of immunoglobulin. Normally, there is a very low quantity of IgE in healthy subjects, so that in a large number of cases the increased IgE level indicates the atopic status.[14] In our results, only one subject did not have the increased total IgE.

There was one case of bronchial asthma in with enormously high level of immunoglobulin E in the study, but all other parameters wer normal (prick test, stool findings on intestinal parasites).

The concentration of immunoglobulin E in blood was very low. Immunoglobulin E in the umbilical cord is usually lower than IU/ml. The children with high level of IgE in the umbilical cord had an allergic manifestation at the age of four in the percentage of up to 80%.[8] In general, at the age of 5-7 children achieve the same IgE concentration as the adults. Between the age of 10 and 14 the IgE level is higher than in adults.

After 70 years of age, the IgE level slightly decreases and it is lower than in adults younger than 40.[17]

In our results, the increased total IgE value in children suffering from bronchial asthma was in 97.14% cases (34 subjects), while normal value was in 2.85% (1 subject).

Mast cells are present in all tissues getting in contact with external environment and with the production of number of neutrophils, proinflammatory citokines, chemokines and growth factors, enable the fast response of the body to the allergens intaken through air or food. The differ from each other in shape, density and granular content. There is a high affinity IgE receptor on their surface.

Mast cells have several roles in body defence. The inflammation taking place via mast cells through proinflammatory substances released from degranulated mast cells enable the circulating cells and plasma proteins to increase the access to interstitial space where they fight the infection.

When the degranulation of mastocytes begins, hystamin and other biological substances are released, which can induce cough, sneezing, vomiting or diarrhea and all that for the purpose of throwing the pathogenes out of the body. The parasites can also be the content of such patho-

genes. Parasitic infections may whelm the gastrointestinal tract, lungs, bloodstream or solid tissues. The consequences of parasitic infection can be: anemia, ellergic reactions, the expansion of granulomas in solid tissues, obstruction of blood and lymph vessels, cancer induction, blindness and diarrhea. During the infestation of parasites, IgE stimulates the occurence of antibody-dependent, cell-mediated citotoxic reaction against helminths and parasites. IgE binds to parasites and focuses the eosinophils against the parasites. At the moment of IgE binding to parasites, the eosinophils bind to IgE, when they release the toxic products against them because the helminths and parasites are too big to be fagocitated. These toxic products may kill, damage or dislocate the parasites for the purpose of protecting the host. [2.17]

One of the tasks in this study was to check the stool on intestinal parasites when the IgE is high in order to exclude the parasitosis but none of the patients had positive findings.

The avoidance of allergens to which the patient is sensitive takes a very important place in the therapy for all allergic diseases, and therefore the allergic asthma as well. That is why the great consideration is given to the identification of allergens. So far, the skin test is considered the most sensitive and most usable method of examining the value of allergens.

The techniques of performing allergy tests are different: scarification method, where the small scratches are made with lancet of 0.5-1cm length, and if the finding is positive, a papula of 3 cm in diameter occurs, and the trial is read after 10-15 minutes; intradermal method where 0,05-0,1 ml of allergen extract is injected intradermally, and the reaction is also read after 10-15 minutes, where the trial is positive, the papula diameter must be at least 5 mm.

We tested our subjects by using prick-technique. The advantage of this method compared to the previous skin tests is the performance compared to scarification and intradermal path. It is less painful and less unpleasant and the risk of unwanted reactions is insignificantly low, because the small quantities will be resorbed.

Sometimes a delayed allergic reaction 6-8 hours and 24 hours after conducting the test can occur and this second phase of the immune response is caused by IgE antibodies.

ELISA is also the method we used in the study in order to detect the specific IgE from patient's serum. It represents a method for the detection and measurement of antigenes (allergens) or antibodies where the enzime reaction with substrate is used and where enzime is used as a marker. Enzimes most commonly bind to antibodies specific for a particular antigene (allergen). The amount of enzimes in antigene-antibody complex is determined directly by measuring the quantities of the appropriate substrate decomposition products which is injected in the final phase of the test. The enzimes whose substrates give the coloured decomposition products and whose quantity can be easily measured callorimetrically are used. The most commonly used enzime is alcaline phosphatase. Its substrate p-nitrophenil phosphate gives intensively yellow p-nitrophenol through decomposition.[9]

The advantage of this method compared to skin tests is that we take the blood sample from a patient and the patient goes home and takes his/her findings on scheduled time. There is no fear from anaphilactic reaction and we get the exact concentration of allergens in blood.

The most common allergens, leading to the occurence of allergic sensibilisation and those that will cause the occurence of asthma caracteristic symptoms during the repeated exposure can be divided in two groups: those that can be met at home and those present outside.

All known inhalant allergens are glikoproteins, with the molecular weight between 10 and 14 kd, and the degree of antigene activity determines the configuration of surfaces un tertiary aminoacid structure and the degre of hydrophylicity determines the solulibility of amino acid side chains in water and the movability of groups of atoms in a molecule.

The moment of first contact with the allergen is important and critical. It is known that the sensibilisation can occur prenatally, but its clinical importance is minor. A contact with large quantities of allergens in first three to six months of life has a great importance for sensibilisation in later life. The expression of atopy starts with the age. The allergens that can be found at home are: home dust, animal hair, mites, mold, insects, cockroach and mice urin, and there are different types of pollen, mould and bees and wasps products in the air outside.

Asthma is mostly caused by the following respiratory allergens: house dust, pollen, mould, dermatophagoideus, animal ephitelium, feathers, insects and industrial chemicals.

Chinoy B, Yee E i Bahna SL 2005 compared the skin test with serum-specific IgE antibodies (radioallergosorbent test (RAST)) using common allergens in patients with respiratory allergic diseases. They had 118 ptients (from 3-month-old to 58-year-olds, with the average of 12 years of age) suffering from allergic rhinitis and/or bronchial asthma. The common allergens were Dermatiphagoides farine (118) cockroach (60), cat epidermis (90), dog epithelium (90). Two tests matched in 52.2% of cases (dog epithelium) up to 62.2% (cat epithelium). When RAST was positive, ST was positive in 80-100% cases (cocroach). When ST was positive, RAST was positive in 16.3% (dog epithelium) to 50.0% (Dermatophagoides farine).

When RAST was negative ST was positive in 48.5% (cat epithelium) to 69.6% (Dermatophagoides farine). When ST was negative RAST was positive in 0% (cockroach) to 5.6% (cat epithelium). For all jointly tested allergens ST was more sensitive than RAST. When both tests were positive their correlation was weak.[5]

In our study, the largest number of positive responses with prick test was at Dermatofagoides pteronyssinus 30 (85.71%), house dust 15 (42.85%), grass pollen 14 (40%), weeds pollen 5 (14.29%), and with ELISA method: Dermatofagoideus pteronyssinus 29 (82,85%), house dust 15 (42.85%), grass pollen 14 (40%) and weeds polen 4 (11.43%).

Saracevic and Mulaosmanovic in their study in 2004, where 450 children were tested with standard inhalation allergens prick method had house dust and Dermatophagoide pteronyssinus as the most common allergens, i.e. 270 (59%), then the animal allergens in 28 children (6.22%) namely 20 children (4.44%) on animal hair and 8 children (1.78%) on feathers.[14]

Chauveau A. at all also researched the correlation between the prick test and specific IgE where they obtained the results with the prick test more sensitive than the specific IgE.[5]

From this research we can see that positive prick test and specific IgE in children performed with ELISA method on the device coincide with house dust and grass polen while at Dermatophagoides pteronyssinus and weeds pollen there were more positive results with the prick test.

Conclusion

The aim of the research was the determination of total and specific immunoglobulin E in children suffering from bronchial asthma.

Number of children included in the study was 35, and they were divided in 3 groups, i.e. by normal total IgE size 3-6 years (11.43%), then 6-10 years (51.43%) and 10-14 years (37.14%).

The diagnosis of asthma was determined on the basis of anamnesis, clinical picture, physical examonation and functional-diagnostic tests at University Clinical Center Tuzla, either through outpatient service or during hospitalisation.

The control group consisted of 10 randomly selected subjects who did not have allergic diseases in their anamnestic data.

Total IgE was determined with nephelometric method and the skin test was performed with prick method in all subjects, and then the stool test on intestinal parasites was done on subjects having increased total IgE. In positive skin allergens, we performed the specific IgE on Hytec 288 device with ELISA method.

After the processed data, 97.14% (34) subjects had the increased IgE, while 2.86% (1) subject had normal findings.

The stool test on intestinal parasites was negative in all subjects who had the increased total IgE.

The positive allergens with prick method were Dermatophagoides pteronyssinus 30 (85.71%), house dust 15 (42.85%), grass pollen 14 (40%), weeds pollen 5 (14.29%), and with ELISA method: Dermatofagoideus pteronyssinus 29 (82.85%), house dust 15 (42.85%), grass pollen 14 (40%) i weeds pollen 4 (11.43%)

The most common allergen was Dermatophagoides pteronyssinus.

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Midazolam induced sedation provides safety and amnesia during bronchoscopy

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Abstract

Aim: We aimed to explore the effects of preoxygenation and premedication with midazolam on cardiovascular stability and amnesia during fiberoptic bronchoscopy.

Patients and methods: We conducted a prospective study at the Clinic for Pulmonary Diseases, University Clinical Centre Tuzla, between May 2017 and May 2018. Experimental group included 30 patients who received 5 mg of Midazolam im. as premedication, 1 hour before bronchoscopy. During the procedure they received 1-3 mg of Midazolam iv. We evaluated sedation using sedation scale test. After 30 minutes we evaluated the level of amnesia. Control group included 30 patients who had bronchoscopy in local anesthesia without premedication with midazolam. According to ASA classification all patients were grouped into risk group I or II.

Results: The increase in systolic blood pressure (> 20 mmHg) was noted in 7 (23.33%) patients in experimental group, while in 26 (86.66%) patients in control group. The increase in diastolic blood pressure (10-20 mmHg) was significantly more present in experimental compered to control group (7 vs 20). The increase in heart rate (> 15 beats per minute) showed the same trend (7 patients in experimental group compared to 20 patients in control group). Total of 25 patients (83.33%) from experimental group reported that they do not remember the procedure, while 20 patients (66,67%) would do repeated bronchoscopy if necessary. 9 patients from control group refused repeated bronchoscopy.

Conclusion: Adequate preoxygenation and premedication with midazolam provides sedation, amnesia, safety and comfort for patients and physicians performing bronchoscopy.

Key words: midazolam, amnesia, bronchoscopy.

Introduction

The most common indications for bronchoscopy are: lung tumors, coughing blood, sudden onset unexplained problems with breathing, prolonged cough of unknown etiology, repeated pneumonias at the same localization. [1,2]

Fiberoptic bronchoscopy (FOB) provides direct visualization of trachea, bronchial tree, helps in diagnosing, staging and treatment of pulmonary diseases. [3,4.]

Thanks to relatively small diameter (5.0 to 6.0 mm), fiberoptic bronchoscope occupies 6 to 11 % of bronchial diameter and does not produce any problems with ventilation in patients who are not intubated. In contrary, during intubation with tube number 8 we reduce 50% of free bronchial lumen. We additionally reduce 25% of free bronchial lumen while putting fiber optic bronchoscope.[5] While doing the aspiration, patients experience so called "stealing phenomenon" which decreases the respiratory volume by 40- 75 %. It has been established that hypoxemia (20 mmHg of decrease in pO2) happens among healthy volunteers while putting fiber optic bronchoscope because of: ventilation perfusion mismatch (V/Q), changes in the patients position, suction, cough with increased resistance within bronchial tree. Serious hypoxemia can be avoided by using nasal catheter during and after bronchoscopy while recording pulse oximetry. [6] Anesthesiology team provides comfort for patients and physicians performing procedure with anesthesia or sedation[7]. Safe sedation and amnesia during bronchoscopy will decrease unwanted physiology response related to fear of medical intervention. British thoracic society guidelines recommend sedation during bronchoscopy for all patients except ones with contraindications[8]. The best way of

achieving amnesia during bronchoscopy is giving iv. medications during the procedure with im. premedication[9]. While choosing sedatives Diazepam has several disadvantages as: long half-life, less induced amnesia, local pain and thrombophlebitis[10]. Using Midazolam in dose of 0.05mg/kg provides amnesia in 60% of patients, while using dose of 0.10mg/kg provides amnesia in 96% of patients and faster recovery from sedation[11]. The greatest effect of amnesia happens 2-5 minutes after iv application and lasts for 20-30 minutes[12] . For bronchoscopy we need light, or moderate sedation (> 2 on sedation scale) so called awaked sedation when patient is able to give verbal response, response to physical stimulation, can protect his airway, has adequate oxygenation, with preserved reflexes and stabile cardiovascular function.[13] During the sedation it is very important to monitor oxygenation, heart rate, respiratory rate, systolic, diastolic, mean arterial pressure, land evel of consciousness. Pulls oximetry warns if desaturation happens before the skin becomes blue and cyanotic with accuracy of +/- 2%.[15] Good patient communication and assessment of patient`s psychological stability before the procedure results with less patient`s anxiety. Premedication before procedure decreases fear, anxiety, reflex irritability, nausea, vomiting, and provides amnesia and comfort for patients.

We aimed to explore the influence of preoxygenation and premedication with midazolam during fiberoptic bronchoscopy on cardiovascular stability and amnesia.

Patients and methods

We conducted a prospective study at the Clinic for Pulmonary Diseases, University Clinical Centre Tuzla, between May 2017 and May 2018. We included 60 patients who underwent diagnostic fiberoptic bronchoscopy. 44 of them were male (73,3%) while 16 (26,7%) were female. All patients were in group I or II, according to ASA classification. Experimental group had 30 patients (24 males, 6 females) with mean age of 58.52+/- 8.6. All of them received 5 mg of Midazolam 1 hour before the procedure. Additional 1-3 mg of Midazolam is given intravenously in the operating room to induce light sedation. We assessed sedation using sedation scale (Table 1.) Control group had 30 patients (20 male, 10 female), with mean age of 56.07 +/- 6.02. They had fiberoptic bronchoscopy in local anesthesia without premedication.

Level of consciousness may or may not be decreased. Patient is able to give meaningful answers to verbal or physical stimuli. Patient is able to protect the airway, has adequate oxygenation and preserved

Table 1. Sedation scale

	Level of sedation	Level of consciousness	Verbal response	Tactile response	Airway	Ventilation with O_2
0	No sedation	Awake and alert	P	P	P	P
1	Light sedation	Mostly awake and alert but sedated	P-L	P	P	P
2	Moderate sedation	Poorly awake and alert, somnolent	L-A	P-L	P-L (small help may be needed)	P-A
3	Deep sedation	Unaware of himself and environment, weak response to stimuli	A	L (no pain)	L-A	L
4	General anesthesia	Without consciousness no response to stimuli	A	A (no pain)	L-A	L-A

P: present; L: limited; A: Absent;
Moderate sedation (sedation scale > 2)= awake sedation

Table 2.

1. What is the last thing you remember before you received sedatives for procedure?
2. What is the first thing you remember after procedure?
3. Do you remember anything in between?
4. What was the most uncomfortable during the procedure?
5. Would you accept to do procedure again in case needed?
6. For physician doing the procedure: Do you feel more comfortable doing the procedure if patient is sedated?

reflexes. Patients were prepared for local anesthesia according to standard protocol (application of local anesthetic in nares). We did trans nasal bronchoscopy with flexible fiberoptic bronchoscope Olympus (Olympus bronchovideoscope evis exera type 1T160, Tokyo, Japan). Using trans nasal catheter we applied oxygenation (3l/min) 5 minutes before bronchoscopy and 2 l/min during the procedure. Vitals were monitored constantly using Manual Patient Monitor Operator (33720 Monitor GIMA SPA B3, Bionet Co, Ltd, South Korea). 30 minutes after the procedure we did the test regarding the level of amnesia. We used the questionnaire made of 6 questions evaluating comfort, negative effects, acceptability, and accepting re bronchoscopy.

We used standard methods of descriptive statistics (student T test) to calculate mean value, standard deviation. Data were presented using central tendency and dispersion. The difference in variables between control and experimental group was considered significant if $p< 0.05$.

Results

We noted significantly different ($p<0.0001$) mean systolic blood pressure measured just before bronchoscopy among patients who received Midazolam premedication (125.33 +/- 9.37 mmHg) compared to patients from control group (139.16 +/- 6.66 mmHg). During bronchoscopy among patients from experimental group the increase in systolic blood pressure was 16,0 +/- 7,13 mmHg, while among patients from control group it was 26,73 +/- 13,61mmHg (mean value). We had an increase in SBP up to 10 mmHg in 3 patients from experimental group, but none from control group. Among patients who received Midazolam premedication an increase in SBP up to 20 mmHg was noted in 20 patients (74.07%); an increase in SBP from 21 to 30 mmHg in 5 patients (18,51), while an increase in SBP above 30 mmHg was noted in 2 patients (7,4%). The results showed significant difference between mean values of increase in SBP among patients from experimental and control group ($p<0.001$). An increase in SBP up to 20 mmHg was noted in 4 patients from control group (13,33%), while 17 (56,67%) of them had an increase in SBP from 21 to 30 mmHg. 9 patients (30%) who did not receive Midazolam premedication had an increase in SBP more than 30 mmHg.

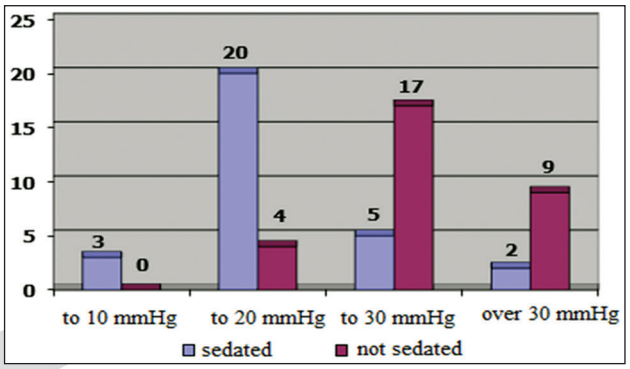

Figure 1. The change of systolic pressure during bronchoscopy in both groups

Initial measurements of diastolic blood pressure (DBP) were similar among patients from experimental and control group (76,27 +/- 5,72 mmHg, vs. 77,33 +/- 6,64 mmHg) p=0.1. During bronchoscopy among 5 patients (16,67 %)from experimental group we noted an increase in DBP up to 5 mmHg. In contrary, all patients from control group had an increase in DBP more than 5 mmHg.

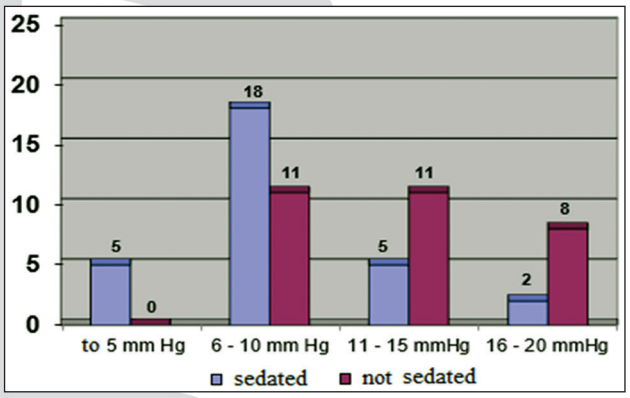

Figure 2. The change of diastolic pressure during bronchoscopy in both groups

18 patients (60%) from experimental group had an increase in DBP of 6-10 mmHg. The same level of increase in DBP was noted in 11 patients (36,67%) from control group. An increase in DBP of 11 to 15 mmHg happened among 5 patients (16,67%) from experimental group and 11 patients (36,67%) from control group. The highest increase in DBP (16-20 mmHg) was noted among 2 patients (6,67%) who received Midazolam premedication and 8 patients (26,67%) who did not (Figure 2). The mean value of increase in DBP during bronchoscopy among patients from experimental group was 7,73 +/- 3,14 mmHg, while among patients from control group it was 12,96 +/- 4,06 mmHg (p=0.002). Initial heart

rate before bronchoscopy was 78,67 +/- 11,03 beats/ per minute in experimental group, while 75,42 +/- 7,23 beats/per minute in control group. The lowest value of heart rate was 55 beats/per minute, while the fastest heart rate was 95 beats/per minute. 13,33% patients from experimental group did not have any changes in heart rate during bronchoscopy, or had insignificant decrease in heart rate. An increase in heart rate of 10 beats/ minute happened among 36,67 % patients from experimental group and 13,33% from control group. 5 patients from experimental group had an increase in heart rate of 16 to 20 beats/ minute, while the same increase was noted among 13 patients from control group. The highest increase in heart rate (more than 21 beats per minute) was noted among 6,67% patients from experimental group and 23,33% patients from control group. Our results showed significantly higher increase in heart rate (p= 0.01) among patients from control group (18,16 +/- 4,78 beats/minute) compared to patients from experimental group (13,33 +/- 4,66). In both groups the highest increase in hear rate was noted while bronchoscope was placed between vocal cords and at the beginning of trachea. Patients who received Midazolam premedication had shorter duration of increased heart rate and their heart rate become normal sooner compared to patients who did not have Midazolam premedication. Patients from control group experienced tachycardia at the end of the procedure while bronchoscope was removed from trachea followed by intensive cough that was way less present in experimental group.

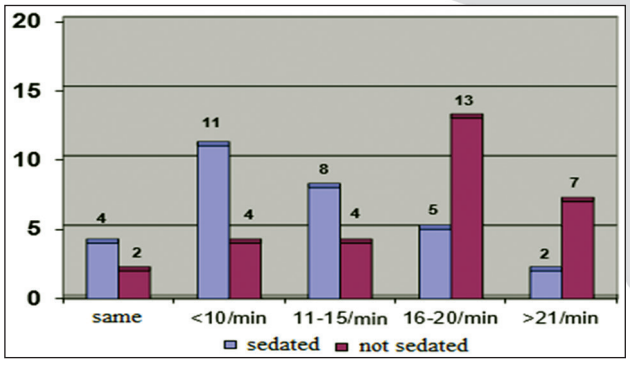

Figure 3. The change of puls rate frequence during bronchoscopy in both groups

Good patient-physician communication including patient education regarding procedure itself, and premedication 1 hour before bronchoscopy provides good physical and psychological support and makes procedure more comfortable for both, patients and physician performing procedure. Pleasant environment, and adequate team communication are also very important for successful outcomes. Thanks to above mentioned as well as premedication with midazolam 25 patients in our study (83,33%) reported amnesia regarding procedure and 20 of them (66.67%) would accept repeated bronchoscopy, if needed.

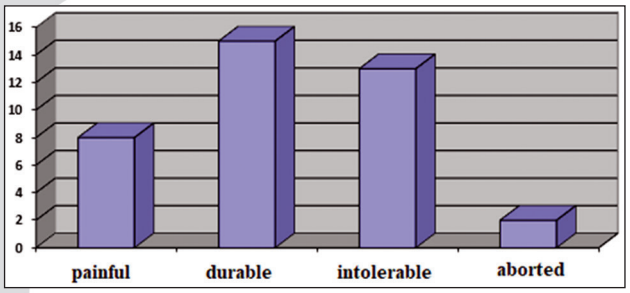

Figure 4. Acceptability assessment of FOB

4 patients (26,67 %) from control group reported bronchoscopy as painful. For 15 patients (50%) procedure was uncomfortable, but they were able to manage, 13 patients (43,33%) reported bronchoscopy as extremely unpleasant "almost impossible to make it" 2 patients could not make it through because it was too uncomfortable. (Figure 4.) 17 patients (56,67 %) would not probably accept repeated bronchoscopy if needed, while 9 of them (30%) confirmed they would certainly not do it again. We did not find statistically significant difference between duration of bronchoscopy among patients from experimental and control group. (19,5+/- 3,4 min, vs. 18,7 +/- 4,4 min. p=0,3).

Discussion

There are different approaches regarding patient`s preparation for fiberoptic bronchoscopy. Some of them do not use sedation at all, while in contrary some protocols use sedatives, opioids and antiemetics. Many hospitals use sedation as a routine, but ideal protocol is so far unknown. [16] Using high dose of sedatives is risky, but low dose does not make procedure more tolerable for patients comparing to no sedation at all. [17] It is necessary to take patient`s weight and general health in consideration while assessing initial dose with good collaboration between anesthesiologist and physician performing bronchoscopy. Sometimes it

is necessary to administer low initial dose of sedative and add additional doses during bronchoscopy itself until adequate sedation is acquired. Our results showed that premedication administered 1 hour before bronchoscopy decreased blood pressure measured just before the procedure for approximately 13,83+/- 2,71 mmHg. Mean value of SBP during initial measurement in experimental group was 125,33 +/- 9,37 mmHg, while in control group it was 139,16 +/- 6,66mmHg (p< 0.0001). Similarly, Mohana et al. reported significant increase in SBP, DBP and heart rate in patents, 5 minutes after starting the procedure (FOB) without significant change in oxygen saturation[18]. Several studies used high dose of Midazolam inducing deeper sedation which required anesthesiology team[19]. Williams et al.[20] used 5 mg of Midazolam and continuous O2 supplementation (3l/min.) in 123 patients that were sedated at the level of "light dream". 2 patients required Flumazenil because of prolonged desaturation and recovery period lasted for 2,5 hours. The authors concluded that level of acceptability of FOB is in direct correlation with sedation. Putinati et al. [21] used The Mental Alertness and Drowsiness protocol and they sedated their patients until Index 3 (somnolent). Their results showed that bronchoscopy was better tolerated with less side effects if sedation was used. Patient recovery period after sedation was 3 hours. These previously published results support our findings that sedation provides better acceptability of FOB and better induced amnesia. In contrary, other studies reported that FOB without sedation is also acceptable for patients and does not bring sedation risk. Pastisa et all. published a study that explored 3 groups of patients doing FOB (one with placebo, other using midazolam for sedation and the third one using remimazolam). The procedure started significantly earlier in patients who received remimazolam (6,4 +/- 5,82 minutes) compared to patients that received placebo (17,2 +/- 4,15 minutes)(p<0.0001) or midazolam (16,3 +/- 8,60 minutes). Time required for patient to become fully awake and alert was significantly shorter in patients who were treated with remimazolam (median 6,0 minutes; 95% CI: 5,2; 7,1) compared to placebo (13,6 minutes; 95% CI: 8,1; 24,0; p=0.0001) or midazolam (12 minutes; 95%CI: 5,0; 15,0.) [22] A systematic review that included 30 studies and 2319 participants explored the safety of midazolam (oral and parenteral) compared to other sedatives or placebo during endoscopy procedures including FOB. Comparing Midazolam and Diazepam (14 studies, 1069 participants) they did not find difference in the level of anxiety (RR: 0.8; 95% CI: 0,39) or pain (RR: 0.6; 95%CI: 0,24; 1,49; I^2: 67%). Midazolam induced better anterograde amnesia (RR; 0,45; 95% CI 0,30; 0,66). In comparison with placebo patients who were sedated with iv. Midazolam (5 studies, 493 participants) had less fear and anxiety (3/47 vs. 15/35). When using oral route of administration Midazolam still acquired less pain (2,56 +/- 0,49 vs. 4,62 +/- 1,49; p<0.005) and anxiety (1,52 +/- SD 0,3 vs. 3,97+/- SD 0,44; p<0.0001) compared to placebo. In contrary, two studies did not find significant difference in the level of anxiety when comparing Midazolam and placebo (1,7 +/- SD 2,4 vs. 2,6 +/- SD 2,9; p=0,216) [23] . Dreher at al. compared the effects of Midazolam alone and Midazolam in combination with alfentanil during FOB. Results showed that initial pO2 was higher in the group treated with Midazolam alone and the difference was even greater 2 hours after FOB (68,5 +/- 11.1 vs. 60.9 +/-8.2 mmHg; 95% CI-15/-0.4; p=0.039). 24 hours later there was no significant difference among groups (p>0.05). Combined sedation showed better tolerance during FOB (p<0.0001). Total dose of Midazolam was significantly lower (twice) if combined with alfentanil. [24] A few randomized controlled studies regarding sedation during bronchoscopy showed that patients often received inadequate dose of sedatives. In those cases, patients experienced bronchoscopy as very uncomfortable and 25 % of them did not want to repeat the procedure if needed. [25]

The results of our study showed that 25 patients from experimental group (83,33%, 22 male and 3 female) did not remember the procedure itself, and 20 of them (66,67%) would accept eventual repeated bronchoscopy. Hirosea et al. published a study conducted on 129 patients who did FOB and 65,8% of them reported that they would repeat bronchoscopy if needed (12,4% of them would do it for sure, while 53,45% would probably do it again). Interestingly, men were more satisfied regarding the fact that sedation reduced cough, pain, fear and induced amnesia. [26]. In study published

by Neumana et al.[27] duration of FOB (20 +/- 4SD) was similar to duration reported in our study.

Conclusion

Midazolam induced sedation during bronchoscopy is safe method and should be recommended to patient when indicated. Although amnesia regarding bronchoscopy has huge importance for patients, current literature is lacking studies that compare using sufficient dose of sedative to induce complete amnesia and lower dose of sedatives that provides less sedation but incomplete amnesia. Our study showed that good patient`s preparation for bronchoscopy provides better cardiovascular stability, less increase in heart rate and blood pressure. Patients who receive sedation during bronchoscopy are more likely to accept repeated procedure if needed. Sedated patient also enables faster and more precise work done by physician performing bronchoscopy.

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The effect of a 6 weeks aerobic and resistance training program on weight and body composition of sedentary University students

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Abstract

Introduction: In recent year's obesity has been increasing in an alarming rate, moreover obesity and overweight pose a significant public health problem for South Africa.

Aim: The aim of the study was to investigate the effectiveness of two commonly undertaken modes of exercise training (aerobic and resistance) in reducing body weight and improving body composition in sedentary university students.

Methods: A total of forty students who met the selection criteria, were randomly divided into two groups (n=20), this was achieve by random number generating. Subjects were ranging between ages of 20-30 years, with a BMI of above 25 and whom have had a stable body weight (+ or -2kg) in last three months and sedentary.

Results: Following an intervention Aerobic group showed no significant improvement in Body Mass Index and Body fat percentage (p>0,027) however there was significant improvement in body weight and Waist Hip circumference (p<0, 003). There was no significant improvement in body fat percentage in fat percentage and BMI as p-value was greater than 0, 05 (p= 0, 607113 and 0, 0815266 respectively).

Conclusion: The present study showed that a 6-week supervised aerobic training intervention is more effective in decreasing BMI, WHR and weight in students.

Key words: Overweight, Obesity, Sedentary, Aerobic exercise program, Resistance exercise program.

Introduction

Throughout the history of human beings, weight gain and increased body fat percentage have been viewed as signs of health and prosperity. However, with the continued rise in standard of living, weight gain and obesity is becoming a serious threat to public health concern in most countries (Savona-ventura and Savona-ventura, 2015). Most countries that are in economic transition from undeveloped to develop such as Brazil, China, India and South Africa in the last decade have experienced an increase in rate of obesity across all economic levels and age groups (Kruger, Puoane, Senekal and van der Merwe. 2005). A study conducted by Savona-Ventura (2015) show that there are a number of genetic determinants which have been identified as major contributing factors towards the development of obesity leading to insulin resistance. World Health Organisation (WHO) in 2016 reported that the prevalence of obesity amongst individuals that are 18 years and older was about 13% of the entire population of the world (650 million adults), furthermore 39% (1.9 billion) of adults worldwide are classified as overweight (Greydanus, Agana, Kamboj, Shebrain, Soares, Ake and Patel 2018). Statistics released by the South African department of health in 2016 shows that South Africa has an average of 30% obesity rate among adults with close to 70% of this statistic relating to women and 40% men, with almost 9% of South African children being considered obese or overweight. South African woman have the highest percentage obesity rate in the Sub-Saharan region.

In recent year's obesity has been increasing in an alarming rate (Lim and Mahmood (2015), moreover obesity and overweight pose a significant public health problem for South Africa (Averett, Stacey and Wang, 2014). WHO listed overweight as one of 10 leading risk factors for high mortality in developing and developed countries (Kruger *et al*., 2005). One of the major contributors to overweight/

obesity is globalisation which has created an environment that promotes consumption of food high in fat and sugar (Kruger *et al.*, 2005). Other main concern among South Africans especially women is sedentary lifestyle as reported by Mohammadi, Khoshnam & Khoshnam, (2018).

Sedentary lifestyle may lead to an increase in cardiovascular diseases and almost two million deaths around the world are caused by lack of exercising and hence exercise plays an essential role in reducing the risks of cardiovascular diseases (CVD) for example hypertension and diabetes. Many South African women even though they are aware of obesity they do not want to lose weight, this may hinder effective weight control management through physical activity (Kruger *et al*., 2005). Physical activity and diet has always been used as the way of lifestyle change; however with abundance high fat food supply and sedentary lifestyle (due to modern technology advancements) people eat and fail to maintain energy balance (Averett, Stacey and Wang, 2014). In trying to decrease obesity level people commonly choose dieting which leads to weigh-loss in short term, which resulting in a decrease in fat free mass leading to decrease in energy expenditure. Whereas with exercise, having post weight loss weight maintenance through the increasing fat free mass, resting energy expenditure and physical activity energy expenditure (Hintze, Messier, Lavoie, Brochu, Lavoie, Prud'homme, Rabasa-Lhoret and Doucet, 2018) and Beavers, Beavers and Martin, (2017) noted that the performance of regular musculoskeletal loading which is resistance training and also weight bearing exercise that fall under aerobic training have shown to lead to weight loss. In solving this health problem, it is highly recommended to participate in physical activity as both weight loss therapy and weight loss maintenance and for more pronounced result combination of exercise and diet is more advisable (Boule, Doucet and Tremblay, 2002).

Both aerobic training and resistance training can effectively improve obesity factors yet previous studies have specified that aerobic training is responsible for providing favourable effects on reducing obesity risks factors, whereas resistance training has shown results in increasing muscle mass and strength (Chen, Chung, Chen, Ho, & Wu, 2017). Research by (Chiu, Yang, Yang, & Chang, 2018) suggested that resistance training's effect on obesity have not been clearly quantified as a result of lacking extensive research, as only a few resistance training studies have been specifically designed for obesity, thus only a few have reported an increase in lean mass, and a few others have reported a reduction of body fat, while others declare that the effects were statistically insignificant. Thus, the importance of this is unquestionable as there are visible gabs that further need to be investigated. The present study aims investigate the effectiveness of both aerobic and resistance training programs in reducing body weight and improving body composition in sedentary university students.

Methods

To meet the three objectives described earlier, this study was consisting of two distinct parts, a descriptive survey to address the first objective and an intervention programme (experiment) to address the second objective. Every subsection under the research methodology was therefore including a reference to each of these.

Description and Selection of Participants

A selection criterion was developed, that the students need to meet to participate in the study. Information pamphlets were distributed randomly to the students attending the University of Zululand. A total of forty students who met the selection criteria, were randomly divided into two groups (n=20) to ensure balance in both exercise program, this was achieve by random number generating.

Subjects were ranging between ages of 20-30 years, with a BMI of above 25 and whom have had a stable body weight (+ or -2kg) in last three months and sedentary (no moderate to high intensity physical activity in the past three months). Lastly and most importantly subjects who were previously diagnosed with diabetes, metabolic, cardiovascular and musculoskeletal disorders were excluded from the study.

Measurement Instruments

Aerobic training (AT) was conducted three days a week at 60-85% of their maximum heart rate; each of AT sessions lasted for sixty minutes. As for Resistance Training (RT) we used the Derlome and Watkins technique of ten repetitions

Maximums (RM) and ii was done on alternate days for six weeks (Bailey, 2012).

Pre- and post-test sessions

After the completion of the informed consent forms, we performed a series of baseline tests i.e. heart rate, blood pressure, height, circumferences tests (weight, waist & hip ratio) and seven site skinfolds. We performed the same tests for post; same as pre-test sessions.

Resistance Training Program

The resistance training program was done 3-days/week, workouts were designed to train both the upper body and lower body once a week each respectively and on the alternative day it will be a full body workout with gradually increasing volume and intensity. The workout was consisting of multiple exercises including but not limited to the following: bench press, lateral pull-down, shoulder press, seated row, shoulder shrug, dip, biceps curl, triceps pushdown, leg press, squat, lunge, leg curl, leg extension and calf raise. These exercises were started at a very low intensity gradually increasing intensity with adaptations.

The upper body strength was assessed in assessing the progress, due to previous experiences by other researchers indicating that greater stability and reliability of data is present when assessing upper body vs. lower body activities. Subjects performed a general warm-up of 3-5 minutes light activity and static stretching exercises. Thereafter, subjects performed multiple lifts at a progressive manner to heavier weights ensuring that no overload occurs. The workouts were supervised by the study investigators and the study participants were given training exercise programmes to familiarise themselves with techniques of the workout.

Aerobic training

The aerobic training program was also a 3 day/week workout designed to train the cardiovascular system in a full body workout. The program included a warm-up for 5 minutes of stretching exercises, 45 minutes of aerobic training exercises like running at the first two days of the six-week program followed by intense running & sprinting between the maximum heart rate of 65%-85% and the cool down phase was 10 minutes of stretches.

The running & sprinting exercises were incorporated in a number of test; namely the yoyo test, illion agility test, 12-minute cooper test and sprinting shuttle run. The participants were introduced to more complex exercises once they had adapted to exercising and they were ready for agility testing. Workout were supervised and participants were given a week's exercise programme to familiarise themselves with the workout techniques.

Testing protocol

At commencement subjects were tested before they started the resistance training program and they were also required to perform all the tests done at the baseline tested every week in order to keep record of any changes in their body composition and body weight. Prior to pre-testing subjects were instructed not to do any strenuous exercise for 48 hours and also not to consume any foods or fluids with caloric contact 2 hours before testing because this was to interfere with their resting values due to metabolic processes. Tests were done in the following order; heart rate, blood pressure, height, circumferences tests (weight, waist & hip ratio) and seven site skinfolds.

The tests that were done before, during and after the intervention program. In week 6 subjects completed a questionnaire to monitor individual changes in delayed-onset muscle soreness, appetite and thirst, muscle cramping, stomach distress. Also blood pressure and heart rate was assessed.

Measurement Instruments

- Scale
- Skinfold calliper
- Stadiometer
- Mercury sphygmomanometer
- Cuff
- Stethoscope
- Tape measure

Results

Following an intervention, the aerobic group showed no significant improvement in BMI pre (31, 56) and post (32, 44) (figure 1 below), p-value (0.815) and body fat aerobic group had pre (33.09), post (29.22) (figure 2 below) and p-value (0.607). However, there was significant improvement in body weight aerobic pre (71.12), post

Table 1. Pre and Post average values for body composition and body weight

Variables	Resistance training group			Aerobic training group		
	Pre-Training	Post-training	P- Value	Pre-Training	Post-Training	P- Value
Body Weight	71,12	68,31	0.437	80,11	80,34	0.027
Waist Hip Ratio	0,74	0,70	0.363	0,79	0,77	0.003
Body Mass Index	27,20	25,95	0.328	31,56	32,44	0.815
Body Fat Percentage	29,58	23,74	0.123	33,09	29,22	0.607

(68.31) (figure 3 below) and p-value (0.027) and Waist Hip Ratio pre (0.79), post (0.77), (figure 4 below) and p-value (0.003). These results consist of the difference between values taken in pre-testing and post-testing. There was no significant improvement in body fat percentage fat percentage and BMI as p-value was greater than 0, 05 (p= 0, 607, and 0, 0815 respectively).

Resistance group showed no significant improvement in all body weight and body composition components, with values for body fat percentage being; pre (29.58) post (23.74) (figure 2), and a p-value of 0,123, while Waist Hip Ratio values were pre (0.74) post (0.70) (figure 4 below), with a p-value of 0,363. The Body Mass Index values were; pre (27.20) post (25.95) (figure 1 below) and a p-value of 0, 328, and body weight values being for pre (71.12), post (68.31) (figure 3) and have p-value of 0, 437. The results mentioned above can be viewed in table 1.

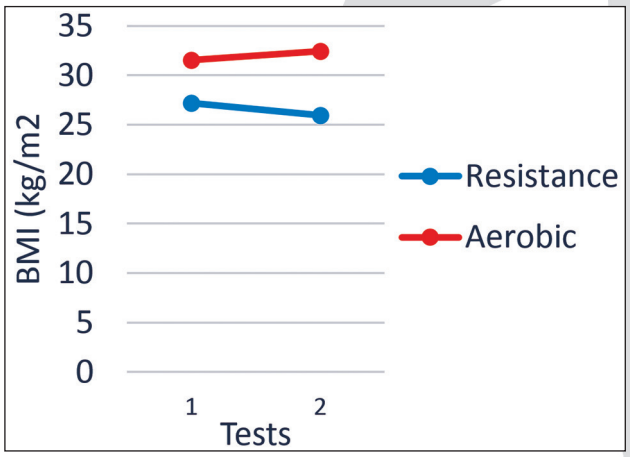

Figure 1. Pre and Post average comparison of BMI

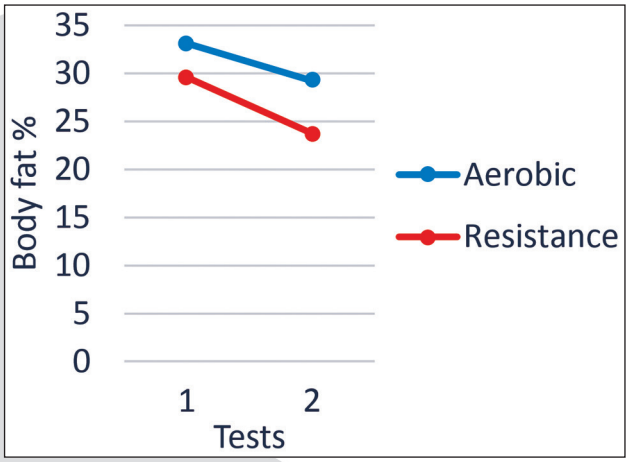

Figure 2. Pre and Post average comparison of Body Fat %

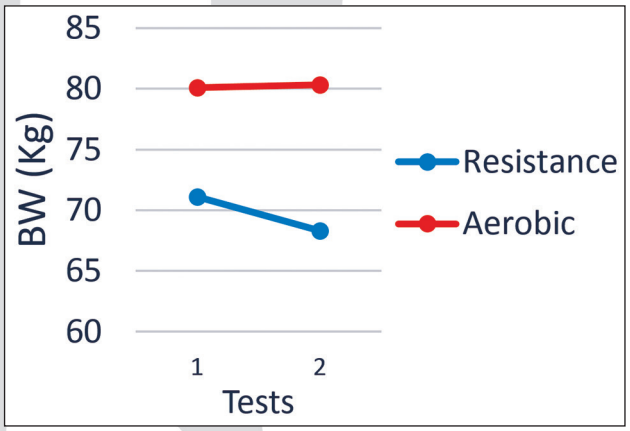

Figure 3. Pre and Post average comparison of Body Weight

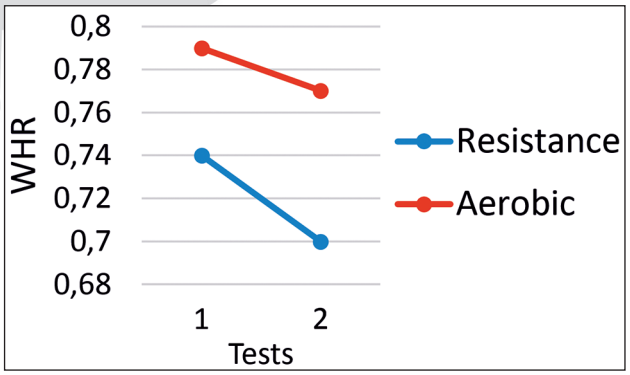

Figure 4. Pre and Post average comparison of Waist Hip to Ratio

Discussion

The aim of the study was to investigate the effectiveness of two commonly undertaken modes of exercise training (aerobic and resistance) in reducing body weight and improving body composition in sedentary university students. The primary findings showed that there were no significant changes in all component of resistance training which concur with the research by (Chiu, Yang, Yang, & Chang, 2018) which suggested that, for resistance training only a few have reported an increase in lean mass, and a few others have reported a reduction of body fat, while others declare that the effects were statistically insignificant. Bateman *et al.,* (2011) reported that resistance training expends fewer calories in each duration of exercise sessions compared to aerobic training, probably owing to the rest periods in between bouts of exercises therefore this inconsistency reduces total body fat thus supports the initial findings of the present study, overall little evidence exists that resistance training alone promotes weight loss.

Although there was no significant changes in body fat percentage in aerobic training but the exercise program prevented body fat percentage gain thus meaning significant body fat percentage loss is possible with aerobic training with no diet restriction but it requires a high training volume which may not be practical or sustainable for general population (Bateman *et al.,* 2013*)*. The current study showed that there was no significant changes in the body mass index from aerobic training which contradicts with Yang *et al.,* (2013) report that aerobic exercises may be associated with the greater body mass index reduction, this could be as a result of large sample size they had compared to the present study and also the duration of their intervention which was slightly longer than that of the present study.

The primary findings of this study showed that with aerobic training there was a significant change in the waist hip ratio and also significant changes in body weight which corresponds to the findings of Beavers and Martin, (2017) which alluded to observable weight loss as mentioned above earlier in text.

Conclusion

The present study shows that a 6-week supervised aerobic training intervention is more effective in decreasing WHR and weight in students. Although resistance training contributed to the reduction of body fat, the effect on overall weight loss was minimal. Overall little evidence exists that resistance training alone promotes weight loss. Most importantly subjects wishing to lose weight should participate in physical activity and have diet restrictions, with overall volume of exercise well above the minimum recommended levels.

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Contribution of depression and anxiety in explaining students' emotional, social and academic adjustment

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Abstract

An increased risk of mental health problems among students around the world has motivated this research, which focuses on the nature of the relationship between different indicators of student mental health and the construct of student adjustment. The main aim of the present paper is to examine the contribution of depression and anxiety in explaining emotional, social and academic adjustment of student from University of Tuzla.

The sample includes 811 respondents, of which 62.9% are female and 37.1% are male respondents. The age of respondents varies between 19 and 43 (M = 21.88; SD = 2.35; Sk = 2.97; Ku = 17.70). The respondents completed Beck's Depression Inventory (BDI), Spielberger's Stait-Trait Anxiety Inventory (STAI) and Student Adaptation to College Questionnaire (SACQ).

When it comes to the role of depression and anxiety in explaining student adjustment, the results show that depression and anxiety are significant predictors of all aspects of student adjustment, with the highest contribution to emotional adjustment (R = 0.67). Anxiety and depression can explain aspects of student adjustment in the range of 13% to 45%. Anxiety is consistent in all aspects of student adjustment as a more relevant predictor than depression.

Key words: depression, anxiety, emotional, social and academic adjustment

Introduction

Psychological distress among students has implications for many aspects of their well-being and may adversely influence their academic performance and quality of life. College students are in a transitional age, young adulthood, which is associated with numerous stressors. Academic pressure, irregular sleep patterns, flux in personal relationships, together with many other stressors typical for starting and attending college, may precipitate the first onset of mental health problems or an exacerbation of symptoms. Emotional problems manifested as general psychological distress, anxiety, low self-esteem, and depression are seen as main reasons for dropping out of college (Gerdes & Mallinckrodt, 1994). The results of numerous studies (Ibrahim, Kelly, Adams, Glazebrook, 2013, Roussis and Wells, 2008, Eisenberg, Gollust, Golberstein and Hefner, 2007, Kessler, et al. 2005) suggest an increase in the more severe symptoms of stress and anxiety, a double increase in the number of depressed students, and a triple increase in incidence of suicidal attempts. The research conducted in the US by the American Association for Mental Health Students (2006, American College Health Association Survey), suggests that anxiety and depression are consistently at the top of the student problem scale (Tartakovsky, 2008). Tartakovsky (2008) finds that even those students who do not have clinical symptoms of the disorder have major problems in their day-to-day functioning caused by a chronic feeling of depression and anxiety.

College adjustment can be considered a domain-specific form of subjective well-being because it addresses a student's functioning in college, adjustment to the college environment, and feelings of global satisfaction while in college (Schmidt and Welsh, 2010). Despite the relatively complex nature of the college environment to which students must adapt, there is currently substantial agreement among educational researchers as to the structure of the broad adjustment to college construct (Credé & Niehorster, 2012). Specifically, most research in this domain explicitly relies on the theoretical taxonomy of Baker and Siryk (1984), or examines adjustment to college constructs that form a subset of this taxonomy. Baker and Siryk base their taxonomy on a review of the adjustment to college literature, and argue that adjustment to college is characterized by four

types of adjustment: academic adjustment, social adjustment, personal–emotional adjustment and attachment to institution. Many educational researchers have viewed adjustment to college as an important outcome in its own right and have subsequently examined a wide array of factors as possible predictors or correlates of students' adjustment to the college environment, including depression and anxiety. A number of studies have examined the relation between depression and anxiety as a particular aspect of psychopathology and different aspects of student adjustment to college.

The studies of student adjustment that used Beck's Depression Inventory to assess level of depressive symptoms (Merryman & Zelezny, 1993, Wang & Smith, 1993, Dodgen-Magee, 1992, Montgomery & Haemmerlie, 2001, cited in Baker, 2004; Vivona, 2000, Wintre & Yaffe 2000) find that depression significantly contributes to adjustment to college, especially to the personal-emotional aspect of adjustment. In these studies, significant negative correlation is found between depression and adjustment to college, showing that students with a lower degree of depression are more adapted. These results are also consistent with numerous studies (Smith, 1994, Shibazaki, 1999, Hunsberger, 2000, Pratt, 2001, Veit & Hutto, 2001, cited in Baker, 2004, Beyers & Goossens, 2002) which use other depression measures. These studies also find a significant negative association between depression and various aspects of adjustment to study, consistently with the highest values in personal-emotional adjustment. When it comes to the relationship of anxiety and adjustment to college, the results of most of the research conducted are quite consistent with the research on depression and adjustment to college, and show the expected association with adjustment aspects. The most significant correlation is again found with the personal-emotional adjustment (Baker, 2004). Unlike the studies that treat depression as a unique phenomenon, the studies on anxiety and its role in adjustment treat various types of anxiety: anxiety as a state, as a trait, separation anxiety, and anxiety disorders. Using State-Trait Anxiety Inventory (STAI; Spielberger, 1983) and all the aspects of adjustment to college, Adan and Felner (1987) and Wang and Smith (1993, cited in Baker, 2004) find significant and substantial correlations in the expected direction between measures of both state and trait anxiety, the highest values tending to occur for personal-emotional adjustment, as did Kline (1992) using only state anxiety and Carlson (1986) trait anxiety. Shilkret and Nigrosh (1997) find significant correlations between the Brief Symptom Inventory's Phobic Anxiety subscale (Derogatis & Melisaratos, 1983) and personal-emotional adjustment.

Although there has been an increasing concern about depression and anxiety in specific groups such as adolescents or the elderly, the problem of university students' depression and anxiety has received relatively little attention, despite evidence of a steady rise in the number of depressed and anxious university students. Depression and anxiety symptoms are reported to be common among university students in many regions of the world and impact the quality of life and academic attainment, but there is little evidence of prevalence of depressive and anxiety symptoms among students in Bosnia and Herzegovina. Considering the extent of the problem of depression and anxiety, together with students' psychological adjustment, the aim of this study is to explore are depressive and anxiety symptoms factors that contribute to the student's personal-emotional, academic, social and institutional adjustment.

Method

Participants

The sample consisted of 811 students, 510 (62,9 %) females, 301 (37,1%) males) randomly selected from the University of Tuzla, Bosnia and Herzegovina. The mean age of students was 21.88 (*SD* = 2,35). There were no age differences between males and females (M_f = 21.87; M_m = 21,88; t = -.03, *df* = 804, *p* = .97; F_L =3.57; p_L = .06).

Measures

Beck Depression Inventory II (BDI-II; Beck, Steer, & Brown, 1996) was used to measure depressive symptom severity in students. It is a self-report inventory, consisting of 21 items representing symptoms of depression such as sadness, pessimism, self-criticalness, agitation, guilt, irritability, loss of appetite, changes in appetite, fatigue, change in sleep patterns, and loss of interest in sex. The items are rated on a scale ranging from 0 to 3, with higher scores indicating an increasing level of symptom intensity, for example, *I do not*

feel sad (0) to *I am so sad and unhappy I can't stand it* (3). The total BDI-II score ranges between 0 and 63, with the scores between 0 and 11 indicating minimal depression, the scores between 12 and 19 mild depression, the scores between 20 and 27 moderate depression, and the scores between 28 and 63 severe depression, (Beck et al., 2009). The English BDI-II demonstrated excellent internal consistency (Cronbach's alpha = .92). We have used the BDI-II version standardized in Serbian students (Beck, Steer, & Brown, 2009) which has also proved high internal consistency (Cronbach's alpha = .90). Good scale reliability (.89) was also obtained in the present study.

State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1977) consists of a 40item self-evaluation questionnaire which includes separate measures of state and trait anxiety. In this study, only the Trait-Anxiety scale was used. The Trait-anxiety scale consists of twenty statements that assess how people "generally feel" about anxiety. All items are rated on a 4-point scale where trait anxiety items assess frequency of feeling in general (1 = almost never, 2 = sometimes, 3 = often, and 4 = almost always). The range of scores is from 20 to 80, the higher the score, the greater anxiety (Spielberger et al., 1983). In the present study the scale demonstrated very good internal consistency (Cronbach's alpha = .89).

Adjustment to college was assessed using *The Student Adaptation to College Questionnaire* (SACQ) (Baker & Siryk, 1989). The SACQ is a 67 item self-report scale with four subscales that measure academic adjustment, social adjustment, personal emotional adjustment, and goal commitment/institutional adjustment. A shortened version of the questionnaire with 64 items on four subscales (academic adjustment, social adjustment, emotional adjustment, and institutional adjustment) was applied. The original instrument was shortened only for those items that do not refer to all participants (living in campus, roommate relations, and a feeling of loneliness for leaving home). The items are rated on a 9-point Likert scale from (1) *does not apply to me* to (9) *fully applies to me*. Higher scores indicate better college adjustment. The SACQ has a full-scale reliability ranging from .92 to .95, and subscale reliability from .77 to .91 (Kramer & Conoley, 1992). Good full-scale reliability (.90), and subscale reliability from .74 (social adjustment) to .84 (academic adjustment), was also obtained in the present study.

Participants completed a demographic questionnaire and reported on their gender, age, residency status and Grade Point Average. Residency status refers to a possible change in their place of residence because of college attendance, and also information on the current residency cohabitation (alone, with a roommate, with parents, etc).

Procedures

The participants were selected using the method of random sampling according to the official list of students. The participants selected were informed about the study via email and then directed to an online platform for research participation. Those who wished to complete the study followed a link to the online questionnaire that included the measures mentioned above, as well as several other measures. The participants provided informed consents prior to completing the questionnaire. They were informed that the questionnaire was designed to assess personality and individual differences.

Results

Descriptive statistics for variables treated in the study are shown in Table 1. The internal consistency reliability for all scales is satisfactory and above the conventional criterion (Cortina, 1993; Nunnally & Bernstein, 1994; Field, 2017). The distribution of the scales does not deviate from the normal distribution model except in the case of depression scale (D'Agostino-Pearson D = 327.09; p <.001) and attachment to institution scale (D'Agostino - Pearson D = 150.08; p <.001). In accordance with established practice (Sheskin, 2011; Yap & Sim, 2011), data are normalized using root transformation. After normalization of the results, the distribution of the scales was reduced in the normal distribution frames (Sk and K in the range ± 0.5).

The dispersion of the results on social adjustment and attachment to institution is moderately expressed, while the dispersion of the results on the scales of academic and emotional adjustment is more expressed. Relationships between anxiety, depression and adjustment measures are shown in Table 2.

Table 1. Descriptive statistics for results on the depression scale, anxiety scale and scales of student adjustment

	α	Ni	M	SD	S_k	K
STAI	.89	20	40.87	9.38	.57	.17
BDI	.89	21	9.93	8.18	1.29	.12
BDI*	-	21	2.85	1.33	.12	-.08
Academic adjustment	.84	24	140.34	25.94	-.00	-.04
Social adjustment	.74	17	107.54	16.39	-.42	-.00
Emotional adjustment	.82	15	80.69	21.84	-.05	-.44
Attachment to institution	.78	14	93.98	14.22	-1.07	1.14
Attachment to institution*	-	14	4.69	1.42	.30	-.05

*Legend: α – Guttman-Cronbachov coeficient ; N_i – number of items; M – arithmetic mean; SD – standard deviation; S_k – skewnes; K – kurtosis; *scale results after root transformation.*

Table 2. Intercorellations among anxiety, depression and adaptation to college measures

	Academic adjustment	Social adjustment	Emotional adjustment	Attachment to institution	STAI	BDI
Academic adjustment	-	.58**	.50**	.65**	-.44**	-.42**
Social adjustment		-	.29**	.73**	-.38**	-.33**
Emotional adjustment			-	.34**	-.62**	-.60**
Attachment to institution				-	-.35**	-.31**
STAI					-	.68**
BDI						-

** *p<.01*

All measures of student's adjustment are statistically significantly correlated, and variance redundancy ranges from 8% to 50%. Anxiety and depression significantly negatively correlate with all adjustment to college measures, what is expected because depression and anxiety scales indicate symptoms of maladjustment.

Simultaneous Multiple Regression is used to determine the influence of depression and anxiety on aspects of student adjustment. A separate predictive model was created for each aspect of adjustment to college.

The combination of anxiety and depression influences has a statistically significant impact on the explanation of academic adjustment ($R = .47$, $F(2,804) = 115.01$, $p < .001$, $\Delta R^2 = .22$ with 95% IC(.17-.27)), and regression model explains around 22% of variance. A somewhat modest influence of predictive variables is detected in the estimation of social adjustment, and about 15% of variance is explained ($R = .39$, $F(2,804) = 73.01$, $p < .001$, $\Delta R^2 = .22$ with 95% IC(.10 -.20)). The smallest potential for prediction was achieved in the estimation of attachment to institution, where the prediction model explained only around 13% of variance ($R = .36$, $F(2,804) = 61.03$, $p < .001$, $\Delta R^2 = .13$ with 95% IC(.09-.17)). Due to the relatively high negative correlation of emotional adjustment with depression ($r = -.60$) and anxiety ($r = -.62$), the most potent regression model was an evaluation of emotional adjustment where about 45% of variance was explained ($R = .67$, $F(2,804)=336.88$, $p<.001$, $\Delta R^2=.45$ with 95% IC(.40-.49)).

Partial contributions of predictors anxiety and depression for all four regression models are shown in Table 3.

Partial contribution of predictors in all regression models is statistically significant ($p <.001$), although it should be emphasized that in general partial contributions of anxiety and depression are very modest (except in the case of emotional adjustment). Consistently, anxiety proved to be a more relevant predictor than depression to all aspects of student adjustment. The squared part correlation sr^2 (Tabachnik & Fidell, 2013, Warner, 2013) was separated as a measure of a unique effect size in explaining student adjustment. The depression scale has a individual contribution to explaining as-

Table 3. Partial contribution of depression and anxiety to student's adjustment

		B	SE B	β	t	p	sr^2
Academic adjustment	BDI	-4.45	.82	-.23	-5.40	.001	0.03
	STAI	-.797	.11	-.29	-6.82	.001	0.04
Social adjustment	BDI	-1.57	.54	-.13	-2.88	.001	0.01
	STAI	-.51	.07	-.30	-6.71	.001	0.04
Emotional adjustment	BDI	-5.29	.59	-.32	-8.99	.001	0.05
	STAI	-.94	.08	-.40	-11.29	.001	0.09
Attachment to institution	BDI	-1.32	.48	-.12	-2.75	.001	0.01
	STAI	-.41	.07	-.27	-6.01	.001	0.04

Legend. B – unstandardized regression coefficient; SE B – standard error of B; β – standardized regression coefficient; t – t ratio; sr^2 - squared part correlation

pects of adjustment to study in the range of 1% to 5%; while anxiety contributes from 4% (academic, social, and attachment to institution) to 9% of variance, in the case of emotional adjustment.

Although regression models do not look particulary significant and interesting, it should be taken into account that prediction is based on only two measures. Therefore, the influence of anxiety and depression in explaining individual aspects of student adjustment (in the range of 13% to 45%) is not negligible. Despite the individual influence of predictors is relatively modest considering the prognostic effect size, the individual contribution of depression and anxiety in all analyzed models is statistically significant in explaining student adjustment. The high redundancy of predictors anxiety and depression (about 46%) suppressed the greater individual contribution of these variables in explaining individual aspects of adjustment to the study.

Discussion

Baker (2004) states that numerous researchers have examined the relationship between depression and adjustment to college. Most of these studies find that, as expected, there is a relationship with all aspects of the adjustment to college, the highest being the emotional adjustment. The results of most the studies conducted on the relationship between anxiety and adjustment to college are quite consistent with the studies on depression and adjustment to college, and show the expected correlation with the aspects of adjustment. The most important relationship was again found with the emotional aspect of adjustment (Baker, 2004).

The results of this research do not deviate from the previous results. It has been shown that intercorrelations between all adjustments to colleeg measures are statistically significant. Redundancy variance ranges from 8% to 50%. The negative correlation of anxiety and depression and all adjustment to college measures is statistically significant. The degree of correlation between anxiety and various aspects of college adjustment ranges from -.35 (attachment to institution) to -.62 (emotional adjustment), and depression and various aspects of adjustment measures ranges from -.31 (attachment to institution) to -.60 (emotional adjustment). Significant negative correlations show that students with a lower degree of depression and anxiety are more adapted, especially emotionally. These results are consistent with the finding of numerous other studies (Smith, 1994, Shibazaki, 1999, Hunsberger, 2000, Pratt, 2001, Veit & Hutto, 2001, cited in Baker, 2004, Beyers & Goossens, 2002)

The predictor effect of the anxiety and depression on various aspects of adjustment to college was examined through the regression analysis. Of all aspects of adjustment emotional adjustment is best explained with anxiety and depression, and the above constructs can explain around (R = .67) 44% of the variance of emotional adjustment. The scores on anxiety and depression scales enables the prediction of 22% (R = .47) variance of academic adjustment. Social and institutional adjustment are more modestly explained: social adjustment is explained with around 15% (R = .39), while institutional adjustment is explained with around 13% (R = .36) variance. These findings are congruent with the results of numerous studies in which Beck's in-

ventory was used to assess depression (Merryman & Zelezny, 1993, Wang & Smith, 1993, Dodgen-Magee, 1992, Montgomery & Haemmerlie, 2001, cited in Baker, 2004; Vivona, 2000, Wintre & Yaffe, 2000), which also claimed that depression significantly contributes to adjustment to college, in particular to the emotional aspect of adjustment. These results also agree with numerous studies (Smith, 1994, Shibazaki, 1999, Hunsberger, 2000, Pratt, 2001, Veit & Hutto, 2001, cited in Baker, 2004, Beyers & Goossens, 2002) which used other measures of depression, where a significant negative correlation between depression and various aspects of adjustment to college was found, again consistent with the highest values in the aspect of emotional adjustment.

Unlike the studies that treated depression as a unique phenomenon, the studies that examined anxiety and its role in adjustment to college addressed different types of anxiety: anxiety as a condition, a trait, separation anxiety, and anxiety disorders. Using Spielberger State-Trait Anxiety Inventory Adan & Felner (1995) and Wang & Smith (1993, cited in Baker, 2004), found a significant negative correlation between (and contribution to) all aspects of adjustment and anxiety as a state, but also anxiety as a trait. The more significant correlation was shown with an emotional adjustment to the college. Kline (1992, cited in Baker, 2004) found the same relation, using the anxiety as a state only, and Carlson (1986, cited in Baker, 2004) using the anxiety as a trait only. In some studies, the relationship between individual aspects of adjustment and anxiety was examined. Using only the dimension of academic adjustment, Lopez (1989) found a significant negative correlation with anxiety as a trait, while Oliver, Reed and Smith (1998), using the scale of emotional adjustment only, also found a significant correlation with anxiety.

In this study, the partial contribution of the anxiety and depression predictors in all regression models is statistically significant ($p < .01$), although it should be noticed that in general the partial contributions of anxiety and depression are very modest (except in the case of emotional adjustment). Anxiety proved to be a more relevant predictor than depression in all aspects of student adjustment.

It should be emphasized that in this study and in most of the studies that examined the relationship of depression and adjustment to study, the measures of depression and anxiety were applied after the enrollment and later semesters, that is, at a time when students had already had the experience with examinations, assessment, and various aspects of student life. The findings of such studies observe depression and anxiety as the outcome of the adjustment to the college, and not as its cause. It is necessary to apply these measures in the longitudinal design of the research, because only the implications of such research could answer the question of whether depression and anxiety are the causes of poor adjustment to the college, or its outcomes. Such a study would also be useful for further clarification of the relationship between emotional problems and adjustment to college, and would give implications for appropriate corrective measures.

Symptoms of anxiety and depression often occur in transitional periods of life, such as starting kindergarten, primary school, going to high school, entering college, employment, marriage, and other situations in which major changes occur, as these are new and unknown situations, and they require adjustment. This phenomenon is particularly noticeable at the beginning of college education, during college and its completion, which can be said to be altogether a cumulative normative event.

College requirements are a major source of stress, since academic achievement is important for the future, further education or employment. During this period, an increase in the symptoms of anxiety and depression occurs. A fear of failure in the studies, anxiety related to taking exams, difficulties related to learning and taking exams, irrational use of study time and a feeling of ineffective studying, the pressures and expectations of the family, all arise as problems that students find quite burdensome and worrisome. If these states, with their character and intensity, correspond to the given situation and when their effects are not such that they make the individual disoriented or unadjusted, they can represent normal phenomenology. However, if a person cannot do anything in a situation when he/she becomes anxious and depressed, he/she becomes burdened with his/her condition, useless, and dysfunctional. These conditions are exhausting and create difficulties in adjustment of the individual to their everyday

life. Such conditions, with a sense of personal inefficiency in the study, can seriously interfere with the quality of studying. They can be an obstacle to achieving appropriate academic success, and possibly be a factor that significantly influences the decision to leave college.

Considering the representativeness of the sample used in this research, it is justified to generalize the results to the student population. Therefore, the findings of this research should be taken into account in order to direct the attention of school psychologists and mental health professionals to design adequate learning programs and accomplishing academic requirements, prevention, treatment and assistance to students during admission to college and continuation of studies.

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A randomized-controlled clinical study of Telos95®, a novel antioxidative dietary supplement, on the shortening of telomere length in healthy volunteers

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Abstract

Introduction: The objective of this study was to determine the deodorant effectiveness of a dietary supplement to halt the shortening of telomere length as measured through blood samples before and after product use.

Patients and methods: This study was a randomized controlled design in fifty (50) healthy adult subjects. Qualified subjects provided a small blood sample via finger stick at their baseline visit. The blood samples were sent to Telomere Diagnostics Inc. for analysis of telomere length which is used to calculate average telomere length (ATL). Subjects were randomized into one of two treatment groups: test article once per day (group A) or twice per day (group B). Subject were provided the test article, a diary to ensure compliance and instructions for use of the test product.

Results and discussion: A total of fifty (50) subjects completed all aspects of the study. Group A decreased in their TeloYear age on average by 7.43 years. Group B decreased in their TeloYear age by 8.52 years. Average telomere length (ATL), also represented as the T/S ratio, showed a baseline measurement of 0.85 but after taking the product for 6 months it increased to 0.95. Group B baseline ATL measured 0.82 at baseline and increased to 0.94 post 6 months of test article usage.

Conclusion: These results suggest that Telos95® supplementation can be an effective and safe approach to halt the shortening of telomere length.

Key words: antioxidative, clinical study, dietary supplement, telomerase, telomere, Telos95, randomized-controlled.

Introduction

Telomeres are functional complexes at the base of eukaryotic chromosomes. They help to maintain and prevent deterioration of the cells[1]. Shorter telomeres have been shown to be the cause of aging and become shorter due to the aging process and an unhealthy lifestyle[2]. The shorter the telomere becomes, the less it can prevent cell death due to the cell no longer being able to divide properly[3].

Telomerase consists of RNA subunits and proteins which aids in elongating telomeres. This lengthening process occurs by adding DNA base-pair sequences to the ends of chromosomes[4].

The objective of this study was to assess the efficacy of a dietary supplement to prevent the telomere shortening when taken over a six-month period. The study compared the baseline telomere results for subjects to results obtained after six months of test article usage[5].

The analysis measures individual's average telomere length (ATL) and provides a "TeloYear" age based on how the subject's Average Telomere Length (ATL) compares to others of the same age and gender[6].

Patients and methods

Selection of Subjects

Screening

An adequate number of subjects were screened and enrolled so that a minimum of 50 subjects would complete the study. Subjects had to satisfy the following inclusion and exclusion criteria, and had to give written informed consent.

The suitability of each subject to participate was confirmed prior to their acceptance onto the study by completion and review of a study specific inclusion and exclusion criteria as well as an assessment by the expert grader and subjective assessments.

Inclusion criteria

a. Healthy volunteers, 30 to 60 years of age;
b. Completed written informed consent containing HIPAA authorization.

Exclusion criteria

a. Female subject is pregnant, nursing or planning to become pregnant (Verbal response only)
b. Subject is an insulin-dependent diabetic.
c. Subject has a disease which, in the opinion of the Investigator, would compromise the safety of the subject or the validity of the study outcome.
d. Subject has a heart condition or a history of heart issues, which, in the opinion of the Investigator, would compromise the safety of the subject or the validity of the study outcome.
e. Subject has a medical history which, in the opinion of the Investigator, would compromise the safety of the subject or the validity of the study outcome.
f. Subject has a history of any malignancy or tumor.

Prohibitions and Restrictions for the Duration of the Study

a. Subject agrees to attend all test facility appointments and follow all study instructions.
b. Subject agrees not to use any antioxidant supplements or dietary supplements that are intended for telomere enhancements for the duration of the study.

Subject Withdrawal

The participation of a subject in this study may have been discontinued for any of the following reasons:

- An adverse event that requires study article to be discontinued;
- Withdraws consent to continue participation in the study;
- Protocol violation (including lack of compliance);
- Other reasons, such as administrative reasons.

Subjects who prematurely dropped out of the study were not replaced.

Test articles

The test article was: Telos95® Dietary Supplement.

Table 1. Parameters of randomization.

Randomization – Amount of Daily use of Test Article	
A	Usage of product once a day (1 capsule of 65 mg each)
B	Usage of product twice a day (2 capsules of 65 mg each)

The test article was used according to the use instructions.

Study procedure

Visit 1 – Screening

A sufficient number of were screened and enrolled on to the study to ensure that a minimum of 50 subjects completed all phases of the study. Qualified subjects had a small blood sample collected via finger stick using the telomere diagnostic collection kit provided by the diagnostic lab (Telomere Diagnostics Inc.). Subjects were assigned to one of two treatment groups (A or B) according to the randomization. Group A was instructed to take one capsule once a day, preferably with a meal. Group B was instructed to take one capsule twice a day, one capsule in the morning and one capsule in the evening and each preferably with a meal. Blood sample collection kits were sent to Telomere Diagnostics Inc. for analysis. The test products were to be taken daily at home for the following 6 months of the study according to the usage instructions provided. Subject were given a diary to fill out every day each time they took the product.

Visit 2 – Month 6 – End of Study

Subjects returned to the test facility following six months of test product use. Adverse events were reviewed and recorded. Subjects were asked to return any unused test product including empty

bottles. Subject compliance with the study instructions and restrictions were assessed and completed diaries were reviewed. A second blood sample (via finger stick) was collected from each subject using the telomere diagnostic collection kit. These samples were sent to the diagnostic lab (Telomere Diagnostics Inc.) for analysis. After the visit was completed, the subject's participation was considered final and they were compensated for their participation.

Study Evaluations

Blood samples (via finger stick) were collected using a collection kit provided by the telomere analysis lab. Samples were collected at baseline and sent to Telomere Diagnostics Inc. for analysis. After 6 months of test article use (either once a day or twice a day) subjects returned to PCR for blood sample collection. Blood samples Analysis of the telomere to determine the average telomere length (ATL) was conducted using the Cawthon qPCR assay.

The basic theory is that the ratio of the telomeric signal vs. the single copy gene signal reflects the average length of the telomeres per cell in the sample (Telomere Diagnostics, Inc.). The results of the ATL were used to assign a TeloYear age to each subject based on the comparison of their ATL to others in their same age and gender.

Study ethics

Ethical Conduct

This study was conducted in compliance with applicable Good Clinical Practice (GCP) Regulations, the Standard Operating Procedures of Princeton Consumer Research and the Sponsor's Protocol and Protocol Amendments.

The Sponsor was responsible for the ongoing safety evaluation of the investigational products and will promptly notify participating Investigators and regulatory authorities of findings that could have adversely affected the safety of subjects, impact the conduct of the study, or alter the IRB's approval to continue the study.

Subject Information and Consent

Subject consent was obtained prior to participation in any study conduct as required by the regulatory guidelines (21 CRF Part 50). Subjects were given ample opportunity to read the consent form and have all questions regarding study conduct answered prior to signing the consent form. Each subject was provided with a copy of the ICF to retain for his or her records. The original signed ICF was retained on file at the study center.

Authorization to Disclose Protected Health Information

Subjects were informed of the following information: The purpose of the protected health information (PHI) being collected, the possibility that the PHI may be re-disclosed, the duration of the authorization, the right to revoke the authorization, and the right to refuse signature and limit access to PHI during and following the conduct of the trial. Written authorization to disclose PHI was incorporated into the informed consent process and was obtained prior to the subjects entering the study per Princeton Consumer Research standard operating procedures (SOPs). Each subject was provided with a signed copy of the authorization and the original was retained on file at the study center.

Indemnity provision

The Sponsor was responsible, without regard to legal liability, and would indemnify Princeton Consumer Research Corp., or any of their respective officers or employees in the event of claims for compensation from subjects suffering injury or other deterioration in health or wellbeing as a result of participation in this study, except and insofar as such claims arise as a result of any negligent act or omission on the part of Princeton Consumer Research Corp. employees or any persons undertaking or involved in the study by arrangement with Princeton Consumer Research Corp.

Results and discussion

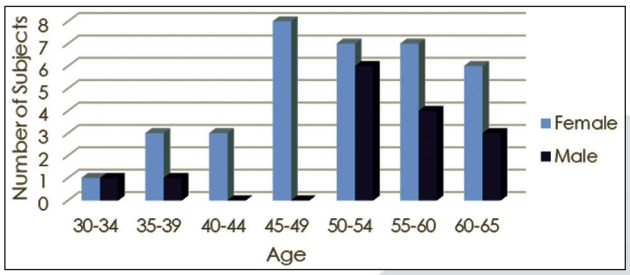
Study data

Location and dates of the study

The study was conducted according to the protocol at Princeton Consumer Research Corp. from 23 March 2017 to 25 April 2018. The original screening was on 23 Mar 2017. The subjects were reconsented and additional subjects were screened and consented for those subjects that didn't reschedule their second visit on 24 Oct 2017.

Subjects

Fifty-nine (59) subjects were screened and enrolled and fifty (50) subjects completed the study. The graphic 1 shows the subjects demographics details.



Graph 1. CERTEL1F Demographics

Adverse Events and Severe Adverse Events

There was one Serious Adverse Event on the study that was not related to the Test Product. Subject 45 was hit by a car and was admitted to the hospital. She suffered a broken pelvic bone and a fractured femur.

Discontinued Subjects

Twenty-six (26) subjects discontinued participation on the study. The table above shows the details on discontinued subjects.

Table 2. Discontinued subjects.

Subject number	Reason for discontinuation
09, 29	PI dropped due to medical history
06	Subject withdrew consent
11, 12, 13, 14, 16, 17, 20, 21, 22, 27, 34, 35, 36, 43, 47, 48, 51, 52, 55, 58, 73, 80	Lost to follow-up. Unable to attend all visits.
49	Subject dropped due to actions on another study. Subject no longer allowed to participate in studies at PCR Corp.

Average TeloYears age decrease

Group A decreased in their TeloYear age on average by 7.43 years. Group B decreased in their TeloYear age by 8.52 years. The tables below show the details on age decrease in both groups.

Table 3. Decrease in TeloYears Age.

Subject number	Group A
2	-11
3	-3
4	1
18	-4
26	-21
32	0
37	-4
38	-13
40	-12
44	-9
45	-6
46	-30
53	1
54	-10
59	-27
61	0
65	-22
74	18
75	-20
77	0
79	1
81	0
82	0
Average	-7.43

Subject number	Group b decrease in teloyears age
1	0
5	-23
7	1
8	2
10	-21
15	0
19	-12
23	15
24	0
25	-1
28	-30
30	-17
31	-28
33	-8
39	0
41	-12
56	0
57	-11
62	-24
63	0
64	-11
66	-7
68	0
70	0
71	-22
76	6
78	-27
Average	-8.52
P value	7.42E-01

Conclusion

A total of fifty (50) subjects completed all aspects of the study. Group A decreased in their TeloYear age on average by 7.43 years. Group B decreased in their TeloYear age by 8.52 years. Average telomere length (ATL), also represented as the T/S ratio, showed a baseline measurement of 0.85 but after taking the product for 6 months it increased to 0.95. Group B baseline ATL measured 0.82 at baseline and increased to 0.94 post 6 months of test article usage.

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In vitro study in Caco-2 cells shows high bioavailability of ATAMg, a novel source of magnesium acetyl taurate

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Abstract

Introduction: Magnesium absorption is essential for the maintenance of magnesium levels in the body, since excretion is poorly regulated. Dietary factors can influence magnesium absorption including low molecular weight substances. Both *in vivo* and *in vitro* assays may be carried out to study magnesium absorption. Studies *in vivo* have the drawback of dealing with a complex system in which it is difficult to determine the relative importance of different factors. In *vitro* cell culture models can overcome this difficulty.

Material and methods: The Caco-2 line, derived from a colon carcinoma, is able to differentiate spontaneously when grown in standard culture conditions. The differentiated cells polarized, formed microvilli and T-junctions associated with the duodenal enterocytes brush border.

Results and discussion: This cell line represent an appropriate model for the study of transport mechanisms related to the intestinal barrier and can be used to study the absorption of nutrients especially magnesium, in relation to dietary intake in particular pertaining to dietary factors that may affect absorption. In this work, we have used differentiated Caco-2 cells grown in bicameral chambers as an intestinal cell model to study the absorption of magnesium from different sources.

Conclusion: Transfer of magnesium across the monolayers in the apical-to-basolateral direction has been found to be greater from ATAMg, a new source of magnesium acetyl taurate. This study has also shown that Caco-2 method can be applied as an *in vitro* method to investigate not only magnesium bioavailability but to other minerals as well.

Key words: ATAMg, absorption, bioavailability, Caco-2 cells, dietary supplement, magnesium acetyl taurate.

Introduction

Bioavailability refers to the proportion of an ingested nutrient from foods or meals that is absorbed and utilized for normal physiological function and/or storage. It is affected by various physiological parameters in the animal and human that consumes the food, but more important from the practical point of view, bioavailability of many nutrients is modulated by other dietary components[1].

These dietary components may be an organic compound that binds the nutrients or another metal which interacts with it. Prior treatment of food and food products such as in cooking and processing may also affect bioavailability of nutrients[1,2].

Apart from dietary factors, physiological factors unrelated to the properties of the foodstuffs are also likely to influence the properties of a nutrient being absorbed. These include such factors as the efficiency of digestion, the previous intake of the nutrient, gut transit time and the presence of gastrointestinal disorder or disease. Nutritional status of an individual is of paramount importance in determining bioavailability as absorption often parallels to the individual's needs. In general, absorption is increased in deficiency and depressed in overload[2].

From practical view point, determining the bioavailability of a mineral is complicated by the fact that once a food is consumed, it mixes in the gastro-intestinal tract with other foods that are consumed at about the same time or may be because the minerals are present in a mixture of sources available in the diet or meal. Furthermore, minerals contained in a meal may also mix with the remains of the previous meal. The use of extrinsic radio minerals tagging and stable isotopes in humans has partly offers a solution to this problem[3].

Apart from the advantage of using an intact biological system, it has greatly advanced our

knowledge on factors affecting the absorption of minerals especially that of magnesium. However, these measurements are cumbersome, time consuming and costly to perform. Furthermore, it is difficult to determine the relative importance of different factors if environmental conditions are not controlled properly[4].

These limitations have restricted the use of mineral absorption studies in human for the purpose of identifying specific biochemical components of the diet that explain the inhibitory or enhancing nature of certain foods. The measurements would be greatly facilitated by the development of a simple *in vitro* screening method to assist in characterizing the biochemical basis of food magnesium bioavailability. In addition, understanding metabolic pathways of minerals as affected by dietary factors could be further elucidated in order to formulate diets[5].

Caco-2 cells line, derived from a colon carcinoma, is able to differentiate spontaneously when grown in standard culture conditions. The differentiated cells polarize, form microvilli and secrete enzymes associated with the duodenal enterocyte brush border such as sucrase-isomaltase, alkaline phosphatase, alkaline phosphatase, lactase and aminopeptidase[6].

This cell line represents an appropriate model for the study of transport mechanisms related to the intestinal barrier and for investigating diverse problems of nutrients bioavailability and absorption without concern for differences between human and animals.

Material and methods

Caco-2 cells, colon carcinoma cell line was obtained from American Type Culture Collection (ATCC), USA. Caco-2 cell were routinely cultured in 25 cm2 tissue culture flask (Costar, UK) with Dulbecco's modified Eagle medium (DMEM) (Gibco, Paisley, UK) supplemented with 10% fetal calf serum (North Umbria, Cramlington, UK), 1% non-essential amino acids (Flow, Rickmansworth, UK), 1 mg/ml bovine insulin (Sigma, Dorset, UK) and antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin (Flow) at 37 °C with 5% CO2. INT 407 cells were cultivated for routine culture in RPMI medium (Flow, UK) without HEPES with similar supplementations. Cells were sub-cultured with 5 ml EDTA (1 mM, BDH, UK) and 5 ml trypsin (0.25% Sigma) for every 6-7 days.

For bioavailability studies, cells were cultured in Transwell Bicameral Chamber (Costar, High Wycombe, UK) until they form complete monolayers. Inside each Transwell bicameral chamber is an insert with the inside wall being treated for uniform cell attachment. The surface area of the membrane is 0.33 cm^2 with o.3 mm pore size and 6.5 mm diameter size. The transwell bicameral chamber has an upper and lower chamber.

Results and discussion

The comparison of the bioavailability of different magnesium salts through the amount of magnesium found in the blood compartment, using Caco-2 cells, was assessed. The various magnesium sources are magnesium acetyl taurate (ATAMg), magnesium citrate, magnesium glycerophosphate, magnesium taurate.

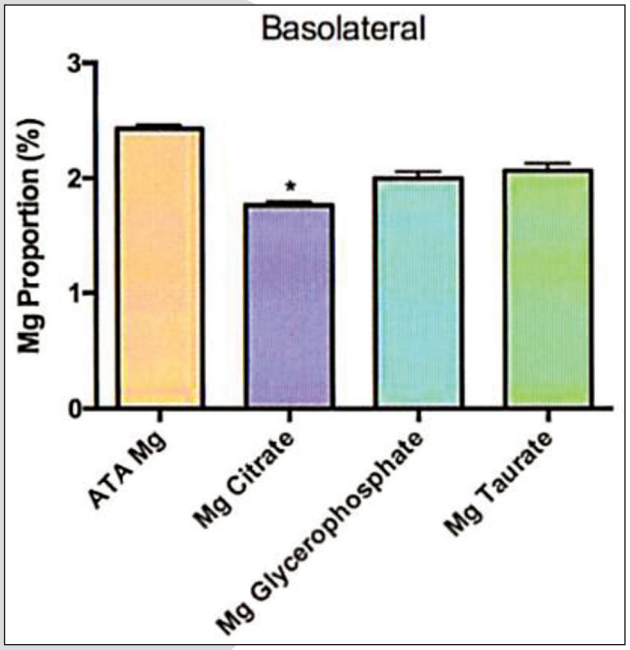

Figure 1. Proportions of magnesium found on the basolateral side of the cells relative to the total amount of magnesium (expressed as percentage)

These values correspond to the percentages of the amount of magnesium found in the basolateral compartment relative to the total magnesium found in the three compartments and represents the quantity of magnesium which has passed through the layer of cells by means of active transport or paracellular passage (predominant rout for magnesium in the ileum).

Table 1. Amounts of magnesium assayed in basolateral media

Magnesium source	Acetyl taurate	Citrate	Glycerophosphate	Taurate
Concentration	2.41%	1.77%	2.03%	2.06%

The highest uptake (expressed in nanomoles) is observed for ATAMg (762.75% ± 6.49 nmol) (Figure 2).

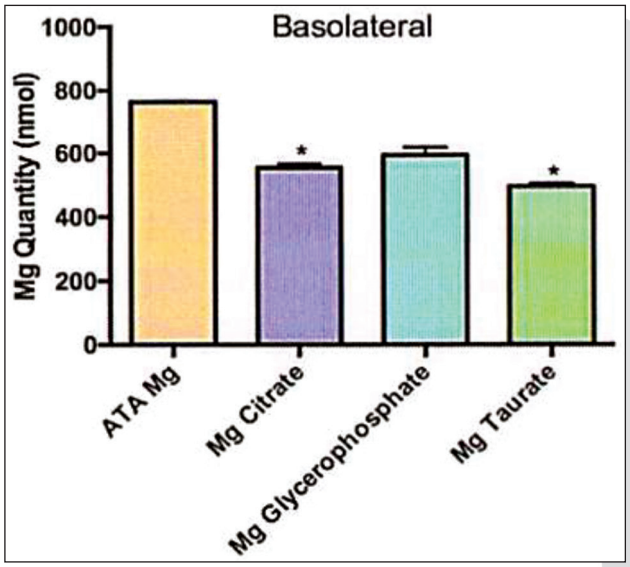

Figure 2. Quantities of magnesium found on the basolateral side of the cells relative to the total amount of magnesium (expressed in nanomoles)

The assay in µg per cm^2 per minute shows the kinetics of the passage of magnesium through the intestinal cells (Figure 3).

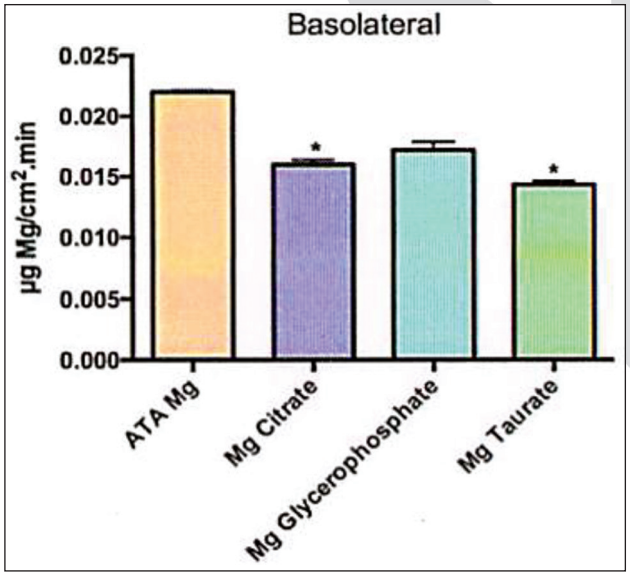

Figure 3. Quantities of magnesium found on the basolateral side, standardized by the surface of the insert and incubation time

ATAMg presents the most efficient kinetics (0.022 µg/cm^2/min), followed by: magnesium citrate (0.0177 µg/cm^2/min); glycerophosphate (0.0171 µg/cm^2/min); and taurate (0.0143 µg/cm^2/min).

Conclusion

This comparative study of the bioavailability of magnesium in this Caco-2 cells model shows a more important passage of magnesium vectorized in the form of N-acetyl taurate form (ATAMg) with respect to citrate, glycerophosphate, and taurate.

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Instructions for the authors

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Abstract

In this paper the instructions for preparing camera ready paper for the Journal are given. The recommended, but not limited text processor is Microsoft Word. Insert an abstract of 50-100 words, giving a brief account of the most relevant aspects of the paper. It is recommended to use up to 5 key words.

Key words: Camera ready paper, Journal.

Introduction

In order to effect high quality of Papers, the authors are requested to follow instructions given in this sample paper. Regular length of the papers is 5 to 12 pages. Articles must be proofread by an expert native speaker of English language. Can't be accepted articles with grammatical and spelling errors.

Instructions for the authors

Times New Roman 12 points font should be used for normal text. Manuscript have to be prepared in a two column separated by 5 mm. The margins for A4 (210×297 mm2) paper are given in Table 1.

Table 1. Page layout description

Paper size	A4
Top margin	20 mm
Bottom margin	20 mm
Left margin	20 mm
Right margin	18 mm
Column Spacing	5 mm

Regular paper may be divided in a number of sections. Section titles (including references and acknowledgement) should be typed using 12 pt fonts with **bold** option. For numbering use Times New Roman number. Sections can be split in subsection, which should be typed 12 pt *Italic* option. Figures should be one column wide. If it is impossible to place figure in one column, two column wide figures is allowed. Each figure must have a caption under the figure. Figures must be a resolution of 300 DPI, saved in TIFF format, width 10 cm min. For the figure captions 12 pt *Italic* font should be used. (1)

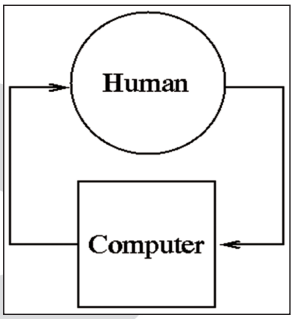

Figure 1. Text here

Conclusion

Be brief and give most important conclusion from your paper. Do not use equations and figures here.

Acknowledgements (If any)

These and the Reference headings are in bold but have no numbers.

References

1. *Sakane T, Takeno M, Suzuki N, Inaba G. Behcet's disease. N Engl J Med 1999; 341: 1284–1291.*
2. *Stewart SM, Lam TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243- 50.*

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