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Proofreader: M. Boyanova

Computer design: D. Alexandrova

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tel. +359 2 952 31 71

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Type of publication	Word count in the main text	Word count in the abstract	Number of references
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Original scientific articles necessarily review a unique material according to thoroughly described methods. The format of *Introduction/preface +objective – Materials and Methods – Results – Discussion – Findings/Conclusion is* of a normative character. In the end of the paper, authors can express acknowledgements to individuals or authorities providing intellectual contributions or a material or financial support. Statistical methods must be described sufficiently so the informed reader with access to original data can check the presented results. The results must be presented quantitatively (if possible) by appropriate indicators for the measurement error or uncertainty (e.g., confidence intervals). Avoid using only p-values in the verification of a hypothesis since this approach does not generate sufficient quantitative information. Quoting the correct p-values in addition to the appropriate confidence intervals is desirable. The number of measurements (sample size) is mandatorily given and the method of calculation is justified. Statistical programs for the performance of analysis must be described. The used statistical terms, abbreviations and symbols should be defined unambiguously. Illustrative material (tables, figures, images) is positioned at the corresponding places within the text with captions and legends. Captions of figures must not be shown within the image. Images with good quality (at least 300 dpi) and appropriate file format (.jpg, tif, .png) are required. Tables must be presented in an editable format rather than as images.Specific abbreviations used in the text are introduced in round brackets with the first appearance of the entire designation. Measurement units should follow the SI system.

Reviews summarize the contents of a number of sources dedicated to a single subject during a defined period of time. Discussion of authors and text should be in compliance with the thematic and problematic relevancy to the study aim. It is intended to review the experience, current state and trends in a given problem, asses the material by an argued manner and suggest the specific, practicable conclusions and recommendations. A logically linked, consistent narration, without mechanically retelling the sources, is expected. Subtitles are thematic.

Clinical Case Reports consist of Introduction, Clinical Case Description, Discussion and Conclusions. An extended review section is used to demonstrate the significance of the presented case. The telegraphic style with duplication of a case history, is unac-ceptable.

Short communication genres follow approximately the structure of an original article. **Letters to the editor** discuss a scientific issue, which is unsolved at the time, in a critical manner or consider other papers.

The list of literature references at the end of the work has to embrace only the publications virtually used and required to delineate the basis, on which the study is designed. Avoid presenting abundant references at the account of their up-to-dateness. Minimize self-citation. *Citing (familiarity with) Bulgarian sources is strongly recommended, too*.

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ORIGINAL ARTICLE

BIOCHEMICAL CONTROL OF 78 PATIENTS WITH CHRONIC HYPOPARATHYROIDISM REFERRED BETWEEN 2006 AND 2020 – WHERE DO WE ACTUALLY STAND?

M. Boyanov[1], D. Zamfirova[1], D. Bakalov[1], V. Karamfilova[1], A. Gateva[1], Y. Assyov[1], E. Zaharieva[1], K. Atanassova[1], G. Sheinkova[1], A. Tsakova[2], Z. Kamenov[1]

[1]Clinic of Endocrinology and Metabolism, University Hospital Alexandrovska, Department of Internal Medicine, Faculty of Medicine, Medical University – Sofia, Bulgaria
[2]Central Clinical Laboratory, University Hospital Alexandrovska, Department of Clinical Laboratory and Clinical Immunology, Medical University – Sofia, Bulgaria

Abstract. *Hypoparathyroidism (hypoPT) is a relatively rare endocrine disease, mainly due to thyroid surgery. The classical supplementation with calcium and active vitamin D may represent a challenge to the clinician.* ***Objective:*** *To describe the level of biochemical control in patients with chronic hypoPT and to look for differences between postsurgical and non-surgical cases referred between 2006 and 2020.* ***Materials and Methods:*** *This was a retrospective cross-sectional study with data review from the database of a tertiary endocrine clinic from the last 15 years. Cases with hypocalcemia not related to PTH were excluded. The patients' medical history was reviewed as well as concomitant diseases and medications. Serum calcium (total, albumin-corrected and ionized; sCa, corrCa, iCa^{+}) and phosphates (P), magnesium, creatinine, alkaline phosphatase together with 24hr urinary calcium and phosphate were measured. The intact parathyroid hormone (iPTH) was determined by electro-hemi-luminescence (Elecsys, Roche Diagnostics). Thyroid and abdominal ultrasound (US) were both performed.* ***Results:*** *Seventy-eight patients met the study criteria – 69 were females. Most of them were between 30 and 60 years (mean age 50.6 ± 14.5 years). Albumin-corrected calcium was in target in 20.5% of the patients, ionized calcium – in 36.5%, serum phosphate – in 46.3%, serum magnesium – in 87.9%. When all four parameters were taken together, less than 20% were in target. Hypercalciuria was registered in 11.8%, while 57.1% of the patients had nephrolithiasis and 27.3% had CKD grade 3-4. Thus, a high proportion of patients with kidney involvement was identified. Calcium carbonate and calcitriol were the preferred replacement choices. Comparing patients with post-surgical and non-surgical hypoPT significant differences were found only for age, total serum calcium, serum magnesium and TSH.* ***Conclusion:*** *Our study is the first of its kind in our country during the last two decades describing the contemporary clinical and biochemical picture of chronic hypoPT in patients referred for specialized care. Low supplementation doses leading to hypocalcemia and hyperphosphatemia were a common finding. Low patient's adherence may be just one possible explanation. Non-surgical cases tend to have even lower calcium and magnesium levels. The patients, their families and treating physicians should be better informed about up-to-date management of chronic hypoPT and the possible impact of suboptimal treatment on morbidity and mortality of the affected subjects.*

Key words: *parathyroid glands, chronic hypoparathyroidism, laboratory findings, comorbidities*

Corresponding author: *Prof. Mihail Boyanov, Clinic of Endocrinology and Metabolism, University Hospital Alexandrovska; 1, G. Sofiyski Street, 1431 Sofia, Bulgaria, e-mail: mihailboyanov@yahoo.com*

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INTRODUCTION

Hypoparathyroidism (hypoPT) is a relatively rare endocrine disorder characterized by inappropriately low secretion of parathyroid hormone (PTH) with resulting low calcemia [1, 2]. In the last two decades its most common etiology is iatrogenic injury to the parathyroid glands during thyroid surgery, which accounts for more than 75% of all cases of chronic hypoPT [3, 4]. However, idiopathic and autoimmune forms of hypoPT can still be diagnosed in clinical practice. Chronic hypoPT after thyroid/parathyroid surgery is diagnosed if hypocalcemia / low PTH levels persist for longer than 6 months [3, 5-7]. Postoperative hypoPT might be expected in 6-12% of all thyroidectomies [7, 8]. Risk factors for the development of postsurgical hypoPT are big-size goiters, Graves' disease and extensive neck explorations due to malignant disease.

The replacement treatment for hypoPT is lifelong and consists of calcium supplements and active vitamin D metabolites [3, 5-7, 9, 10]. More recently, recombinant human PTH (rhPTH) has been introduced in the USA and several other countries for the treatment of resistant cases requiring very high doses of traditional calcium / vitamin D replacement [11, 12]. The goal of treatment is to maintain albumin-corrected serum calcium in the lower reference range (2.1-2.3 mmol/l) together with normal serum phosphates and magnesium [5, 7].

The treatment success is tightly bound to the patients' compliance. A few medications can alter the absorption of calcium salts (like proton-pump inhibitors, PPI). Biochemical follow-up is very important for proper adjustment of calcium/vitamin D doses [7].

The **aim of the present study** was to describe the level of biochemical control in patients with chronic hypoPT and to look for differences between postsurgical and non-surgical cases.

MATERIALS AND METHODS

Design

This was a retrospective observational cross-sectional study based on hospital chart review of routine clinical care in our tertiary endocrine clinic. All patients had given their informed consent for hospitalization and their data handling. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The inpatients' hospital records for the last fifteen years were searched through with the keyword "chronic hypoparathyroidism".

One-hundred and two positive patient records were identified between 2006 and 2020. Chronic hypoPT was defined as a combination of low albumin-corrected or ionized serum calcium and inappropriately low parathyroid hormone (PTH) before supplementation with calcium/active vitamin D [1, 2]. Postsurgical hypoPT was confirmed if the above changes had persisted for at least 6 months after thyroid/parathyroid surgery [3]. Other causes for hypocalcemia were excluded including but not limited to: CKD grade 5, malabsorption syndromes, hypercalciuria due to other causes (loop diuretics, idiopathic, etc.), pseudohyperparathyroidism [4, 13]. Ten cases had incomplete data and were not included in the analyses. Seventy-eight patients had complete records and were included. The primary reason for their referral to the clinic had been the need for biochemical follow-up and for proper adjustment of the patients' calcium/vitamin D supplementation.

Laboratory and hormonal data

Total serum calcium had been measured by a photometric assay (reference range 2.12-2.62 mmol/l) and whenever possible by atomic absorptiometry – Ca-AAS. Albumin-corrected calcium was calculated based on a mean albumin of 44 g/l (local lab reference range for albumin 35-52 g/l; and for corrected sCa 2.10-2.60 mmol/l). Ionized calcium (iCa+) had been measured by atomic absorptiometry (reference range 1.1-1.3 mmol/l). Data on serum magnesium (reference range 0.65-1.10 mmol/l) and phosphates (0.81-1.45 mmol/l) were also present.

Intact PTH (iPTH) had been measured by a second generation assay (Elecsys, Roche Diagnostics, Switzerland) and expressed in pmol/l (reference range 1.59-6.89 pmol/l).

Serum creatinine had been measured by a colorimetric method and the estimated glomerular filtration rate (eGFR) calculated according to the MDRD-formula in ml/min/1.73 m². Urinary excretion of calcium and phosphate were measured from 24h urinary samples in mmol/24 hr (reference ranges 2.5-8.0 mmol for calciuria and 13-42 mmol for phosphaturia).

Imaging

The imaging included neck ultrasound of all patients (using a linear 9-12 MHz transducer, including Doppler imaging). In cases of a visible thyroid parenchyma its volume was calculated by multiplying the three diameters (anterior-posterior, medial-lateral, proximal-distal) and dividing the product by a factor of 0.48 (formula for ellipsoids).

Statistical analysis

All analyses were performed with the SPSS 13.0 for Windows platform (SPSS Corp., Chicago, IL). Descriptive statistics and frequency analysis were performed. Missing data were not replaced. Most of the data were positively skewed, thus medians and quartile ranges were preferred. The Mann-Whitney and Wilcoxon tests were used for comparisons of independent and dependent samples. Statistical significance was set at $p < 0.05$.

Results

Among the 102 screened patient records with the diagnosis of hypoparathyroidism, 78 patients met the definition of chronic hypoPT and their records contained sufficient data for analysis (69 females and 9 males). Their mean age was 50.6 ± 14.5 years (range 23 – 81 years). Their distribution according to age was as follows: 21-30 yrs – 5.1%; 31-40 yrs – 25.7%; 41-50 yrs – 20.5%; 51-60 yrs – 23.1%; 61-70 years – 12.8% and > 70 yrs – 12.8% (see also Fig. 1).

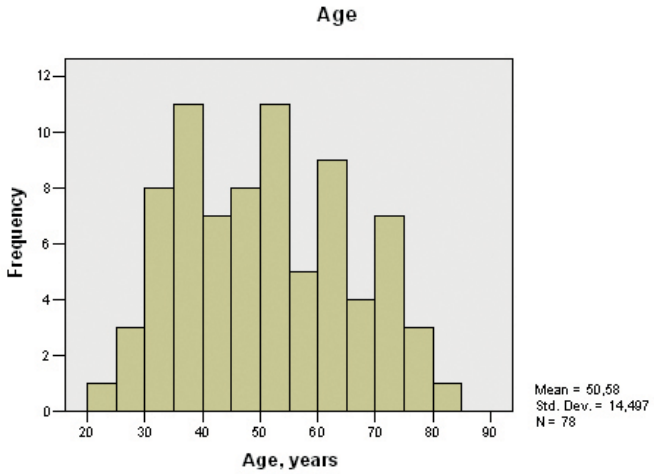


Fig 1. Age distribution of the 78 included patients with chronic hypoPT

The etiology of the hypoPT was post-surgical due to thyroidectomy in 60 cases (76.9%) and due to parathyroidectomy – in 3 cases (3.8%). The remaining 15 cases (19.2%) were classified as non-surgical (possibly idiopathic) hypoPT. The mean duration of the hypoPT was 9.8 years (± 12.2 yrs) with a median of 5.5 years. It was less than 1 year in 35.9%; between 1 and 5 years – in 14.1%; between 5 and 10 years – in 15.4%; ≥ 10 years – in 34.6%.

The biochemical and hormonal data of the patients on their first day in hospital are presented in Table 1. The frequency distribution of normal/abnormal laboratory values is displayed in Table 2. Albumin-corrected calcium was ≥ 2.10 mmol/l in 30.3% only and in the target range of 2.10-2.30 mmol/l in 26.5% only. None of the patients presented with hypercalcemia (> 2.60 mmol/l). 84.1% of the patients had values of ionized calcium below the lower limit of the

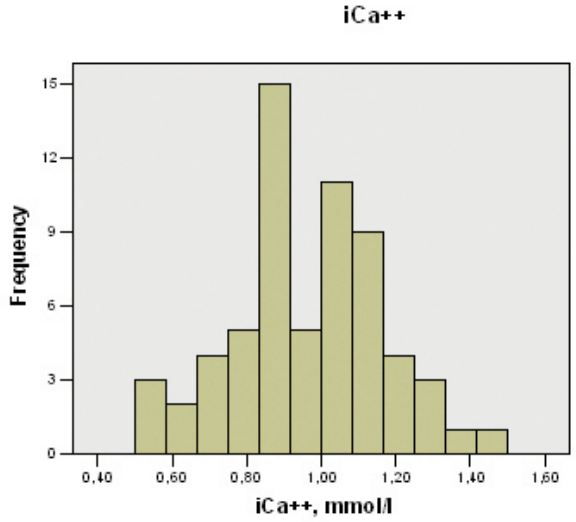
Table 1. The biochemical and hormonal data of the patients with hypoPT during their hospital stay for biochemical follow-up and therapy adjustment are presented as means ± SD and medians with quartiles

		sCa mmol/l	iCa++ mmol/l	sP mmol/l	sMg mmol/l	Creatinine µmol/l	Albumi ng/l	Ca X P, mmol²/l²	PTH pmol/l	eGFR ml/min/m²	uCa mmol/24 hr	Phosphate mmol/24 hr	Calcium daily mg	Levothyroxine µg	TSH UI/L
N with data		77	63	77	66	76	74	67	73	75	68	68	60	52	66
Mean		1.92	0.96	1.50	0.71	86.9	40.5	2.90	1.03	69.1	4.33	14.25	1417.8	100.3	7.32
Std. Dev.		0.32	0.20	0.27	0.08	27.2	5.0	0.61	1.03	21.8	6.67	14.15	547.7	42.7	15.70
Median		1.96	0.98	1.44	0.72	83.0	41.0	2.86	0.80	66.7	2.69	11.68	1200.0	100.0	2.54
Minimum		1.19	0.54	1.05	0.45	47.0	25.0	1.74	0.10	19.6	0.22	0.10	180.0	25.0	0.00
Percentile	25	1.68	0.85	1.30	0.68	69.0	37.0	2.47	0.41	55.4	1.15	7.63	1200.0	63.8	1.07
	75	2.20	1.13	1.69	0.76	97.8	44.0	3.39	1.39	82.4	4.67	17.56	1800.0	125.0	4.72
Maximum		2.58	1.45	2.30	0.85	218.0	49.0	4.76	6.24	145.0	48.30	100.00	3000.0	200.0	100.0

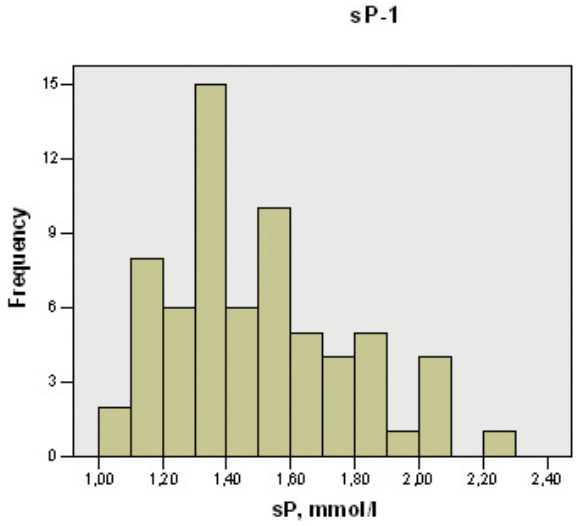
local lab of 1.16 mmol/l with 2 patients (3.2%) having values ≥ 1.32 mmol/l (upper normal limit of local lab). The distribution of ionized calcium levels and serum phosphates is shown in Fig. 2. Serum phosphates were elevated in more than half of the study sample with 5 patients (6.4%) showing alarming values ≥ 2.0 mmol/l. The calcium phosphate product exceeded 4.4 $mmol^2/l^2$ in only 1 patient out of 78.

Table 2. The frequency distribution in percentages of normal/abnormal (in target/outside target) laboratory values is displayed

Laboratory parameter	Frequency, %	Local lab range
Albumin-corrected calcium, mmol/l		2.10-2.60
≤ 1.9 mmol/l	47.1%	mmol/l
1.90-2.09 mmol/l	22.6%	
2.10-2.29 mmol/l	20.5%	
2.30-2.59 mmol/l	9.8%	
Ionized calcium, mmol/l		1.16-1.32
< 1.0 mmol/l	54.0%	mmol/l
1.10-1.20 mmol/l	36.5%	
> 1.20 mmol/l	9.5%	
Serum phosphates, mmol/l		0.81-1.45
In normal range (≤ 1.45 mmol/l)	46.3%	mmol/l
Mildly elevated (>1.45 – ≤ 1.70 mmol/l)	30.9%	
Severely elevated (> 1.70 mmol/l)	22.8%	
Serum magnesium		0.65-1.10
< 0.65 mmol/l	12.1%	mmol/l
In range	87.9%	
iPTH, pmol/l		1.20-6.90
< 1.20 (below normal range)	83.6%	pmol/l
≤ 2.30 (low normal)	13.7%	
> 2.30 (in range)	2.7%	
Urinary calcium, mmol/24 hr		♀: 2.50-6.50
< 2.50 mmol/24 hr	45.6%	mmol/l
In range	32.6%	♂: 2.50-7.00
> UNL (6.50 ♀; 7.00 mmol/l ♂)	11.8%	mmol/l
Urinary phosphate, mmol/24 hr		13.0-42.0
< 13.0 mmol/l	55.9%	mmol/24hr
In range	41.1%	
> UNL	3.0%	
eGFR, ml/min/1.7 m^2 (MDRD-formula)		
15-29 (CKD grade 4)	2.7%	
30-59 (CKD grade 3)	24.6%	
60-89 (CKD grade 2)	56.7%	
90-119 (CKD grade 1)	13.3%	
≥ 120	2.7%	
TSH, UI/L	66 patients	0.3-4.2 UI/L
< 0.3 UI/L	10.6%	
In range	56.1%	
4.2-10 UI/L (subclinical hypothyroidism)	21.2%	
> 10 UI/L (overt hypothyroidism)	11.1%	



A



B

Fig. 2. Frequency distribution in mmol/l of: **A**) ionized calcium; and **B**) serum phosphates

Data on TSH were available from all 66 patients with post-surgical hypoPT. A total of 33.3% showed values above the ULN (> 4.2 mIU/l) indicating possible insufficient thyroid hormone replacement.

Imaging data

Data from renal ultrasound were available in 68 patients (80.8%). Kidney stones were diagnosed in 57.1% of them with 9.5% having microcalculi (≤ 4.0 mm), 42.9% – calculi > 4.0 and ≤ 8.0 mm; and 4.8% – bilateral nephrolithiasis.

All patients with post-surgical hypoPT had thyroid remnants visible on US – their volumes ranging from 1.1 ml to 19.6 ml. Solitary thyroid nodules were seen in 3 cases (3.8%) and multiple nodules – in 4 ones (5.1%).

Calcium/vitamin D replacement

All participants received vitamin D supplementation with nine of them (11.5%) taking cholecalciferol only. The preferred active metabolite was calcitriol (62 pa-

tients – 79.5%), while the remaining seven patients on active metabolites (9.0%) were receiving calcidiol (alfacalcidiol). The majority were taking 0.5 and 0.75 mcg calcitriol daily – 28.6% and 34.9% of the whole sample, followed by 1.0 mcg daily – 11.1%. Three patients only were taking > 1.0 mcg and ≤ 2.0 mcg daily with 2.0 mcg being the highest dose prescribed.

Calcium carbonate was the preferred calcium supplement (69 pts – 88.5%). The mean daily dose was 1417 mg, while the median -1200 mg. Eight patients (10.3%) were taking ≤ 600 mg elementary calcium daily, while twenty-five (32%) were taking 1200 mg and another twenty (25.6%) – 1800 mg daily. The highest daily dose was 3.0 grams (1 patient).

Recombinant PTH is not registered in our country and no data on its use could be found in our sample.

Fifty-two patients (82.5% of all post-thyroidectomy patients) were taking levothyroxine. The mean and median of the daily dose were equal – 100 mcg. The distribution of the daily calcium supplementation and levothyroxine dose is displayed in Fig. 3.

Concomitant diseases

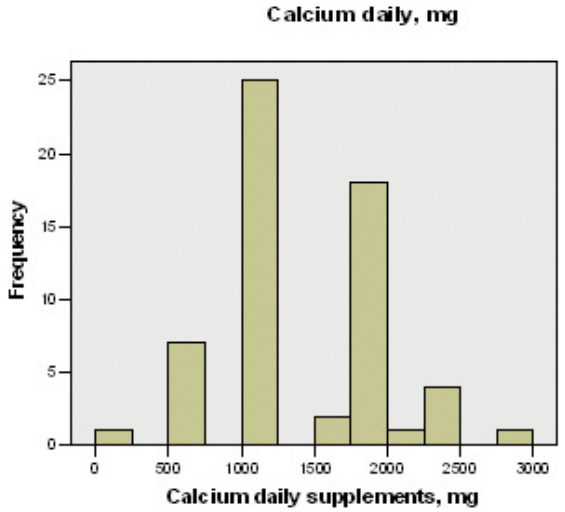
Data on thyroid autoimmunity were available from 55 patients (70.5% of the whole sample). Seventeen patients had been operated due to Graves' disease (21.8%), while 6 had autoimmune thyroiditis (7.7%).

The most frequently reported co-morbidity was arterial hypertension – 44 participants (56.4%), which was an isolated abnormality in 20 of them (45.5% of the hypertensive subgroup). Eleven patients (14.1%) had coronary artery disease with 5 of them having survived myocardial infarction. Two patients (2.6%) reported heart failure and another two (2.6%) – history of stroke.

Malignancy as the cause for the previously performed thyroidectomy was identified in 15 patients (22.0% of the thyroid postsurgical group): papillary thyroid carcinoma (PTC) in 12 patients (15.4% of the thyroid postsurgical group), follicular thyroid carcinoma (FTC), sarcoma and thymoma – 3 patients in total, each one in 1 patient (6.7% of the postsurgical group). One patient had been operated for lymphoma of the thyroid. Radioiodine ablation had been reported by 7 patients.

Surgical versus non-surgical hypoPT

Comparing patients with post-surgical and non-surgical (idiopathic) hypoPT, significant differences were found only for age, as well as for levels of total serum calcium, serum magnesium and TSH (see Table 3).



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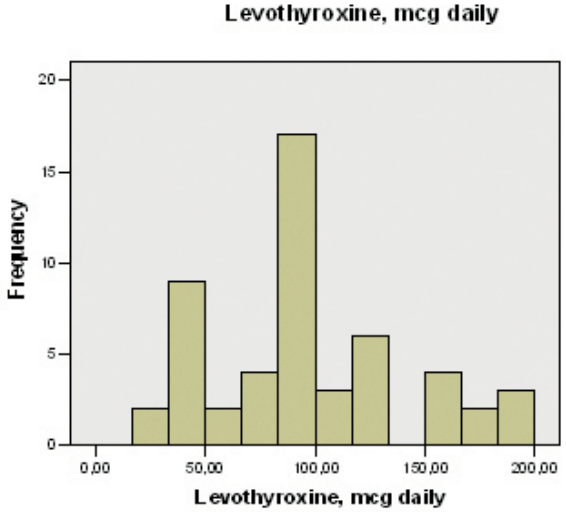

B

Fig. 3. Distribution of the daily calcium carbonate dose (**A**) and the levothyroxine dose (**B**) in the study sample

Table 3. Significantly different parameters between the surgical and non-surgical subgroups of hypoPT

Significantly different parameters	Type of hypoPT	Mean	Standard Deviation	Std. Error Mean	Significance, p
Age, years	Post-surgical	52.5	14.0	1.8	0.023 a
	Non-surgical	43.0	14.7	3.8	
Total sCa, mmol/l	Post-surgical	1.99	0.28	0.04	0.030 b
	Non-surgical	1.67	0.36	0.10	
Serum Mg, mmol/l	Post-surgical	0.73	0.06	0.01	0.034 a
	Non-surgical	0.68	0.086	0.024	
TSH, UI/L	Post-surgical	8.57	17.12	2.33	0.006 b
	Non-surgical	1.79	1.42	0.47	

a – equal variances assumed, b – equal variances not assumed.

The non-surgical patients with hypoPT were of younger age and had significantly lower levels of serum calcium, magnesium and TSH.

DISCUSSION

We did a retrospective hospital chart review of patients with chronic hypoPT referred for regular follow-up to our tertiary endocrine clinic in the time period 2006-2020. We registered a very low proportion of patients with normal calcium-phosphate metabolism: albumin-corrected calcium was in target in 20.5%, ionized calcium – in 36.5%, serum phosphate – in 46.3%, magnesium – in 87.9%. If all four parameters taken together, less than 20% were in target. More than half of the patients (57.1%) had nephrolithiasis and 27.3% had CKD grade 3-4 indicating a surprisingly high proportion of patients with kidney involvement. Calcium carbonate and calcitriol were the preferred replacement drugs. Our study indicated insufficient calcium/vitamin D supplementation warranting thorough data review and evolving management strategies for better adherence and quality of life.

We compared our results on reaching targets of calcium/vitamin D supplementation with already published data from other cohorts of patients with chronic hypoPT (see Table 4). Table 4 shows that serum levels of total (albumin-corrected) calcium and phosphates in our sample were less well corrected than in the studies under comparison. On the contrary, data on serum magnesium, urinary calcium and glomerular filtration did not differ substantially.

Considering the insufficient biochemical control of hypoPT in our study population a number of reasons might be listed. First, all patients were hospitalized ones, thus introducing a bias toward more severe and uncontrolled disease. Second, for the study period active vitamin D metabolites were reimbursed in our country while calcium salts were prescribed out-of-pocket. This predisposes patients' adherence to vitamin D metabolites only. And third, one should not underestimate the patients' concerns about side effects. In the case of calcium salts these include gastrointestinal complaints (e.g. obstipation, flatulence and others), risk for vascular calcifications and renal stones [17-19]. Different strategies have been implemented by other authors to overcome these worries – alternate calcium intake or even "no calcium" regimens [20, 21]. The most common worries about calcitriol are kidney damage and polyuria as already reported in a different study with 64 patients [20]. Calcitriol has actually been linked to declining renal function and risk for polyuria and nephrolithiasis [22-25]. Coming back to poor biochemical control of our patients, one must keep in mind that it is clearly linked to a higher risk for complications and worse quality of life as reported elsewhere [25-27].

The supplementation doses reported by the patients in our study might also be a bit lower than the optimal ones [3, 5]. Forty-two percent of the subjects on calcium carbonate were taking ≤ 1200 mg daily while 63.5% of those on calcitriol were taking daily doses in the range 0.5/0.75 μg which might not always be sufficient as indicated in up-to-date clinical guidelines on hypoPT [3, 5, 6]. The suboptimal dosage might be due to both clinicians' and patients' preferences and, of course, to financial reasons. Another preliminary study revealed that one-third of the patients may lack motivation to use calcium whereas half of all may be anxious about possible side effects [19]. In this particular study one third of the patients were taking oral calcium and calcitriol less than the recommended dose [18]. A UK national chronic hypoPT audit identified 80 individual patients' cases and reported compliance with treatment standards between 98.8% and 60%, whereas the compliance with monitoring standards fell even lower – 91.3-20% [28]. In a German survey on management of hypoPT by family physicians only 75.8% of the patients had data on serum calcium and one third of them still showed hypocalcemia [29].

Table 4. Data on reaching targets of calcium/vitamin D supplementation – comparison with already published observations

Parameter under study*	Our data	Russian Registry [14]	Canadian Registry [15]	Turkish study [16]
Number of subjects	78	544	130	107
Hypocalcemia	69.7%	44.2%	58%	68.1%
High phosphates	53.7%	63.1%	29%	46.2%
Low magnesium	12.1%	23.6%	12%	
Hypercalciuria	11.8%	37.6%	36%	43.7%
eGFR < 60 ml/min/1.73 m^2	27.3%	15.8%	23%	

* According to specific lab reference values applied in the studies

Concerning possible differences between surgical and non-surgical cases we confirmed the tendency for non-surgical patients to be younger and to present with deeper hypocalcemia and hypomagnesiemia. This finding has already been reported in previous studies by other authors [14, 30]. The reasons behind these differences are not yet clearly elucidated but surely refer to the different etiology of hypoPT (lack of parathyroid tissue versus autoimmune or invasive destruction of the parathyroid cells and secretion) [31]. As expected TSH was normal in our non-surgical cases while it was suboptimal in a great number of post-surgery participants meaning that poor adherence to levothyroxine treatment might parallel that to the calcium/vitamin D replacement.

We were struck by the high prevalence of nephrolithiasis in our patients studied with renal ultrasound – 57.1%. At the same time hypercalciuria was relatively uncommon (in 11.8%). In other detailed studies the prevalence of renal stones had been reported as low as 32.5% [14] or even < 27% [15]. The detrimental impact of the hypoPT itself on the kidney has already been documented [23]. However, our results on such a high rate of renal stones warrants further analysis and follow-up.

Our study has some limitations. First, we included patients referred to a specialized endocrine center introducing a bias towards more difficult to control cases. Second, the presented data are cross-sectional ones and longitudinal follow-up might have added further value. Third, the number of patients was rather modest therefore not allowing detailed sub-analyses.

CONCLUSION

The major strength of our study was that it described the contemporary clinical and biochemical picture of chronic hypoPT in patients referred for specialized care. Low supplementation doses leading to hypocalcemia and hyperphosphatemia were a common finding. Low patient's adherence may be just one possible explanation. The non-surgical hypoparathyroid patients were of younger age and had significantly lower levels of serum calcium and magnesium. All the hypoparathyroid patients, their families and treating physicians should be better informed about up-to-date guidelines on hypoPT [32] and the possible impact of suboptimal treatment on morbidity and mortality in hypoparathyroid subjects [33, 34]. Unfortunately, we do not know with certainty yet, whether the introduction of treatment of hypoPT with rhPTH might be of substantial benefit to our not well-controlled patients [35].

Ethical considerations

All patients had given their informed consent for hospitalization and data handling.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Bilezikian JP. Hypoparathyroidism. J Clin Endocrinol Metab 2020; 105(6): 1722-1736.
2. Hakami Y, Khan A. Hypoparathyroidism. Front Horm Res 2019; 51:109-126.
3. Orloff LA, Wiseman SM, Bernet VJ, et al. American Thyroid Association Statement on Postoperative Hypoparathyroidism: Diagnosis, Prevention, and Management in Adults. Thyroid 2018; 28(7): 830-841.
4. Cianferotti L. Classification of Hypoparathyroid Disorders. Front Horm Res 2019; 51: 127-138.
5. Bollerslev J, Rejnmark L, Marcocci C, et al; European Society of Endocrinology. European Society of Endocrinology Clinical Guideline: Treatment of chronic hypoparathyroidism in adults. Eur J Endocrinol 2015; 173(2): G1-20.
6. Khan AA, Koch CA, Van Uum S, et al. Standards of care for hypoparathyroidism in adults: a Canadian and International Consensus. Eur J Endocrinol 2019; 180(3): P1-P22.
7. Bollerslev J, Rejnmark L, Zahn A, et al. 2021 PARAT Working Group. European Expert Consensus on Practical Management of Specific Aspects of Parathyroid Disorders in Adults and in Pregnancy: Recommendations of the ESE Educational Program of Parathyroid Disorders. Eur J Endocrinol 2022; 186(2): R33-R63.
8. Rao SD. Epidemiology of parathyroid disorders. Best Pract Res Clin Endocrinol Metab 2018; 32(6): 773-780.
9. Maeda SS, Moreira CA, Borba VZC, et al. Diagnosis and treatment of hypoparathyroidism: a position statement from the Brazilian Society of Endocrinology and Metabolism. Arch Endocrinol Metab 2018; 62(1): 106-124.
10. Marcucci G, Brandi ML. Conventional Treatment of Hypoparathyroidism. Front Horm Res 2019; 51: 160-164.
11. Marcucci G, Masi L, Cianferotti L, et al. Chronic hypoparathyroidism and treatment with teriparatide. Endocrine 2021; 72(1): 249-259.
12. Mihai R, Thakker RV. Management of endocrine disease: Postsurgical hypoparathyroidism: current treatments and future prospects for parathyroid allotransplantation. Eur J Endocrinol 2021; 184(5): R165-R175.
13. Pepe J, Colangelo L, Biamonte F, et al. Diagnosis and management of hypocalcemia. Endocrine 2020; 69(3): 485-495.
14. Kovaleva EV, Eremkina AK, Elfimova AR, et al. The Russian Registry of Chronic Hypoparathyroidism. Front Endocrinol (Lausanne) 2022; 13: 800119.
15. Khan AA, AbuAlrob H, Punthakee Z, et al. Canadian national hypoparathyroidism registry: an overview of hypoparathyroidism in Canada. Endocrine 2021; 72(2): 553-561.
16. Onder CE, Kuskonmaz SM, Koc G, Firat SN, Omma T, Culha C. Evaluation of management of patients with postoperative permanent hypoparathyroidism. How close are we to the targets? Minerva Endocrinol 2020. doi: 10.23736/S0391-1977.20.03291-5.
17. Lopera JZ, Tabares SAL, Herrera DÁ, et al. Characteristics of hypoparathyroidism in Colombia: data from a single center in the city of Medellín. Arch Endocrinol Metab 2020; 64(3): 282-289.

18. Mazoni L, Matrone A, Apicella M, et al. Renal complications and quality of life in postsurgical hypoparathyroidism: a case-control study. J Endocrinol Invest 2022; 45(3): 573-582.
19. Bilginer MC, Aydin C, Polat B et al. Assessment of calcium and vitamin D medications adherence in patients with hypoparathyroidism after thyroidectomy. Arch Osteoporos 2022; 17(1): 22.
20. Akkan T, Dagdeviren M, Koca AO et al. Alternate-day calcium dosing may be an effective treatment option for chronic hypoparathyroidism. J Endocrinol Invest 2020; 43(6): 853-858.
21. Al-Sharefi A, Glenister E, Morris M, Quinton R. Is calcium supplementation always needed in patients with hypoparathyroidism? Clin Endocrinol (Oxf) 2019; 90(6): 775-780.
22. Coudenys E, Van Meerhaeghe T, Unuane D et al. Long-Term Treatment with Calcitriol in Postsurgical Hypoparathyroidism Leads to Renal Function Decline. Horm Metab Res 2019; 51(6): 362-366.
23. Peacock M. Hypoparathyroidism and the Kidney. Endocrinol Metab Clin North Am 2018; 47(4): 839-853.
24. Ketteler M, Chen K, Gosmanova EO, et al. Risk of Nephrolithiasis and Nephrocalcinosis in Patients with Chronic Hypoparathyroidism: A Retrospective Cohort Study. Adv Ther 2021; 38(4): 1946-1957.
25. Underbjerg L, Sikjaer T, Rejnmark L. Long-Term Complications in Patients With Hypoparathyroidism Evaluated by Biochemical Findings: A Case-Control Study. J Bone Miner Res 2018; 33(5): 822-831.
26. David K, Moyson C, Vanderschueren D, Decallonne B. Long-term complications in patients with chronic hypoparathyroidism: a cross-sectional study. Eur J Endocrinol 2019; 180(1): 71-78.
27. Anaforoglu I, Sancak S, Akbas EM, et al. Effects of Treatment Adherence on Quality of Life in Hypoparathyroid Patients. Exp Clin Endocrinol Diabetes 2021; 129(12): 918-925.
28. Kiam JS, Sharma V, Glenister L, Fraser WD, Turner JJO. UK national chronic hypoparathyroidism audit. Clin Endocrinol (Oxf) 2022; 97(5): 562-567.
29. Allemeyer EH, Kossow MS, Riemann B, Hoffmann MW. Ambulante Versorgungsqualität für den permanenten postoperativen Hypoparathyreoidismus [Outpatient quality of care for permanent postoperative hypoparathyroidism]. Dtsch Med Wochenschr 2019; 144(21): e130-e137.
30. Zanchetta MB, Robbiani D, Oliveri B, et al. Hipopara-Red, Real Life Experience in 322 Patients With Hypoparathyroidism. J Endocr Soc 2020; 5(2): bvaa181. doi: 10.1210/jendso/bvaa181.
31. Cianferotti L, Marcucci G, Brandi ML. Causes and pathophysiology of hypoparathyroidism. Best Pract Res Clin Endocrinol Metab 2018; 32(6): 909-925.
32. Ministry of Health, Bulgarian Society for Endocrinology. Recommendations for good clinical practice in parathyroid disease. Sofia, 2019 (in Bulgarian).
33. Hadedeya D, Kay J, Attia A, et al. Effect of postsurgical chronic hypoparathyroidism on morbidity and mortality: a systematic review and meta-analysis. Gland Surg 2021; 10(10): 3007-3019.
34. Almquist M, Ivarsson K, Nordenström E, Bergenfelz A. Mortality in patients with permanent hypoparathyroidism after total thyroidectomy. Br J Surg 2018; 105(10): 1313-1318.
35. Edafe O, Mech CE, Balasubramanian SP. Calcium, vitamin D or recombinant parathyroid hormone for managing post-thyroidectomy hypoparathyroidism. Cochrane Database Syst Rev 2019; 5(5): CD012845. doi: 10.1002/14651858.CD012845.pub2.

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ORIGINAL ARTICLE

DIETARY INTAKE OF VITAMIN D AND DENTAL CARIES INCIDENCE IN PEOPLE WITH OVERWEIGHT AND OBESITY

B. Stoichkov[1], L. Doichinova[2], P. Bakurdjiev[2], E. Radeva[3], D. Kirov[4], M. Nikolova[5]

[1]Department of Dental, Oral and Maxillofacial Surgery, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria
[2]Department of Pediatric Dental Medicine, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria
[3]Department of Conservative Dentistry, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria
[4]Department of Prosthetic Dental Medicine, Faculty of Dental Medicine, Medical University – Sofia, Bulgaria
[5]Department of Epidemiology and Hygiene, Faculty of Medicine, Medical University – Sofia, Bulgaria

Abstract. Aims: *The aims of the present study are to investigate the relationship between the anthropometric indicators and peculiarities of the nutritional intake in people with overweight and obesity and the risk factors for the development of dental caries in adults.* ***Materials and Methods:*** *The study involved 264 individuals aged between 18 and 60 years. The following risk factors for the development of dental caries were traced: diet (carbohydrate intake), oral hygiene habits and social status in overweight and obese people. The dental caries incidence was determined through the DMFT index, by assessing the total number of teeth which are decayed (D), missing (M) due to caries, or filled (treated, F).* ***Results:*** *The age-related distribution was as follows: from 18 to 25 years – 14.4%; from 25 to 35 years – 16.7%; from 35 to 50 years – 42.4%; and over 50 years – 26.5%. The established average BMI was 25.60 ± 4.359, with the lowest and highest measured values – 18.5 and 37.55, respectively. The value of DMFT was 12.55 ± 5.545. A direct correlation between the elevated incidence of dental caries in patients with overweight and obesity was revealed. No significant relationship was found between the decreased nutritional intake of vitamin D and the incidence of dental caries in individuals with Class I and Class II obesity.* ***Conclusion:*** *Dental caries and obesity have a similar etiology – improper dietary habits, excessive consumption of foods containing low molecular weight carbohydrates and carbonated beverages. The reported higher levels of dental caries in these groups could be explained by an improper diet and more frequent snacking.*

Key words: *overweight, obesity, dental caries incidence, vitamin D*

Corresponding author: *M. G. Nikolova, Department of Hygiene, Medical Ecology and Nutrition, Faculty of Medicine, Medical University of Sofia, 2 Zdrave Street, 1431 Sofia, Bulgaria; e-mail: marianikolova@mail.bg*

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INTRODUCTION

The World Health Organization (WHO) has defined obesity as a chronic recurrent disease which is included in the international classification of diseases [1, 2]. The disease is polyetiological, having physiological, biochemical, metabolic, anatomical, psychological, social, genetic, and hereditary factors. It is believed that the disturbed equilibrium between the bacterial species in the intestinal microflora, especially Bacteroidetes and Firmicutes, is often associated with overweight and obesity [3]. In the intestinal microbiome of obese people, a predominance of the Firmicutes bacterial species was found, and vice versa, in healthy individuals, there were more Bacteroides and greater species diversity [4]. Many authors consider that obesity results from an energy imbalance related to increased caloric intake and decreased caloric expenditure [5]. The basic risk factors include a sedentary lifestyle and consumption of foods rich in fats and refined sugars [6, 7, 8]. The higher incidence of obesity is closely connected to: (1) significant dietary changes; (2) consumption of carbonated beverages; (3) excessive intake of low molecular weight carbohydrates, and (4) changed lifestyle.

Obesity and dental caries share a multi-factor etiology. Their incidence results from the dietary and oral hygiene habits, as well as the type of food consumed. Dental caries is a consequence of the usage of fermentable carbohydrates, including sucrose, glucose, fructose, lactose, maltose, and starch [9]. These fermentable carbohydrates have a local effect on dental caries development [10,11]. The interactions between the dental structure, a biofilm formed over the tooth's surface, sugars, the salivary, and genetic factors are the main prerequisites for development of dental caries. The damage begins with the demineralization of the hard dental tissues as a result of the exposure of the tooth enamel to fermentable carbohydrates. Streptococcus mutans and Streptococcus sorbrinus synthesize organic acids (lactic, acetic, and formic acids) from the adopted sugars and create insoluble extracellular dextran, which contributes to bacterial colonization around the tooth surface [12]. When the acidity in the dental plaque decreases during the process of bacterial fermentation below the level of pH 5.5-5.7, the demineralization of the tooth enamel begins. This appears after consuming too much added sugar found in sweets, carbonated beverages, fruit juices, or sugar added to coffee or tea [13]. The consequences of dental caries, especially if left untreated, can lead to tooth loss, reduced chewing ability, low self-esteem, impaired quality of life, and a generally negative effect on nutrition and general health. The severity of this problem has been supported by a recent systematic review by Chan et al., where more than 50% of adults had a minimum of one untreated dental caries [14]. On the other hand, the amount and composition (lysozyme, lactoferrin, peroxidase enzymes, histatin, proline-rich proteins, mucins, glycoproteins, fibronectin, beta-macroglobulin, lysozyme, secretory immunoglobulin) of saliva act as natural protective mechanisms against this process [12].

The data on the relationship between overweight and the elevated dental caries incidence among adults is controversial. Idrees et al. [2], as well as Sharma et al. [15], do not establish a statistically significant correlation between the increased body mass index (BMI) and the increased frequency of dental caries, but in many other studies, a positive correlation between these indicators has been reported [13, 16, 17]. The main factors related to the increased caries incidence in overweight patients are those resulting from nutritional habits as: frequent meals, excessive consumption of fermentable carbohydrates in the food, including carbonated beverages, eating of unhealthy and high-calorie and cariogenic foods [18]. On the other hand, the concentration in the saliva of some micronutrients, such as vitamin D, has a suppressive effect on bacterial metabolism and dental plaque maturation [19]. Thus, they could have a protective effect on the dental surface demineralization [20]. In some studies, an elevated dental caries incidence in patients with low serum vitamin D concentrations has been revealed [21]. Currently, there is no direct evidence of an inversely proportional relationship between the low serum levels of the vitamin D and the increased dental caries incidence in adults [7].

Aims of the study: To investigate the relationship between anthropometric indicators and the peculiarities of the nutritional intake in overweight and obese individuals and the risk factors for developing dental caries in adults aged between 18 and 60 years.

MATERIALS AND METHODS

This is a cross-sectional observational study. It was approved by the responsible authorities at the Medical University of Sofia and complied with the ethical standards and the Declaration of Helsinki. Informed consent from each participant prior to his/her participation in any procedure was obtained. The following inclusion criteria for selecting the participants in the study were applied: (1) age from 18 to 60 years (the age limit was chosen in order to avoid the influence over the body mass of age-related sarcopenia); (2) BMI ≥ 18.5 kg/m²; (3) the minimal level of oral hy-

giene – twice a day. The exclusion criteria were as follows: (1) body weight over 150 kg; (2) obesity resulting from the administration of glucocorticoids or psychotropic medications; (3) hypercorticism; (4) hypothalamic or hereditary syndromes; (5) decompensated hypothyroidism and hyperthyroidism; (6) other secondary causes – heart inefficiency, respiratory inefficiency, chronic renal failure, cirrhosis of the liver, pancreatitis, and musculoskeletal disorders.

The following risk factors for dental caries development were traced: diet (carbohydrate intake), oral hygiene habits, social status, routine dental visiting, and development of new caries over the last 36 months. During the clinical examination the number of affected teeth was recorded as follows: (1) dental caries; (2) extracted teeth due to dental caries; and (3) filled teeth, applying the recommendations of the World Health Organization published in 1997 and 2013. In cases where the diagnosis of dental caries was difficult for clinicians, sectoral (periapical) radiographs were used. The number of Decayed (D), Missed (M), and Filled (F) teeth (DMFT) was calculated. The height and weight of the patients were measured. The body mass index (BMI) was calculated according to the following formula: BMI = Weight (kg) / Height^2 (m^2). BMI was categorized according to the standard criteria: underweight (BMI < 18.5); normal weight (BMI 18.5-24.9); overweight (BMI 25-29.9), and obesity (BMI > 30 kg/m^2). In addition, information about nutritional habits – consumption of carbonated beverages and foods containing refined sugar; physical activity data; smoking, and oral hygiene habits were recorded.

STATISTICAL ANALYSIS

The data were processed using IBM SPSS Statistics 25 (Armonk, NY, 10504-1722, USA). For the level of significance at which the null hypothesis (H0) was rejected, it was accepted $p < 0.05$. Methods of descriptive statistics, correlational analysis, as well as methods for testing of hypotheses were applied. The following working hypothesis was defined: overweight and unhealthy dietary habits affected the incidence of dental caries in adults. To monitor the existence (or absence) of a correlation between the frequency of dental caries and the impact of overweight, we used a non-parametric Spearman's test.

RESULTS

A total of 264 patients (128 men and 136 women), aged between 18 and 60 years, were examined. According to the age groups, the distribution is as follows: from 18 to 25 years – 14.4%; from 25 to 35 years – 16.7%; from 35 to 50 years – 42.4%; and over 50 years – 26.5%. We found a high incidence of dental caries. It was revealed that the DMFT index values were ranging between 1 and 21. The average value of DMFT index was 12.55 (SD = 5.54). We used the Kruskal-Wallis test to check if the DMFT index was distributed evenly between all age groups. The data showed a statistically significant difference in the distribution of DMFT values in the among age groups (h 89.353; df 3; P = 0.001, n 264). An increase in the incidence of dental caries was also established as age increased (Figure 1).

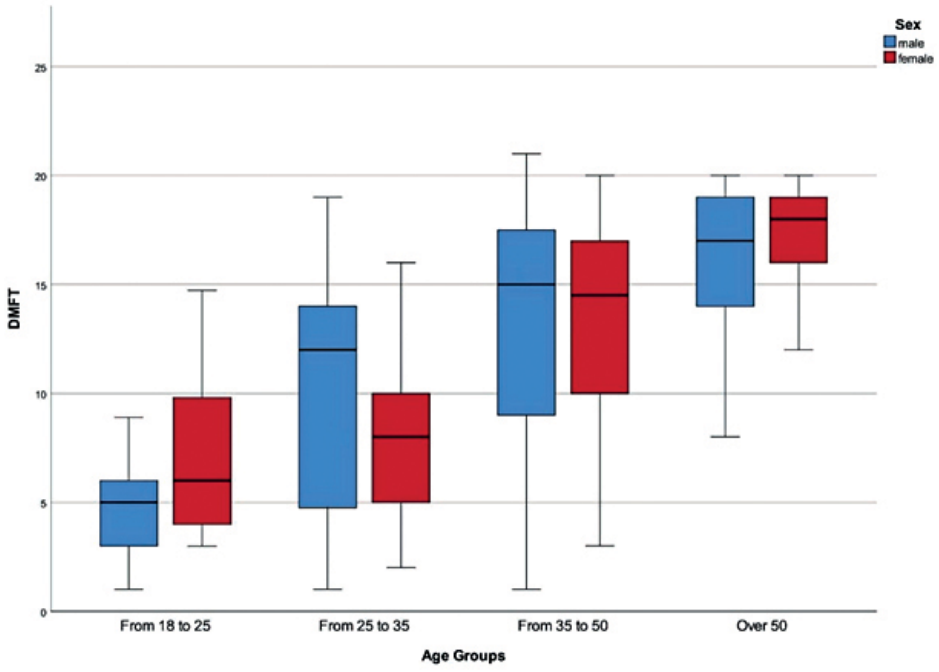


Fig. 1. Distribution of DMFT* is defined by age groups and sex. An increase in the incidence and the number of teeth affected by dental caries is observed as age increases. In patients under 25 years, the average DMFT values are approximately the same in men and women, in the group with participants aged 25-35 years it is higher in men, whereas in the next two groups, they are distributed almost similarly. As age increases, the trend related to increasing the number of teeth affected by caries is stable

* DMFT – Decayed, Missing, and Filled Teeth

The mean value of BMI in the cases was 25.60 (n 264), St. Dev = 4.359. The lowest BMI was 18.5, whereas the highest BMI – 37.55. The distribution of the indicator tracked, according to the age groups and sex, is presented in Table 1.

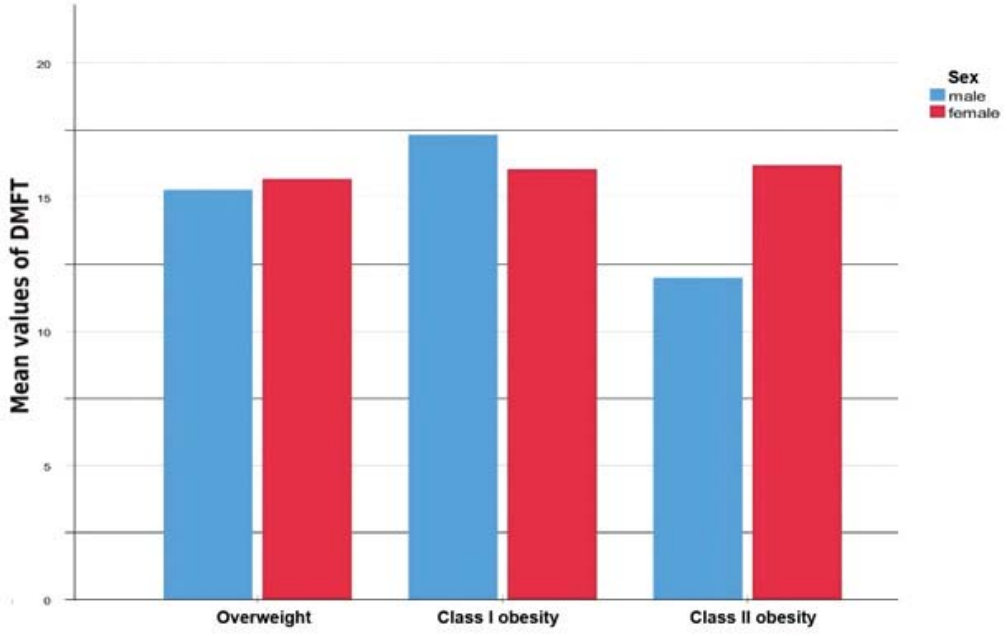
The results showed that overweight and obesity have a statistically significant correlation with the elevated incidence of dental caries in the studied cases (Rho = 0.592; p = 0.001, n 264). We did not find statistically significant differences related to the increased DMFT values in the overweight and obese patients according to their sex (Mann-Whitney U = 8628.000; Z = -0.123; p = 0.902, n 264) (Figure 2).

The data in Figure 2 show that the mean values of DMFT levels in the selected sample of overweight cases (BMI 25-29.9) demonstrate close values to the Class I obesity (BMI from 30 to 34.9) and Class II obesity. DMTF is similarly distributed between men and women.

The average daily intake of vitamin D in all groups of studied participants was lower than the recommended dietary intake of 15 μg/day (the average daily intake of vitamin D for the entire group was 6.6 μg/day). In the groups with a BMI higher than 30, the nutritional intake of Vitamin D was the lowest – 6.0 μg/day, affecting mostly women with Class II (BMI >

Table 1. Distribution of BMI according to the age groups and sex

BMI Groups			Sex		
			Male	Female	Total
Normal weight	Age Groups	From 18 to 25	16	16	32
		From 25 to 35	13	15	28
		From 35 to 50	12	30	42
		Over 50	4	6	10
	Normal weight group total:		45	67	112
Overweight	Age Groups	From 18 to 25	4	0	4
		From 25 to 35	7	6	13
		From 35 to 50	27	23	50
		Over 50	23	13	36
	Overweight group total:		61	42	103
Obese	Age Groups	From 18 to 25	0	2	2
		From 25 to 35	0	3	3
		From 35 to 50	9	11	20
		Over 50	13	11	24
	Obese group total:		22	27	49
Total for all groups:			128	136	264



* DMFT – Decayed, Missing, and Filled Teeth

Fig. 2. Distribution of DMFT* between the overweight and obesity groups (n = 152)

35) and Class III obesity (BMI > 40) [39]. We tried to find evidence regarding the existence or absence of a relationship between the higher DMFT values and the low nutritional intake of Vitamin D among these groups. No statistically significant difference in DMFT distribution and reduced intake of vitamin D was found in these groups.

DISCUSSION

The results of our study give us grounds to accept the null hypothesis (H0). We found a direct correlation between increasement in the dental caries incidence and the cases of overweight and obesity. The dental caries incidence in men and women from the studied groups is similarly distributed. The mentioned data fully complies with the findings of Abbass M. et al. [16], Pereira A. et al. [18], Iwasaki T. et al. [22], and Hamasha AA. et al. [23]. The foregoing studies have been conducted among adults in Japan, Saudi Arabia, Egypt, and Brazil. Such a relationship has not been found in the study conducted in the Republic of Korea by Song IS. et al. [24]. The authors did not establish a connection between the increased BMI values and the elevated incidence of dental caries. This difference indicates the polyethiology of the dental caries, which is mainly dominated by dietary habits and the food composition used in various geographical regions.

Other studies by Słotwińska S. et al. [20] and Alswat K. et al. [25] have found that overweight patients have a greater incidence of dental caries than the patients from other groups. Increased incidence of dental caries (DMFT > 6.5) is associated with more frequent food intake and is not related to the sex of the participants in the studied group. The study of Modéer T. et al. [21] has shown that overweight and obese patients are more affected by dental caries than those with normal weight, which may be explained by the frequent snacking in these groups and consumption of foods containing low molecular weight carbohydrates.

Our data show that the elevated dental caries incidence in patients with overweight and obesity affects equally both sexes, being in accordance with the foregoing studies. Furthermore, like Abbass M. et al. [16], we have found a significant increase in the incidence of the DTMF index as age increases.

The influence of serum vitamin D concentrations as a protective factor in the etiology of dental caries in adults has not been fully clarified so far. However, we have found very close average values in the incidence of dental caries related to overweight and obesity groups. These results are in full compliance with the studies of Hujoel P. et al. [9] and Hu Z. et al. [19]. Although, the intake of vitamins (including vitamin D) is often recommended as a protective measure against oral diseases and dental caries, there is currently no persuasive evidence of their protective function in terms of dental caries in adults.

In our study, we found higher values of dental caries distribution, and, the coefficient of DMFT (12.55 ± 5.545) compared to the data obtained from other geographical regions. Higher values of DMFT index for our country have been reported by Bonev B. et al.: 17.8 ± 7.98 [26]. These differences are probably related not only to the size of the sample used but also to the place of living of the studied groups. Unlike our study, in their sample, 47% of the cases were residents of small towns and villages, where the access to health care differs from that in the big cities.

CONCLUSION

This study has shown a direct relationship between the increased dental caries incidence and BMI in patients with overweight and obesity. Men and women are equally affected by this condition. Both diseases have similar etiology – improper dietary habits, excessive consumption of foods containing low molecular weight carbohydrates and carbonated beverages. Overweight and obese patients are at greater risk of dental caries development. The more frequent snacking can explain the higher DMFT values in this group.

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REFERENCES

1. Mok A, Ahmad R, Rangan A, Louie JCY. Intake of free sugars and micronutrient dilution in Australian adults. Am J Clin Nutr, 2018, 107(1):94-104.
2. Idrees M, Hammad M, Faden A, Kujan O. Influence of body mass index on severity of dental caries: cross-sectional study in healthy adults. Ann Saudi Med, 2017, 37(6):444-448.
3. Jandhyala S, Talukdar R, Subramanyam C et al. Role of the normal gut microbiota, World J Gastroenterol. 2015, 21(29):8787.
4. Verdam F, Fuentes S, de Jonge C et al. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity, 2013, 21(12):607-615.
5. Han J, Lawlor D, Kimm SY. Childhood obesity. Lancet, 2010, 375(9727):1737-1748.

6. Wright J, Kennedy-Stephenson J, Wang C et al. Trends in intake of energy and macronutrients – United States, 1971-2002. MMWR Morb Mortal Wkly Rep, 2004, 53:80-82.
7. Sallis JF, Glanz K. Physical activity and food environments: solutions to the obesity epidemic. Milbank Q, 2009, 87(1):123-154.
8. Speakman JR. Functional Analysis of Seven Genes Linked to Body Mass Index and Adiposity by Genome-Wide Association Studies: A Review. Hum Hered, 2013, 75(2-4):57-79.
9. Hujoel PP, Lingström P. Nutrition, dental caries and periodontal disease: a narrative review. J Clin Periodontol. 2017, Suppl 18:S79-S84.
10. Jayasinghe TN, Harrass S, Erdrich S et al. Protein Intake and Oral Health in Older Adults-A Narrative Review. Nutrients, 2022,14(21):4478.
11. Atkinson FS, Khan JH, Brand-Miller JC, Eberhard J. The Impact of Carbohydrate Quality on Dental Plaque pH: Does the Glycemic Index of Starchy Foods Matter for Dental Health? Nutrients, 2021,13(8):2711.
12. Moynihan P, Petersen PE. Diet, nutrition and the prevention of dental diseases. Public Health Nutr. 2004 Feb;7(1A):201-26.
13. Rahman MT, Hossain A, Pin CH, Yahya NA. Zinc and Metallothionein in the Development and Progression of Dental Caries. Biol Trace Elem Res. 2019, 187(1):51-58.
14. Chan AKY, Tamrakar M, Jiang CM et al. A Systematic Review on Caries Status of Older Adults. Int J Environ Res Public Health, 2021, 18(20):10662.
15. Sharma A, Subramaniam P. Association of Salivary Zinc Levels to Dental Caries and Body Mass Index. A Comparative Study. J Clin Pediatr Dent, 2021, 45(4):265-268.
16. Abbass MMS, AbuBakr N, Radwan IA et al. The potential impact of age, gender, body mass index, socioeconomic status and dietary habits on the prevalence of dental caries among Egyptian adults: a cross-sectional study. F1000 Res. 2019, 8:243.
17. Akarsu S, Karademir SA. Association Between Body Mass Index and Dental Caries in a Turkish Subpopulation of Adults: A Cross-Sectional Study. Oral Health Prev Dent, 2020, 18(1):85-89.
18. Pereira ALP, Frias AC, Hasegawa CCT et al. Assessment Between Dental Caries Index and Body Mass Index among Adults. Oral Health Prev Dent. 2018, 16(6):563-569.
19. Hu Z, Zhou F, Xu H. Circulating vitamin C and D concentrations and risk of dental caries and periodontitis: A Mendelian randomization study. J Clin Periodontol. 2022, 49(4):335-344.
20. Słotwińska SM, Słotwiński R. Host response, obesity, and oral health. Cent Eur J Immunol. 2015, 40:201-205.
21. Modéer T, Blomberg CC, Wondimu B et al. Association between obesity, flow rate of whole saliva, and dental caries in adolescents. Obesity (Silver Spring), 2010, 18(12):2367-73.
22. Iwasaki T, Hirose A, Azuma T et al. Associations between caries experience, dietary habits, and metabolic syndrome in Japanese adults. J Oral Sci, 2019, 61(2):300-306.
23. Hamasha AA, Alsolaihim AA, Alturki HA et al. The relationship between body mass index and oral health status among Saudi adults: a cross-sectional study. Community Dent Health, 2019, 36(1):217-222.
24. Song IS, Han K, Ryu JJ, et al. Obesity is inversely related to the risks of dental caries in Korean adults. Oral Dis, 2017, 23(8):1080-6.
25. Alswat K, Mohamed WS, Wahab MA, Aboelil AA. The Association Between Body Mass Index and Dental Caries: Cross-Sectional Study. J Clin Med Res, 2016, 8(2):147-52.
26. Bonev B, Avramova N, Yaneva K, Filchev D. Dental caries and associated socio-demographic factors in adult people in Bulgaria. Balk J Dent Med. 2015; 19:33-7.

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ORIGINAL ARTICLE

POLYMORPHIC VARIANT RS11206510 IN PCSK9 AND RISK OF CORONARY ARTERY DISEASE IN BULGARIANS

R. Tzveova[1], T. Yaneva-Sirakova[2], G. Naydenova[3], S. Vandeva[4], D. Pendicheva-Duhlenska[5], P. Atanasov[6], V. Mitev[7], R. Kaneva[7]

[1]Department of General and Clinical Pathology, University Hospital "Tsaritsa Yoanna – ISUL" – Sofia, Bulgaria
[2]Acibadem City Clinic Cardiovascular Center – Sofia, Bulgaria
[3]Department "Propaedeutics of Internal Diseases", Medical University – Pleven, Bulgaria
[4]Clinical Center of Endocrinology and Gerontology, Medical University – Sofia, Bulgaria
[5]Department of Pharmacology and Toxicology, Medical University – Pleven, Bulgaria
[6]National Sports Academy – Sofia, Bulgaria
[7]Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University – Sofia, Bulgaria

Abstract. Objective: *The aim of this study was to investigate the potential association of rs11206510 in PCSK9 gene with coronary artery disease (CAD) and myocardial infarction (MI) in Bulgarians.* ***Materials and Methods:*** *The current analysis included 261 patients with angiographically documented CAD (153 with MI and 108 without MI) and 496 population – based controls. Genomic DNA was extracted from venous blood samples. The selected polymorphism was genotyped by TaqMan SNP Genotyping Assay. The genotype and allele frequencies were compared between cases and controls using χ^2 test.* ***Results:*** *In this study, the presence of the T allele of rs11206510 in the PCSK9 gene was found to be associated with elevated risk for MI in patients with already existing myocardial ischemia (allele T, OR1.78,CI95:1.16-2.73, p = 0.007). The result was enhanced in the male subgroup (allele T, OR1.74, CI95:1.02-2.96, p = 0.038). Also, we found reduced risk of CAD (without MI) for T allele (OR0.70, CI95:0.49-0.99, p = 0.04). This trend was stronger in the male subgroup (OR0.56, CI95:0.35-0.90, p = 0.02). There was not any relationship of the studied genetic variant with the levels of total cholesterol, triglycerides, low density lipoproteins and high-density lipoproteins, or with systolic and diastolic blood pressure values.* ***Conclusion:*** *Our study found a difference in the frequencies of rs11206510 genotypes and alleles in the PCSK9 gene between cases and controls, and the relationship of the investigated polymorphism to the risk of cardiac injury in the Bulgarian population was demonstrated. Further investigations with a larger number of cases and controls will be needed in order to evaluate a possible association between this variant and CAD/MI in Bulgarians.*

Key words: *PCSK9, polymorphic variant, coronary artery disease, Bulgarians*

Corresponding author: *Reni Tzveova, Department of General and Clinical Pathology, University Hospital "Tsaritsa Yoanna – ISUL", 1527 Sofia, Bulgaria, 8 Byalo more Street, e-mail: renitzveova@abv.bg*

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INTRODUCTION

The gene PCSK9 codes the protein proprotein convertase subtilisin/kexin type 9. The enzyme reduces the number of low-density lipoprotein receptors (LDLR) at the surface of the hepatocyte [1]. This is followed by changes in lipid profile and elevated levels of LDL cholesterol in the blood. In 2003 year the first mutation in the PCSK9 gene was found - dominant form of familial hypercholesterolemia, and this initiated the understanding of the cholesterol metabolism [2].

A meta-analysis of 32 scientific studies showed significant association between the allele variant G of rs505151 in PCSK9 and higher levels of LDL cholesterol in the serum of Caucasian people. The same polymorphism was also associated with higher incidence of cardio-vascular incidents. On the other hand, the T allele of variant rs11591147 in PCSK9 was associated with lower levels of serum cholesterol and LDL cholesterol, as well as with lower risk for cardio-vascular incidents [3].

Other meta-analyses show association between the variant rs505151 in PCSK9 with higher levels of cholesterol, LDL-cholesterol, and cardio-vascular risk [4, 5].

These variants, as well as others in the gene PCSK9, can be used as genetic biomarkers for evaluation of cardio-vascular risk. This will help the diagnostic and prognostic process and will enhance the success of treatment in cardio-vascular diseases associated with dyslipidemia. That is why the indebt study of the polymorphisms of PCSK9 in the Bulgarian population is necessary in the context of successful future genetically based prophylaxis of socially important diseases.

MATERIALS AND METHODS

The study included patients with angiographically proven coronary artery disease (CAD), with or without myocardial infarction, who were hospitalised in one of the affiliated cardiology clinics. All population controls were from the DNA biobank of the Molecular Medicine Center, Medical University, Sofia, Bulgaria. The National University Complex for Biomedical and Translational Research (NUCBTR) is a strategic network of infrastructures for fundamental and translational biomedical research and includes partners from two of the largest medical universities in Bulgaria, the Medical University of Sofia and the Medical University of Plovdiv, as well as a number of hospital and research centers. At the core of the University Complex is the unification of the largest biobanks for the storage of biological material and clinical data in the country.

Inclusion criteria for the patients with CAD: 1. Age between 18 and 75 years; 2. Systolic blood pressure ≤ 120 mm Hg; 3. Diastolic blood pressure ≤ 80 mm Hg; 4. CAD proven by coronary angiography; 5. Hospitalized or ambulatory patients. 6. Signed written informed consent for participation in the current study.

Exclusion criteria: 1. Age below 18 years or above 75 years; 2. Absence of CAD established by coronary angiography; 3. Absence of signed informed consent; 4. Acute renal failure; acute liver failure; 5. Severe anemia, requiring transfusion; 6. Chronic dialysis; 7. Epilepsy.

The demographic and clinical characteristics of the studied groups of patients with CAD with and without MI and the population controls, are given in Table 1.

The basic laboratory panel recommended by the World Health Organization for the precise assessment of cardiovascular risk (serum glucose levels, total cholesterol, ASAT, ALAT, GGT, IGF-1 – insulin like growth factor 1, TSH – thyroid stimulating hormone) known only for 1/3 of the population controls and for all patients with CAD.

All participants in the study were genotyped for polymorphic variant rs11206510 in gene PCSK9 (Table 2). The

Table 1. Demographic and clinical characteristics of the studied groups of patients and the population controls

	CAD (N = 108)	MI (N = 153)	Population controls (N = 496)
Age (years)	66.27 ± 8.81	66.34 ± 10.39	36.08 ± 12.99
Sex (males)	60 (55.56)	92 (60.53)	241 (48.59)
BMI (kg/m2)	29.66 ± 5.72	28.36 ± 4.89	25.66 ± 4.91
Total cholesterol (mmol/l)	5.64 ± 1.04	5.94 ± 0.75	4.99 ± 0.94
Triglycerides (mmol/l)	1.32 ± 0.67	2.07 ± 0.56	0.93 ± 0.56
LDL-cholesterol (mmol/l)	4.23 ± 1.06	4.14 ± 0.79	3.18 ± 0.88
HDL-cholesterol (mmol/l)	1.22 ± 0.37	1.39 ± 0.28	1.62 ± 0.40
Systolic blood pressure (SBP) (mmHg)	147.86 ± 21.83	135.92 ± 10.96	-
Diastolic blood pressure (DBP) (mmHg)	87.50 ± 13.64	83.64 ± 6.60	-

Table 2. Genome information for the polymorphic variant rs11206510 in gene PCSK9

Chromosome	Position	Gene	Identification number	Change	Type of change	Localization
1p32.3	55496039	PCSK9	rs11206510	T > C	transition	intron

genomic DNA was isolated from peripheral venous blood. The studied polymorphic variant was genotyped with the use of TaqMan SNP Genotyping Assay (Applied Biosystems).

The comparative statistical analysis of the genotypic and allelic frequencies between patients with CAD and healthy controls was done with the use of $\chi 2$ test. We also conducted a check of the Hardy – Weinberg equation. For statistically significant difference was accepted $p < 0.05$. The analysis was done with SPSS version 19.0 (IBM).

Ethical Aspects: the study was approved by the Ethical committee of Medical University Sofia, Bulgaria and the Ethical Committee of Medical University Pleven. The study was conducted in accordance with ethical principles of the Declaration of Helsinki for human rights form 1975 year.

RESULTS

The current analysis included 261 patients with angiographically proven CAD (153 patients with MI [6, 7] and 108 without MI) and 496 population controls. All participants were genotyped for the polymorphic variant rs11206510 in gene PCSK9.

The allelic and genotypic frequencies of rs11206510 in gene PCSK9 in the group of patients with CAS and the population group of Bulgarians is given in tables 3-6.

As a result of the current genetic analysis we found, that in Bulgarians with CAD without MI, the more frequent T allele of the polymorphic variant rs11206510 in gene PCSK9 could not be associated with higher risk for CAD incidence (OR 0.70, CI95: 0.49-0.99, p = 0.04). This tendency was stronger for males as compared to females (OR 0.56, CI95:0.35-0.90, p = 0.02) (Table 4).

The polymorphic allele T was a risk one for the incidence of MI in patients with already developed CAD – allele T, OR 1.78, CI95: 1.16-2.73, p = 0.007. This effect was stronger for males as compared to females – allele T, OR 1.74, CI95: 1.02-2.96, p = 0.038 (Table 6).

As addition, we conducted associative analysis for a potential association between several clinical variables and a certain genotype of rs11206510 in gene PCSK9 (Table 7).

Table 3. Allelic and genotypic distribution of the studied polymorphic variant rs11206510 in gene PCSK9 in the group of patients with CAD (with or without MI) and the population controls in Bulgarians

Chr	Gene	Variant	Model	Genotype/ allele	all			Males			Females		
					CAD n (%)	controls n (%)	P	CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P
1	PCSK9	rs11206510	genotypic	CC	8 (2.49)	15 (3.02)	0.77	4 (1.95)	5 (2.07)	0.56	4 (3.45)	10 (3.92)	0.58
				CT	87 (27.10)	125 (25.20)		59 (28.78)	54 (22.41)		28 (24.14)	71 (27.84)	
				TT	226 (70.41)	356 (71.78)		142 (69.27)	182 (75.52)		84 (72.41)	174 (68.24)	
			allelic	C	103 (16.04)	155 (15.63)	T 0.82 (OR 0.97, CI95:0.74-1.27)	67 (16.34)	64 (13.28)	T 0.20 (OR 0.78, CI95:0.54-1.14)	36 (15.52)	91 (17.84)	T 0.44 (OR 1.18, CI95:0.78-1.80)
				T	539 (83.96)	837 (84.37)		343 (83.64)	418 (86.72)		196 (84.48)	419 (82.16)	

Table 4. Distribution of the allelic and genotype frequencies of the studied polymorphic variant rs11206510 in gene PCSK9 in the group of patients with CAD without MI and population controls

Chr	Gene	Variant	Model	Genotypic/allelic	All			Males			Females		
					CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P
1	PCSK9	rs11206510	Genotypic	CC	5 (4.03)	15 (3.02)	0.11	3 (4.00)	5 (2.07)	0.03	2 (4.08)	10 (3.92)	0.76
				CT	42 (33.87)	125 (25.20)		26 (34.67)	54 (22.41)		16 (32.65)	71 (27.84)	
				TT	77 (62.10)	356 (71.78)		46 (61.33)	182 (75.52)		31 (63.27)	174 (68.24)	
			allelic	C	52 (20.97)	155 (15.63)	T 0.04 (OR 0.70, CI95:0.49-0.99)	32 (21.33)	64 (13.28)	T 0.02 (OR 0.56, CI95:0.35-0.90)	20 (20.41)	91 (17.84)	T 0.57 (OR 0.84, CI95:0.49-1.46)
				T	196 (79.03)	837 (84.37)		118 (78.67)	418 (86.72)		78 (79.59)	419 (82.16)	

Table 5. Distribution of the allelic and genotype frequencies of the studied polymorphic variant rs11206510 in gene PCSK9 in the group of patients with CAD with MI and population controls

Chr	Gene	Variant	Model	Genotypic/allelic	All			Males			Females		
					CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P	CAD n (%)	Controls n (%)	P
1	PCSK9	rs11206510	genotypic	CC	3 (1.52)	15 (3.02)	0.46	1 (0.77)	5 (2.07)	1	2 (2.99)	10 (3.92)	0.26
				CT	45 (22.84)	125 (25.20)		33 (25.38)	54 (22.41)		12 (17.91)	71 (27.84)	
				TT	149 (75.64)	356 (71.78)		96 (73.85)	182 (75.52)		53 (79.10)	174 (68.24)	
			allelic	C	51 (12.94)	155 (15.63)	T 0.21 (OR 1.24, CI95:0.89-1.75)	35 (13.46)	64 (13.28)	T 1.00 (OR 0.98, CI95:0.63-1.53)	16 (11.94)	91 (17.84)	T 0.10 (OR 1.60, CI95:0.91-2.83)
				T	343 (77.06)	837 (84.37)		225 (86.54)	418 (86.72)		118 (88.06)	419 (82.16)	

Table 6. Distribution of the allelic and genotype frequencies of the studied polymorphic variant rs11206510 in gene PCSK9 between the groups of patients with CAD with and without MI

Chr	Gene	Variant	Model	Genotypic/allelic	All			Males			Females		
					With MI n (%)	Without MI n (%)	P	With MI n (%)	Without MI n (%)	P	With CAD n (%)	Without n (%)	P
1	PCSK9	rs11206510	genotypic	CC	3 (1.52)	5 (4.03)	0.01	1 (0.77)	3 (4.00)	0.08	2 (2.99)	2 (4.08)	0.21
				CT	45 (22.84)	42 (33.87)		33 (25.38)	26 (34.67)		12 (17.91)	16 (32.65)	
				TT	149 (75.64)	77 (62.10)		96 (73.85)	46 (61.33)		53 (79.10)	31 (63.27)	
			allelic	C	51 (12.94)	52 (21.0)	T 0.007 OR 1.78 (CI95: 1.16-2.73)	35 (13.46)	32 (21.33)	T 0.038 (OR 1.74, CI95: 1.02-2.96)	16 (11.94)	20 (20.41)	T 0.08 (OR 1.89, CI95:0.92-3.87)
				T	343 (87.06)	196 (79.0)		225 (86.54)	118 (78.67)		118 (88.06)	78 (79.59)	

Table 7. Associative analysis for potential association between the given clinical variables, blood pressure values, and a certain genotype of rs11206510 in gene in patient with CAD

Genotype		Total cholesterol (mmol/L)	HDL-cholesterol (mmol/L)	LDL-cholesterol mmol/L)	TGs (mmol/L)	SBP (mmHg)	DBP (mmHg)
CC	mean	5.26	1.46	3.46	1.69	130.00	80.00
	Standard Deviation (± SD)	0.997	0.307	1.033	0.891	14.032	5.742
	minimum	4.00	1.00	3.00	1.00	130.00	80.00
	maximum	7.00	2.00	5.00	3.00	13.00	80.00
	Standard error (± SE)	0.446	0.137	0.462	0.398	3.523	2.132
CT	mean	5.22	1.46	3.41	1.44	154.55	87.73
	Standard Deviation (± SD)	0.834	0.357	0.795	0.772	15.076	6.842
	minimum	3.00	1.00	2.00	1.00	140.00	80.00
	maximum	7.00	2.00	5.00	3.00	180.00	100.00
	Standard error (± SE)	0.101	0.044	0.096	0.094	4.545	2.063
TT	mean	5.39	1.41	3.54	1.54	142.41	85.93
	Standard Deviation (± SD)	1.139	0.403	1.150	0.905	20.493	14.280
	minimum	3.00	1.00	2.00	2.00	90.00	60.00
	maximum	11.00	3.00	8.00	5.00	200.00	130.00
	Standard error (± SE)	0.076	0.027	0.078	0.061	3.944	2.748
P		0.509	0.586	0.667	0.647	0.163	0.815

We could not find any statistically significant difference between the mean values of total cholesterol value, HDL-cholesterol (HDL), LDL-cholesterol (LDL), triglyceride levels (TGs), systolic blood pressure (SBP), diastolic blood pressure (DBP) in the groups of patients with and without the genetic variants CC, CT and TT of rs11206510 in PCSK9 (Table 7).

DISCUSSION

Dyslipidemia is one of the major risk factors for development and progression of cardio-vascular diseases [8]. The statins play a basic role in the treatment of dyslipidemia, however, they may have different clinical and laboratory side effects with respect to the genetic profile of the given patient. The finding of mutations in the PCSK9 gene recently, and its association with the levels of LDL-cholesterol was related to cardio-vascular incidents. The gene PCSK9 encodes an enzyme, which reduces LDLR on the hepatocyte membrane. This induces changes in the lipid profile and elevation of LDL-cholesterol [2]. The polymorphic variants of PCSK9 have a complex significance for the pathogenesis and regulation of LDL levels [2, 9]. This induced the development of a whole new drug class for the treatment of severe dyslipidemia [9, 10].

A multilocus study, aimed at assessment of the genetic risk on the basis of 27 chromosome loci, incl. PCSK9, identified individuals with elevated risk for cardio-vascular events. These findings were based on the results from the Malmo Diet and Cancer study, and four additional randomized controlled trials: JUPITER, ASCOT, CARE and PROVE IT-TIMI 22 [11].

The aim of the current trial was to test if there was association between polymorphic variant rs11206510 in gene PCSK9 and the risk for CAD and MI in Bulgarians. We did not find T allele associated with a higher risk for CAD, but in patients with already developed ischemia, it was associated with elevated risk for MI.

A possible explanation for the discrepancy with the literature because of lack of association of T allele and CAD can be explained with two factors, which can be corrected in future trials. First, we used population controls and not age-matched control individuals. It can be suspected that a large number of genetically predisposed but clinically still not manifested young individuals were included in the control group. Second, the patients with angiographically proven CAD in the study were relatively elderly, with multiple concomitant cardio-vascular and socio-economic risk factors that may modify the added, non-genetically associated cardio-vascular risk. The contemporary conception for the genetic predisposition of CAD is that it is based on multiple genes and loci with relatively small individual significance [12]. In a relatively small sample size, some discrepancies with the findings from GWAS may arise. However, it is important to emphasize that the study of the genetic aspects of CAD in Bulgarians is important, because this is a high-risk group and specific genetic variants may potentially play a role. It should also be noted, that a variety of modifiable risk factors that form the environment could influence the genetic expression of certain variants and further modify the local phenotypic spectrum [13, 14].

It was impossible from a practical point of view to find a control group of healthy individuals with a mean age, corresponding to the mean age of the CAD patients. Because of this reason, in the present study we recruited a twice larger sample of population controls compared with patients with cardiovascular disease. Their DNA was from the national biobank of the Molecular Medical Center, Medical University Sofia, Bulgaria. The use of a much larger control group was meant to match the polymorphic variant frequencies of the general population. It was expected that the frequencies in the patients' group would be much higher.

In the literature, the precise association between a given allele/genotype is not one. In a study of Zhu et al. was found that CC and CT genotypes were associated with elevated CAD mortality rate in patients on statins [15].

The result for a higher risk for MI in T allele corresponded to the major findings in the literature [16].

The difference in the plasma LDL levels and the total cholesterol levels did not differ significantly between the wild type and the heterozygous to the polymorphic allele C. The potential explanations were two: either the sample size is not big enough, or the effect of statins treatment masks the precise genetic association with a given levels of cholesterol. According to the general recommendations, all the patients with CAD have indications for statin treatment [17, 18, 19]. In the literature there are many positive results for a potential association of rs11206510 in gene PCSK9 with the values of lipids in blood. There are studies in the literature for a potential association with the given polymorphism and the risk for CAD.

A meta-analysis from 2013 of Zhou et al showed that the polymorphic variant was a risk factor for CAD in

Caucasians (P = 0.007, OR = 1.09, 95% CI = 1.03-1.17), but not in Asians (P = 0.167, OR = 1.16, 95% CI = 0.94-1.43) [20]. Earlier studies in the field proved the role of the TGs, LDL cholesterol, HDL cholesterol as independent predictors for CAD. Some genetic variants may be exposed to the influence of serum lipids and their expression thus, modified [22].

In 2010, Teslovich et al. published association study of case-control type, that included more than 100,000 Europeans. They test the association between cardiovascular disease and several polymorphic variants in genes PCSK9 and LDLR [23]. The results showed that the alleles rs11206510-T in gene PCSK9 and rs1122608-G in gene LDLR were associated with higher levels of LDL cholesterol and total cholesterol, and thus were risk factors for cardio-vascular disease.

Guella et al. in 2010 year found that the rare C allele of rs11206510 in gene PCSK9 significantly correlated with lower LDL levels – OR = 0.82, 95% CI = 0.73-0.93, P = 1.89×10-3) and the total cholesterol (OR = 0.80, 95% CI = 0.72-0.89, P = 8.12×10-5) in Italians [24].

According to Willer et al., rs11206510 was positively associated both with the concentration of LDL cholesterol and the risk for CAD in Europeans [25].

Another three genome association studies confirmed the significance of rs11206510 (T > C) in PCSK9 as a risk factor for CAD in Europeans [26-28].

Contrary to the above results were the findings of Reilly et al in 2011 year, who could not confirm such an association in Europeans in a genome wide study [29].

CONCLUSION

This is the first study of the potential association of polymorphic variant rs11206510 in gene PCSK9 and CAD with or without MI in Bulgarians. The data cannot confirm the role of this polymorphism in cardio-vascular pathology because of the small effect on the studied disease and the possibly insufficient number of patients and controls. The further study of other polymorphic variants in PCSK9 will help build the whole picture for the possible role of this gene in the cardio-vascular pathology, locally in Bulgarians.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Fitzgerald K et al. A Highly Durable RNAi Therapeutic Inhibitor of PCSK9. N Engl J Med, 2017, 376(1):41-51.
2. Abifadel M et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. Nat Genet, 2003,34(2):154-6.
3. Qiu C et al. What is the impact of PCSK9 rs505151 and rs11591147 polymorphisms on serum lipids level and cardiovascular risk: a meta-analysis. Lipids Health Dis, 2017,16(1):111.
4. Cai G et al. The associations between proprotein convertase subtilisin/kexin type 9 E670G polymorphism and the risk of coronary artery disease and serum lipid levels: a meta-analysis. Lipids Health Dis, 2015,14:149.
5. Adi D et al. Relationships between genetic polymorphisms of E670G in PCSK9 gene and coronary artery disease: a meta-analysis. Int J Clin Exp Med, 2015, 8(8):13251-8.
6. Thygesen K, Alpert J, Jaffe A, et al. Third universal definition of myocardial infarction. Circulation. 2012;126;2020-35.
7. Thygesen K, Alpert J, Jaffe A, et al. Fourth universal definition of myocardial infarction (2018). European Heart Journal. 2019; 40: 237-69.
8. Mach F, Baigent C, Catapano A, et al. 2019 ESC/EAS Guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk: the Tak Force for the management of Dyslipidaemias of the European Society of Cardiology {ESC} and European Atherosclerosis Society (EAS). European Heart Journal. 2020; 41: 111-88.
9. Peterson AS, Fong LG, Young SG. PCSK9 function and physiology. J Lipid Res, 2008,49(7):1595-9.6.
10. Stein R, Ferrari F, Scolari F, Genetics, Dyslipidemia, and Cardiovascular Disease: New Insights. Curr Cardiol Rep, 2019,21(8):68.7.
11. Mega JL et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. Lancet, 2015,385(9984): 2264-2271.8.
12. McPherson R, Tybjaerg-Hansen. Generics of Coronary Artery Disease. Circulation Research. 2016; 118: 564-78.
13. Findley A, Richards A, Petrini C, et al. Interpreting coronary artery disease risk through gene-environment interactions in gene regulation. Genetics. 2019; 213: 651-63.
14. Findley A, Monziani A, Richards A, et al. Functional dynamic genetic effects on gene regulation are specific to particular cell types and environmental conditions. eLife. 2021; 10: e67077.
15. Zhu L, Ji X, Jiang L, et al. Utility of genetic variants to predict prognosis in coronary artery disease patietns receiving statin treatment. Int J Clin Exp Pathol. 2017; 10: 8795-8803.
16. Kathiresan, S. et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet. 2009; 41, 334-41.
17. Knuuti J, Wijns W, Saraste A, et al. 2019 ESC Guidleines for the diagnosis and management of chronic coronary syndromes: The Task Force for diagnosis and management of chronic coronary syndromes of the European Society of Cardiology (ESC). Eur Heart J. 2020; 41: 407-77.
18. Ibanez B, James S, Agewall S, et al. 2017 ESC Guidlienes for the management of acute myocardial infarction in patients presenting with CT segment elevation: The Task Force for the management of acute Myocardial infarction in patients presenting with ST – segment elevation of the

European Society of Cardiology.Eur Heart J, 2018; 39: 119-77.
19. Collet J, Thiele H, Barbato E, et al. 2020 ESC Guideliens for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). 2021; 42: 1289-1367
20. Lloyd-Jones DM et al. Lifetime risk of coronary heart disease by cholesterol levels at selected ages. Arch Intern Med, 2003,163(16): 1966-72.9.
21. Kathiresan S et al. A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. BMC Med Genet, 2007,8 Suppl 1: S17.10.
22. Zhou Li, He M, Mo Z, et al. A genome wide association study identifies common variants associated with lipid levels in the Chinese population. PLoS One. 2013; 8(12): e82420.
23. Teslovich TM et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature, 2010.,466(7307):707-13. 11.
24. Guella I et al. Effects of PCSK9 genetic variants on plasma LDL cholesterol levels and risk of premature myocardial infarction in the Italian population. J Lipid Res, 2010,51(11):3342-9.12.
25. Willer CJ et al. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet, 2008,40(2):161-9.13.
26. Schunkert H et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet, 2011,43(4):333-8.14.
27. Waterworth DM et al. Genetic variants influencing circulating lipid levels and risk of coronary artery disease. Arterioscler Thromb Vasc Biol, 2010,30(11):2264-76. 15.
28. Kathiresan S et al. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet, 2009,41(1):56-65. 16.
29. Reilly MP et al. Identification of ADAMTS7 as a novel locus for coronary atherosclerosis and association of ABO with myocardial infarction in the presence of coronary atherosclerosis: two genome-wide association studies. Lancet, 2011,377(9763):383-92.17.

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ORIGINAL ARTICLE

ANTIPROLIFERATIVE ACTIVITY OF NATURAL FLAVONOID FUSTIN ISOLATED FROM THE HEARTWOOD OF *COTINUS COGGYGRIA* SCOP. AGAINST BREAST AND COLON CANCER CELL LINES

Z. Gospodinova[1], G. Antov[1], M. Novakovic[2], V. Tesevic[3], N. Krasteva[4], D. Pavlov[5], S. Valcheva-Kuzmanova[6]

[1]Laboratory of Genome Dynamics and Stability, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences – Sofia, Bulgaria
[2]University of Belgrade-Institute of Chemistry, Technology and Metallurgy, National Institute of the Republic of Serbia – Belgrade, Serbia"
[3]University of Belgrade-Faculty of Chemistry – Belgrade, Serbia
[4]Department of Electroinduced and Adhesive Properties, Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences – Sofia, Bulgaria
[5]Department of Biochemistry, Molecular Medicine and Nutrigenomics with Laboratory of Nutrigenomics, Functional Foods and Nutraceuticals, Faculty of Pharmacy, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, Bulgaria
[6]Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov" – Varna, Bulgaria

Abstract. Background and objective: *Cotinus coggygria Scop. is a valuable medicinal plant species with pronounced pharmacological potential due to its numerous biological activities. The herb is characterized by a high content of polyphenols among which is fustin. The anticancer activities of fustin, however, are extremely weakly studied. The aim of the present study was to investigate the in vitro antiproliferative potential of fustin isolated from the heartwood of C. coggygria against cell lines originating from two of the most common cancer types – breast (MDA-MB-231 and MCF7), and colon cancer (Colon 26).* ***Materials and methods:*** *Cell growth inhibitory properties of fustin were examined by MTT assay. Subsequently, phase-contrast and fluorescence microscopy analysis as well as colony-forming assay were carried out on the most sensitive to the cytostatic action of the fustin cell line.* ***Results:*** *The obtained results showed that fustin reduced the proliferation of all studied cell lines. The highest cytostatic effect was registered towards breast cancer MDA-MB-231 cells with a half maximal inhibitory concentration (IC_{50}) value of 56.02 μg/ml followed by colon cancer cells with an IC_{50} of 78.07 μg/ml. MCF7 cell proliferation was least affected with a calculated IC_{50} of 187.8 μg/ml. Further investigations on breast cancer MDA-MB-231 cells indicated decreased density of cell monolayer and some morphological alterations, significant attenuation in the number of viable cells, and diminished clonogenic ability of cells after fustin exposure.* ***Conclusion:*** *It could be concluded that fustin isolated from the heartwood of medicinal plant C. coggygria possesses marked antiproliferative properties against breast cancer cell line MDA-MB-231 which will be a subject of our more detailed future investigations.*

Key words: *fustin, C. coggygria Scop., antiproliferative effect, MDA-MB-231, MCF7, Colon 26*

Corresponding author: *Zlatina Gospodinova, Laboratory of Genome Dynamics and Stability, Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 21 Acad. G. Bonchev Street, 1113 Sofia, Bulgaria; e-mail: zlatina.go@abv.bg*

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INTRODUCTION

Cancer is one of the leading causes of mortality worldwide with about 10 million deaths reported in 2020 [1]. Considering the adverse and often life-threatening side effects of cancer treatment, the need for more effective, non-toxic, well-tolerated, and at the same time cost-effective and readily available anticancer agents is imperative.

Medicinal plants play an increasingly important role in the therapy and prevention of a number of diseases, including cancer, both independently or complementary to conventional therapy. *Cotinus coggygria* Scop. (Anacardiaceae), also known as European smoke tree, is a medicinal plant with a wide range of distribution from southern Europe, Central Asia and the Himalayas to northern China. The herb is traditionally used in folk medicine for treatment of skin ailments, paradontosis, gastric and duodenal ulcers, diarrhea, cardiac and renal diseases, diabetes, asthma, cough, liver disease, cancer, and many others. The plant also possesses various proven biological and therapeutic properties, such as antihaemorrhagic, antipyretic, antiseptic, immunomodulatory, antioxidant, anti-inflammatory, antigenotoxic, anticancer, hepatoprotective, antibacterial, antiviral, etc. [2, 3].

C. coggygria is well-distinguished by the presence of wide range of polyphenolic secondary metabolites, including sulfuretin (3′,4′,6-trihydroxyaurone), fisetin (3′,4′,7-trihydroxyflavonol), taxifolin (5,7,3’,4’-flavanon-ol), quercetin (3,3’,4’,5,7-pentahydroxyflavone), butein (trans-2’,3,4,4’-tetrahydroxychalcone), butin (3’,4’,7-trihydroxyflavanone), rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside),liquiritigenin(4’,7-dihydroxyflavanone), isoliquiritigenin (trans-2’,4,4’-trihydroxychalcone), gallic acid (3,4,5-trihydroxybenzoic acid), 4’,5,7-trihydroxyflavanone, eriodictyol (3′,4′,5,7-tetrahydroxyflavanone), fisetinidol-(4α→8)-(+)-catechin, epifisetinidol-(4β→8)-(+)-catechin, epoxide (2,10-oxy-10-methoxysulfuretin), cotinignan A, etc. [4-6]. Among the polyphenolic compounds in the medicinal shrub is fustin (3’,4’,7-trihydroxyflavanol), also known as “dihydrofisetin”. Fustin is a flavanonol subtype of flavonoid.

Flavonoids are a major class of phytochemicals with about 4000 types reported in plants [7] and are intensively studied in recent years due to their chemopreventive and chemotherapeutic properties against different kinds of cancer [8].

Fustin is weakly studied in regard to its anticancer and chemopreventive qualities. There are only a few reports stating that fustin promoted cell death of multiple myeloma cells and suppressed tumor cell growth *in vivo* in BALB/c mice in combination with epigallocatechin-3-O-gallate [9], and possesses protective activity against chromosome aberrations in peripheral human lymphocytes [10].

AIM OF THE STUDY

Taking into account the limited data about the anticancer potential of the natural flavonoid fustin the aim of the present research was to assess the *in vitro* antiproliferative capacity of fustin isolated from the heartwood of *C. coggygria* against cell lines originating from two of the most common cancer types – breast and colon cancer.

MATERIALS AND METHODS

Plant material and extraction

The *C. coggygria* heartwood was collected at Deliblatska Peščara (Deliblato Sand), Vojvodina province, Serbia, in May 2019. Plant material was identified by Prof. Milan Veljic, Faculty of Biology, University of Belgrade, and voucher specimen BEOU 17422 was deposited at the Herbarium of the Institute of Botany and Botanical Garden “Jevremovac”, Belgrade, Serbia. The heartwood was air-dried and milled to a fine powder. Wood powder, 1 kg, was extracted three times with 10 l of methylene chloride/methanol 1:1 for 24 h at room temperature to give 76 g of the crude extract which was subjected to fractionation by Si gel CC.

Isolation and identification of fustin

For column chromatography (CC) Merck silica gel (Si gel) (particle size 0.063-0.200 mm), methanol and methylene chloride were used. Analytical TLC was performed on aluminium plates precoated with Merck silica gel 60 F254 (0.25 mm thickness). The NMR spectra were obtained on a Bruker Avance III 500 (500 MHz for ^{1}H; 125 MHz for ^{13}C), in CD_3OD as solvent. Chemical shifts (d) were expressed in

ppm and coupling constants (J) in hertz (Hz). Semi-preparative reversed phased HPLC was performed on Agilent Technologies 1100 Series HPLC-DAD and Zorbax Eclipse XDB C18 column (150 × 9.4 mm, i.d. 5 µm) was used.

Crude extract was chromatographed on a Si gel CC column (750 × 45 mm), with methylene chloride/methanol (gradient elution – from 97/3 to 60/40). This step was repeated seven times to obtain high amounts of fustin. Column chromatography was monitored by TLC, and the fractions with similar Rf values were combined. Fustin was found in fractions eluted with methylene chloride/methanol approximately 80:20. Pure fustin was isolated from these fractions by reversed phase semi-preparative HPLC using water/acetonitrile system, 254 nm for detection and the following program: 0-20 min, 20-37% CH_3CN; 20-21 min, 37-50% CH_3CN; 21-27 min, 50% CH_3CN; and 27-30 min, 50-100% CH_3CN. Fustin was purified up to 98% on reversed phase semiprep. HPLC using the following program: 0-20 min, 25-40% CH_3CN (Rt = 5.1 min).

Cell lines and culturing conditions

Two human breast cancer cell lines, MDA-MB-231 (triple negative breast cancer subtype – TNBC) and MCF7 (luminal A breast cancer subtype), and a murine colon adenocarcinoma cell line Colon 26 were purchased from American Type Culture Collection (ATCC, Manassas, Virginia, USA). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atmosphere containing 5% CO_2.

MTT cell proliferation assay

Cell proliferation was assessed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay [11]. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well and after overnight incubation were treated with increasing concentrations of fustin (5-135 µg/ml) in a new medium for 72 h. As a negative control were used untreated cells cultured in a medium for the same time period.

During the last 4 h of the incubation 20 µl MTT solution with a concentration of 5 mg/ml per well was added and the samples were incubated in dark. At the end of incubation the medium was removed, the formazan complex was dissolved in 10% SDS and 0.01 M HCl, and the absorbance was measured at 570 nm on a microplate reader (Thermo Scientific Multiskan Spectrum). The percentage of cell proliferation was calculated using the following formula:

Cell proliferation (%) = (Absorbance test sample/Absorbance control) × 100

Phase-Contrast Light Microscopy

Phase-contrast observation of the cells was done in order to monitor any alterations in their morphology after treatment with fustin at concentrations of 35, 55, and 75 µg/ml for 72 h. Phase-contrast micrographs were taken at magnifications of 10× with a Leitz microscope equipped with a digital camera.

Fluorescent Microscopy

Fluorescein diacetate (FDA) staining of cells was performed for visualization and a quantitative evaluation of viable metabolic active cells after 72 h treatment with the studied bioflavonoid at concentrations of 35, 55, and 75 µg/ml. At the initial step of the analysis, 1.25×10^4 cells were seeded on round coverslips placed in a 24-well plate, incubated overnight for attachment and treated on the next day with fustin. At the end of the incubation time, cells were observed at a magnification of 10× under fluorescent microscope Axiovert 25 (Carl Zeiss, Germany) equipped with a digital camera after staining for 2 min with 0.001% FDA dissolved in acetone and washed with 1× Phosphate Buffered Saline (PBS). Afterward, the micrographs were analyzed by ImageJ software to count the number of viable attached cells.

Colony-forming assay

For the analysis evaluating cell capability to proliferate and form a colony, cells were seeded at a density of 1×10^3 cells per well in 6-well plates, allowed to attach overnight, and treated with 35 µg/ml fustin. After 5 days, cells were fixed and stained with 2% methylene blue in 50% ethanol for 20 min for colony visualization.

Statistical analysis

Data were presented as means ± standard error of the mean (SEM) of three independent experiments each performed in at least three parallel repeats. The half maximal inhibitory concentration (IC_{50}) values were calculated by GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Statistical differences between control untreated and treated groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test and the results were considered significant if $p < 0.05$.

RESULTS

Antiproliferative potential of C. coggygria isolated fustin on MDA-MB-231, MCF7 and Colon 26 cells

The antiproliferative properties of fustin isolated from the heartwood of *Cotinus coggygria* were studied on human breast cancer cell lines MDA-MB-231 and MCF7, and a murine colon adenocarcinoma cell line Colon 26

after 72 h period of treatment in the range of concentrations from 5 to 135 μg/ml through MTT assay.

The obtained results displayed cytostatic effect of fustin on all tested cell lines (Fig. 1) with the highest cell growth inhibitory activity against the MDA-MB-231 cell line representing triple negative breast cancer subtype with an IC_{50} value of 56.02 μg/ml and maximal registered reduction of proliferation to 40.39% at 115 μg/ml. A considerable cytostatic effect was also found for colon adenocarcinoma cells Colon 26 (IC_{50} = 78.07 μg/ml). The antiproliferative effect was weakest for the MCF7 cell line, the luminal A breast cancer subtype, with a calculated IC_{50} concentration of fustin of 187.8 μg/ml. Statistically significant differences between treated and control groups with p-values of less than 0.0001 were found for all of the tested concentrations of fustin, even at the lowest doses of 5 μg/ml, in the three cancer cell lines (Fig. 1).

MDA-MB-231 cell morphological examination

Phase-contrast micrographs showed that 72 h treatment of MDA-MB-231 cells with fustin at concentrations of 35, 55, and 75 μg/ml reduced significantly the density of cell monolayer when compared to the control cells. As can be seen in Fig. 2, most cells displayed a well-spread polygonal shape with the formation of large lamellipodium. Many cells with an elongated shape with long and thin extensions were also observed as well as cells with a round shape mostly in the samples treated with 35 μg/ml fustin.

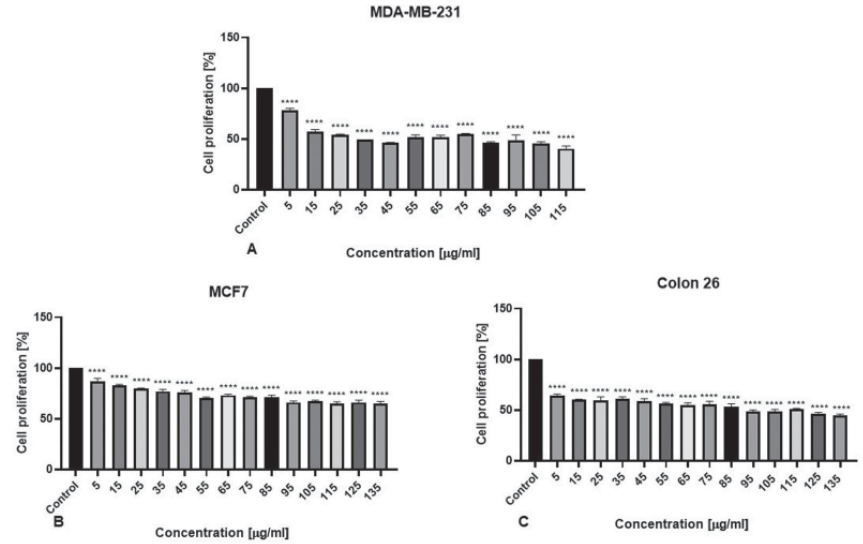

Fig. 1. MTT assay of MDA-MB-231 (A), MCF7 (B) and Colon 26 (C) cells treated for 72 h with increasing concentrations of fustin. Error bars represent standard error of the mean (SEM). **** indicates significant differences from the control group by Dunnett's test (**** $p < 0.0001$)

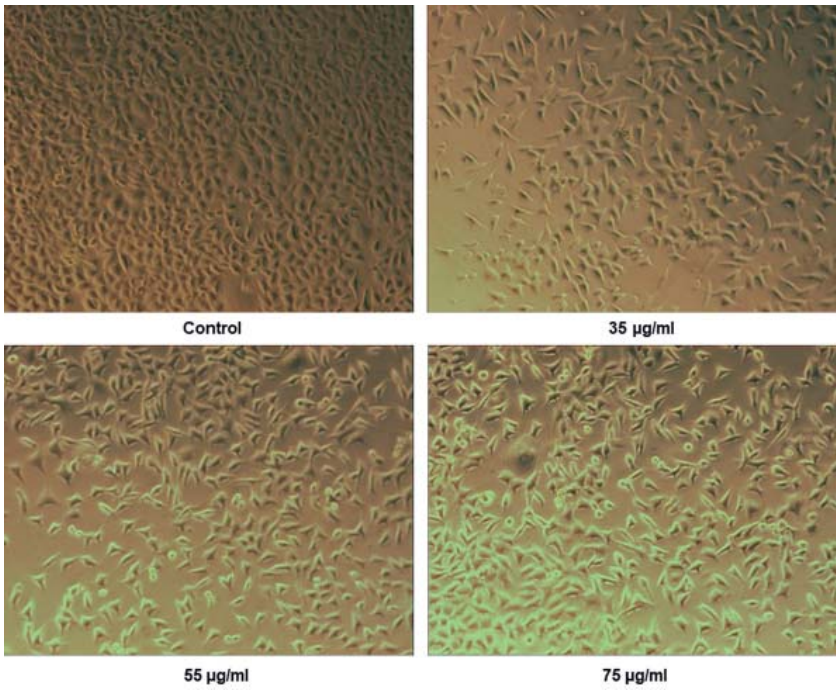

Fig. 2. Phase-contrast micrographs of MDA-MB-231 cells incubated for 72 h with fustin at concentrations of 35, 55, and 75 μg/ml; magnification 10×

Effects of fustin on MDA-MB-231 cell viability

Fluorescein diacetate is an uncharged, non-fluorescent, lipid-soluble dye that crosses biological membranes and can be hydrolyzed by intracellular esterases of the living cells to polar and fluorescent fluorescein. Free fluorescein is retained by intact living cells and its accumulation results in fluorescing cells.

Fluorescence microscopic analysis of cells stained with FDA was carried out to visualize MDA-MB-231 overall cell morphology and to evaluate the number of viable adherent cells 72 h after cell exposure to the fustin in concentrations of 35, 55, and 75 µg/ml.

A quantitative analysis of fluorescent micrographs showed a statistically significant decrease in the number of viable attached cells in comparison to the control containing untreated breast cancer cells which corresponds to the data from the MTT assay (Fig. 3). The registered effect was not dose-dependent and the strongest inhibitory effect on cell viability was observed at the lowest applied concentration of fustin of 35 µg/ml where the percentage of living cells was decreased to 37%, while at the highest applied concentration of 75 µg/ml the survival rate was 61.6%.

Inhibitory effect of fustin on clonogenicity of MDA-MB-231 cells

Clonogenic potential of breast cancer cells MDA-MB-231 was investigated after treatment with a concentration of 35 µg/ml of fustin for a period of 5 days. A considerable anticlonogenic effect in treated cancer cells was observed in comparison to the untreated controls (Fig. 4), which is an indication for inhibitory action of the studied phytochemical on breast cancer cells' reproductive integrity.

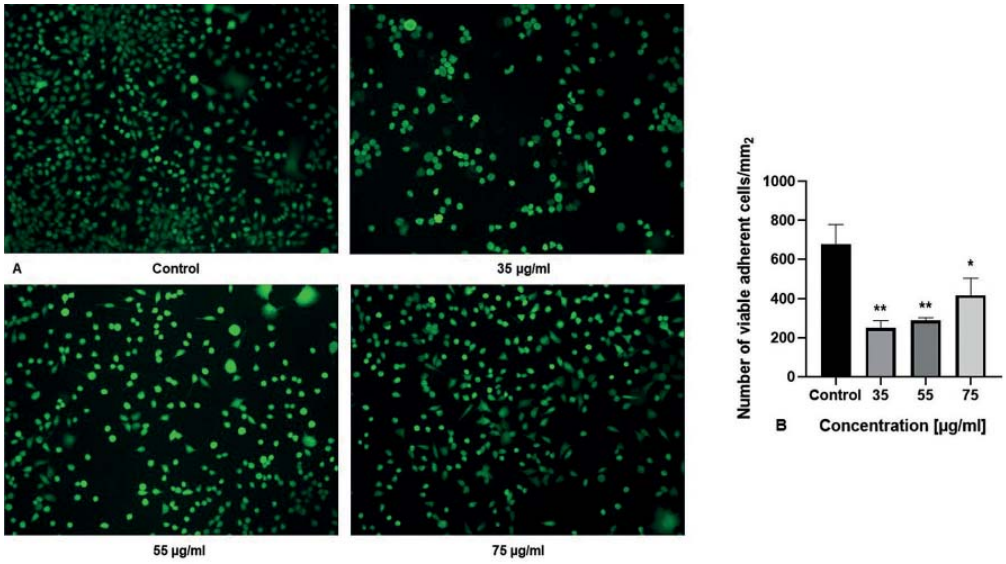

Fig. 3. Fluorescent microscopy analysis of MDA-MB-231 cells stained with fluorescein diacetate after treatment with fustin (35, 55, and 75 µg/ml) for 72 h: A – Fluorescent micrographs; magnification 10×; B – Quantitative evaluation of viable attached MDA-MB-231 cells. Error bars represent standard error of the mean (SEM). * and ** indicate significant differences from the control group by Dunnett's test (* $p < 0.05$, ** $p < 0.01$)

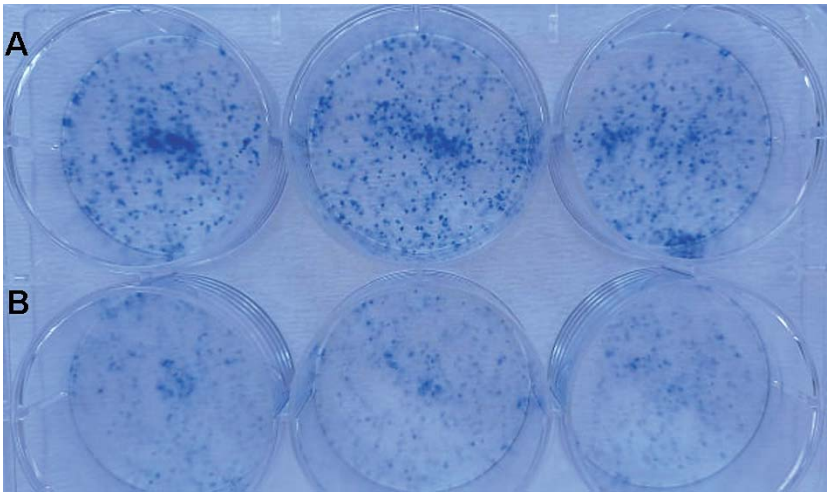

Fig. 4. Effects of fustin isolated from *C. coggygria* on clonogenicity of MDA-MB-231 cells after treatment for 5 days with 35 µg/ml (B) compared to untreated control (A)

DISSCUSSION

In the present research, we studied the antiproliferative potential of the flavonoid fustin isolated from the heartwood of *C. coggygria* in three cancer cell lines – breast (MDA-MB-231 and MCF7), and colon (Colon 26) and found that fustin can significantly inhibit cell growth of all studied cell lines. We chose the above-mentioned cell lines in view of the fact that they originate from two of the most frequent cancer types. According to the World Health Organization (WHO), newly diagnosed cases for 2020 for breast cancer amounted to 2 260 000 and for colon cancer – 1 930 000 [1]. The number of deaths associated with the disease are 684 996 (amounting to 6.9% of all cases) for breast cancer, and 935 173 (9.4%) in regard to colon cancer. Among the studied cell lines, the most sensitive to the fustin action was the MDA-MB-231 breast adenocarcinoma cell line. Therefore, we decided to perform further investigations including microscopic observation and clonogenic assay only of MDA-MB-231 cells after fustin exposure. Fluorescent microscopy observation after staining with FDA of treated MDA-MB-231 cells revealed a pronounced but not dose-dependent decrease in the number of viable attached cells. The results obtained from the clonogenic assay showed a high reduction in the ability of MDA-MB-231 cells to form colonies after treatment with fustin at a concentration of 35 μg/ml. The absence of dose-dependence and reduction of antitumor activity at high doses of application is not unusual for some plant extracts, active compounds, and chemotherapeutic agents [12, 13]. A possible reason for the lack of concentration dependence of the anticancer effects could be the depletion of specific targets of action.

Breast cancer represents a heterogeneous disease with high rates of intra- and intertumoral diversity and variability of response to different treatments. The MDA-MB-231 cell line used in the current research is triple negative breast cancer (TNBC) subtype defined by the absence of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) amplification/overexpression. Even though it accounts for about 15-20% of all breast cancers, it is an aggressive subgroup, associated with poorer prognosis and a higher rate of distant recurrence compared to estrogen receptor-positive subtypes. MCF7 cell line represents the luminal A breast cancer subtype, characterized by ER- and PR-positive and HER2-negative molecular profile. The luminal A subtype is the most common of all breast cancers, accounting for 50-60% of the cases. It is characterized by a low histological grade and a good prognosis with a low relapse rate. TNBC has restricted therapy options when compared to the other types of breast cancer with a growing need for novel naturally-derived treatment approaches with improved efficacy and reduced toxicity.

Flavonoids are polyphenolic compounds with a wide range of pharmacological activities, including anticancer [14]. Among their anticancer effects are induction of programmed cell death, cell cycle arrest, metastasis and invasion inhibition, and modulation of ROS-scavenging enzyme activities [15-17]. The observed in this study *in vitro* anticancer properties of the flavonoid fustin are of great interest in order to evaluate the antineoplastic potential of this still weakly studied flavonoid. To the best of our knowledge, this is the first study reporting an anticancer effect of fustin on breast and colon cancer cell lines. The obtained data, including cytostatic, cell viability inhibiting and anticlonogenic effects, indicate fustin as a perspective candidate for further more detailed analysis of its therapeutic potential against triple negative breast cancer subtype.

In conclusion, the here presented results revealed that the flavonoid fustin isolated from *Cotinus coggygria* heartwood possesses antiproliferative properties against three cancer cell lines: breast (MDA-MB-231 and MCF7), and colon (Colon 26) cancer. The strongest cell growth inhibitory effect was established against the MDA-MB-231 triple negative breast cancer cell line. Fustin caused a significant reduction in the number of viable MDA-MB-231 cells and demonstrated considerable anticlonogenic properties. Further studies will be focused on more detailed characterization of molecular mechanisms and targets of fustin anticancer action.

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Z. Gospodinova and G. Antov have contributed equally to this work.

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REFERENCES

1. Ferlay J, Colombet M, Soerjomataram I et al. Cancer statistics for the year 2020: An overview. Int J Cancer, 2021;149(4):778-789.
2. Antal DS, Ardelean F, Jijie R et al. Integrating Ethnobotany, Phytochemistry, and Pharmacology of *Cotinus coggygria* and Toxicodendron vernicifluum: What Predictions can be

Made for the European Smoketree?. Front Pharmacol, 2021;12:662852.
3. Matić S, Stanić S, Mihailović M, Bogojević D. *Cotinus coggygria* Scop.: An overview of its chemical constituents, pharmacological and toxicological potential. Saudi J Biol Sci, 2016;23(4):452-461.
4. Valianou L, Stathopoulou K, Karapanagiotis I et al. Phytochemical analysis of young fustic (*Cotinus coggygria* heartwood) and identification of isolated colourants in historical textiles. Anal Bioanal Chem, 2009;394:871-882.
5. Antal D, Schwaiger S, Ellmerer-Muller E, Stuppner H. *Cotinus coggygria* wood: novel flavanone dimer and development of an HPLC/UV/MS method for the simultaneous determination of fourteen phenolic constituents. Planta Med, 2010;76:1-8.
6. Novakovic M, Djordjevic I, Todorovic N et al. New aurone epoxide and auronolignan from the heartwood of *Cotinus coggygria* Scop. Nat Product Res, 2019;33(19):2837-2844.
7. Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. Farmaco, 2001;56(9):683-687.
8. Chahar MK, Sharma N, Dobhal MP, Joshi YC. Flavonoids: A Versatile Source of Anticancer Drugs. Pharmacogn Rev, 2011;5:1-12.
9. Kumazoe M, Fujimura Y, Yoshitomi R et al. Fustin, a Flavanonol, Synergically Potentiates the Anticancer Effect of Green Tea Catechin Epigallocatechin-3- O-Gallate with Activation of the eNOS/cGMP Axis. J Agric Food Chem, 2022;70(11):3458-3466.
10. Milosavljevic S, Djordjevic I, Mandic B et al. Flavonoids of the Heartwood of *Cotinus coggygria* Scop. Showing Protective Effect on Human Lymphocyte DNA. Nat Prod Commun, 2021;16(12):1-8.
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods, 1983;65(1-2):55-63.
12. Gospodinova Z, Bózsity N, Nikolova M et al. Antiproliferative Properties Against Human Breast, Cervical and Ovarian Cancer Cell Lines, and Antioxidant Capacity of Leaf Aqueous Ethanolic Extract from *Cotinus coggygria* Scop. Acta Med Bulg. 2017;44(2): 20-25.
13. Malhotra V, Perry MC. Classical chemotherapy: mechanisms, toxicities and the therapeutc window. Cancer Biol Ther, 2003;2:S2-S4.
14. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as Anticancer Agents. Nutrients, 2020;12(2):457.
15. Rodriguez-Garcia C, Sanchez-Quesada C. Dietary flavonoids as cancer chemopreventive agents: an updated review of human studies. Antioxidants, 2019;8(5):137.
16. Abotaleb M, Samuel SM, Varghese E et al. Flavonoids in Cancer and Apoptosis. Cancers, 2018;11(1):28.
17. Perez-Vizcaino F, Fraga CG. Research trends in flavonoids and health. Arch Biochem Biophys, 2018;646:107-112.

ORIGINAL ARTICLE

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THE EFFECT OF SMOKING AND OPIOID CONSUMPTION ON THE SEVERITY OF THE DISEASE AND DURATION OF HOSPITALIZATION IN COVID-19 PATIENTS

H. S. Fayazi[1], A. Naeimi[2], M. Yaseri[1], S. S. M. Khatibani[1]

[1]Assistant Professor of Internal Medicine, Department of Internal Medicine, School of Medicine, Razi Hospital, Guilan University of Medical Sciences – Rasht, Iran
[2]Student Research Committee, School of Medicine, Guilan University of Medical Sciences – Rasht, Iran

Abstract. Objective: *In the Coronavirus 2019 (COVID-19) global pandemic, various studies on the target communities of this virus were widely started and rapidly progressed. Smokers and opioid consumers are one of the virus targets since they have a vulnerable respiratory system. Due to the contradictory results in previous studies and the lack of similar investigations in this area, we aimed to perform this study to investigate the effect of smoking and opioid consumption on the consequences of the COVID-19 disease.* ***Materials and Methods:*** *In this retrospective study, the required information was collected and analyzed from the archives of Razi Hospital, Rasht, Iran. Study variables included age, sex, the need for intubation, hospital length of stay, history of current smoking or opioid consumption, and intensive care unit (ICU) admission, ICU length of stay, admission oxygen saturation, disease severity, and the outcome of death or recovery. Data were collected and divided into the case (including current cigarette smokers, opioid consumers, and cigarette-opioid consumers) and control (non-smokers and non-opioid-consumers) groups. Out of 986 patients, 489 patients met the criteria for inclusion and subsequent analysis. The average age was 69.79 ± 16.06, and 294 (60.1%) patients were male. The median age of the case group (65.15 ± 42.41) was older than the control group (57.45 ± 15.71, P = 0.001). The case group consisted of more male patients than the control group (P = 0.001).* ***Results:*** *The adjusted regression models demonstrated that current cigarette smoking, opioid, and cigarette-opioid consumption did not significantly predict hospital and ICU length of stay, ICU admission, disease severity, and mortality outcomes (P > 0.05). Current cigarette smoking and opioid consumption could not be an independent predictor for the consequences of ICU admission, hospital and ICU length of stay, the need for intubation, disease severity, and mortality in COVID-19 patients.*

Key words: *COVID-19, SARS-CoV-2, opioid abuse, smoking, opioid*

Corresponding author: *Dr. Seyyedeh Sahereh Mortazavi Khatibani, MD, Assistant Professor of Internal Medicine, Department of Internal Medicine, School of Medicine, Razi Hospital, Guilan University of Medical Science, Rasht, Iran, tel/fax: +98 013 33542460, e-mail: ssahere.mortazavi@gmail.com/ smortazavi@gums.ac.ir, ORCID: 0000-0002-7751-884X*

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INTRODUCTION

A new infectious disease caused by the Coronavirus 2019 (COVID-19) first appeared in China in December 2019 and spread worldwide as the World Health Organization (WHO) described it as an epidemic in March 2020 announced. The magnitude of the crisis has made the COVID-19 epidemic the worst health disaster of the century [1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA-enveloped virus, which codes structural and nonstructural proteins [2]. The most important receptor in human host cells is the angiotensin-converting enzyme 2 (ACE2) receptor that provides the entry for SARS-CoV-2 [3]. Both immune impairment and excessive immune response to SARS-CoV-2 result in severe conditions in patients [4].

Clinical manifestations of SARS-CoV-2 infection are diverse, ranging from asymptomatic infection to severe viral pneumonia with respiratory failure and death [5]. Older age, male gender, hypertension, diabetes, cardiovascular disease, and respiratory disease were reported as the COVID-19 risk factors and are associated with a poor outcome in these patients [6, 7]. Although there is general agreement that severe COVID-19 infection is related to the comorbidities listed above, the link between tobacco use and the severity of COVID-19 disease is still debated [8-13]. Smokers and opioid consumers are one of the virus targets since they have a vulnerable respiratory system. It is estimated that there are about 1 billion smokers worldwide, accounting for about 30% of men and 7% of women [14]. Exposure to tobacco leads to pneumonia, increased permeability of epithelial cells, and damage to the mucosal defense barrier [15]. Disorders of mucous secretions are a side effect of smoking, and the risk of underlying diseases such as diabetes and vascular disease is more common among smokers [16].

Additionally, due to frequent hand-to-mouth motions and sharing of tobacco products, smokers are more vulnerable to COVID-19 disease and more likely to become infected [17]. There is concern among the WHO and the USA Food and Drug Administration (FDA) that cigarette smoking may raise the risk of COVID-19 [18]. Although many studies have assessed the effects of smoking on the severity of COVID-19 disease, the results have not been the same; and there are generally controversies in this area [18, 19]. Usman et al. contributed some of these controversies among studies to the lack of distinction between current and former smokers and unadjusted analysis [20].

In this regard, we performed this retrospective adjusted study to evaluate the effects of current smoking and opioid consumption on the COVID-19 patient's outcomes, including intensive care unit (ICU) admission, hospital and ICU length of stay, and duration of intensive mechanical ventilation (IMV), disease severity and mortality.

PATIENTS AND METHODS

Data, Inclusion, and Exclusion criteria

The Ethics Committee of Guilan University of Medical Sciences approved this retrospective research project (IR.GUMS.REC.1399.377). The data of this retrospective study were obtained from the archives of Razi Hospital, Rasht, Iran. All consecutive adult patients (≥ 18 years of age) included in the study presented to the Razi Hospital between March 20th to September 21th, 2020, with a SARS-CoV-2 diagnosis confirmed by positive reverse transcriptase polymerase-chain-reaction (RT-PCR). Patients with a definitive clinical outcome (being discharged to outpatient setting, cured or dead) were included for further study. Patients younger than 18 years of age, without complete history information, or patients who didn't complete their hospital course and ex-smokers were excluded from the analysis. Informed consent was obtained from all individual participants included in the study.

Clinical variables

Demographical data and clinical characteristics of patients including age, gender, history of current cigarette smoking, opioid consumption, and underlying disease (diabetes, hypertension, cardiovascular disease, liver or kidney disorders, asthma, chronic obstructive pulmonary disease (COPD), and cancer), the need for intubation, duration of invasive mechanical ventilation, admission oxygen saturation, ICU admission, hospital and ICU length of stay, lab test results and the discharge outcome (death or recovery) were collected. Patients were categorized into two groups, including case (n = 205) (smokers, opioid consumers, smoker-opioid consumers) and control group (n = 284) (non-smokers and non-opioid-consumers). Severe COVID-19 was defined as the occurrence of each of the consequences of ICU admission, the need for intubation, organ failure, severe hypoxia (O_2sat < 90%), and DIC in patients during the hospitalization.

Statistical Analysis

Categorical variables are described as frequency and percentage, while continuous variables are as median and interquartile ranges. Chi-square test or exact Fisher test were applied to evaluate the association between two categorical variables, while a non-parametric Wilcoxon Mann-Whitney test was used to study differences between two groups of a categorical variable on a continuous variable. The Kaplan-Meier survival curves were used to analyze overall survival, along with 95% confidence intervals (CIs). The Cox proportional hazard models were checked to simultaneously evaluate the effect of independent variables on patient survival and ICU admission. Statistical calculations were performed using the IBM SPSS Statistics for Windows (version 22) and statistical significance was evaluated at the level of 0.05.

RESULTS

Out of 986 patients, 489 patients met the criteria for inclusion and subsequent analysis. A total of 324 patients didn't meet the selection criteria, and 173 did not complete their hospital course. The demographic and clinical characteristics of these patients are summarized in Table 1. The average age was 69.79 ± 16.06, and 294 (60.1%) patients were male. The case group consisted of 205 patients, including fifty-six current smokers, ninety-nine opioid consumers, and fifty cigarette-opioid consumers. The median age of the case group (65.15 ± 42.41) was older than the control group (57.45 ± 15.71, P = 0.001). The case group consisted of more male patients compared to the control group (P = 0.001). Multiple linear regression models analysis adjusted with age, gender, underlying disease revealed that hospital and ICU length of stay outcomes could not be predicted significantly by current smoking ([ß = 0.052, P = 0.959], [β = -0.134, P = 0.867]), opioid consumption ([β = -1.036, P = 0.207], [ß = -0.065, P = 0.920]) and cigarette-opioid consumption ([ß = -0.599, P = 0.559], [β = 0.454, P = 0.575]), respectively (Table 2). Binominal logistic regression models adjusted for age, gender, underlying disease showed that disease severity and the need for intubation consequences could not significantly be predicted by current smoking ([β = 0.212, CI = 0.62-2.44], [ß = -0.021, CI = 0.40-2.39]), opioid consumption ([β = 0.076, P = 0.62-1.85], [β = 0.187, CI = 0.59-2.44]) and cigarette-opioid consumption ([β = 0.142, CI = 0.58-2.27], [β = 0.570, CI = 0.75-4.15]), respectively (Table 3). The Cox regression model after adjustment for confounding factors demonstrated that the risks of death outcome in current smokers (HR = 1.19, 95%CI = 0.65-2.18), opioid consumers (HR = 1.56, 95%CI = 0.97-2.51), and cigarette-opioid consumers (HR = 1.61, 95%CI = 0.89-2.92) were 1.19, 1.56 and 1.61 fold higher than the control group, respectively, although they were not statistically significant (Table 4). Also, the risks of ICU hospitalization in current smokers (HR = 0.63, 95%CI = 0.25-1.59), opioid consumers (HR = 1.05, 95% CI = 0.54-2.05), and cigarette-opioid consumers (HR = 0.66, 95%CI = 0.27-1.61) were 0.63, 1.05 and 0.66 folds higher than the control group, respectively, although they were not statistically significant (Table 4).

Table 1. Baseline characteristics and outcomes of the participants according to case and control groups

Variable	Case (n = 205)	Control (n = 284)	P-value
Age (Year)	65.15 ± 42.41	57.45 ± 15.71	0.001
Gender (Male)	155 (75.6%)	139 (48.9%)	0.001
Underlying disease	129 (62.9%)	84 (64.8%)	0.703
Hospital length of stay (Day)	7.68 ± 5.18	8.33 ± 6.79	0.584
ICU admission	30 (22.6%)	37 (13.3%)	0.022
ICU length of stay (Day)	8.97 ± 7.23	10.11 ± 10.92	0.934
The need for Intubation	39 (19.0%)	37 (13.0%)	0.077
Duration of IMV* (Day)	6.92 ± 8.39	8.70 ± 11.02	0.448
SpO2 (%)**	91.27 ± 7.45	92.74 ± 6.42	0.024
Severely ill patients	124 (66.0%)	139 (56.0%)	0.038
Hospital outcome (Death)	65 (31.7%)	53 (18.7%)	0.001

*IMV = Intensive mechanical ventilation, **SpO_2 = Peripheral oxygen saturation. P-value < 0.05 is statistically significant.

Table 2. Multiple linear regression analysis for predicting hospital and ICU length of stay consequences based on age, gender, underlying disease, case and control groups

Variables		Unstandardized Coefficients β	Unstandardized Coefficients SE**	Standardized Coefficients Beta	t	P-value
Hospital length of stay	Age	0.001	0.022	0.003	0.054	0.957
	Female gender	1.698	0.679	0.131	2.502	0.013
	Underlying disease	0.418	0.695	0.031	0.601	0.548
	Current smoker	0.052	1.010	0.003	0.051	0.959
	Opioid consumer	-1.036	0.820	-0.065	-1.264	0.207
	Cigarette/Opioid consumer	-0.599	1.025	-0.030	-0.584	0.559
	Severe COVID-19	2.059	0.632	0.158	3.258	0.001
ICU length of stay*	Age	-0.024	0.017	-0.076	-1.418	0.157
	Female gender	0.507	0.536	0.050	0.946	0.345
	Underlying disease	-0.140	0.549	-0.013	-0.256	0.798
	Current smoker	-0.134	0.798	-0.009	-0.167	0.867
	Opioid consumer	-0.065	0.648	-0.005	-0.100	0.920
	Cigarette/Opioid consumer	0.454	0.810	0.029	0.561	0.575
	Severe COVID-19	2.229	0.499	0.217	4.463	< 0.001

*ICU = Intensive care unit, **SE = Standard error. P-value < 0.05 is statistically significant

Table 3. Logistic regression for predicting severe COVID-19 and the need for intubation consequences based on age, gender, underlying disease, smoking, and opioid consumption

Variable		B	SE*	Wald	Odds Ratio (OR)	P-value	95%CI for OR** Lower	95%CI for OR** Upper
Severe COVID-19	Age	0.023	0.007	9.684	1.023	0.002	1.00	1.03
	Female gender	-0.483	0.227	4.548	0.617	0.033	0.39	0.96
	Underlying disease	0.336	0.232	2.102	1.399	0.147	0.88	2.20
	Control group	-	-	0.454	-	0.929	-	-
	Current smoker	0.212	0.348	0.370	1.236	0.543	0.62	2.44
	Opioid consumer	0.076	0.277	0.075	1.079	0.785	0.62	1.85
	Cigarette/Opioid consumer	0.142	0.347	0.167	1.152	0.683	0.58	2.27
Need for intubation	Age	0.007	0.10	0.542	1.007	0.462	0.98	1.02
	Female gender	0.171	0.319	0.288	1.187	0.591	0.63	2.21
	Underlying disease	-0.151	0.322	0.219	0.860	0.640	0.45	1.61
	Control group	-	-	1.986	-	0.575	-	-
	Current smoker	-0.021	0.456	0.002	0.979	0.963	0.40	2.39
	Opioid consumer	0.187	0.360	0.268	1.205	0.605	0.59	2.44
	Cigarette/Opioid consumer	0.570	0.435	1.718	1.769	0.190	0.75	4.15

*SE = Standard error, **CI = Confidence interval, OR = Odds ratio. P-value < 0.05 is statistically significant

Table 4. Cox regression analysis for predicting ICU admission and patient's survival based on age, gender, underlying disease, smoking, and opioid consumption

Variable		SE*	Hazard ratio	z	P > | z |	95% CI**	
						Lower	Upper
ICU admission	Age	0.009	1.009	0.95	0.341	0.99	1.02
	Female gender	0.193	0.653	-1.43	0.152	0.36	1.16
	Underlying disease	0.330	1.086	0.27	0.786	0.59	1.97
	Current smoker	0.297	0.630	-0.98	0.329	0.25	1.59
	Opioid consumer	0.357	1.057	0.17	0.867	0.54	2.05
	Cigarette/Opioid consumer	0.301	0.667	-0.90	0.371	0.27	1.61
Patients Survival	Age	0.007	1.013	1.85	0.065	0.99	1.02
	Female gender	0.167	0.709	-1.45	0.147	0.44	1.12
	Underlying disease	0.223	1.016	0.07	0.942	0.66	1.56
	Current smoker	0.366	1.195	0.58	0.561	0.65	2.18
	Opioid consumer	0.378	1.567	1.86	0.062	0.97	2.51
	Cigarette/Opioid consumer	0.489	1.616	1.59	0.113	0.89	2.92
	Severe COVID-19	2.148	5.804	4.75	< 0.001	2.81	11.98

*SE = Standard error, **CI = Confidence interval, P-value < 0.05 is statistically significant

DISCUSSION

In our study, the mean age in the case group was significantly higher than the control group and the majority of patients in the case group were males. According to the culture and conditions in society, it was expected to see more smoking in men and more opium consumption in the elderly. In this regard, in a study by Khalili et al., it was found that the highest number of opium consumers was in the age range of 55-64 years old, and the rate of opium consumption in males was significantly higher than in females [21]. Also, in the study by Mehrabi et al., it was reported that the prevalence of smoking in females is more prone to old age [22]. This present study showed that ICU admission, the need for intubation, and hospital and ICU length of stay didn't differ in the two study groups. Similar to our study, Huang et al. reported that smokers required less hospitalization in ICU [23]. The study by Ho et al. also found that current smoking was not associated with the consequences of intubation, ICU, mortality, and hospitalization [18]. The other finding of our study was the lack of association between current smoking, opioid, and cigarette-opioid consumption with disease severity and survival outcomes. Although our study initially manifested a significant relationship between these groups with disease severity and survival outcome, after adjusting regression models with age, gender, and underlying disease, it became interestingly non-significant. Before our study, Reddy et al. and Ho et al. studies demonstrated that current smoking was not associated with the consequences of intubation, ICU, mortality and hospitalization risk [7, 18]. Our study is consistent with previous studies that found no association between active smoking and disease severity [11, 13, 24] and mortality [18, 19, 25]. However, some studies, such as Alqahtani et al., showed that the severity and mortality of the COVID-19 disease were higher in people who smoked [26]. In addition, Gulsen et al. study stated that the severity of the disease in smokers is 1.5-2 times higher than in non-smokers [12]. However, many studies have been conducted to investigate the consequences of the COVID-19 in smokers, but the results have not been conclusive. The study by Simons et al. revealed that the outcomes of hospitalization, disease severity, and mortality in current smokers were inconclusive, but generally, smoking increased the risk of more severe disease slightly [19]. Theoretically, smokers are expected to have a higher risk of developing the COVID-19 disease due to impaired pulmonary mucociliary clearance system, increased permeability of the airway epithelial membrane, and increased respiratory system inflammation [27]. Yet, a systematic review reported a lower frequency of smokers in COVID-19 patients than in the general population [28]. One mechanism that justifies this disagreement is a higher expression level of ACE2 in the smokers. Nicotine alone can increase ACE2 gene expression [29]. Several studies have shown the opposite effect of nicotine, with nicotine or smoking reducing ACE-2 receptor expression [30-32]. Moreover, nicotine, with its cholinergic agonist properties, in cigarettes exerts anti-inflammatory effects and inhibits the expression of inflammatory cytokines such as IL-1, IL-6, and TNF5, which are activated in the cytokine

storm in COVID-19 patients. Therefore, although smokers are expected to have a greater risk of developing the disease since ACE2 receptors are the only way the COVID-19 virus enters the body, the simultaneous protective effect of ACE2 and the anti-inflammatory effect of nicotine may reduce the potential consequences of the COVID-19 disease. This reason can justify the controversies among studies. In this study, opioid consumption and concomitant use of cigarettes and opioids were evaluated on the consequences of the hospital and ICU length of stay, the need for intubation, disease severity, and survival in COVID-19 patients, which represented no significant relationship. So far, few studies have assessed the relationship between opioid consumption and the COVID-19 outcomes. Consistent with our research, Qeadan et al. found no higher mortality rate among patients taking opioids, although patients were more likely to require ICU care [33]. However, Riahi et al.'s unadjusted study demonstrated that opium consumers experience higher mortality and severity of the COVID-19 disease [34]. According to studies, opium exerts anti-inflammatory properties by suppressing bone marrow leukocyte migration [35]. The immune system can also be dangerously suppressed by high levels of opium [36]. However, some studies have indicated that although opioids like morphine reduce the production of inflammatory cytokines such as IFN- α and IFN-Y, they weaken the immune system and consequently increase virus entry into the body and lungs damage. From another point of view, smokers and opium users have a lower level of socio-economic status and access to health care than other members of society [37]. Concurrent comorbidities such as COPD and cardiovascular diseases, which are associated with higher severity of COVID-19, have been reported more frequently in these patients [12, 38, 39]. Generally, it seems that concomitant factors such as age, gender, and underlying diseases play an important role in worsening the outcomes of these patients.

Our study had some limitations. First, although the Razi hospital was the primary referral center in the Guilan province, Iran, for the COVID-19 patients, due to being a single center, the results may differ in a larger population with different ethnicity. Secondly, according to the COVID-19 pandemic and top referral of the COVID-19 patients, the hospital ICU capacity for patient admission was limited, and therefore, this limitation could affect the comparison of numbers of ICU admission between study groups. Thus, further multi-center studies with a more diverse population are recommended.

CONCLUSION

Current cigarette smoking and opioid consumption could not be an independent predictor of the consequences of ICU admission, hospital and ICU length of stay, the need for intubation, disease severity, and mortality in COVID-19 patients.

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Ethical Approval: *The Ethics Committee of Guilan University of Medical Sciences approved this retrospective research project (IR.GUMS.REC.1399.377).*

Informed Consent: *The authors affirm that human research participants provided informed consent for the publication of the study.*

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Authors Contribution

All authors contributed to study conception and design. Material preparation, data collection, and analysis were performed by Haniyeh Sadat Fayazi, Maryam Yaseri, Arvin Naeimi, and Seyyedeh Sahereh Mortazavi Khatibani. The first draft of the manuscript was written by Haniyeh Sadat Fayazi, Arvin Naeimi, and Seyyedeh Sahereh Mortazavi Khatibani and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Halaji M, Heiat M, Faraji N, Ranjbar R. Epidemiology of COVID-19: An updated review. J Res Med Sci [Internet]. 30 September 2021;26:82. Available at: https://pubmed.ncbi.nlm.nih.gov/34759999
2. Hardenbrook NJ, Zhang P. A structural view of the SARS-CoV-2 virus and its assembly. Curr Opin Virol. 2022;52:123–34.
3. Samavati L, Uhal BD. ACE2, Much More Than Just a Receptor for SARS-COV-2. Vol 10, Frontiers in Cellular and Infection Microbiology. 2020.
4. Zeinali T, Faraji N, Joukar F et al. Gut bacteria, bacteriophages, and probiotics: Tripartite mutualism to quench the SARS-CoV2 storm. Microb Pathog [Internet]. 2022;105704. Available at: https://www.sciencedirect.com/science/article/pii/S0882401022003175
5. Anastassopoulou C, Gkizarioti Z, Patrinos GP, Tsakris A. Human genetic factors associated with susceptibility to SARS-CoV-2 infection and COVID-19 disease severity. Hum Genomics [Internet]. 2020;14(1):40. Available at: https://doi.org/10.1186/s40246-020-00290-4
6. Yaghubi T, Shakoori V, Nasiri S et al. Clinical characteristics and outcomes of COVID-19 patients with a history of cardiovascular disease. J Curr Biomed Reports. 2022.

7. Reddy RK, Charles WN, Sklavounos A et al. The effect of smoking on COVID-19 severity: A systematic review and meta-analysis. J Med Virol. Februarie 2021;93(2):1045-56.
8. Eastin C, Eastin T. Clinical Characteristics of Coronavirus Disease 2019 in China: Guan W, Ni Z, Hu Y, et al. N Engl J Med. 2020 Feb 28 [Online ahead of print] DOI: 10.1056/NEJMoa2002032. J Emerg Med [Internet]. 2020/06/03. April 2020;58(4):711-2. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7266766/
9. Rossato M, Russo L, Mazzocut S et al. Current smoking is not associated with COVID-19. The European respiratory journal. 2020, 55.
10. Hopkinson NS, Rossi N, El-Sayed Moustafa J et al. Current smoking and COVID-19 risk: results from a population symptom app in over 2.4 million people. Thorax. Julie 2021;76(7):714-22.
11. Yu X, Sun X, Cui P et al. Epidemiological and clinical characteristics of 333 confirmed cases with coronavirus disease 2019 in Shanghai, China. Transbound Emerg Dis. Julie 2020;67(4):1697–707.
12. Gülsen A, Yigitbas BA, Uslu B et al. The Effect of Smoking on COVID-19 Symptom Severity: Systematic Review and Meta-Analysis. Pulm Med [Internet]. 08 September 2020;2020:7590207. Available at: https://pubmed.ncbi.nlm.nih.gov/32963831
13. Lippi G, Henry BM. Active smoking is not associated with severity of coronavirus disease 2019 (COVID-19). Eur J Intern Med. 2020;75:107–8.
14. West R. Tobacco smoking: Health impact, prevalence, correlates and interventions. Psychol Health. 2017/05/28. Augustus 2017;32(8):1018–36.
15. Strzelak A, Ratajczak A, Adamiec A, Feleszko W. Tobacco Smoke Induces and Alters Immune Responses in the Lung Triggering Inflammation, Allergy, Asthma and Other Lung Diseases: A Mechanistic Review. Int J Environ Res Public Health. Mei 2018;15(5).
16. Liu W, Tao Z-W, Wang L et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. Chin Med J (Engl). Mei 2020;133(9):1032-8.
17. Alqahtani JS, Aldhahir AM, Oyelade T et al. Smoking cessation during COVID-19: the top to-do list. NPJ Prim care Respir Med. Mei 2021;31(1):22.
18. Ho KS, Narasimhan B, Sheehan J et al. Controversy over smoking in COVID-19-A real world experience in New York city. J Med Virol. Julie 2021;93(7):4537–43.
19. Simons D, Shahab L, Brown J, Perski O. The association of smoking status with SARS-CoV-2 infection, hospitalization and mortality from COVID-19: a living rapid evidence review with Bayesian meta-analyses (version 7). Addiction. Junie 2021;116(6):1319–68.
20. Usman MS, Siddiqi TJ, Khan MS et al. Is there a smoker's paradox in COVID-19? BMJ evidence-based Med. Desember 2021;26(6):279–84.
21. Khalili P, Ayoobi F, Mohamadi M et al. Effect of opium consumption on cardiovascular diseases – a cross-sectional study based on data of Rafsanjan cohort study. BMC Cardiovasc Disord [Internet]. 2021;21(1):2. Available at: https://doi.org/10.1186/s12872-020-01788-4
22. Mehrabi S, Delavari A, Moradi G et al. Smoking among 15- to 64-Year-Old Iranian People in 2005. irje [Internet]. 01 September 2007;3(1):1–9. Available at: http://irje.tums.ac.ir/article-1-165-en.html
23. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet [Internet]. 15 Februarie 2020;395(10223):497–506. Available at: https://doi.org/10.1016/S0140-6736(20)30183-5
24. Chen Q, Zheng Z, Zhang C et al. Clinical characteristics of 145 patients with corona virus disease 2019 (COVID-19) in Taizhou, Zhejiang, China. Infection [Internet]. 2020/04/28. Augustus 2020;48(4):543–51. Available at: https://pubmed.ncbi.nlm.nih.gov/32342479
25. Zhou F, Yu T, Du R et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet (London, England). Maart 2020;395(10229):1054–62.
26. Alqahtani JS, Oyelade T, Aldhahir AM et al. Prevalence, Severity and Mortality associated with COPD and Smoking in patients with COVID-19: A Rapid Systematic Review and Meta-Analysis. PLoS One. 2020;15(5):e0233147.
27. Samet JM. Tobacco Products and the Risks of SARS-CoV-2 Infection and COVID-19. Nicotine Tob Res [Internet]. 01 Desember 2020;22(Supplement_1):S93–5. Available at: https://doi.org/10.1093/ntr/ntaa187
28. Farsalinos K, Barbouni A, Niaura R. Systematic review of the prevalence of current smoking among hospitalized COVID-19 patients in China: could nicotine be a therapeutic option? Intern Emerg Med. Augustus 2020;15(5):845–52.
29. Zureik M, Baricault B, Vabre C et al. Nicotine-replacement therapy, as a surrogate of smoking, and the risk of hospitalization with Covid-19 and all-cause mortality: a nationwide, observational cohort study in France. medRxiv [Internet]. 01 Januarie 2020;2020.07.28.20160630. Available at: http://medrxiv.org/content/early/2020/07/30/2020.07.28.20160630.abstract
30. Farsalinos K, Niaura R, Le Houezec J et al. Editorial: Nicotine and SARS-CoV-2: COVID-19 may be a disease of the nicotinic cholinergic system. Toxicology reports. 2020,7,658-63.
31. Oakes JM, Fuchs RM, Gardner JD et al. Nicotine and the renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol. November 2018;315(5):R895-906.
32. Yue X, Basting TM, Flanagan TW et al. Nicotine Downregulates the Compensatory Angiotensin-Converting Enzyme 2/Angiotensin Type 2 Receptor of the Renin–Angiotensin System. Annal Am Thorac Soc, 2018;15, bl S126-7.
33. Qeadan F, Mensah NA, Tingey B et al. The association between opioids, environmental, demographic, and socioeconomic indicators and COVID-19 mortality rates in the United States: an ecological study at the county level. Arch Public Heal [Internet]. 2021;79(1):101. Available at: https://doi.org/10.1186/s13690-021-00626-z
34. Riahi T, Sadeghzadeh-Bazargan A, Shokri S et al. The effect of opium on severity of COVID-19 infection:An original study from Iran. Med J Islam Repub Iran. 2021;35:115.
35. Chadzinska M, Kolaczkowska E, Seljelid R, Plytycz B. Morphine modulation of peritoneal inflammation in Atlantic salmon and CB6 mice. J Leukoc Biol [Internet]. 01 Mei 1999;65(5):590-6. Available at: https://doi.org/10.1002/jlb.65.5.590
36. Roy S, Loh HH. Effects of opioids on the immune system. Neurochem Res. November 1996;21(11):1375-86.
37. Dolati-Somarin A, Abd-Nikfarjam B. The Reasons for Higher Mortality Rate in Opium Addicted Patients with COVID-19: A Narrative Review. Iran J Public Health. Maart 2021;50(3):470-9.
38. McBride PE. The health consequences of smoking. Cardiovascular diseases. Med Clin North Am. Maart 1992;76(2):333–53.
39. Fang X, Li S, Yu H et al. Epidemiological, comorbidity factors with severity and prognosis of COVID-19: a systematic review and meta-analysis. Aging (Albany NY) [Internet]. 2020/07/13. 13 Julie 2020;12(13):12493–503. Available at: https://pubmed.ncbi.nlm.nih.gov/32658868

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ORIGINAL ARTICLE

THE IMPORTANCE OF MANUAL DETORSION IN INTRAVAGINAL TESTICULAR TORSION

M. Anastasakis[1], I. Gkalonaki[1], C. Doitsidis[1], P. Michou[2], I. Patoulias[1]

[1]First Department of Pediatric Surgery, Aristotle University of Thessaloniki Greece, General Hospital "G. Gennimatas" – Thessaloniki, Greece
[2]Department of Pediatrics, General Hospital "G. Gennimatas" – Thessaloniki, Greece

Abstract. Objective: *The aim of the present prospective clinical study was to highlight the importance of the proper application of the manual detorsion (MD) in cases of intravaginal testicular torsion. Major complications such as ischemia, reperfusion injury and testicular compartment syndrome could be avoided.* ***Materials and Methods:*** *From January 2017 to February 2018, 26 boys aged between 8 and 16 years underwent surgical treatment for intravaginal testicular torsion (ITT) (14 left- and 12 right-sided). Diagnosis was made upon clinical criteria (both symptoms and signs); sudden onset of scrotal pain (n = 26, 100%), nausea and vomiting (n = 25, 96,15%), abdominal pain (n = 3, 11,53%), high testicular position (n = 21, 80,77%), absence of the cremasteric reflex (n = 26, 100%), harshness of the twisted testicle (TT) (n = 24, 87.5%), alteration on axis or orientation of the TT (n = 24, 94,31%), and pain during palpation (n = 26, 100%). Two cases presented with neglected scrotum leading to inability to evaluate the intrascrotal structures. Major ultrasonographic findings were the following: absence of perfusion, heterogeneity of the parenchyma and identification of the Whirlpool sign. Therefore, our study group consisted of 15 out of the 26 cases, in which the initial assessment at the Emergency Department occurred within the first 3-7 hours after the onset of ITT.* ***Results:*** *Based on high clinical suspicion and ultrasonographic documentation of the ITT, MD was performed in all those cases. Pain alleviation followed immediately, while significant improvement of the clinical picture of the suffering scrotum was also observed. Successful detorsion was documented via ultrasonography. After completion of the preoperative assessment, bilateral orchidopexy was performed. All patients had an uneventful postoperative course and were discharged home on the second postoperative day.* ***Conclusion:*** *In conclusion, we hereby document that MD is a safe, non-invasive method, easy to learn for every clinician. It can be applied immediately after the diagnosis of the ITT, converting a highly urgent surgery into an elective one. Of course, surgical exploration of intrascrotal structures constitutes a crucial final step.*

Key words: *intravaginal testicular torsion, manual detorsion, ischemia, reperfusion injury, testicular compartment syndrome, male*

Corresponding author: *Anastasakis Michail, MD, Amalias 37, 54640 Tessaloniki, Greece, Phone: +30 6938130252, e-mail: mixalis.pao@windowslive.com*

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INTRODUCTION

The annual incidence of testicular torsion in pre-adolescents and adolescents in the United States is estimated to be 3.8-4.5 cases/100,000/year [1].

Underlying causative factors for an intravaginal testicular torsion (ITT) include bell clapper deformity, broad mesorchium, sexual activity, physical exercise and especially cycling, overactive cremasteric reflex, cold weather resulting in asymmetric clamping of the cremasteric muscle, malignancy and finally rapid testicular development [2, 3, 4]. In 4-8% of ITT cases, traumatic injury of the affected scrotum is identified as the causative factor [6]. There are indications of a genetic background via the action of the insulin-like 3 hormone and its cell membrane receptor (Rx1f2) [6, 7].

DaJusta et al. and Sozubir et al. have also highlighted the high familiar predisposition to testicular torsion [6, 7]. Based on a report by Martin et al., bell clapper deformity is observed in 12% of the affected boys [8]. This anatomic variance has been recognized in up to 78% of all cases of ITT [1, 2, 3, 4].

Treatment of the testicular torsion is "a battle against time" as the anatomic alterations begin from the first few minutes after the onset of ischemia [9]. The basic parameters for the development of permanent alterations on the suffering testis are the duration and the degree of the torsion [10, 11, 12]. It has been now established that 4 to 6 hours after the onset of the TT, irreversible ischemic lesions develop [13, 14, 15]. Sharpet et al. referred to the correlation between survival of the twisted testis and testicular torsion duration, as shown in Table 1 [16].

Table 1. Relationship between the duration of testicular torsion and survival rates of the twisted testicle

Duration of TT in hours	Survival rates of the twisted testicle (%)
24	10
12	50
4-8	90-100

The aim of the present clinical study was to highlight the importance of the timely application of manual detorsion (MD) in highly suspected cases of ITT, which represents a safe, non-invasive treatment approach, simplifying the subsequent surgical treatment and improving the overall prognosis for the patient, with the avoidance of major, related to ischemia complications.

MATERIALS AND METHODS

From January 2020 to February 2021, 26 boys aged between 8 and 16 years (mean: 11.5 years) underwent surgical treatment in the context of ITT. Fourteen cases were left- and 12 cases were right-sided. Diagnosis was based upon patient's reported symptoms and the findings of physical examination, as shown in Table 2.

Brunzel's sign, defined as the high and transverse position of the twisted testis, was identified in 21 cases. Two patients were admitted to the Emergency Department with a delay of 24 hours after the onset of the symptoms, resulting in the development of intense localized inflammation, leading to a clinical picture of a neglected acute scrotum and restricting our ability to evaluate adequately the intrascrotal structures.

Table 2. Signs and symptoms of the affected patients

Clinical sign or symptom	Number of patients
Sudden onset of pain	26
Nausea or/and vomiting	25
Abdominal pain	3
High position of the suffering testicle	21
Absence of cremasteric muscle reflex	26
Harshness of the affected testicle	24
Change in the axis or orientation of the affected testicle	24
Painful palpation of the affected testicle	26
Earlier episodes of testicular pain	8

In all cases, gray scale ultrasonography with the addition of Doppler color flow was performed, confirming the presence of testicular torsion. Major ultrasonographic findings are summarized in Table 3.

Table 3. Major ultrasonographic findings

Ultrasonographic finding	Result
Absence of perfusion of the affected testicle	Positive: 25/26 Doubted: 1/26
Loss of testicular homogeneity	Positive: 10/16 Negative: 15/16
Whirlpool sign (Figure 1)	Positive: 26/26

Our study group consisted of 15/26 cases of ITT (6 right- and 9 left-sided), which met the following prespecified criteria: a) pain duration less than 6 hours and b) homogeneous texture of the affected testicle

in ultrasonographic examination. It is obvious that our study group included reversible cases of ITT, regarding the effects of ischemic trauma and the subsequent reperfusion, including testicular compartment syndrome development.

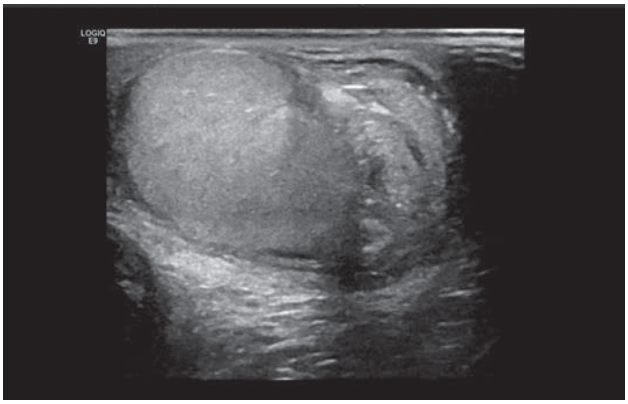

Fig. 1. Ultrasound image highlighting Whirlpool sign in ITT (5/6 patients)

The degree of the ITT ranged from 360-720° (Table 4).

Table 4. Degrees of ITT in each included case

Localization	Degree of ITT
Right (5/6)	360°
Right (1/6)	540°
Left (3/9)	540°
Left (2/9)	360°
Left (3/9)	720°
Left (1/9)	540°

Based on the high clinical suspicion of ITT and the subsequent ultrasonographic documentation, MD of the affected testicle was performed. The whole procedure was carried out without anesthesia requirement in the Emergency Department room.

Methodologically, the gradual detorsion of the affected testicle with each step of 180° was initially attempted, having as clinical criterion of the successful completion of the procedure the immediate pain relief as reported by the patient and the restoration of the normal (vertical) axis and the orientation of the testicle (the head of the epididymis located at the upper pole of the testis with epididymis body and tail being palpable on its posterior surface). A final clinical criterion for the successful detorsion was the palpation of the suffering testicle on the base of the ipsilateral hemiscrotum.

MD of a right twisted testicle was performed like someone is scrolling from the beginning to the end pages of a book, while the reverse procedure was followed in those cases of left-sided ITT. However, if after the completion of the first phase of 180o detorsion there was difficulty in completing the procedure or the pain deteriorated, then the process was carried out on a reverse trend.

RESULTS

MD was successful in all cases. In the nine cases of the left-sided ITT the MD was performed in a clockwise direction, while in the six cases of the right-sided testicular torsion the MD was carried out in an anticlockwise direction. The successful and complete MD was confirmed by color Doppler ultrasonography, documenting the restoration of symmetric arterial blood supply to both testicles (Figures 2 and 3).

Surgical exploration of the scrotum was performed within the next day after the completion of the preoperative assessment, under general endotracheal anesthesia, with mid-scrotal incision. Initially, the previously twisted testicle was retracted into the surgical field.

In all cases, successful MD was confirmed. No biopsy or orchiectomy was required.

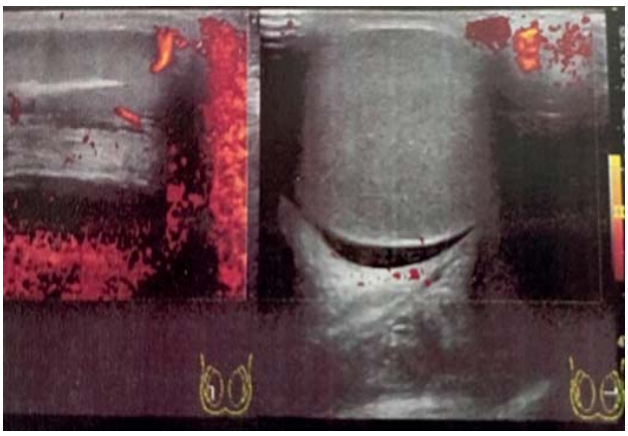

Fig. 2, 3. US confirmation of arterial blood disruption in the torsed testis (Fig. 1), and restoration of arterial supply after MD (Fig. 2)

In 12 out of 15 cases (80%), bell clapper deformity was identified (Figure 4).

In 7 out of 15 cases the macroscopic appearance of the detorsed testis was normal, while venous congestion of the spermatic cord was observed in the rest (8/15) cases (Figure 5).

The effects of mild to moderate ischemia were evident in 12/15 cases. In those cases, 2-3 elongated sections of the tunica albuginea of the affected testicle were overlapped with the parietal layer of the tunica vaginalis to prevent testicular compartment syndrome (Figure 6).

Testicular fixation was performed in a created subcutaneous pouch. Then the contralateral testis was retracted into the surgical field.

In 3 cases bell clapper deformity was identified bilaterally. The operation was completed with fixation of the contralateral testis in a dartos pouch. All patients received perioperatively chemoprophylaxis with second-generation cephalosporin in a therapeutic dose. All patients had an uneventful postoperative course and they were discharged home in good general condition within the first two postoperative days.

The postoperative follow-up schedule consisted of visits at six month intervals for one year. Follow-up consisted of clinic and ultrasonographic assessment.

Clinically, testicular size and turgidity were evaluated. The decrease in size and the harshness of the previously twisted testicle were considered as signs of permanent damage. By ultrasonographic evaluation, testicular dimensions and volume were evaluated along with the parenchyma echogenicity. We considered as criteria of irreversible testicular damage the smaller size of the previously affected testicle, at least 15%, compared to the contralateral and the heterogeneity of the testicular parenchyma (Table 5).

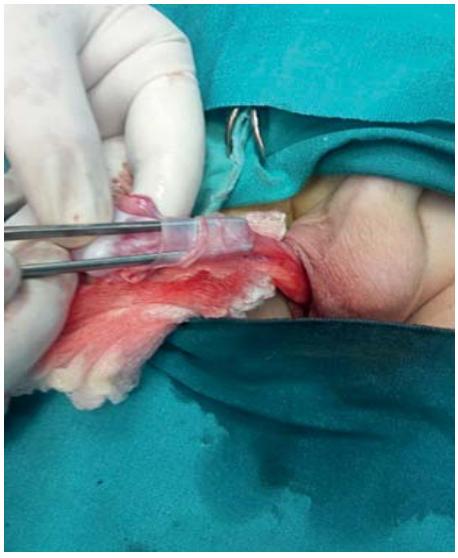

Fig. 4. Bell clapper deformity in the 2/15 study cases

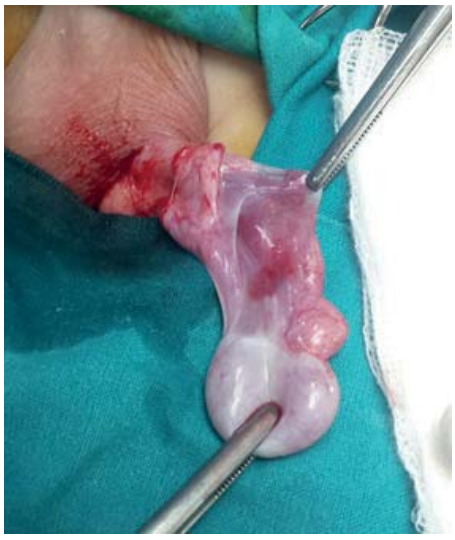

Fig. 5. Remaining venous congestion of the spermatic cord after successful MD (3/15 cases)

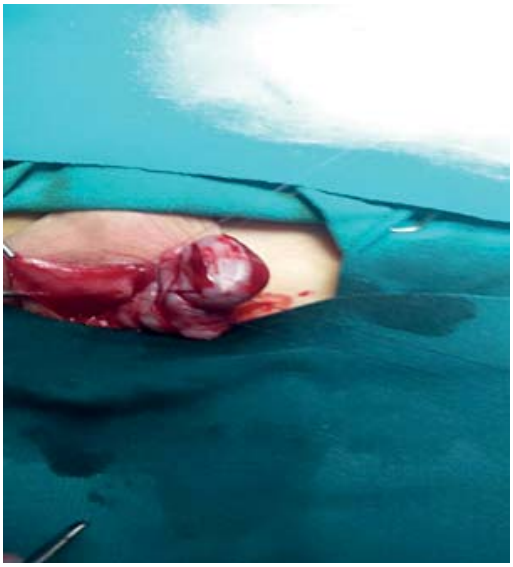

Fig. 6. Elongated sections on the tunica albuginea of the suffering testis (5/15 cases)

Table 5. Clinical and ultrasonographic criteria assessed during the follow-up period

Rating Criterion	Finding (patients)
Turgidity of the twisted testis	Normal (n = 15)
Comparative testicular size estimation	Normal (n = 14), almost normal (n = 1)
Volume of the twisted testis	Normal (n = 14), testicular volume < 20% compared to the contralateral (n = 1)
Echogenicity of the twisted testis	Normal (n = 15)

DISCUSSION

In the initial phase of TT, testicular vascular congestion and worsening of intercellular edema developed. However, no change in cellular morphology was established. These alterations are considered as reversible and are not detected by the performed ultrasonography. Beyazal et al. depicted these initial and reversible ischemic changes by performing Diffusion-weighted imaging (DWI) sequence – a specialized magnetic resonance imaging (MRI) technique [17].

Successful detorsion is followed by apoptosis of the spermatogenic cells, accumulation of neutrophils and oxidative stress development. Neutrophils chemotaxis is induced by the increased expression of testicular vascular E-selectin [18]. Because of the pathophysiological effects of ischemia and the subsequent reperfusion of the twisted testicle, intercellular edema and increased intra-testicular pressure is developed. After these alterations, reduced capillary pressure is exerted, with simultaneous exacerbation of those ischemic effects. All those events constitute the so-called testicular compartment syndrome [19]. Along with the blood-testis barrier and the extended release of cytokines, ischemia induces several alterations in the contralateral testicle, including extensive cell apoptosis of the seminal epithelium, Leydig cells atrophy, dysplasia of the spermatozoa, as well as alterations in the Sertoli cells [20].

Nash was the first to apply and describe MD, in order to rapidly restore the arterial blood supply to the affected testicle [21, 22]. Later, Van der Poel described another case of a young man, being a doctor himself, who suffered from recurrent episodes of TT; he was applying MD to himself [23].

Urgent surgical treatment is the basic therapeutic approach to restore blood flow, so as to rescue the testicle from ischemia and prevent recurrent torsion of both testicles. Unfortunately, patients most times visit the hospital with a delay, after the first, critical 6 hours from the onset of TT. According to Demirbas et al., vital time equal to 80-90 minutes is usually spent from the initial patient's examination until the beginning of the surgery [24].

MD is apparently not indicated in an extra-vaginal testicular torsion, observed mainly during the perinatal period and in patients with cryptorchidism. Dewan et al. demonstrated that MD is not widely accepted by both urologists and young surgeons, who consider this technique as ineffective [25]. This perception was in fact the motive for this study.

No anesthesia induction was required for the performance of MD in any of our patients. The recession of pain occurred quickly after the successful and complete detorsion, representing a significant clinical criterion for the evaluation of the method [24].

Cattolica describes the application of MD in 35 out of a total of 104 cases of testicular torsion over a decade [26]. No analgesia or anesthesia was required in any of the above-mentioned cases. On the contrary, Gatti et al. considers analgesia as necessary in order to reassure the cooperation of the pediatric patient, while maximizing the effects of MD with the relaxation of the cremasteric muscle, as well [27]. Another clinical criterion that could be utilized for the assessment of a successful MD is the palpation of the suffering testicle on the base of the ipsilateral scrotum [28].

The TT can be lateral, outward, median or inward [28, 29, 30]. There are no established parameters to determine the direction of TT [31]. In 60-70% of cases, torsion occurs in the midline and in 30% in the lateral line [32, 33, 34]. Yecies et al. in their study including 104 boys with ITT that underwent surgical treatment, found that ITT was lateral in 38 cases (46%) [35]. In our study, in four out of five cases the ITT was left-sided and thus the MD was performed in a clockwise direction, while in the right-sided case the detorsion was carried out in an anti-clockwise direction. The overall rotation can usually range from 180º-1080º [24, 35]. In our study it ranged from 360-720º.

Beyazal et al. carried out the surgical exploration of the scrotum by an intrascrotal approach, which constitutes the typical methodology, which we also followed [17]. Demirbas et al. performed a complete and successful MD in 20 cases out of a total of 26 patients with ITT (76.92%) [24]. Cornel and Karthaus described successful MD in 14 out of the 17 patients experiencing an ITT [36]. Similar results were also demonstrated by Cattolica et al. [26] and Kiesling et al [37].

An essential risk of MD is the incomplete detorsion, which could be easily mistaken as successful. Sessions et al. reported that in 32% of all the cases they managed, MD was incomplete [29]. We believe that by selecting the patients with specific and strict criteria, by applying the technique appropriately and by evaluating the outcome objectively, the potential risk of unsuccessful detorsion is diminished.

In the context of the surgical investigation, immediately after completion of the preoperative assessment of the patient, it is essential to perform longitudinal incisions in the tunica albuginea of the suffering testicle to minimize the potential risk development of testicular compartment syndrome. The development of intercellular edema within the testicular parenchyma due to ischemia, restoration of arterial blood

supply, and the rigidity of testicular fibroids leads to increased intra-cortical pressure and restriction of microcirculation, resulting in the exacerbation of ischemia [38]. Figueroa et al. found that treatment success, simultaneously with the rescue of the affected testicle increased from 64% to 85% by performing longitudinal incisions in the tunica albuginea [39]. However, a delayed surgical exploration of the scrotum is not indicated, as it endangers the development of compartment testicular syndrome. We believe that the surgical investigation should be performed as early as possible, immediately after the completion of a meticulous preoperative evaluation of the patient.

CONCLUSION

In conclusion, MD is a safe and non-invasive method, easy to learn for every physician.

It can be applied immediately after the diagnosis of the ITT, with high success rates, aiming to the restoration of arterial blood supply of the affected testicle.

After the performance of a successful MD, an extremely urgent surgical exploration of the intrascrotal structures is converted into an elective one.

Emergent surgical intervention is required when MD fails, according to specific clinical and ultrasonographic criteria.

Surgical exploration of the scrotum is absolutely the necessary final step for the correction of a potentially incomplete detorsion, the prevention of reperfusion complications and mainly of the testicular compartment syndrome, along with future episodes of TT affecting either the same or the contralateral testicle.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Williamson RC. Torsion of the testis and allied conditions. Br J Surg 1976; 63(6): 465-76.
2. Zhao LC, Lautz TB, Meeks JJ, Maizels M. Pediatric testicular torsion epidemiology using a national database: incidence, risk of orchiectomy and possible measures toward improving the quality of care. J Urol 2011; 186(5): 2009-13.
3. Feher Miklos A, Bajory Z. A review of main controversial aspects of acute testicular torsion. J Acute Disease 2016; 5(1): 1-8.
4. Leibovitch I, Mor Y. The vicious cycling: bicycling related urogenital disorders. Eur. Urol 2005; 47(3): 277-86.
5. Seng YJ, Moissinac K. Trauma induced testicular torsion: a reminder for the unwary. J Accid Emerg Med 2000; 17(5): 381-2.
6. DaJusta DG, Granberg CF, Villanueva C, Baker LA. Contemporary review of testicular torsion: new concepts, emerging technologies and potential therapeutics. J Pediatr Urol 2013; 9(6 Pt A): 723-30.
7. Sozubir S, Barber T, Wang Y et al. Loss of Insl3: a potential predisposing factor for testicular torsion. J Urol 2010; 183(6): 2373-9.
8. Martin AD, Rushton HG. The prevalence of bell clapper anomaly in the solitary testis in cases of prior perinatal torsion. J Urol 2014; 191(5 Suppl): 1573-7.
9. Kapoor S. Testicular torsion: a race against time. Int J Clin Pract. 2008; 62:821-7.
10. Barthold JS. Abnormalities of the testes and scrotum and their surgical management. In: Wein AJ, editor. Campbell-Walsh urology.10th ed. Philadelphia, Pa: Saunders Elsevier; 2011. p.3587-92.
11. Castaneda-Sanchez I, Tully B, Shipman M, Hoeft A et al. Testicular torsion: A retrospective investigation of predictors of surgical outcomes and of remaining cotroversies. J Pediatr Urol. 2017 Oct;13(5): 516.e1-516.e4.
12. Visser AJ, Heyns CF. Testicular function after torsion of the spermatic cord. BJU Int. 2003; 92:200-3.
13. Tryfonas G, Violaki A, Tsikopoulos G, et al. Late postoperative results in males treated for testicular torsion during childhood. J Pediatr Surg. 1994; 29:553-6.
14. Yang C, Song B, Tan J et al. Testicular torsion in children: a 20-year retrospective study in a single institution. ScientificWorld Journal 2011; 11: 362-8.
15. Drlik M, Kocvara R. Torsion of spermatic cord in children: a review. J Pediatr Urol 2013; 9(3): 259-66.
16. Sharp VJ, Kieran K, Arlen AM. Testicular torsion: diagnosis, evaluation and management. Am Fam Physician 2013; 88(12): 835-40.
17. Beyazal M, Celiker FB, Inecikli MF et al. The monitoring of preoperative external detorsion with diffusion-weighted imaging in a patient with acute testicular torsion. Hindawi Case Reports in radiology vol. 2017, article ID 3702873, 3 pages.
18. Turner TT, Bang HJ, Lysiak JL. The molecular pathology of experimental testicular torsion suggest adjunct therapy to surgical repair. J Urol 2004,176 (6 Pt 2): 2574-8.
19. Paddy D, Bhavish K, John L. Survey of manual detorsion of the testicle Mini review. 2016;1(2) JO Uro & Nephron, 555556.
20. Hadziselimovic F, Geneto R, Emmons LR. Increase apoptosis in the contralateral testes of patients with testicular torsion as a factor for infertility. J Urol 1998;160(3Pt 2):1158-60.
21. Nash WG. Acute torsion of the spermatic cord: reduction: immediate relief. Br Med J. 1893; 1:742.
22. Lee LM, Wright JE, McLoughlin MG. Testicular torsion in the adult. J Urol. 1983; 130:93-4.
23. Van der Poel J. Strangulation of the testis and epididymis from the torsion of the spermatic cord. Medical Records, 1895,47:737.
24. Demirbas A, Demir D, Ersoy E et al. Should manual detorsion be a routine part of treatment in testicular torsion? BMC Urol 2017,17:84.
25. Dewan P, Kowlessur B, Lazarus J. A Survey of Manual Detorsion of The Testicle Mini Review 2016; 1(2): 555556. JO Uro & Nephron.
26. Cattolica EV. Preoperative manual detorsion of the torsed spermatic cord. J Urol. 1985 May;133(5):803-5.
27. Gatti JM, Patrick Murphy J. Current management of the acute scrotum. Semin Pediatr Surg 2007; 16(1): 58-63.
28. Siu Uribe A, Garrido Perez JI, Vazquez Rueda F et al. Manual detorsion and elective orchiopexy as an alternative treatment for acute testicular torsion in children. Cir Pediatr. 2019 Jan 21;32(1):17-21.

29. Sessions AE, Rabinowitz R, Hulbert WC, et al. Testicular torsion: direction, degree, duration and disinformation. J Urol. 2003;169:663-5.
30. Gunes M, Umul M, Altok M. Atypical testicular torsion: We must be cautious in the course of manual detorsion. Pediatr Emerg Care. 2016 Oct;32(10):691-692.
31. Cannon ML, Finger MJ, Bulas DI. Manual testicular detorsion aided by color doppler ultrasonography. J Ultrasound Med. 1995 May. 14(5):407-9.
32. Estremadoyro V, Meyrat BJ, Birraux J et al. Diagnosis and management of testicular torsion in children. Rev Med Suisse. 2017,13(550):406-410.
33. Gunes M, Umul M, Celik AO et al. A novel approach for the manual de- torsion of an atypical (outward) testicular torsion with bedside doppler ultrasonography guidance. Can Urol Assoc J. 2015 Sep-Oct. 9 (9-10):E676-8.
34. Saxena AK, Castellani C, Ruttenstock EM, Hollwarth ME. Testicular torsion: a 15-year single-center clinical and histological analysis. Acta Paediatr. 2012 Jul. 101(7): e282-6.
35. Yecies T, Bandari J, Schneck F, Cannon G. Direction of rotation in testicular torsion and identification of predictors of testicular salvage. Urology. 2018 Apr;114: 163-166.
36. Cornel E, Karthaus H. Manual derotation of the twisted spermatic cord. Br J Urol, 1999. 83 (6):672-4.
37. Kiesling V, Schroeder D, Pauljev P, Hull J. Spermatic cord block and manual reduction: primary treatment for spermatic cord torsion. J Urol, 1984 132(5):921-3.
38. Kutikov A, Casale P, White MA, Meyer WA, et al. Testicular compartment syndrome: a new approach to conceptualizing and managing testicular torsion. Urology 2008; 72(4):786-9
39. Figueroa V, Pippi Salle JL, Braga LH et al. Comparative analysis of detorsion alone versus detorsion and tunica albuginea decompression (fasciotomy) with tunica vaginalis flap coverage in the surgical management of prolonged testicular ischemia. J Urol 2012; 188(4 suppl):1417-22.

ORIGINAL ARTICLE

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INTRASCROTAL INCISION: AN ALTERNATIVE TECHNIQUE FOR THE MANAGEMENT OF INGUINOSCROTAL PATHOLOGIES, EXPERIENCE FROM 76 CASES

I. Gkalonaki[1], M. Anastasakis[1], V. Moutsanas[2], T. Feidantsis[3], M. Mitroudi[1], I. Patoulias[1]

[1]First Department of Pediatric Surgery, Aristotle University of Thessaloniki – Greece, General Hospital "G. Gennimatas", Thessaloniki – Greece
[2]Pediatric Department, General Hospital "G. Gennimatas", Thessaloniki – Greece
[3]Pediatric Surgery Department, General Hospital of Larisa, Larisa – Greece

Abstract. Introduction: *The aim of this clinical study was to present our experience gained by using the intrascrotal incision through the mid raphe for the management of a variety of inguinoscrotal pathologies.* ***Materials and Methods:*** *A total of 76 male patients, between 2 and 16 years, underwent surgical treatment through a mid raphe intrascrotal incision, for a wide range of inguinoscrotal diseases, including torsion of the spermatic cord, torsion of the testicular appendages, non-communicating hydrocele, communicating hydrocele, ectopic testis, retractile testicles, palpable undescended testis, testicular trauma and testicular prosthesis placement.* ***Results:*** *All the patients had an uneventful postoperative recovery, with none mentioned complication, and none of them required convention to the traditional inguinal method. The follow-up examination ranged from 6 months to 3 years, with no surgical complication highlighted.* ***Conclusions:*** *We recommend that the intrascrotal incision through the mid raphe may be considered as an alternative technique to inguinoscrotal pathologies, instead of other approaches. The ability to treat a variety of pathologies regarding both two hemi-scrotums and the inguinal region at the same time, the provision of adequate surgical site, while succeeding much less dissection and disruption of tissue, the excellent cosmetic result, the greater comfort for the 'day-case' child, the ability to use the scrotal septum in order to fix the testis in the scrotum and the avoidance of an extra incision are the main advantages of the intrascrotal incision.*

Key words: *intrascrotal incision, midline incision, mid raphe orchiopexy, inguinoscrotal pathologies*

Corresponding author: *Ioanna Gkalonaki, MD, Papafi 178, 54453 Thessaloniki, Greece; phone: +306972529608; e-mail: iongalonaki@gmail.com, Orchid ID: https://orcid.org/0000-0002-0815-8377*

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INTRODUCTION

Intrascrotal incision through the mid raphe may be considered as an alternative technique to inguinoscrotal pathologies, instead of other approaches, which include the laparoscopic approach, the inguinal incision, the high single scrotal incision (Bianchi) and the circumcision incision – as described by M. Chua et al for palpable, low inguinal undescended testes [1].

According to similar studies related to the intrascrotal incision, the approach has been used for the surgical management of inguinal hernia, webbed penis, scrotal exploration, tumors of the inguinoscrotal area and testicular biopsy [2, 3, 4].

We report our experience from 76 patients operated using a vertical intrascrotal incision, through the mid raphe for the management of inguinoscrotal pathologies. At the same time, after systematic and comprehensive review of the relevant international literature, the wider range regarding the type of the performed procedures is documented.

MATERIALS AND METHODS

This was an observational study. All patients included in the study were referred to our hospital for the management of inguinoscrotal pathologies during a specific period of time – between January, 2018 and December, 2018. The patients were treated by a one and the same surgical team led by Dr Patoulias Ioannis.

A total of 76 boys aged between 2 and 16 years underwent surgery for inguinoscrotal pathologies, through a vertical intrascrotal incision (Figure 1).

Eight out of 76 (8/76) patients, aged 12 years, suffered a spermatic cord torsion. The surgical management included detorsion, testicular fasciotomy by making a longitudinal incision of the tunica albuginea for the prevention of the testicular compartment syndrome, and bilateral orchiopexy by fixing the lateral surface of the testis on the intrascrotal septum (Figures 2, 3).

Twelve out of 76 (12/76) patients suffered torsion of the testicular appendage, where the torsed appendage was excised through an intrascrotal incision.

A total of 4 out of 76 (4/76) patients were operated due to communicating hydrocele. High ligation of the processus vaginalis and creation of a “window” through the tunica vaginalis was performed.

Four out of 76 (4/76) patients underwent surgical operation due to non-communicating hydrocele. Inversion of the tunica vaginalis and orchiopexy of the homolateral testis was performed.

One out of 76 (1/76) patients was operated because of testicular trauma. The surgical management included inspection of the intratesticular structures, control of the bleeding and drainage of the hematoma. Two penrose drain tubes were positioned respectively with the upper and lower pole of the homolateral testis (Figure 4).

A total of 34 out of 76 (34/76) patients suffered palpable undescended testis, where Bianchi procedure and fixation of the testis on the scrotal septum was performed.

Ten out of 76 (10/76) patients had and ectopic testis. The surgical steps included dissolution of the fibrous adhesions to the height of the external inguinal ring, inspection for an open processus vaginalis (negative) and placement of the unilateral testicle in a pouch between the scrotum and the dartos (sub-dartos pouch) (Figure 5).

A total of 3 out of 76 (3/76) patients had a testicular prothesis placement, where preparation and expansion of the scrotum, for the prothesis placement without tension, was performed (Figure 6).

Table 1. List of inguinoscrotal pathologies where the intrascrotal incision was applied, number of patients and chosen surgical technique

Disease	Number of patients	Surgical treatment
Torsion of the spermatic cord	8	Detorsion, prevention of the testicular compartment syndrome, bilateral orchiopexy
Torsion of the testicular appendages	12	Excision of the torsed appendage
Adult-type hydrocele	4	Inversion of the tunica vaginalis
Communicating hydrocele	4	High ligation of the processus vaginalis
Testicular trauma	1	Inspection of the intratesticular structures, control of the bleeding and drainage of the hematoma
Ectopic testis	10	Orchiopexy
Palpable undescended testis	34	Bianchi procedure and fixation of the testis on the scrotal septum
Testicular prothesis placement	3	Preparation and expansion of the scrotum, for the prothesis placement without tension

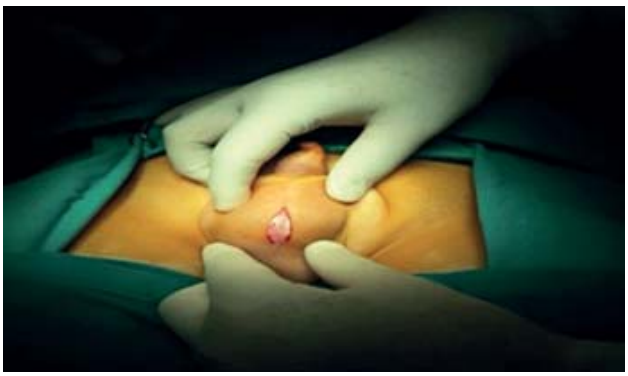


Fig. 1. Intrascrotal (vertical) incision through the midline raphe

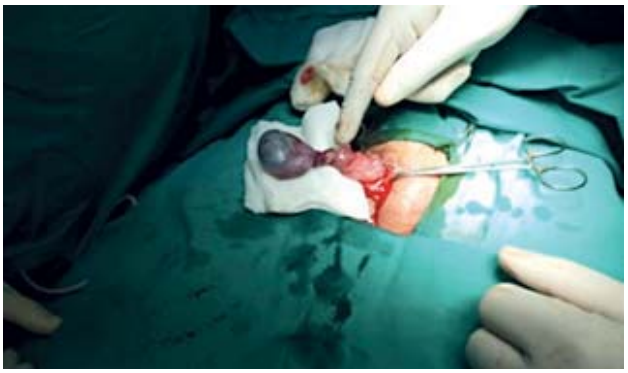


Fig. 2. Torsion of the testicular cord

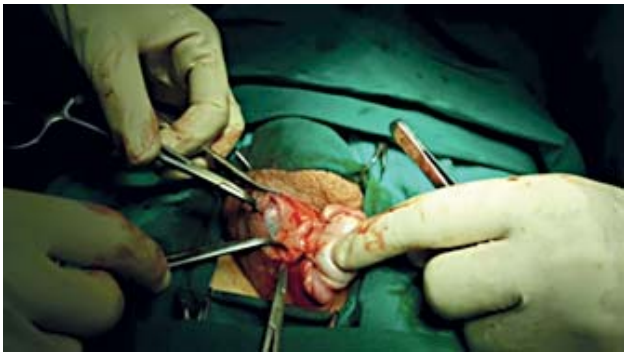


Fig. 3. Bilateral orchiopexy through one intrascrotal incision

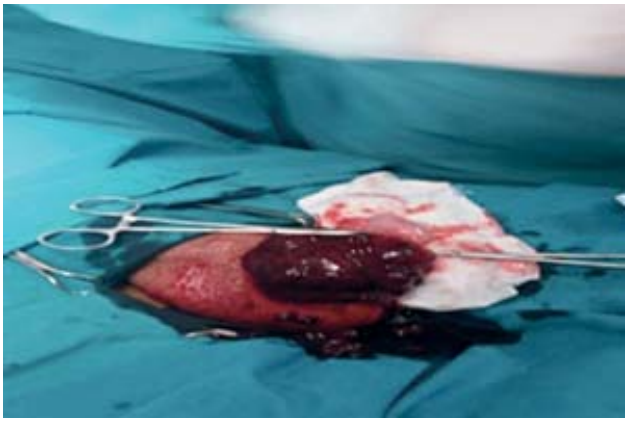


Fig. 4. Testicular trauma

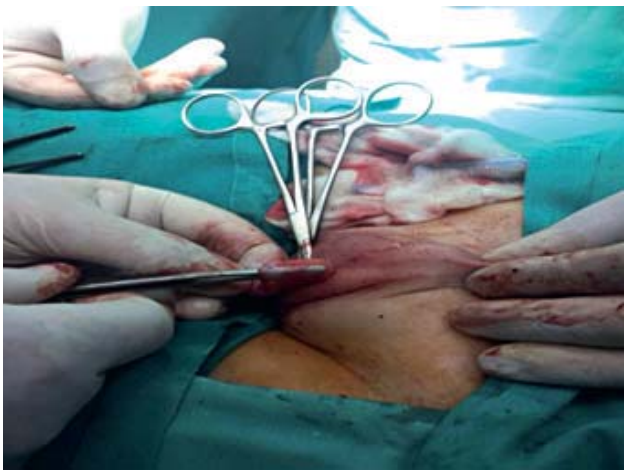


Fig. 5. Check for an open processus vaginalis

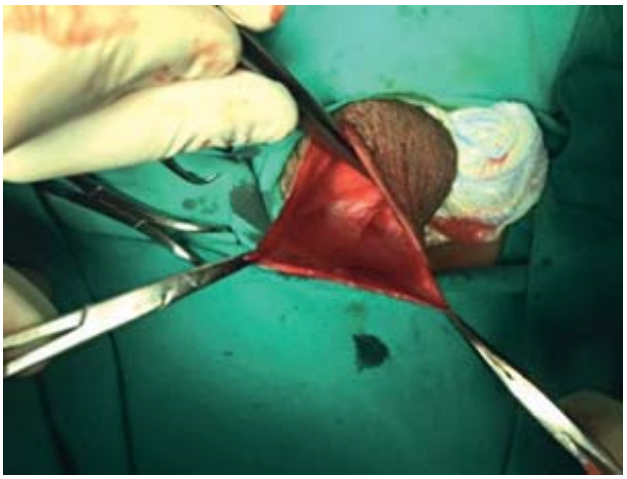


Fig. 6. Expansion of the scrotum, for the prothesis placement without tension

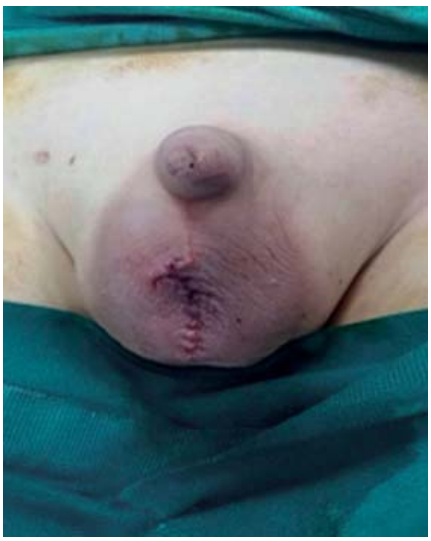
RESULTS

All the patients had an uneventful postoperative recovery. Besides the patient with the testicular trauma, the rest were discharged from the hospital on the first postoperative day. None of the patients required convention to the traditional inguinal method. The follow-up period ranged from 6 months to 2 years, with no surgical complication noted. The aesthetic result of the incision was considered satisfactory (Figures 7, 8, 9).

DISCUSSION

It is worth highlighting that the laparoscopic approach has a limited use, in the inguinal pathologies, and therefore cannot be applied in scrotal pathologies, such as testicular torsion, testicular prothesis placement and trauma of the testes.

Moreover, laparoscopic hydrocelectomy has been reported, but the operation time was longer than that of



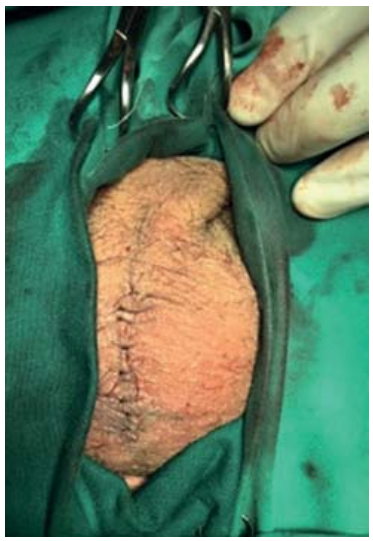


Fig. 7, 8. The intrascrotal incision postoperatively, after suture placement

Fig. 9. Intrascrotal incision during follow-up. Excellent cosmetic result

scrotal hydrocelectomy and the incision scars were more noticeable than that of the scrotal approach. Also, the cost it incurred was higher than that of the scrotal approach [5, 6].

The circumcision incision technique is available for patients who undergo orchidopexy and request or have indications for circumcision performance, in the same setting.

However, it is a restricted method, due to its limited use in concurrent circumcision and palpable, low inguinal undescended testes and the need for great experience [1].

The conventional inguinal approach is still preferred in the management of high inguinal undescended testis, as it allows better retroperitoneal mobilization of the testicular vessels, such that their new and straighter course towards the scrotum permits additional testicular descent. The basic principles of this method involve making a second scrotal incision to place the testis in a dependent sub- dartos pouch without tension. That means that it requires two standard skin incisions, while in contralateral inguinal hernias the number of the incisions is doubled. This results in longer operative time, lower cosmetic results and higher rates of postoperative pain.

The high single scrotal incision is similar to the intrascrotal incision, as regards to the success rate and the postoperative results. As described by Seong Woong Na et al., the success rate in the single incision orchiopexy group was as high as 92.5% (135/146 testes). Only 11/ 107 patients required conversion to traditional inguinal incision orchiopexy or had postoperative complications (scrotal hematoma, wound dehiscence), in comparison to the traditional inguinal incision orchiopexy group, which was 96.5% (136/141 testes) [7]. Parson JK et al. describes that only 20% of the patients (out of 56 patients) required concomitant inguinal hernia repair through a standard inguinal incision [3]. Bianchi and Squire reported a 95.8% success rate while they performed a single scrotal incision orchiopexy in 120 patients [8]. Dayanc et al. evaluated the success rate with or without inguinal hernia in patients with an undescended testis within the inguinal canal or beyond the external inguinal ring. Scrotal orchiopexy had a success rate of 97.6% in the distal to the external inguinal ring group, while only 1/34 patients required conversion to traditional inguinal incision. The success rate in the within the inguinal canal group was 89.7%, while 3/ 22 cases needed additional groin incision [9]. This means that a palpable undescended testis may be surgically relocated into the dependent scrotum without sacrificing the traditional principles of orchiopexy [10]. The main reason for conversion remains the insufficient length of cord. It is suggested that when the testis is located in the inguinal canal or higher, a traditional inguinal incision should be considered before the operation.

A possible controversy regarding the scrotal approach is whether the dissection is high enough to easily allow for adequate lengthening of the cord and placement of the testis into the scrotum without tension. Also, there is concern that a single scrotal approach may not allow sufficient ligation of the processus vaginalis to avoid hernia or hydrocele formation after the operation. However, the majority of the undescended testes are palpable distal to the inguinal canal.

Furthermore, in the pediatric age group, the inguinal canal is short, with the internal and the external rings almost superimposed to one another. This couple with the relative morbidity of the skin in the inguinal region allows retraction of the skin incision, thereby enabling dissection through the scrotum without opening the inguinal canal. Moreover, palpable undescended testes appear to be held up by a shorter than normal processus vaginalis that often has high insertion, as observed by Bianchi and Squire in 1989.

When additional cord length is required, additional dissection through the scrotal incision is possible, by opening the external inguinal ring and canal, as we have already performed in cases of cryptorchidism.

According to these facts, the scrotal incision is considered adequate for orchidopexy in most palpable undescended testes.

In addition to that, it is possible to successfully correct other inguinoscrotal abnormalities by using the scrotal incision method, such as hernia and hydrocele, as it gives such excellent access to the processus vaginalis, external inguinal ring and inguinal canal [2, 7, 11].

Another controversy is whether the older the patient, the higher the failure rate of high scrotal orchidopexy in terms of successful placement of the testis in a dependent position in the scrotum. According to a study of Talabi et al., the increasing age of patients and location of palpable undescended testes had no influence on successful placement of the testes into the scrotum via both scrotal and inguinal technique [12].

The scrotal incision is also considered preferable in redo orchidopexy procedure in cases of recurrent and iatrogenic undescended testes, after primary traditional orchiopexy, as it allows early entry into unscarred, previously unexplored layer of the canal, which allows a safer mobilization of the testis and then the cord [13].

A scrotal incision is associated with shorter operative time, less postoperative pain, no added morbidity and improved cosmetic outcomes.

As for the noted complications, theoretically these include transient scrotal oedema, wound hematoma, and testicular renascent, which had no significant difference while using the traditional orchiopexy. Our patients had an uneventful postoperative recovery, with none of the mentioned complications, and none of them required convention to the traditional inguinal method.

The main difference of the intrascrotal incision, compared to the high single scrotal incision, is that the second one requires two incisions for contralateral pathologies, on the contrary to the intrascrotal approach, that allows the examination and repair of the impairment through one mid raphe incision, which allows access to both sides of the scrotum. Additionally, there is a minimal chance, though, of injuring the superficial branches of the genitofemoral and the ilioinguinal nerves, using the high single scrotal incision [14].

As for the testicular prothesis placement, after systematic and comprehensive review of the relevant literature, we did not find a similar study. Traditionally, the specific procedure is established through an inguinal incision. In our opinion, the intrascrotal incision facilitates the exploration of the unilateral scrotum and inguinal region, enables the expansion of the unilateral hemi-scrotum for the prothesis placement, while at the same time allows the performance of preventive orchiopexy of the bilateral testis.

Initially, we managed four cases of testicular torsion using the intrascrotal incision. These cases encouraged us to broaden the use of this technique in a range of inguinoscrotal pathologies, as we believed it was possible, from a surgical and technical aspect.

The main disadvantages of our study are:

1. The relatively limited number of patients we managed, although greater than most of the corresponding studies in the recent literature [4].
2. The restricted period of time, this study refers to.
3. Finally, this is an observational study, thus it lacks the compared results of a double-blind study.

CONCLUSIONS

The results of our study show that the intrascrotal incision may be used in the following pathologies: torsion of the spermatic cord, appendiceal torsion, adult-type hydrocele, communicating hydrocele, ectopic testis, retractile testicles, palpable undescended testis, testicular trauma and testicular prosthesis placement.

The advantages that encouraged us to use the intrascrotal incision as the technique of choice are:

1. The ability to treat a variety of pathologies regarding both two hemi scrotum and the inguinal region at the same time.
2. The provision of adequate surgical field, while succeeding much less dissectonal tissue disruption.
3. The excellent cosmetic result.
4. The greater comfort for the ‘day-case’ child
5. The ability to use the intra scrotal septum in order to fix the testis in the scrotum and the avoidance of an extra incision.

Finally, besides the disadvantages mentioned above, we believe that the number of cases described, being greater than most of the relative studies of the recent literature, are reliable and valuable.

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All the authors contributed equally to the writing of this article.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Chua ME, Silangcruz JM, Gomez O et al. Circumcision-incision orchidopexy: A novel technique for palpable, low inguinal undescended testis. Invest Clin Urol, 2017, 58(6):468.
2. Lais A, Ferrow F. Trans-scrotal approach for surgical correction of cryptorchidism and congenital anomalies of the processus vaginalis. Eur Urol, 1996, 29:235–239.
3. Parsons JK, Ferrer F, Docimo SG. The Low Scrotal Approach to the Ectopic or Ascended Testicle: Prevalence of a Patent Processus Vaginalis. J Urol, 2003, 169(5):1832-1833.
4. Soualili Z, Achouri D, Haif A et al. The interscrotal approach to inguinoscrotal pathologies. Arab J Urol, 2015, 13(3):176-178.
5. Alp BF, Irkilata HC, Kibar Y et al. Comparison of the inguinal and scrotal approaches for the treatment of communicating hydrocele in children. The Kaohsiung Journal of Medical Sciences, 2014, 30(4):200-205.
6. Braga LH, Lorenzo AJ, Romao RL. Canadian Urological Association-Pediatric Urologists of Canada (CUA-PUC) guideline for the diagnosis, management, and follow up of cryptorchidism. Canad Urol Assoc J, 2017, 11(7).
7. Na SW, Kim S-O, Hwang EC et al. Single Scrotal Incision Orchiopexy for Children with Palpable Low-Lying Undescended Testis: Early Outcome of a Prospective Randomized Controlled Study. Korean J Urol, 2011, 52(9):637.
8. Bianchi A, Squire BR. Transscrotal orchidopexy: orchidopexy revised. Pediatr Surg Int, 1989, 4(3)
9. Dayanç M, Kibar Y, Tahmaz L et al. Scrotal incision orchiopexy for undescended testis. Urology, 2004, 64(6):1216-1218.
10. Caruso AP, Walsh RA, Wolach JW et al. Single scrotal incision orchiopexy for the palpable undescended testicle. J Urol, 2000, 164: 156-159.
11. Iyer KR, Kumar V, Huddart SN et al. The scrotal approach. Pediatr Surg Int, 1995, 10(1).
12. Talabi AO, Sownde OA, Adejuyigbe O. High (trans) scrotal orchidopexy for palpable undescended testes in children: Influence of age and testicular position. African J Urol, 2018, 24(2):114-119.
13. Fares AE, El Barbary MM. High scrotal approach for secondary orchidopexy in cases of recurrent and iatrogenic undescended testes. Ann Pediatr Surg, 2011, 7(3):108-110.
14. Talabi AO, Sowande OA, Anyanwu L-JC et al. Trans-scrotal single-incision orchidopexy compared with conventional orchidopexy in palpable undescended testis in a Nigerian Tertiary Hospital. Ann Pediatr Surg, 2015, 11(1):25-29.

CASE REPORT

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INDOCYANINE GREEN FLUORESCENCE FOR LIVER ASSESSMENT AND IMAGING-GUIDED RESECTION OF COLORECTAL METASTASES: A CASE REPORT

E. Arabadzhieva[1], S. Bonev[1], D. Bulanov[1], L. Simonova[1], E. Zhivkov[1], G. Korukov[1], Zh. Shavalov[1], M. Velizarova[2], D. Svinarov[2], A. Yonkov[1]

[1]Department of General and Hepato-pancreatic Surgery, University Hospital "Alexandrovska", Sofia, Medical University – Sofia, Bulgaria
[2]Department of Clinical Laboratory and Clinical Pharmacology, University Hospital "Alexandrovska", Sofia, Medical University – Sofia, Bulgaria

Abstract. Introduction: *Liver resection is widely accepted as a treatment option for primary liver cancer and metastases. The goal of surgery is to resect all tumours with negative histological margins while preserving sufficient functional hepatic parenchyma and reducing postoperative complications. The use of Indocyanine green (ICG) for liver function assessment and fluorescence image-guided surgery could be used to achieve that goal.* ***Clinical Case Description:*** *We present the case of a 62-year-old female patient with diagnosed sigmoid colon cancer with four bilobar liver metastases who underwent a simultaneous sigmoid resection and ICG fluorescence image-guided liver resection 3 days after preoperative ICG liver function assessment. We decided to perform liver-sparing resection having in mind the liver metastases' number, size and location and the slightly impaired liver function (ICG retention rate 15 – ICGR15 was 14,02%). All liver tumours were removed without complications, and the resected margins were all microscopically free of tumour tissue (R0 resection). The postoperative period was uneventful, without any signs of postoperative liver failure.* ***Conclusions:*** *ICGR15 can be considered a safe and informative marker for liver function and indirectly for the degree of portal hypertension. ICG fluorescence provides an additional method to assist intra-operative tumour identification. The best timing of injection requires further study.*

Key words: *liver resection, ICG fluorescence image-guided surgery, liver metastasis, liver function, ICGR15*

Corresponding author: *Elena Arabadzhieva, MD, PhD, Department of General and Hepato-pancreatic Surgery, University Hospital "Alexandrovska", Medical University – Sofia, 1 Sv. G. Sofiiski Street, 1431 Sofia, Bulgaria; mobile: +359886376071; e-mail: elena_arabadjieva@abv.bg*

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INTRODUCTION

Contemporary liver oncological surgery is based on three main principles: performing R0 liver resections, preserving healthy liver parenchyma, and reducing postoperative complications [1]. Postoperative liver failure (PHLF) is one of the most severe complications [2]. Its occurrence is closely related to the volume and functional capacity of the residual liver [2]. Conventional basic liver function tests (AST, ALT, bilirubin, albumin, GGT) can assess the absence or presence of liver damage but cannot provide a quantitative assessment of liver function [3]. Dynamic liver function tests are more sensitive and provide a qualitative and quantitative assessment of the functional liver capacity [3]. Worldwide, the Indocyanine green (ICG) 15-minute retention rate (ICGR15) is one of the most popular methods [3, 4].

In addition, ICG near-infrared fluorescence imaging can be used to improve the achievement of R0 liver resection and prevent missing tumour tissue. Ishizawa et al. reported in 2009 that intravenous injection of ICG caused “bull’s eyes” of fluorescence to be formed around primary or metastatic tumours in the liver [5, 6]. The preoperatively injected ICG is retained inside them due to biliary excretion disorders in the cancerous tissues [6]. The dose of ICG most frequently used to identify liver tumours is 0.5 mg/kg body weight, administered 1-14 days before surgery [6, 7].

We present the case of a patient with sigmoid colon cancer with liver metastases who underwent a simultaneous sigmoid resection and ICG fluorescence image-guided liver resection after preoperative ICG liver function assessment. As far as we know, this is the first case in Bulgaria reporting the combined usage of these methods. The case is a part of a study funded by GRANT – 2021 of the Medical University of Sofia (D-110/ 04.06.2021).

CLINICAL CASE DESCRIPTION

A 62-year-old female was referred to our hospital for sigmoid colon cancer with liver metastases diagnosed at another hospital. Her main complaints were mild pain and discomfort in the right hypochondrium. A colonoscopy revealed an ulceropolypic tumour at a distance of 35 cm from the anus. The CT images demonstrated an eccentrically thickened wall of the sigmoid colon, with high-grade lumen stenosis of about 4.8 cm in length. Prestenotic dilatation of 7.35 cm and locoregional lymphadenomegaly were also registered. The liver was enlarged and fatty, and hypodense lesions of various sizes were visualized in the parenchyma of both lobes. The tumour in segment VIII was 7 cm in size, and that in segment VI was about 2.4 cm. The patient had a history of previous appendectomy and laparoscopic cholecystectomy. Her concomitant diseases included obesity and ischemic heart disease. Laboratory tests were within the normal range, except for gamma-glutamyl transferase (GGT) – 62 U/l. Tumour markers were elevated – CA 19-9 – 195.8 U/mL; CEA – 43.9 ng/mL.

She received a preoperative ICG 15-minute retention test (ICG-R15 test) for liver function assessment at an ICG dose of 0.5 mg/ kg body weight. The result was 14,02%. She underwent a simultaneous sigmoid colon resection, omentectomy and liver-sparing hepatic resection 3 days after the ICG-R15 test. We used an ICG fluorescence imaging system and intraoperative ultrasound to identify liver tumours during surgery. Intraoperatively, we found a fatty liver with several metastatic lesions. The largest one, with a diameter of about 7.5 cm, was located in the VIII segment (Fig. 1). One was in the liver segment III about 3cm in diameter, and two of similar size were located in the VI and VII segments (Fig. 2). The primary tumour was in the middle third of the sigmoid colon. The lesion was about 4 cm in diameter, with hard cartilaginous density, almost entirely stenosing the intestibnal lumen. We decided to perform liver-sparing hepatic resection having in mind the liver metastases’ number, size and location and the slightly impaired liver function.

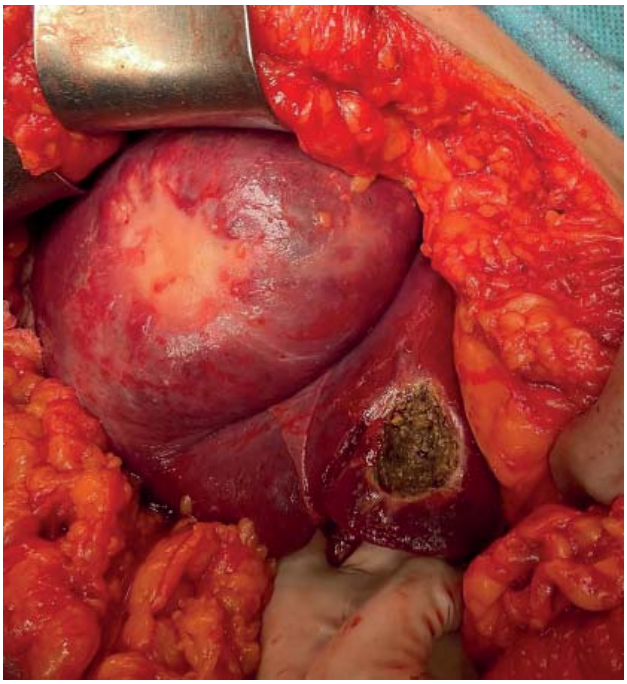

Fig. 1. Liver metastasis in VIII liver segment. The picture also shows the resection surface after the removal of the lesion in the III segment

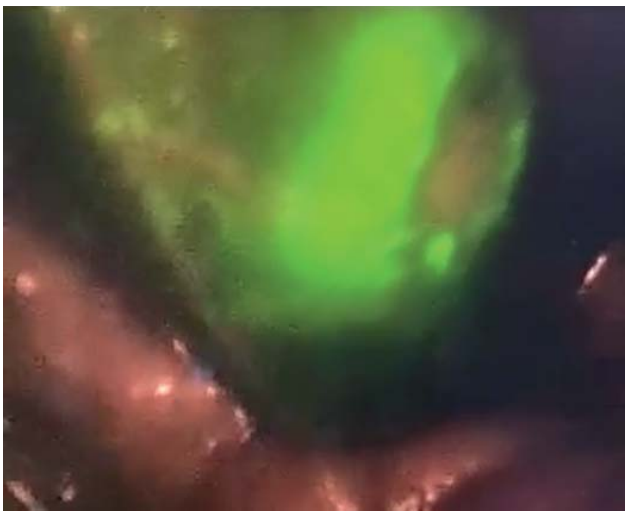

Fig. 2. ICG fluorescence imaging of the metastasis in VI liver segment

Before the liver transection, the entire liver pedicle was encircled with tape, and an intermittent period of inflow clamping using the Pringle manoeuvre was performed to prevent haemorrhage. During the surgical resection, ICG fluorescence imaging was also used to distinguish in real-time between tumour borders and normal liver parenchyma. Guided by the fluorescent signal, at least a 1-cm resection margin was marked, and hepatic transection was performed through a clamp-crushing method employing the Ligasure and bipolar forceps (Fig. 3). All the tumours were entirely removed without complications (Fig. 4).

Finally, the resected margins were all microscopically free of tumour cells (R0 resection). The postoperative period was uneventful, without any signs of PHLF. One month after the operation, adjuvant chemotherapy was initiated.

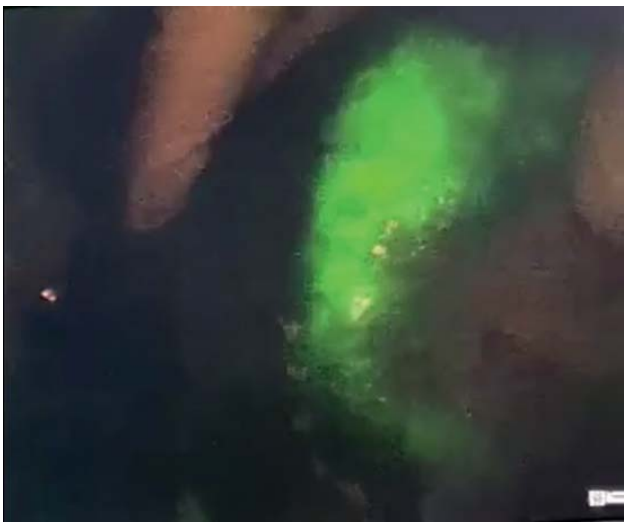

Fig. 3. ICG fluorescence imaging of the metastasis in VIII segment, distinguishing tumour borders and normal liver parenchyma

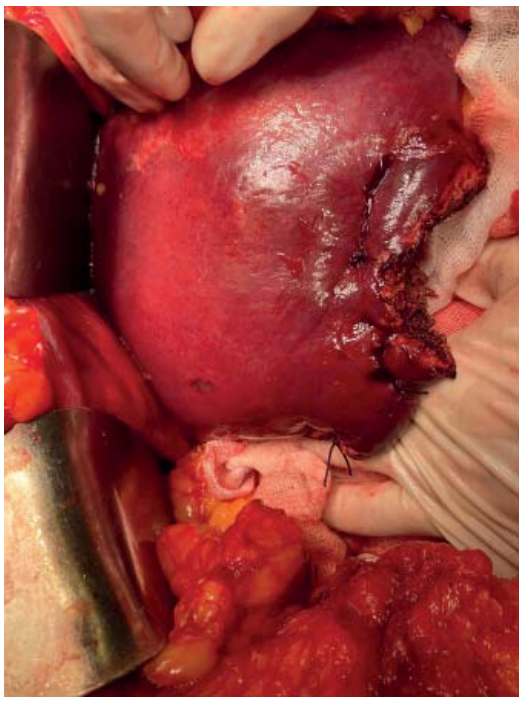

Fig. 4. The patient's liver after resection of the lesions in VI and VII liver segment

DISCUSSION

Liver resection is widely accepted as a treatment option for primary liver cancer and liver metastases (most commonly colorectal and from neuroendocrine tumours) [8]. Colorectal liver metastases (CLC) are the most common reason for liver resection in Western countries, and results in recent years have been encouraging – postoperative mortality in large series ranging from 0.2% to 3.5%, 1-year survival – 89%-97%, and the 5-year survival being between 15% and 50% [8].

The diagnostic and treatment approach in patients with liver tumours must be complex, multidisciplinary and individualized in order to achieve optimal results. The goal of surgery should be to resect all tumours with negative histological margins while preserving sufficient functional hepatic parenchyma [6, 9].

The appearance of PHLF is closely related to the volume and functional capacity of the residual liver tissue (RF) [2]. To prevent it, according to modern algorithms, a FLR > 20% is considered the minimum safe volume for patients with normal liver function, while a FLR above 30% is necessary for patients who have already received chemotherapy, and 40% is considered the minimum required for patients with cirrhosis [2].

Therefore, an accurate assessment of liver function is crucial. ICG has been widely used in clinical settings to estimate cardiac output and liver function since its approval by the US Food and Drug Admin-

istration in 1954 [10]. It is excreted unchanged and almost entirely (97%) in the bile [3]. Toxicity is extremely low, with allergic reactions and side effects reported in less than 1/40,000 cases [3, 4]. An easily performed method for evaluating liver function is the spectrophotometric determination of the ICG blood concentration 15 minutes after its application (ICG R15). Established reference values are 0-10% [3].

The indications for the ICG R15 test are: 1) evaluation of the functional liver reserve in patients before liver resection (the method is precise in patients with underlying liver disease – cirrhosis, steatosis) [3, 4]; 2) prediction of postoperative complications in liver surgery [3]; 3) evaluation of the liver function of the donor/cadaveric liver when planning a liver transplant [3]; 4) non-invasive assessment of portal hypertension and oesophagal varices [3, 11]; 5) early functional assessment of the graft after liver transplantation [3].

The benefits of this method are evident from the many publications on the subject. In 2014, ICGR15 was included in a modified liver damage grading system [Liver Damage Grading System (LDGS)] [12]. The Japanese Liver Cancer Study Group of Japan proposed and applied LDGS instead of Child-Pugh grading as a more accurate and appropriate tool for functional assessment of liver reserve [12, 13]. Many authors suggest different surgical approaches depending on the ICGR15 values. According to the accepted Makuuchi criteria, large-volume liver resections should be performed in cirrhotic patients with ICGR15 < 15%. Suitable candidates for right hemihepatectomies are patients with ICGR15 up to slightly > 10%, while left hepatectomies are also discussed in patients with slightly-high ICGR15 (range 10% to 19%) [14]. At 20-29%, ICGR15 segmentectomy could be performed, and at 30-39%, only partial, atypical, limited resection is feasible. The significance of using ICGR15 to assess the liver reserve and predict postoperative outcomes is also evident from the published experience of the Makuuchi group: zero mortality in 1056 hepatectomies performed between 1994 and 2002 [15]. Other authors reported that the lower ICG R15 limit for performing a safe large-volume hepatectomy is between 14% and 17%, the latter being accurate in young patients with milder liver disease [16]. In our case, we preferred the liver-sparing hepatectomies (limited resections) due to the number of liver metastases, their size and bilobar location, impaired liver function and the need for simultaneous bowel resection.

As mentioned before, precise detection of liver tumours and their resection in negative margins are crucial. Preoperative conventional imaging techniques as ultrasound, contrast-enhanced CT, and magnetic resonance imaging are currently used to diagnose cancer and are an aid in guiding the resection [6, 17]. However, liver metastases of colorectal cancer might be multi-focal, and small intrahepatic tumours are difficult to diagnose [6].

ICG is retained in tumorous tissues even after excretion from the background hepatic parenchyma, leading to unambiguous identification of liver tumours by intraoperative ICG fluorescence imaging [6]. In contrast to intraoperative ultrasound, it can identify superficial hepatic lesions with an excellent sensitivity of 96-100% [6, 18]. It can also detect small or occult tumours, which could be missed by conventional imaging techniques [10]. All lesions missed by intraoperative ultrasound usually are superficially located, whereas the lesions missed by ICG fluorescence imaging are profound because of the fluorescence tissue penetration. This suggests a potential role in combining intraoperative ultrasound and ICG fluorescence imaging to increase sensitivity and the chance of complete resection [6, 19]. Purich et al. demonstrated that when intraoperative ICG fluorescence imaging is used in conjunction with intraoperative ultrasound, ICG could detect additional superficial malignant lesions in 11.6% of patients [18]. Intraoperative histopathological analysis of frozen tumour margins is expensive, time-consuming, and may be inadequate in large lesions [6]. Defective biliary clearance in the transition area between tumour and normal liver tissue and liver tumours may result in ICG retention, which can be visualized using an ICG fluorescence imaging system [19]. The benefit of ICG fluorescence is easily seen during laparoscopic surgery since its fluorescent property compensates for the lack of tactile feedback during this procedure [6]. Regarding the timing of injection, it has been variously described [7]. Generally, ICG is administered 14 days before the operation day, and specifically within 3 days in most studies. An additional administration (0.02-0.5 mg/kg) is an option in case of a long interval between the administration and operation day [7]. In cirrhotic or fibrotic liver with impaired liver function, slower metabolic elimination of ICG leads to an increased false positive rate of tumour detection [20]. Studies, including a high rate of cirrhotic patients, tended to present intervals between ICG administration and surgery longer than 7 days [20].

CONCLUSIONS

ICGR15 can be considered a safe and sound marker for liver function and indirectly for the degree of portal hypertension. ICG fluorescence provides an

additional method to assist intra-operative tumour identification. The best timing of injection requires further study.

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Ethics approval: *This study was approved by the hospital ethics committee and was carried out following the principles outlined in the Declaration of Helsinki. Consent from the patient was obtained. This report does not contain any information by which they could be identified.*

REFERENCES

1. Marino MV, Di Saverio S, Podda M et al. The Application of Indocyanine Green Fluorescence Imaging During Robotic Liver Resection: A Case-Matched Study. World J Surg. 2019;43(10):2595-606.
2. Kim HJ, Kim CY, Park EK, et al. Volumetric analysis and indocyanine green retention rate at 15 min as predictors of post-hepatectomy liver failure. HPB (Oxford). 2015;17: 159-67.
3. De Gasperi A, Mazza E, Prosperi M. Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery? World J Hepatol. 2016;8(7): 355-67.
4. Vos JJ, Wietasch JK, Absalom AR et al. Green light for liver function monitoring using indocyanine green? An overview of current clinical applications. Anaesthesia. 2014;69:1364-76.
5. Ishizawa T, Fukushima N, Shibahara J et al. Real-time identification of liver cancers by using indocyanine green fluorescent imaging. Cancer 2009;115:2491-504.
6. Kang CH, Chen IS. Indocyanine green fluorescence imaging-guided resection of colorectal liver metastasis. J Cancer Res Pract. 2021;8:109-12.
7. Wakabayashi T, Cacciaguerra AB, Abe Y et al. Indocyanine Green Fluorescence Navigation in Liver Surgery: A Systematic Review on Dose and Timing of Administration. Ann Surg. 2022;275(6):1025-1034.
8. Tsim NC, Frampton AE, Habib NA, Jiao LR. Surgical treatment for liver cancer. World J Gastroenterol 2010;16(8): 927-933.
9. Poultsides GA, Schulick RD, Pawlik TM. Hepatic resection for colorectal metastases: the impact of surgical margin status on outcome. HPB (Oxford). 2010;12(1):43-9.
10. Nakaseko Y, Ishizawa T, Saiura A. Fluorescence-guided surgery for liver tumors. J Surg Oncol. 2018;118:324-31.
11. Lisotti A, Azzaroli F, Buonfiglioli F et al. Indocyanine green retention test as a non-invasive marker of portal hypertension and esophageal varices in compensated liver cirrhosis. Hepatology. 2014;59:643-650.
12. Mizuguchi T, Kawamoto M, Meguro M, et al. Preoperative liver function assessments to estimate the prognosis and safety of liver resections. Surg Today. 2014;44:1-10.
13. Seyama Y, Kokudo N. Assessment of liver function for safe hepatic resection. Hepatol Res. 2009;39:107-116.
14. Kokudo N, Makuuchi M. Evidence-based clinical practice guidelines for hepatocellular carcinoma in Japan: the J-HCC guidelines. J Gastroenterol 2009;44 Suppl 19:119-121.
15. Imamura H, Sano K, Sugawara Y et al. Assessment of hepatic reserve for indication of hepatic resection: decision tree incorporating indocyanine green test. J Hepatobiliary Pancreat Surg. 2005;12:16-22.
16. Yau T, Tang VYF, Yao TJ et al. Development of Hong Kong liver cancer staging system with treatment stratification for patients with hepatocellular carcinoma. Gastroenterology. 2014;146:1691-700.e3.
17. Nowicki TK, Markiet K, Szurowska E. Diagnostic Imaging of Hepatocellular Carcinoma – A Pictorial Essay. Curr Med Imaging Rev. 2017;13(2):140-53.
18. Purich K, Dang JT, Poonja A et al. Intraoperative fluorescence imaging with indocyanine green in hepatic resection for malignancy: a systematic review and meta-analysis of diagnostic test accuracy studies. Surg Endosc. 2020;34(7):2891-903.
19. Boogerd LS, Handgraaf HJ, Lam HD et al. Laparoscopic detection and resection of occult liver tumors of multiple cancer types using real-time near-infrared fluorescence guidance. Surg Endosc. 2017;31(2):952-961.
20. Mehdorn AS, Beckmann JH, Braun F, et al. Usability of indocyanine green in robot-assisted hepatic surgery. J Clin Med. 2021;10:456.

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CASE REPORT

A NEW CASE OF CHILDHOOD ACUTE LYMPHOBLASTIC B-CELL LEUKEMIA FROM PRISTINA

V. Graiqevci-Uka[1], E. Behluli[1], L. Spahiu[1], T. Liehr[2], G. Temaj[3]

[1]Department of Pediatric, University Clinical Center, Prishtina – Kosovo
[2]Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena – Germany
[3]College UBT, Faculty of Pharmacy, Prishtina – Kosovo

Abstract. *Acute lymphoblastic leukemia (ALL) is a malignant disease caused by mutations in B- or T-cell precursors of bone marrow cells. Childhood acute lymphoblastic leukemia (ALL) is a subtype of pediatric cancer with a 1 in 2000 incidence. Here we present a new childhood ALL in a 3-year-old girl. As CD45/19, CD10/19, CD3, CD8, CD10, and CD19 were positive in immunohistochemically analyses of blast cells, a B-ALL was diagnosed with a causative ETV6-RUNX1 gene fusion. The patient was treated based on standard protocols BMF-ALL 2009. Interestingly, an aunt and a grandfather of the patient had experienced malignancies as well, which may be carefully interpreted as a hint on a familial cancer syndrome.*

Key words: *acute lymphoblastic leukemia, cancer syndrome, childhood gene fusion*

Corresponding author: *Assoc. Prof. Dr. Gazmend Temaj, University for Business and Technology/ American-European University; Lipjan Kampus, 10000 Prishtina, Kosovo, Mob.: 00383 45803450; Tel.: 00383 38541400, e-mail: gazmend.temaj@ubt-uni.net*

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) accounts for 30% of cancer diagnoses in children under the age of 15 years [1], with a cumulative risk of 1 in 2,000 children up to the age of 15 years [1]. The disease is diverse, considering both biological and clinical aspects, presenting different subtypes; each subtype has been shown to be associated with various genetic alternations with own prognostic relevance [2]. The most common chromosomal rearrangement observed in ALL is the translocation t(12;21)(p13.2;q22.1) leading to an ETV6-RUNX1 gene fusion [3, 4]. During recent decades, a significant improvement in treatment was achieved being due to such factors as intensive chemotherapy, targeted therapy with monoclonal antibodies, and hematopoietic stem cell transplantation. However, in adults with B-ALL, prognosis remains inferior, as those have adverse outcomes and survival rates decrease with age, being as low as 10-20% in the elderly [5].

To the best of our knowledge, this case with gene fusion *ETV6-RUNX1* due to a translocation t(12;21) presented here is the first childhood B-ALL case diagnosed and treated in Kosovo.

CASE PRESENTATION

A 3-year-old female presented with weakness, headache, nausea, vomiting, and fever. She was the fourth child from the fourth pregnancy of her parents; pregnancy completed at term, with birth weight of 3,900 g.

There was normal development until actual hospitalization. Body weight at time of diagnosis was 20.5 kg, and she showed normal psychomotor development. However, the skin was very pale without turgor efflorescence and preserved elasticity. Liver and spleen were not palpable. The family history indicated that the patient's paternal grandfather had had malignant lung disease, and the patient's paternal aunt had breast cancer (Figure 1).

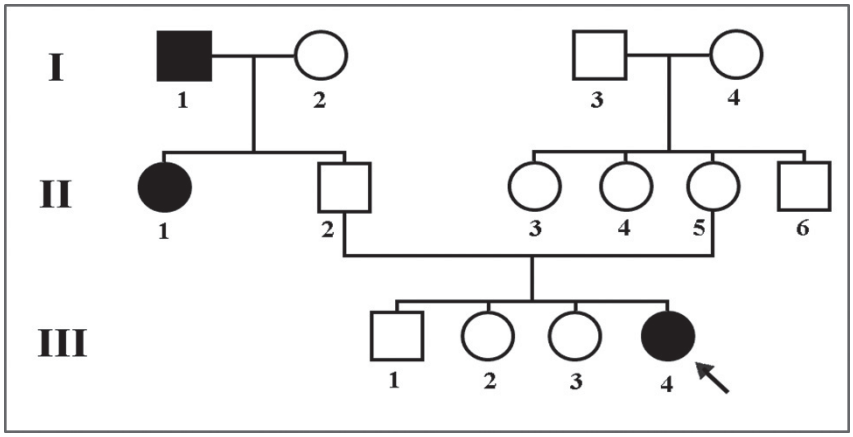

Fig. 1. Pedigree of the patient with childhood ALL (arrow, III-4). Grandfather (I-1) was affected with lung cancer, an aunt (II-1) had breast cancer. Remainder family members were not affected by cancer, yet

Flow cytometry/FACS analysis of peripheral blood samples was positive for cluster differentiation CD45/19, CD10/19, CD3, CD8, CD10, and CD19, but negative for CD20, CD22, CD2, CD7, CD56, CD13, CD14, CD15, CD30, CD33, CD34, and sIgM (Table 1). A reverse transcription (RT-) PCR based molecular screening for gene fusions typically observed in ALL (*BCR-ABL; MLL-AF4; ETV6-RUNX1, E2A-PBX1*) and AML (*MLL-AF4; MLL-AF9; MLL-ENL; RUNX1-RUNX1T1; PML-RARA; CBFb-MYH11*) according to the methods developed by Europe Against Cancer Program, showed that the present case had a *ETV6-RUNX1* gene fusion. Banding cytogenetics revealed a karyotype 46,XX, which means the translocation t(12;21)(p13.2;q22.1) was cryptic, as being typical for this rearrangement. Immunohistology was positive for Pax-5, and immunological staining turned out positive for CD99, CD10, and TdT, with 80% of infiltrates with ALL-blast of L2 morphology. Laboratory analysis of erythrocytes identified anisochromia and hyperchromia; leukocytes expressed leukopenia, neutrophils were absent; the number of thrombocytes was enhanced; granulocytes were much reduced apart from some eosinophils. Biochemical data obtained during the ten days of hospitalization are shown in. Immunological analysis showed that ANA (anti-nuclear antibody), MPO (pANA – perinuclear anti-neutrophil cytoplasmic antibody), and Pr3 (cANCA – antineutrophil cytoplasmic antibody) were negative (Table 1). Bone marrow aspiration has shown morphological changes in blast which are presented in Figure 2.

Table 1. Results of immunological analysis for our patient

Analysis	Results	Reference values	Unit
ANA (Anti-Nuclear Antibody)	0.2 Negative	< 0.8 COI Negative	COI
		0.8-1.2 COI suspected	
		> 1.2 COI Positive	
MPO (pANCA)	<3.0 Negative	< 12 AU/ml Negative	AU/ml
		12-18 AU/ml suspected	
		> 18 AU/ml Positive	
Pr3 (cANCA)	<3.0 Negative	< 12 AU/ml Negative	AU/ml
		12-18 AU/ml suspected	
		> 18 AU/ml Positive	
Complement C3	186.8H	90-180	mg/Dl
Complement C4	29.7	Oct-40	mg/Dl
Immunoglobulin E (IgE)	55.5	< 100	U/Ml
Immunoglobulin A (IgA)	1.8	0.7-4.0	g/L
Immunoglobulin M (IgM)	0.9	0.4-2.3	g/L
Immunoglobulin G (IgG)	10.3		g/L

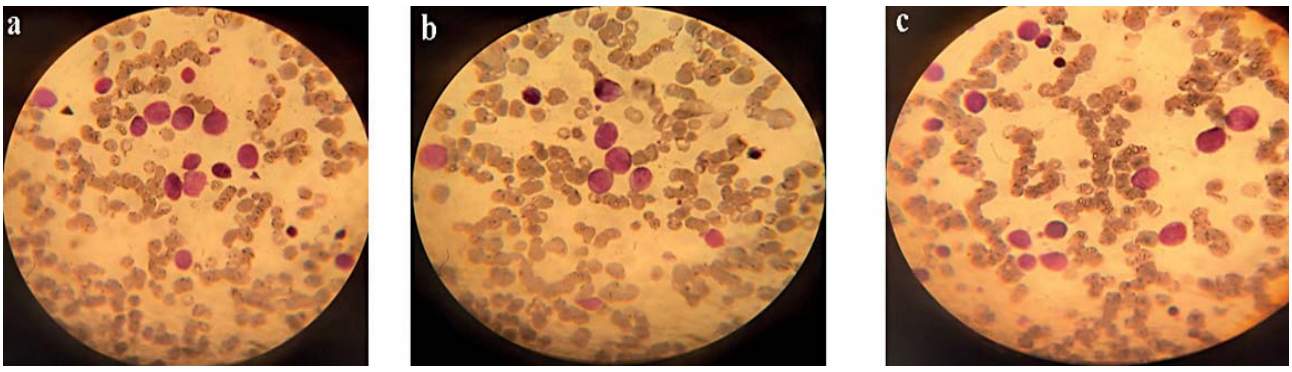

Fig. 2. Bone marrow aspiration was done twice; blasts were below 25% at initial punction, however, 1 month later leukemia was diagnosed. L2 cell morphology (according to FAB classification) are large cells, with heterogeneous chromatin, less cytoplasm, larger nuclei and irregular shape

Our patient was treated according to the AIEOP-BFM ALL 2009 protocol, with some modification (first phase: 6-mercaptopurine 20 mg/m^2/dl; methotrexate 12 mg; leucoverin-rescue i.v. 15 mg/m^2; second phase: prednisone 48 mg; vincristine 1.2 mg; daunorubicin 24 mg; methotrexate 12 mg; third phase: cyclophosphamide 80 mg; ARA-C, cytarabine 75 mg/m^2/d; 6-mercaptopurine 48 mg/m^2/dl; methotrexate (MTX) 12 mg. According to the protocol, (MTX) should be given 5 g/m^2 and we have given 3 g/m^2. Since MTX should be measured in serum and based on serum, MTX values the antidote Amp Leucoverin should be given. Due to the high toxicity of the drug we started with the dose 1/m^2, then 2 g/m^2 and finally we now have 3 g/m^2. We will gradually increase the dose to 5 g/m^2 for other patients; Daunorubicin has been replaced with Doxorubicin because we do not have it on the market in Kosovo; Peg-Asparaginase has been replaced by L-Asparaginase due to its high toxicity. All these changes were made in consultation with two European University Clinics: University Clinic of Graz – LKH and the University Clinic of the Netherlands in Utrecht. She underwent the induction phase with chemotherapy. The patient is now in the consolidation phase with chemotherapy. In the induction phase on day 33, the bone marrow was punctured and resulted in 0% blasts, an indication for a complete remission being achieved.

DISCUSSION

Acute lymphoblastic leukemia (ALL) is a common pediatric malignancy. It is caused by bone marrow abnormalities leading to massive production of white blood cells being harmful for other bone marrow cells and leading to anemia, thrombocytopenia, and even neutropenia. The classic symptoms of children with ALL are fever, easy bruising or bleeding, flat, pinpoint, dark-red spots under the skin caused by bleeding, weakness, feeling tired, or looking pale, bone or joint pain, shortness of breath, painless lumps in the neck, underarm, stomach, or groin, pain or feeling of fullness below the ribs, and loss of appetite [6]. From data obtained in Japan, the male sex is slightly predominant (1.2-fold), with a peak incidence at 1-4 years of age [7]. During the last years, clinical course of childhood ALL improved with an overall surveillance rate exceeding 90%.

Different genetic factors identified by conventional karyotyping can be used for risk stratification. Hyper- and hypodiploidy and several other specific chromosomal rearrangements are typically observed in childhood ALL. Genomic studies furthermore demonstrated a close connection of inherited and somatic genetic alternations in ALL [8]. Diagnoses occur once symptoms appear and include physical examination, complete blood cell count, and bone marrow biopsy. The latter are assessed by microscope for morphological changes and in parallel undergo cultivation, cytogenetic preparation, and analyses for chromosomal changes, which can be supplemented by fluorescence in situ hybridization (FISH) and/or molecular analyses like RT-PCR [9, 10].

Family risk for ALL were assessed when any member of family were diagnosed with any kind of cancer [11]. During the genetic consult, parents reported that the paternal grandfather had suffered from pulmonary cancer and an aunt from breast cancer. Interestingly, in rare cases a familial predisposition for leukemia and other cancer types has been reported: different variants including TP53 mutation in germline (LiFraumeni-syndrome), ETV6 variants and hyperdiploidy in ALL, or PAX5 mutation and B-ALL with dicentric/ischoromosome 9 [12, 13, 14, 15]. Thus, here a familial component predisposing for malignancy cannot be excluded.

In our case, a child with karyotype 46,XX was diagnosed with B-ALL. Together with RT-PCR-result, she had a cryptic translocation t(12;21)(p13.2;q22.1). Previous studies showed that patients with this kind of translocation do extremely well. BFM group showed that the incidence of translocation t(12;21) in relapse cases was identical to that seen at the initial phase of diagnosis [16].

In conclusion, our study underscored the Pession hypothesis that treatment of patients with B-ALL based on BFM-ALL 2009 protocols, with four drug inductions for ALL patients, results in maximal therapeutic efficacy.

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Statement of ethics: *Samples from the patients were obtained in accordance with the Helsinki Declarations. Written informed consent for genetic testing was obtained from patient and/or their parent/guardian.*

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Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Dickinson HO. The causes of childhood leukaemia. BMJ. 2005; 330: 1279-1280.
2. Rowley JD, Le Beau MM, Rabbitts TH. Chromosomal translocations and genome rearrangements in cancer. Cham: Springer 2015.
3. Golub TR, Barker GF, Bohlander SK, et al. Fusionof the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. Proc Nat Acad Sci U S A. 1995; 92: 4917-4921.
4. Romana SP, Mauchauffe M, Le Coniat M, et al. The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. Blood. 1995; 85: 3662–3670.
5. Geyer MB, Hsu M, Devlin SM, et al. Overall survival among older US adults with ALL remains low despite modest improvement since 1980: SEER analysis. Blood. 2017; 129: 1878-81.
6. Clarke RT, Van den Bruel A, Bankhead C, et al. Clinical presentation of childhood leukaemia: a systematic review and meta-analysis. Arch Dis Child. 2016; 101(10): 894-901.
7. Horibe K, Saito AM, Takimoto T et al. Incidence and survival rates of hematological malignancies in Japanese children and adolescents (2006-2010): Based on registry data from the Japanese Society of Pediatric Hematology. Int J Hematol. 2013; 98: 74–88.
8. Inaba H, Mullighan CG. Pediatric acute lymphoblastic leukemia. Haematologica. 2020; 105(11): 2524-2539.
9. Pui CH, Yang JJ, Hunger SP, et al. Childhood Acute Lymphoblastic Leukemia: Progress Through Collaboration. J Clin Oncol. 2015; 33(27): 2938-48.
10. Van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia. 1999; 13(12): 1901-28.
11. Seeger K, Buchwald D, Peter A, et al. TEL-AML1 fusion in relapsed childhood acute lymphoblastic leukemia. Blood. 1999; 94(1): 374-376.
12. Li X, Sundquist K, Sundquist J et al. Family history of early onset acute lymphoblastic leukemia is suggesting genetic associations. Sci Rep. 2021; 11(1): 12370.
13. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013; 45(3): 242-252.
14. Moriyama T, Metzger ML, Wu G, et al. Germline genetic variation in ETV6 and risk of childhood acute lymphoblastic leukaemia: a systematic genetic study. Lancet Oncol. 2015; 16(16): 1659-1666.
15. Shah S, Schrader KA, Waanders E, et al. A recurrent germline PAX5 mutation confers susceptibility to pre-B cell acute lymphoblastic leukemia. Nat Genet. 2013; 45(10): 1226- 1231.
16. Noetzli L, Lo RW, Lee-Sherick AB, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015; 47(5): 535-538.

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CASE REPORT

A CLINICAL CASE OF TONSILLAR LYMPHANGIOMATOUS POLYP

A. Vlaykov[1], A. Atanasov[1], M. Hadzhi[2], M. Gulubova[2]

[1]Department of Otorhinolaryngology, Faculty of Medicine, Trakia University – Stara Zagora, Bulgaria
[2]Department of General and Clinical Pathology, Faculty of Medicine, Trakia University – Stara Zagora, Bulgaria

Abstract. Introduction: *Tonsil lymphangiomas are extremely rare benign tonsil tumors. They could be asymptomatic, especially when they are small, and in these cases, they are most often found by chance on physical examination.* ***Clinical case:*** *The authors present a 67-year-old man with complaints of discomfort, a sore throat, and an unspecific formation on his right tonsil. Upon microscopic examination, we found a polypoid mass covered by squamous epithelium with a stroma composed of lymphoid tissue.* ***Discussion:*** *Benign tonsillar tumors are significantly more common than malignant ones. Lymphangiomatous polyps located in the tonsillar region, on the other hand, have been described as very rare, and their etiology and pathogenesis remain unclear. They tend to occur in areas where lymph vessels are abundant, with more than 90% of all lymphangiomas occurring in the head and neck region.* ***Conclusion:*** *Lymphangiomatous polyps should be considered in the differential diagnosis of all benign tumors, and it is extremely important to differentiate them from malignant tonsil lesions.*

Key words: *tonsillar lymphangiomatous polyps, benign tumors*

Corresponding author: *Atanas Vlaykov, Department of Otorhinolaryngology, Faculty of Medicine, Trakia University, 2 "Armeiska" Street, 6000 Stara Zagora, Bulgaria, e-mail: at.vlaykov@gmail.com, mob. ph. 00359886072199, ORCID iD: https://orcid.org/0000-0002-4980-9631*

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INTRODUCTION

Benign tonsil tumors are very rare compared to malignant tumors. There are various types of benign polypoid neoplasms, such as angiomas, polypoid tonsil lymphangiomas, hamartomatous tonsil polyps, lymphoid polyps, and tonsil lymphangiomatous polyps, that have been described in the literature. According to Kardon et al., there are ten well-documented cases of tonsillar lymphangiomatous tumors [1]. Due to the different descriptions of the same or similar histological forms, it is difficult to determine the actual frequency of the tumor itself [2, 3].

The pathogenesis of tonsillar lymphangiomatous polyps is controversial. Chronic tonsillogenic inflammation and the associated obstruction of the draining lymph canals are discussed as the most likely mechanisms of occurrence. These disorders of lymphedema eventually lead to mucosal prolapse and the appearance of polypoid edema [4].

Tonsil lymphangiomas are most commonly asymptomatic when they are small in size, and in these cases, they are found by chance on physical examination. Rarely, symptoms could be manifested by complaints, e.g., dysphagia or a feeling of a foreign body in the throat, which can cause an unproductive cough. Reaching larger sizes can compress the sur-

rounding structures, creating breathing difficulties, stridor, or nausea. Difficulty swallowing may progress and lead to profuse salivation [5].

Mesopharyngoscopy most often reveals the tumor formation located on the surface of the palatine tonsils. They are almost always unilateral, but in isolated cases could be bilateral [6]. It's not typical, but when they are symptomatic, as in our case, they can hardly be evaluated as benign lesions before pathohistological examination and provoke a wide differential-diagnostic process.

CLINICAL CASE

This case is about a 67-year-old man from Stara Zagora with complaints of discomfort and sore throat, more pronounced on the right side 4 months ago. Two weeks before the visit, he noticed a formation on his right tonsil that began to interfere with food intake.

A physical examination of the oral cavity was conducted and showed a pale pedicular formation originating from the right palatal tonsil. Upon palpation, the formation presented as a painful, dense mass with a smooth surface. No crepitations, surrounding hyperemia, exudates, or ulcerations were observed.

No pathological changes were found in the rest of the oral, pharyngeal, and laryngeal cavities. No cervical lymphadenopathy was detected, and no deviations from the norm were found in the blood count.

The neoplasm was assessed as an inflamed tonsillar papilloma. Intra-oral excision/biopsy was performed under local anesthesia. The macroscopic dimensions were 14x11 mm. The tumor mass was firm and smooth, with a small foot base. No recurrences had been observed upon follow-up until 11 months after the procedure.

Microscopic findings

As shown in the images below, the polypous mass was covered by squamous epithelium. In the stroma, lymphoid tissue is observed, resembling lymphoid follicles. In the connecting tissue stroma, many vessels are found. The vessels are of lymphatic and blood origin (Figure 1 a, b).

Special staining

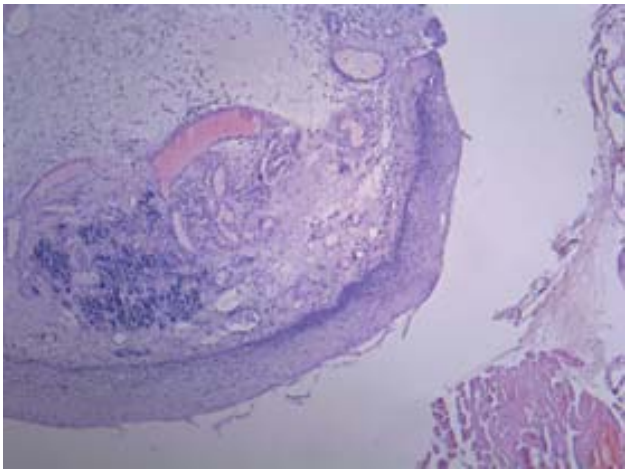
The polyp was stained by markers for blood vessels (CD31 and CD34), lymph vessels (D2-40) (Figures 2a and 2b), lymphocyte cell markers (CD3, CD20, CD45) (Figures 3 a and 3b), and a marker for myoepithelial and myofibroblastic cell markers (α-SMA).

The blood vessels showed reactivity with anti-CD31 and anti-CD34. Lymphatic vessels – with anti-D2-40. Smooth muscle actin is present in the walls of dilated lymphatic blood vessels. The hematologic markers (CD3 [T-cell], CD20 [B-cell], and CD45) are expressed in the lymphoid cells in the lymphoid tissue.

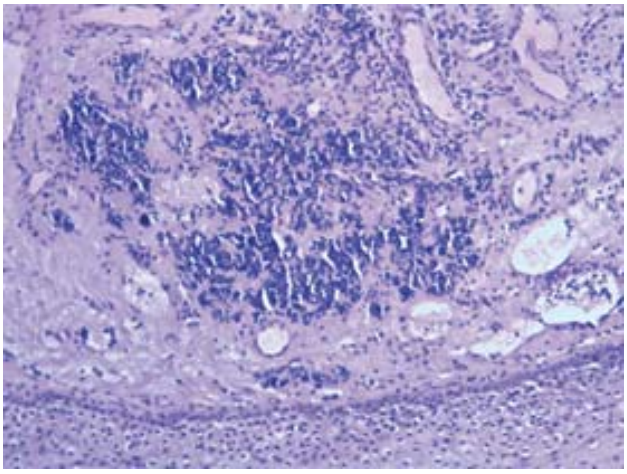
DISCUSSION

Lymphangiomatous polyps located in the tonsillar region have been described as extremely rare, and their etiology and pathogenesis remain unclear. In most cases, they are asymptomatic and are found incidentally.

These tumors tend to occur in areas where lymph vessels are abundant, with more than 90% of all lymphangiomas occurring in the head and neck region. Most commonly, they involve the skin and sub-

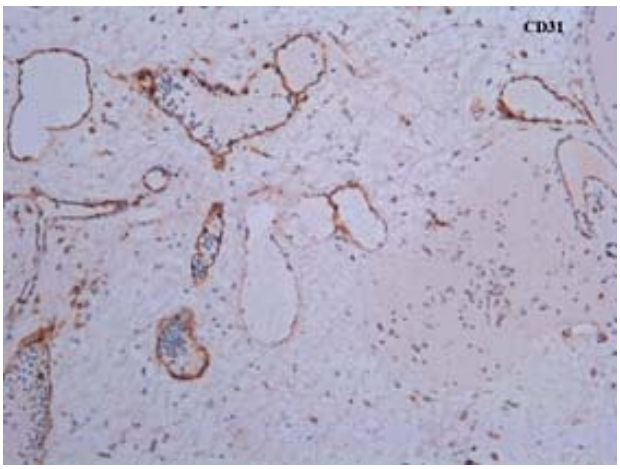


a. Lymphangiomatous polyp HE

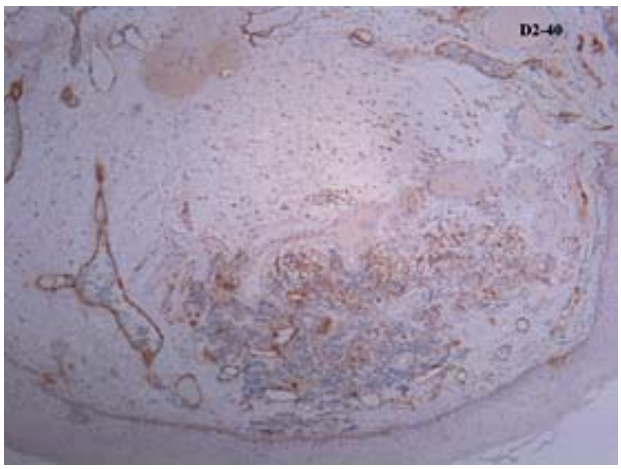


b. Lymphangiomatous polyp HE0001

Fig. 1 a, b. Microscopic findings



a. Lymphangiomatous polyp CD0031-0003



b. Lymphangiomatous polyp D2-40

Fig. 2 (a, b). The polyp was stained by markers for blood vessels (CD31 and CD34) (a) and for lymph vessels (D2-40) (b)

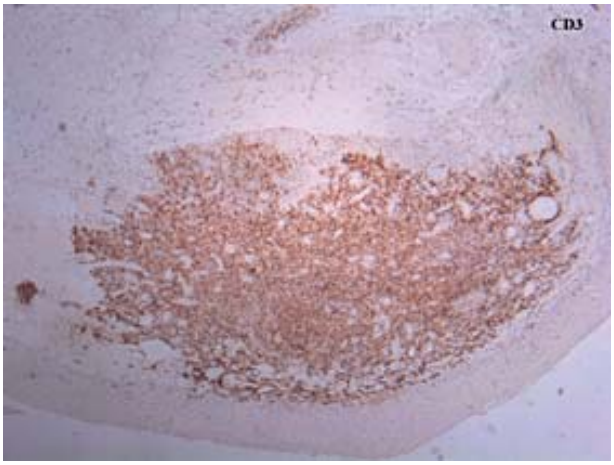


Fig. 3a. Lymphangiomatous polyp CD3

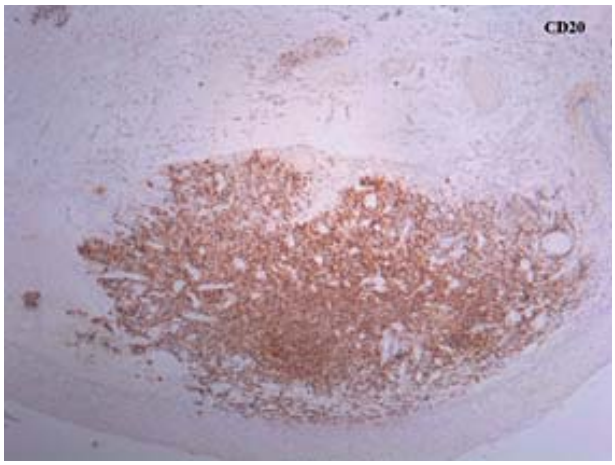


Fig. 3b. Lymphangiomatous polyp CD20

cutaneous tissues, but some articles describe localizations like the nasal cavity, larynx, parotid gland, mouth, and tongue [7].

Histologically, formations are covered with squamous epithelium. The stroma of the polyp consists of various components, mainly collagen tissue with varying degrees of density of collagen fibers, adipose tissue, dilated lymph vessels, and lymphoid tissue [8].

Immunohistochemically, CD34 is less frequent than CD31 for lymphatic vessels [9]. In our case, CD31 and CD34 were detected both in blood and lymph vessels. The hematologic markers, including CD3, CD20, and CD45, showed polymorphous infiltration of the vascular channels and stroma.

There is no consensus on the pathogenesis of these benign tumors, but the most widely discussed hypothesis is that their occurrence is due to obstruction and dilation of lymph vessels provoked by chronically persistent inflammatory processes in the tonsils. The prognosis for tonsillar lymphangiomas is good. In the available literature, we did not find a described case of malignant degeneration. Treatment is either partial excision or, most often, extensive surgical removal, with tonsillectomy being the preferred procedure to ensure complete removal. This is because of the lack of encapsulation, and recurrences of lymphangiomas are generally common if not completely removed [10].

Attempts have been made to treat them with ionizing radiation, but most of the authors conclude that these lesions are not radiation-sensitive, so radiotherapy is ineffective and only unduly increases the risk of developing various complications, including malignancies of the head and neck [11].

CONCLUSION

Lymphangiomatous polyps of the palatine tonsils are very rare, benign lesions that manifest as tonsil-

lar growths. The anamnesis and the clinical examination are integral parts of the diagnostic process, but for their definitive establishment, histological examination and immunohistochemical analysis are required. Lymphangiomatous polyps should be considered in the differential diagnosis of all benign tumors, and it is extremely important to differentiate them from malignant tonsil lesions with detailed histological analysis.

Treatment is only surgical removal with a partial or total tonsillectomy. If partial, the patient should be followed for recurrence.

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Animal and human rights statement: *All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.*

REFERENCES

1. Kardon D, Wenig B, Heffner D, Thompson L. Tonsillar Lymphangiomatous Polyps: A Clinicopathologic Series of 26 Cases. Mod Pathol. 2000 Oct;13(10):1128-33.
2. Barreto I, Costa AF, Martins MT et al. Immunohistochemical study of stromal and vascular components of tonsillar polyps: High endothelial venules as participants of the polyp's lymphoid tissue. Virchows Arch. 2011;459(1):65-71.
3. Ormerod F. Angioma of the tonsil. J Laryngol Otol. 1926;41:797-800.
4. Visvanathan PG. A pedunculated tonsillar lymphangioma. J Laryngol Otol. 1971;85(1):93-96.
5. Balatsouras DG, Fassolis A, Koukoutsis G et al. Primary lymphangioma of the tonsil: a case report. Case Rep Med. 2011(3):183182.
6. Chen HH, Lovell MA, Chan KH. Bilateral lymphangiomatous polyps of the palatine tonsils. Int J Pediatr Otorhinolaryngol. 2010;74(1):87-88.
7. Kardon DE, Wenig BM, Heffner DK, Thompson LD. Tonsillar lymphangiomatous polyps: A clinicopathologic Series of 26 cases. Mod Pathol. 2000 Oct;13(10):1128-33.
8. Heffner D. Pathology of the tonsils and adenoids. Otolaryngol Clin North Am. 1987;20:279-86.
9. Duggal P, Chakravorty S, Sharma S, Ahluwalia RK. Pedunculated hamartomatous polyp of palatine tonsil in a child: A new presentation. Int J Pediatr Otorhinolaryngol. 2008;3:120-3.
10. Zadvinskis DP, Benson MT, Kerr HH, et al. Congenital malformations of the cervicothoracic lymphatic system: embryology and pathogenesis. Radiographics. 1992;12(6):1175-1189.
11. Hazra TK, Dutta SK, Mondal SK. Lymphangioma of the tonsil. Indian J Otolaryngol Head Neck Surg. 1996;48(3)225-227.

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CASE REPORT

A RARE CASE OF ACUTE STANFORD TYPE A AORTIC DISSECTION PRESENTING WITH ANTERIOR ST-ELEVATION MYOCARDIAL INFARCTION

N. G. Ivanova[1,2]

[1]Department of Urology and General Medicine, Medical University – Plovdiv, Bulgaria
[2]"Sveta Karidad" Hospital – Plovdiv, Bulgaria

Abstract. Introduction. *Aortic dissections occur as a result of a tear in the intimal layer, continued longitudinal splitting within the intima and media, and formation of false lumen. This could lead to sudden death or severe aortic regurgitation and cardiogenic shock. The presented case here describes a patient with acute anterior ST-elevation myocardial infarction due to acute Stanford type A ascending aortic dissection.* ***Case presentation.*** *A 55-year-old male presented with severe chest pain, radiating into the back, jaw, and left arm, and signs of cardiogenic shock. Electrocardiography showed acute anterior ST-elevation myocardial infarction and echocardiography confirmed that there was a reduced left ventricle ejection fraction (38% calculated using the Simpson method), severe aortic regurgitation, and wall motion abnormalities. Based on these findings, we made a diagnosis of acute myocardial infarction. In accordance with the current guidelines, we opted for an interventional therapeutic approach. Angiography showed left main trunk dissection extending to the left anterior descending coronary artery caused by ascending aorta dissection. This finding altered the diagnosis and treatment plan and the patient was immediately sent to the operating room for emergency surgery.* ***Conclusions.*** *Aortic dissection should be suspected in patients presenting with acute anterior ST-elevation myocardial infarction, severe aortic regurgitation, and cardiogenic shock. Involvement of the left main trunk and left anterior descending artery occurs much more rarely than that of the right coronary artery, which causes inferior myocardial infarction.*

Key words: *ascending aorta dissection, acute myocardial infarction, intimal flap, false lumen left main trunk, left anterior descending coronary artery, cardiogenic shock, severe aortic regurgitation, angiography, surgical treatment*

Corresponding author: *Nevena Ivanova, e-mail: nevenai@yahoo.com*

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INTRODUCTION

An aortic dissection is caused by a tear in the intimal layer of the aortic wall. As a result, the blood flow provokes a continuing longitudinal splitting within the intima and media and formation of false lumen. This could lead to sudden death or development of further complications such as severe aortic regurgitation, cardiogenic shock, myocardial infarction, and arrhythmias. Aortic dissection is considered to be the most common cause of aorta-related death. These dissections are associated with

some known risk factors such as hypertension, atherosclerotic disease, male gender, connective tissue disorders, especially in younger patients (Marfan's syndrome or Ehler's-Danlos syndrome), and bicuspid aortic valve.

Aortic dissections may be categorized according to the Stanford classification into type A and type B dissections. Type A aortic dissections affect the ascending aorta and involve any part of the aorta proximal to the origin of the left subclavian artery. Type B dissections do not involve the ascending aorta and arise distal to the origin of the left subclavian artery.

A rare but life-threatening complication of type A dissection is acute myocardial infarction, which is related to the extension of the dissection flap into the ostium of the coronary artery in 1-2% of cases. Involvement of the left main trunk and left anterior descending artery is much rarer compared to that of the right coronary artery. Myocardial infarction occurs more often than aortic dissection in emergency departments [1]. Most ST-elevation myocardial infarctions are caused by a rupture of an atherosclerotic plaque, subsequent thrombosis, and coronary occlusion so patients benefit from urgent percutaneous coronary intervention. Patients presenting with type A aortic dissection need emergency surgery [2]. Therefore, when acute dissection is complicated with acute myocardial infarction, the correct diagnosis of dissection can be elusive and these patients might be treated with primary percutaneous coronary intervention (PCI) instead of surgical operation [3]. In these cases, the outcome can be catastrophic [4].

The reported case is a rare presentation of acute anterior myocardial infarction due to an acute Stanford type A aortic dissection involving the left main trunk of the coronary artery, causing cardiogenic shock, and severe aortic regurgitation.

CASE PRESENTATION

A 55-year-old male from Plovdiv, Bulgaria, was admitted for a hospital treatment due to sudden severe sharp chest pain radiating into the back, jaw, and left arm, accompanied by sweating, nausea, and vomiting. The complaints started 40 minutes before admission. The patient suffered from arterial hypertension but no current treatment was provided. He presented at the emergency department in poor general condition, pale and sweaty. The physical examination of cardiovascular system revealed sinus tachycardia (120 beats per minute) and hypotonic blood pressure (80/50 mm Hg). The diagnostic process continued with recording of an **electrocardiogram (ECG)** which revealed ST-depression in standard leads I, II, III (Fig. 1A), ST-elevation and Q wave in aVR, aVL, ST-depression in aVF (Fig. 1B), ST-elevation and Q wave in the precordial leads V1-V3 (Fig. 1C), and ST-elevation in V5-V6 (Fig. 1D).

The initial diagnosis based on the clinical presentation and ECG findings was acute anterior myocardial infarction and the patient was transported to the cardiology department. During monitoring at the intensive care unit, a ventricular ectopic beats R-on-T phenomenon (V Lown class) was registered. To assess cardiac function, urgent echocardiography was performed. It showed left ventricular hypertrophy (septum and left ventricle posterior wall 1.2 cm) as a result of untreated arterial hypertension. Left ventricle systolic function was assessed by measuring the left ventricle ejection fraction (LVEF) using two separate methods. It was found to be significantly reduced – LVEF 40% measured by the Teichholz method and 38% measured by the Simpson method (normal range 55 to 70%). Valve assessment performed using color Doppler showed moderate mitral regurgitation (II grade), severe aortic regurgitation (IV grade), severe tricuspid regurgitation (III grade), and elevated tricuspid valve gradient (52 mHg) (normal range 12.6 to 29. 3 mm Hg). Elevated systolic pulmonary artery pressure of 62 mm Hg (normal range 18 to 25 mm Hg) was indicative for pulmonary hypertension and was registered by using pulse wave Doppler in the parasternal short axis view. Wall motion abnormality such as apical dyskinesia was observed in apical four-chamber view and was taken as a result of regional myocardial ischemia due to coronary artery occlusion.

The laboratory blood tests revealed leukocytosis (13.29×10^9/l) (normal range 3.5 to 10.5×109/l) and elevated blood glucose (8.76 mmol/l) due to severe stress reaction. Myocardial injury was suspected on the basis of elevated creatine phosphokinase (CPK, 178 U/l) (normal range 0 to 171 U/l) and elevated CPK-MB (25.4 U/l) (normal range 0 to 25 U/l). The lipid profile revealed an additional risk factor for atherosclerotic disease, a concomitant dyslipidemia: elevated total cholesterol (6.14 mmol/l) (normal range 3.5 to 5.17 mmol/l) and triglycerides (5.38 mmol/l) (normal range up to 1.69 mmol/l). The initial diagnosis based on clinical presentation, ECG changes, echocardiography data, and laboratory results was acute anterior myocardial infarction. In accordance with the European guidelines for the treatment of acute coronary syndrome, interventional strategy was undertaken. The percutaneous coronary intervention (PCI) using radial access showed left main trunk dissection involving proximal left anterior descending artery (LAD) (Fig. 2) with partial perfusion and thrombolysis in myocardial infarction (TIMI) flow grade II.

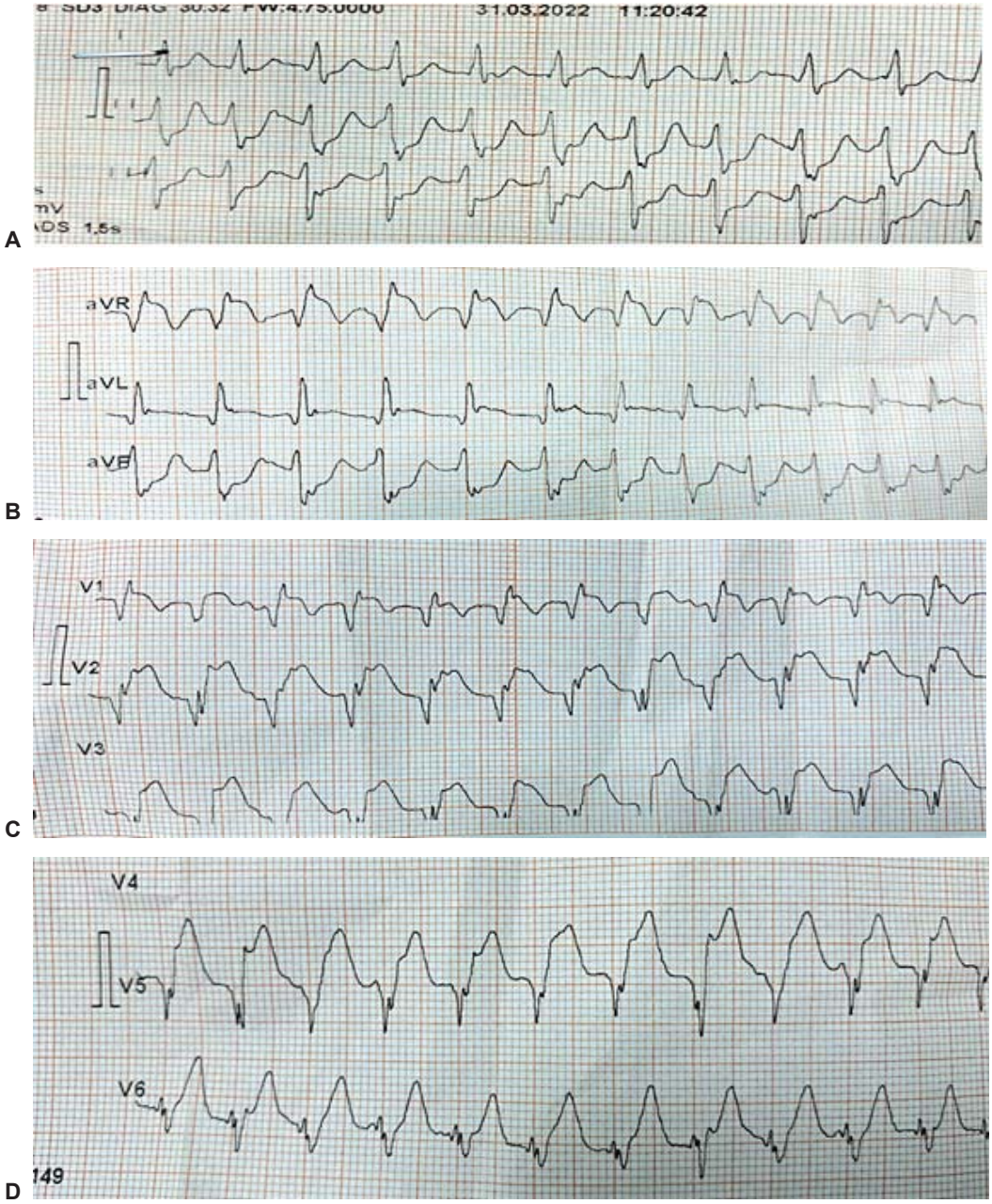

Fig. 1. Electrocardiogram. **A)** ST-depression in standard leads I, II, III; **B)** ST-elevation and Q wave in standard leads aVR, aVL, ST-depression in aVF; **C)** ST-elevation and Q wave in precordial leads V1-V3; **D)** ST-elevation in precordial leads V5-V6

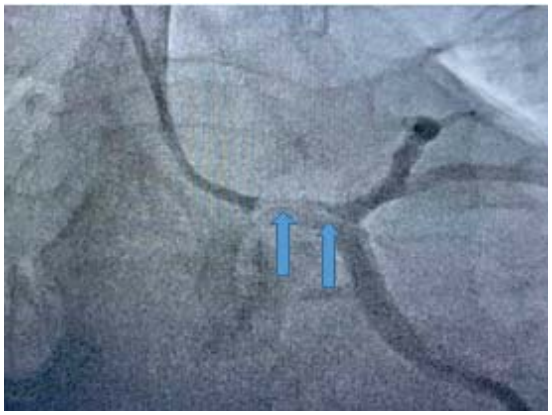

Fig. 2. Percutaneous coronary intervention. Left main trunk dissection

The left circumflex and right coronary arteries were intact with normal blood flow.

A decision to undertake an aortography investigation was made based on the echo findings of severe aortic regurgitation. The investigation revealed dilated aortic root and ascending aorta (Fig. 3A) and presence of intimal flap (Fig. 3B). The pigtail catheter was placed into the true lumen (Fig. 3C).

All these findings confirmed the accuracy of the diagnosis of aortic dissection previously misdiagnosed with acute myocardial infarction due to coronary atherothrombosis. Treatment was based on the initial diagnosis. As the patient presented with typical

complains, ECG changes, echo data of wall motion abnormalities, and reduced left ventricle ejection fraction, laboratory data indicating acute anterior STEMI, the initial treatment included anticoagulant (unfractionated heparin 5000 E loading dose followed by an infusion of 1000 E/hour) and antiplatelet agent (acetylsalicylic acid 250 mg loading dose). Due to hemodynamic instability that led to the developed cardiogenic shock, inotropic drugs were administered (dopamine and dobutamine at an infusion rate of 2.5 μg/kg/min). The complex ventricular arrhythmias were treated with amiodarone infusion and magnesium. Urgent transportation to cardiac surgery clinic was arranged right after confirmation of severe ascending aorta dissection of Stanford type A. Unfortunately, the patient died in the ambulance during the transportation.

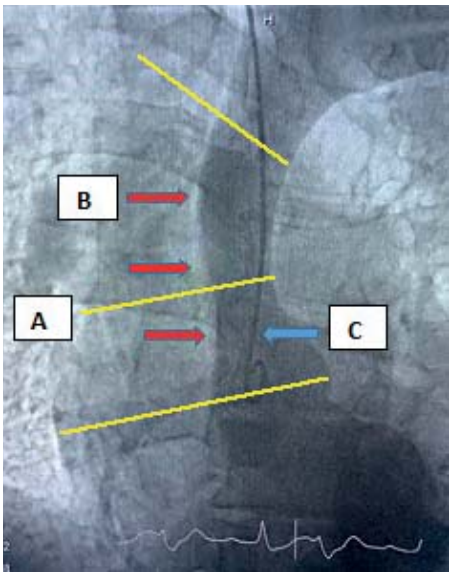

Fig. 3. Aortography. **A)** Dilated aortic root and ascending aorta; **B)** Presence of intimal flap; **C)** Catheter placed into true lumen

DISCUSSION

The acute aortic syndrome includes aortic dissection, intramural hematoma, and a penetrating aortic ulcer. Although dissection is one of the most common disorders associated with aortic disease, it is also the most fatal one, for a variety of reasons, including the patient presenting with unusual symptoms or severe acute heart disease. Therefore, healthcare professionals are faced with a serious challenge leading to difficulties in making an accurate diagnosis and, as a result, delaying the commencement of the correct surgical therapy, resulting in an increase in mortality rate. Several case reports of a Stanford type A aortic dissection in combination with a myocardial infarction have been published [5-12]. When a type A aortic dissection extends to the left main trunk of the coronary artery, life-threatening hemodynamic instability may occur, including cardiogenic shock, severe systolic LV dysfunction, frequently ending with sudden death [15]. Acute myocardial infarction due to extension of an acute Stanford type A aortic dissection is very rare. When combined with myocardial infarction due to left main trunk involvement, it is one of the most lethal clinical situations. This requires a prompt and accurate diagnosis and urgent surgical treatment to save the patient's life. However, it is difficult to make a differential diagnosis between acute myocardial infarction and myocardial infarction due to the extension of aortic dissection into the coronary artery. Ischemia could be a result of dynamic hemodynamic changes as the intimal flap causes an occasional obstruction of the coronary artery. Another possible mechanism is static, in which the hematoma narrows the lumen of the vessel [2]. Evaluation of the ascending aorta using either transthoracic or transesophageal echography and a computed tomography (CT) scan, when possible, is extremely important for accurate diagnosis. Interventional study using aortography with manual injection of the contrast through a pigtail catheter is effective for detecting the aortic dissection. The presence of an intimal flap and dilation of the aorta are crucial for exact diagnosis of aortic dissection. A resistance noted while advancing the diagnostic catheter during PCI is suspicious for aortic dissection. If the catheter is placed in the true aortic lumen, the movement of the catheter is free and smooth but if placed in the false lumen, the advancement is difficult [1].

The presented clinical case is significant for clinical practice because it is a rare demonstration of acute type A dissection involving the trunk of the left coronary artery, resulting in acute blood flow disruption and the development of another life-threatening condition, acute anterior myocardial infarction with ST-elevation. In comparison, involvement of the right coronary artery in acute aortic dissection is far more common. Aortic dissection must be addressed as an underlying etiological cause when patients present to the emergency room with ST-elevation acute coronary syndrome. Because of its rarity, the current clinical case demonstrates the challenges in reaching an accurate diagnosis, which requires experience and modern technical equipment, including imaging equipment. Severe aortic dissection was not suspected by emergency department physicians even during the performance of transthoracic echocardiography. Percutaneous coronary intervention and aortography provided complete vi-

sualization of the aorta and in this case served as invasive diagnostic imaging modalities for the establishment of the correct diagnosis.

CONCLUSIONS

Patients with acute anterior STEMI, significant aortic regurgitation, and cardiogenic shock should be specifically evaluated for aortic dissection. Although acute myocardial infarction has a characteristic appearance, it is crucial to actively look for symptoms of aortic dissection during physical examination, imaging (echocardiography and CT scan), and interventional tests to obtain an accurate diagnosis. Any delay could result in the patient's death. The only treatment for acute Stanford type A aortic dissection is immediate surgical repair.

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Wang JL, Chen CC, Wang CY, et al. Acute type A aortic dissection presenting as ST-segment elevation myocardial infarction referred for primary percutaneous coronary intervention. Acta Cardiologica Sinica 2016; 32(3): 265-72.
2. Laine M, Grisoli D, Bonello L. An atypical case of acute myocardial infarction. J Invasive Cardiol 2011; 23: 305-6.
3. Elliott MA, David AM. Diseases of the aorta. In: Bonow RO, Mann DL, Zipes DP, Libby P, editors. Braunwald's heart disease. 9th ed. Saunders, 2011: 1319-23.
4. Cook J, Aeschlimann S, Fuh A, et al. Aortic dissection presenting as concomitant stroke and STEMI. J Hum Hypertens 2007; 21: 818-21.
5. Horszczaruk GJ, Roik MF, Kochman J, et al. Aortic dissection involving ostium of right coronary artery as the reason of myocardial infarction. Eur Heart J 2006; 27: 518.
6. Kawahito K, Adachi H, Murata S, et al. Coronary malperfusion due to type A aortic dissection: mechanism and surgical management. Ann Thorac Surg 2003; 76: 1471-6.
7. Neri E, Toscano T, Papalia U, et al. Proximal aortic dissection with coronary malperfusion: presentation, management, and outcome. J Thorac Cardiovasc Surg 2001; 121: 552-60.
8. Pego-Fernandes PM, Stolf NA, Hervoso CM, et al. Management of aortic dissection that involves the right coronary artery. Cardiovasc Surg 1999; 7: 545-8.
9. Weber M, Kerber S, Rahmel A, et al. Acute thoracic aortic dissection with occlusion of the left coronary artery. Herz 1997; 22: 104-10.
10. Ohara Y, Hiasa Y, Hosokawa S. Successful treatment in a case of acute aortic dissection complicated with acute myocardial infarction due to occlusion of the left main coronary artery. J Invasive Cardiol 2003; 15: 660-2.
11. Tominaga R, Tomita Y, Toshima Y, et al. Acute type A aortic dissection involving the left main trunk of the coronary artery – a report of two successful cases. Jpn Circ J 1999; 63: 722-4.
12. Coselli JS. Treatment of acute aortic dissection involving the right coronary artery and aortic valve. J Cardiovasc Surg 1990; 31: 305-9.
13. Hosaka S, Tsuchiya K, Morishita A, et al. Treatment of acute type A aortic dissection with onset of the right coronary insufficiency. Nippon Kyobu Geka Gakkai Zasshi (J Jpn Assoc Thorac Surg) 1995; 43: 236-40.
14. Kyo S, Ueda K, Yokote Y, et al. Surgical treatment of acute aortic dissection (Stanford type A) associated with myocardial ischemia. Jpn J Cardiovasc Surg 1997; 26: 135-40.
15. DeSanctis RW, Doroghagi RM, Austen WG, et al. Aortic dissection. N Engl J Med 1987; 317: 1060-7.

REVIEW

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TELEMEDICINE IN OPHTHALMOLOGY: LESSONS FROM THE COVID-19 ERA AND BEYOND

S. Uzunova[1,2], K. Kilova[3]

[1]Faculty of Public Health, Medical University of Plovdiv – Bulgaria
[2]Clinic of Ophthalmology "Dr. Uzunova", Plovdiv – Bulgaria
[3]Department of Medical Informatics, Biostatistics and e-Learning, Faculty of Public Health, Medical University of Plovdiv – Bulgaria

Abstract. *The progress of information and communication technologies in the era of COVID-19 created an unprecedented opportunity for medicine to adapt to new models of care. Telemedicine and telehealth have enabled medical care at a distance in various fields, including ophthalmology.* ***The aim*** *of this article is to review the current state and the opportunities for telemedicine in ophthalmology.* ***Materials and methods:*** *PubMed, ScienceDirect Database, Google Scholar databases, as well as official sites of various governmental and non-governmental institutions were explored. The search was conducted between May 1, 2022 and July 31, 2022 using as key words "teleophthalmology"; "telemedicine/telehealth and ophthalmology"; "ophthalmology and COVID-19".* ***Results:*** *87 primary sources were reviewed. An exploratory analysis of the current state and application of telemedicine in ophthalmology was made.* ***Conclusion:*** *A great number of innovations have created an environment allowing for teleophthalmology to flourish, whereas the COVID-19 epidemic has accelerated the development and adoption of these digital technologies. Telemedicine has become an extremely valuable tool during a pandemic, and even if it would never fully replace in the person-to-person patient visits, it certainly has an important role in our dynamic and high-tech world.*

Key words: *teleophthalmology, telemedicine, telehealth, COVID-19, information and communication technologies, healthcare*

Corresponding author: *Kristina Kilova, PhD, Department of Medical Informatics, Biostatistics and e-Learning, Faculty of Public Health, Medical University of Plovdiv, Bulgaria; e-mail: k_kilova@yahoo.com*

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INTRODUCTION

The COVID-19 pandemic, announced by the World Health Organization in March 2020, fundamentally changed the practice of medicine. Doctors had to live in a new reality. They had daily to apply incredible, often creative, and innovative methods in increasingly complex conditions. The situation unfolded in real time and the push was to make smart decisions that would keep the public body safe while providing the best possible medical care. Its safe, timely and efficient provision was becoming an increasing challenge. Necessary but non-urgent examinations and operations were postponed, often indefinitely. At the same time, efforts to limit the spread of the virus placed patients in a situation where they had to choose between receiving medical care and risking exposure to the coronavirus infection (COVID-19).

COVID-19 had left no aspect of modern life untouched; its impact was unprecedented and long-lasting. In addition to unexpected consequences for patients with existing eye problems and difficult access to medical care, there were also consequences for everyone else – increased prevalence of dry eye syndrome for computer workers, development of myopia in children. Remote work and learning inevitably lead to increased screen time – a change that had a significant impact on eye health. Studies showed that the average number of blinks dropped from about 18 per minute under normal conditions to three or four per minute while looking at a screen. These flashes were not as strong or complete as those made under normal conditions. Low humidity and restricted indoor air flow had been suggested to induce or exacerbate eye conditions [1].

There are good reasons why progressive myopia has become a global epidemic in the last two or three decades: children are spending much less time outdoors than they used to, at the expense of close viewing at devices such as smartphones and tablets [2].

During the pandemic, especially during the weeks of strict isolation, distance learning became a reality for many children and young people. As a result, they spent even longer hours indoors in front of computer and tablet screens, neglecting both outdoor time and time spent looking at distant points like the classroom blackboard. Meanwhile, studies showed that one hour of active outdoor activity reduced significantly the risk of progressive myopia – by as much as 45% [3]. Children who used electronic devices for longer than six hours a day and had less than three hours of outdoor activity per week had twice the risk of developing near-sightedness. All this led to the increase in incidence of myopia.

Social distancing, quarantine and lockdown periods prevented many patients from receiving regular eye exams, limiting their access to treatment. They were reluctant to leave their homes, and routine examinations were cancelled or postponed. At the same time, planned operations were suspended. Shortages of medical professionals due to illness or quarantine further limited the ability to follow up and monitor patients. Chronic eye conditions such as diabetic retinopathy (DR), age-related macular degeneration (AMD) and glaucoma need routine periodic outpatient screening and monitoring, regular follow-up tests, perimetry exams and optical coherence tomography (OCT). Common eye emergencies such as retinal detachment and acute angle-closure glaucoma, which require timely diagnosis and specialist intervention, were cumbersome and difficult to manage in this setting. Quarantine and stay-at-home prescriptions could lead to adverse psychological consequences, whereby patients may refuse to seek immediate medical care and may limit their access to appropriate eye care [4].

Eye examinations are extremely difficult to perform in compliance with safety protocols. The use of portable diagnostic equipment such as a handheld tonometer or bio-microscope reduces the accuracy compared to gold standards in eye diseases. Preliminary data suggested that nearly one-third of patients with COVID-19 might have nonspecific ocular manifestations consistent with conjunctivitis, such as epiphora, conjunctival hyperemia, and chemosis. Although these manifestations usually occur in patients with a more severe course, those with conjunctivitis as a first symptom were identified [5].

Significant advances in information and communication technologies allow the application of remote medical care in various fields, including ophthalmology. Telemedicine is defined as the use of digital means and information exchange to provide health care with remote access [6]. The presence of powerful hardware, advanced software and fast communication enable doctors to diagnose and treat various emergency and chronic eye conditions remotely. The use of telemedicine in ophthalmology presents a unique challenge. The pandemic has dramatically changed the practice in ophthalmology, and home tele-diagnosis solutions allow to remotely monitor the patients, becoming invaluable tools for many practices.

The simultaneous progress of multiple information and communication technologies in 2020 created an unprecedented opportunity for ophthalmology to adapt to new models of care using telehealth supported by digital innovation. These digital innovations include artificial intelligence (AI), 5th generation telecommunication networks (5G) and the Internet of Things (IoT), creating an interdependent digital ecosystem offering opportunities for developing new models of eye care that address the challenges of COVID-19 [7]. Ophthalmology is advancing in these fields in part because of the many imaging-based studies. Telehealth and AI provide synchronous solutions to challenges faced by ophthalmologists and healthcare providers worldwide [8].

The aim of this article was to review and analyse the current state and the opportunities for the application of telemedicine in ophthalmology.

MATERIALS AND METHODS

PubMed, ScienceDirect Database, Google Scholar databases, as well as official sites of various governmental and non-governmental institutions were explored. The search was conducted in the period May 1, 2022 – July 31, 2022 using key words and phrases

such as “tele-ophthalmology”; “telemedicine/telehealth and ophthalmology”; “ophthalmology and COVID-19”.

RESULTS

In response to the COVID-19 pandemic, the American Academy of Ophthalmology (AAO) published guidelines on March 18, 2020, advising the discontinuation of any non-urgent or urgent care [9]. At the Vanderbilt Eye Institute, daily workloads in April 2020 were reduced by approximately 70%. This coincided with an initiative for patient service while minimizing exposure. A contract was signed with Zoom® (Zoom Video Communications, San Jose, CA) for a virtual meeting on the Epic Electronic Medical Record (EMR) platform, and access for each physician and patient was established through a “my health” link. Within four weeks, telemedicine visits increased to account for 18.5% of all visits performed at the Vanderbilt Eye Institute. During the 12-week period following implementation of the telemedicine platform, pediatric and postoperative visits accounted for the largest share (35.7%) of all telemedicine visits, followed by neuro-ophthalmology (22.4%) and oculoplastics (22.4%). The mean age for the follow-up cohort was 36.5 years. On the other hand, regarding retinal diseases, the overwhelming majority of in-person visits (70%) were for intravitreal injections of anti-vascular endothelial factors in AMD [10]. Patients with early visits in their postoperative course following intraocular surgery represented also a significant part of the examinations, given the inability to assess intraocular inflammation and view of the posterior segment using modern telemedicine technologies. Despite these limitations, every ophthalmology subspecialty can successfully use the new telemedicine format although partially.

Physical eye examination, including visual acuity and intraocular pressure, may be difficult or impossible to perform at home. Remote visual acuity testing is available through various apps, but these are not validated and are incompatible with Snellen visual acuity measurements. However, in a study done at the Moorfield Eye Hospital, London UK, involving 350 patients who used the Home Vision Monitor app, 70% of the pilot group felt reassured knowing their vision was being monitored during a pandemic. Approximately 93% of participants thought the app was easy to use, while 85% used it at least once or twice a week. The technology was designed specifically for patients with macular diseases, including neo-vascular age-related macular degeneration (AMD) and diabetes [11].

Remote measurement of intraocular pressure poses similar challenges. The iCare Home Tonometer (iCare Finland Oy) facilitates measurements, but not all patients have such a device. Home technology for optical coherence tomography has been developed for conditions such as age-related macular degeneration (AMD), but access to such a device is difficult in most cases. There are also major gaps in the use of these remote methods and further research is needed, concerning their validation, to allow their use for routine tele-ophthalmic care. In addition to the challenges inherent in ophthalmology itself, any medical center or clinic wishing to offer telemedicine services must also anticipate staff challenges. Considerable preparation work and training are required to ensure these e-visits run smoothly.

The ForeseeHome® AMD Monitoring program was designed by Notal Vision as a monitoring device that can be prescribed to AMD patients as part of a remote diagnostic service provided by the Notal Vision Diagnostic Clinic. Using automated AI-based alert generation, ForeseeHome helps detecting AMD at an earlier stage, allowing early treatment, minimizing the risk of irreversible visual acuity loss. The AREDS2-HOME study, sponsored by the National Eye Institute (NEI), demonstrated that 94% of patients, whose wet AMD had been detected with the ForeseeHome, preserved better vision, compared to 62% of patients whose clinicians used only standard methods for detection such as the Amsler Grid [12]. Patients were instructed to test their eyes daily and the results were automatically transmitted to the clinic at the end of each test. An AI-based classifier identified changes in visual distortions that might indicate a dry-to-wet AMD conversion, and when a statistically significant change in test patterns was detected, an app alerted the treating physician so they can determine the best way to action. The patient’s test data can be accessed by the doctor through a secure portal at any time.

Caffery et al. described 62 discrete tele-ophthalmic models of care ranging from eye disease screening, various consultation services, triage, remote monitoring, educational purposes, and emergency services [13]. Most tele-ophthalmology services relied on digital images captured by primary care physicians or trained technicians with various modalities for capturing the anterior and/or posterior segment of the eye. Images were transmitted electronically for evaluation to an ophthalmologist. Ophthalmology is a high-volume specialty and telemedicine has the potential to offer a cost-effective alternative to a live consultation. This concept plays an important role in rural and remote areas where medical care is not available. In addition, the use of telemedicine may be useful in other special circumstances where access to medical care is limited, such as during natural disasters or when social distancing is necessary, as in the COVID-19 pandemic.

Telemedicine is a convenient way to reduce face-to-face meetings during times of social distancing and self-isolation. Major advantages of telemedicine during the COVID-19 pandemic are lised below:

1. Virtual triage

This technique allows sorting patients out for treatment before they go on site to the health facility. Any reduction in face-to-face consultations improves the protection of patients, doctors and the wider community. The utility of triage will increase with the growing need for social distancing and self-isolation. The key to its successful implementation is a good risk determination strategy based on a minimal data set. In ophthalmology, this may include elements of medical history, objective measurements (visual acuity, intraocular pressure) and imaging tests (photographs, OCT, visual fields). Triage protocols can be optimized through automated intelligent decision trees guiding the collection of structured data. An example of such a platform is the Big Picture Medical, connecting opticians with ophthalmologists from Moorfelds Eye Hospital, London, UK [14]. There is huge potential for this technology to ease the pressure on eye clinics at times of greatest stress.

2. Bio-microscope examination

Advanced imaging and diagnostic techniques raise the question of whether a bio-microscope examination is necessary in all cases. This examination requires the doctor and patient to sit facing each other at a distance of less than one meter. The barrier shields placed on the split lamps since 2020 could reduce, but cannot eliminate the risk of cross-infection. The evaluation of the benefits and risks of this specific exam is critical today, especially since other equipment can also be used to view eye structures.

3. Eye video consultation

This type of consultation is particularly suitable for minor emergencies, oculoplastics and strabismus, for example. Following in the footsteps of tele-dermatology, platforms such as Consultant Connect [15] and Attend Anywhere [16] are being increasingly used in the National Health Service (NHS) in Great Britain. Patients get more convenient and often earlier access to specialized care. For instance, a virtual waiting room can imitate an eye clinic.

A collaboration between the NHS Forth Valley and Moorfelds Eye Hospital has recently demonstrated the world's first tele-examination of an eye in 4K resolution using 5G broadband, where video of a bio-microscope examination was transmitted live between London and a conference in Edinburgh. This was a turning point in tele-ophthalmology as detailed real-time video was successfully provided using readily available equipment. In this way, a remote examination by an on-call ophthalmologist is possible.

4. Communication

Instant messaging applications allow easier communication [17], making the patient's condition more stable – especially when individual team members unexpectedly need to go into isolation. There is an option to take images using your own device and to send them for immediate review by a specialist. This technology provides a "rough and ready" storage and transmission solution at a time when it is physically impossible to visit a clinic. Communication failure is a known key factor and can cause patient anxiety and stress [18, 19].

5. Remote control systems

During a crisis like the COVID-19 pandemic, it is inevitable that hospitals will rush to implement remote management systems that allow them to provide some care for patients who are unable or unwilling to visit the clinic. However, as the crisis passes, we see many of the innovations designed to meet short-term needs becoming long-term solutions. These new systems must be subject to constant control to ensure their quality, their meeting standards, and future developments.

Despite numerous attempts to find a solution and more and more new and innovative devices and apparatus, common criteria, as well as concise guidelines in tele-ophthalmology have not yet been created. A major obstacle to the diagnosis and treatment of eye diseases through telemedicine is the need for a detailed examination, which requires measurement of visual acuity, intraocular pressure, examination of the anterior and posterior eye segments. Although some diagnoses may require the use of specialized equipment, patients feel better if they share their concerns via phone or video call. Often a red or irritated eye turns out to be a subconjunctival hemorrhage or halation. By allowing us to diagnose these conditions remotely, tele-ophthalmology minimizes any potential exposure to COVID-19 for patients and staff while providing medical care.

The establishment of specialized ophthalmic clinics equipped with remote monitoring devices that are self-managed or that can be operated by minimally trained personnel can overcome the gap in the treatment for periods of prolonged quarantine and isolation for patients with COVID-19. For medical staff, remote care is also a safer alternative to in-person screening, allowing more patients to be tested for a given period of time. Advanced imaging techniques such as ultra-wide-field, artificial intelligence-based algorithms, and automated robotic systems have the potential to accelerate the implementation of tele-ophthalmology and to increase its utility. Nevertheless, telemedicine relies heavily on network and Internet capabilities, integration with current electronic medical

records (EMR) and image quality. Existing barriers in the legal, financial, bureaucratic system, as well as conservatism will have to be reviewed on a country-by-country basis. Technological barriers are still a prerequisite for slow large-scale adoption. With improvements in image processing as well as better integration with EMRs, teleophthalmology is likely to become a far more accepted and utilized modality even more so in circumstances where social distancing is required.

Countless innovations have created an environment ripe for telemedicine in ophthalmology, and COVID-19 has accelerated the development and adoption of these digital technologies. Emerging artificial intelligence systems and telecommunication technologies can potentially transform globally the data-rich and image-dependent specialty of ophthalmology. 5G, IoT and AI are gradually introduced in ophthalmology, but the potential for reliably connected machines such as optical coherence tomography (OCT) and fundus cameras and algorithms transforming the delivery of ophthalmic services is significant. Such technolgy is likely to become more prevalent with the increase of the 5G network coverages, enabling better IoT. These technologies may be able to make a key contribution to providing quality, sustainable eye care to all patients. The experience of the pandemic has revealed the utility of telemedicine even in densely populated and well-resourced places. Challenges to the implementation of these technologies remain, including validation, patient acceptance, and end-user education about these technologies [8].

CONCLUSION

The “new normal” for medicine after this pandemic will include telehealth. Although the technology for remote ophthalmic monitoring is not quite ready, patients are willing to accept this approach. This crisis will urge innovation. In ophthalmology, this may mean home testing. Remotely controlled optical coherence tomography devices, mobile applications, and non-mydriatic eye cameras may become more widely available in public places. Telemedicine has become an extremely valuable tool in the current situation, and while it will never completely replace in-person visits, it certainly has a place in today’s dynamic and high-tech world. This pandemic has urged medical professionals to adapt their practices and protocols in creative ways – certainly for the better. These new lessons and tools need to be put forward into the future so that we can evolve and offer our patients the best possible treatment and care methods.

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REFERENCES

1. Patel S, Henderson R, Bradley L et al. Effect of visual display unit use on blink rate and tear stability. Optom Vis Sci. 1991;68(11):888-892.
2. Hansen MH, Laigaard PP, Olsen EM, et al. Low physical activity and higher use of screen devices are associated with myopia at the age of 16-17 years in the CCC2000 Eye Study. Acta Ophthalmol. 2020;98(3):315-321.
3. Lingham G, Mackey DA, Lucas R, Yazar S. How does spending time outdoors protect against myopia? A review. Br J Ophthalmol. 2020;104(5):593-599.
4. Brooks SK, Webster RK, Smith LE, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. Lancet. 2020;395(10227):912-920.
5. Wu P, Duan F, Luo C, et al. Characteristics of Ocular Findings of Patients with Coronavirus Disease 2019 (COVID-19) in Hubei Province, China. JAMA Ophthalmol. 2020;138(5):575-578.
6. Lurie N, Carr BG. The Role of Telehealth in the Medical Response to Disasters. JAMA Intern Med. 2018;178(6):745-746.
7. Ting DSW, Carin L, Dzau V, et al. Digital technology and COVID-19. Nat. Med. 2020;26:459-461.
8. Li JO, Liu H, Ting DSJ, et al. Digital technology, tele-medicine and artificial intelligence in ophthalmology: A global perspective. Prog Retin Eye Res. 2021;82:100900.
9. American Academy of Ophthalmology. Important coronavirus updates for ophthalmologists. Available from: https://www.aao.org/h eadline/alert-important-coronavirus-context
10. Williams AM, Kalra G, Commiskey PW, et al. Ophthalmology Practice During the Coronavirus Disease 2019 Pandemic: The University of Pittsburgh Experience in Promoting Clinic Safety and Embracing Video Visits. Ophthalmol Ther. 2020;9(3):1-9.
11. Powell S. Moorfields Eye Hospital patients test vision at home using app. Optometry today. Feb 25, 2021. Available from: https://www.aop.org.uk/ot/science-and-vision/technology/2021/02/25/moorfields-eye-hospital-patients-test-vision-at-home-using-app
12. AREDS2-HOME Study Research Group, Chew EY, Clemons TE, et al. Randomized trial of a home monitoring system for early detection of choroidal neovascularization home monitoring of the Eye (HOME) study. Ophthalmology. 2014;121(2):535-544.
13. Caffery LJ, Taylor M, Gole G, et al. Models of care in tele-ophthalmology: A scoping review. J Telemed Telecare. 2019;25(2):106-122.
14. Big Picture. Available from: https://landing.bigpicturemedical.com/
15. Consultant connect. Available from: http://www.consultantconnect.org/
16. Attend Anywhere. Available from: https://www.attendanywhere.com/
17. Alakidi A, Mihaylova V, Liochkova M, et al. Priority spheres and preferences for the general practitioner’s lifestyle. Part I. General Medicine. 2020;22 (4):3-10.
18. Kitova-John M, Tsigarovski G, Kitov B. Risk factors for the development of burnout syndrome by medical specialists. General Medicine. 2020;22(3):73-79.
19. Kitov B, Epifanceva E, Asenova R, Kitova T. Stress and its importance for the human body. General Medicine, 2020;22(6):74-80.