The Terrorist Attacks and the Human Live Birth Sex Ratio: a Systematic Review and Meta-Analysis

Gwinyai Masukume[1,2], Sinéad M. O'Neill[1], Ali S. Khashan[1,3], Louise C. Kenny[1], Victor Grech[4,*]

ABSTRACT

Aim: The live birth sex ratio is defined as male/total births (M/F). Terrorist attacks have been associated with a transient decline in M/F 3–5 months later with an excess of male losses in ongoing pregnancies. The early 21st century is replete with religious/politically instigated attacks. This study estimated the pooled effect size between exposure to attacks and M/F. Registration number CRD42016041220. Methods: PubMed and Scopus were searched for ecological studies that evaluated the relationship between terrorist attacks from 1/1/2000 to 16/6/2016 and M/F. An overall pooled odds ratio (OR) for the main outcome was generated using the generic inverse variance method. Results: Five studies were included: 2011 Norway attacks; 2012 Sandy Hook Elementary School shooting; 2001 September 11 attacks; 2004 Madrid and 2005 London bombings. OR at 0.97 95% CI (0.94–1.00) (I^2 = 63%) showed a small statistically significant 3% decline in the odds (p = 0.03) of having a male live birth 3–5 months later. For lone wolf attacks there was a 10% reduction, OR 0.90 95% CI (0.86–0.95) (p = 0.0001). Conclusion: Terrorist (especially lone wolf) attacks were significantly associated with reduced odds of having a live male birth. Pregnancy loss remains an important Public Health challenge. Systematic reviews and meta-analyses considering other calamities are warranted.

KEYWORDS

population stress; sex ratio; pregnancy; stillbirth; miscarriage

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KEY NOTES

- Male births occur slightly in excess of female but transiently dip following violent events, a potential Public Health issue.
- This study carried out a systematic review and meta-analysis of five recent terrorist events.
- Overall pooled odds ratio (OR) showed a 3% decline in the odds (p = 0.03) of having a male live birth 3–5 months after such events. For lone wolf attacks this was 10%, OR 0.90 95% CI (0.86–0.95) (p = 0.0001).

AIM

For centuries, the relative proportions of male and female births have fascinated and intrigued researchers (1). The human live birth sex ratio, also known as the secondary sex ratio, is defined as male live births ÷ total births. This ratio is quick, easy and cheap to measure and it has been suggested since at least the 1990s that this ratio can be used as a sentinel health indicator in both industrialized and non-industrialized countries to monitor the health of populations (2).

Momentous events of our time exert varying degrees of stress on differing populaces, effects which may last from days to weeks. Such events include the September 11, 2001 (9/11) terrorist attacks (3), and Great East Japan Earthquake of 2011 (4) (which led to the Fukushima nuclear power plant disaster), all of which have been linked to significant declines in the sex ratio at birth in exposed populations. The contention that exogenous stress influences the human live birth sex ratio is underpinned by a large body of evidence from animal models, human epidemiologic studies and evolutionary theory (3). However, because the sex ratio at birth is an easy outcome to measure, multiple studies in this domain are performed with a high risk of publication bias from false positives (5).

While what would be deemed as terrorist attacks have occurred for centuries, we sought specifically to consider a contemporary period in the new millennium and century so that our results would be generalizable to our time. Soon after the turn of the 21st century, the 9/11 terrorist attacks on the United States of America led to unprecedented global media attention and responses from governments (6). These attacks are spreading and increasing (7). A particular chain of events by which terrorist attacks culminate in an excess loss of unborn males via miscarriage or stillbirth leading to a reduced sex ratio at birth via maternal stress has been posited (Figure 1). Pregnancy loss remains an important public health challenge (3). Given the prominence of terrorist attacks, the aim of this systematic review was to investigate the effect of terrorist attacks on the human live birth sex ratio in the published literature to date and to provide a quantitative estimate of the magnitude of the effect in the form of a meta-analysis.

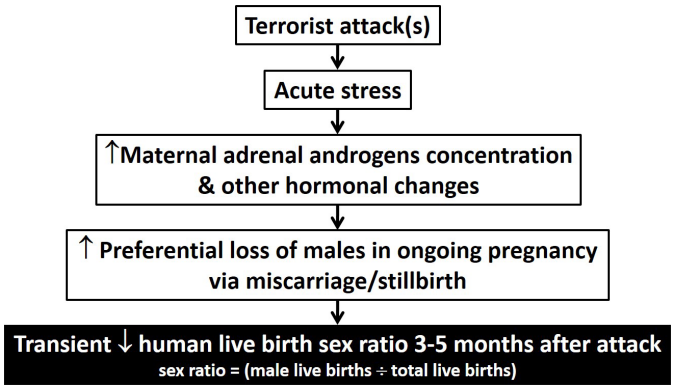

Fig. 1: Conceptual framework of the chain of events from a terrorist attack(s) leading to the excess *in utero* loss of males (3, 16). Transient/acute psychological stress acts via the adrenal glands causing perturbations in hormonal concentrations of androgens, cortisol, catecholamines and downstream reproductive hormones.

METHODS

The protocol for this systematic review and meta-analysis was registered on the International prospective register of systematic reviews (PROSPERO) site, registration number CRD42016041220 (8) and adheres to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines (9).

SEARCH STRATEGY

A systematic search of the literature (without language restrictions) was performed. One reviewer searched for potentially eligible studies published from January 1, 2000 up to June 16, 2016 from the electronic databases PubMed and Scopus, using a comprehensive search strategy (Table 1), applying the principles of Boolean logic and medical subject headings (MeSH). All of the citations were imported into EndNote reference manager (Thompson Reuters version X7) and two reviewers (GM, SON) independently screened the titles and abstracts according to the inclusion/exclusion criteria (Table 2). Full texts of the studies were obtained and agreed on for eligibility. No disagreement regarding inclusion occurred. The search was supplemented by hand-searching the reference lists of included studies.

ELIGIBILITY

The inclusion and exclusion criteria used for this study with search limits and restrictions are as in Table 2.

DATA EXTRACTION

One reviewer (GM) extracted data from the included studies and a second reviewer (SON) checked the data for accuracy. Data extracted included author name and year and country/region of attack.

QUALITY ASSESSMENT

To the best of our knowledge, there is no tool validated to assess the quality of ecological studies (10). Nevertheless, the group exposure (terrorist attack) was assessed as

Tab. 1: Search terms used to identify relevant studies.

Exposure	Outcome
Terror[a]	Birth gender ratio
Bomb[a]	Human gender proportion at birth
Shoot[a]	Human sex odds
	Human sex ratio at birth
	Male to female ratio at birth
	Male:female birth ratio
	Male:female ratio at birth
	Male-female ratio
	Male/female ratio
	Secondary sex ratio
	Secondary sex ratio at birth
	Sex odds
	Sex ratio
	Sex ratio at birth

[a] Denotes wildcard to retrieve for example terror, terrorism, terrorist, terrorizing, etc. The AND operator was used between exposure and outcome while the OR operator was used within the exposure and outcome categories.

Tab. 2: Inclusion and exclusion criteria.

	Inclusion	Exclusion
Participants	Population exposed to terrorist attack	No terrorist attack
Exposure[a]	Terrorist attack	No terrorist attack
Comparison	Control population from immediate prior or future years (same time of the year) in same geographic region not exposed to terrorist attack	No terrorist attack
Outcome	Human live birth sex ratio reported at least monthly	Study without this outcome
Study design	Ecological	Letters, editorials, reviews, etc.
Language	All languages	Not applicable
Date	Terrorist attack from January 1 2000 to present (June 16 2016)	Terrorist attacks before 2000

[a] Direct observation of attack or indirectly via the media.

defined by the population that experienced the attack i.e. there was general consensus that the attack was terrorist in nature and the studies described them as such. Because the human live birth sex ratio of an entire population (and not a sample) was considered and the risk of misclassifying sex at birth is low (2), minimal bias was anticipated in measuring the outcome. The risk of bias in ascertaining the group exposure and group outcome were thus judged to be low.

STATISTICAL ANALYSIS

After identifying the final studies, it became clear it was possible to obtain the original data. By contacting the civil registration and vital statistics systems listed shortly, the reviewers acquired the anonymized primary data underlying the retrieved studies in order to facilitate a uniform analytic approach for the meta-analysis since the original studies used different statistical techniques and considered different analytic periods:

- United States Centers for Disease Control and Prevention, Vitalstats
- The National Institute of Statistics (*Instituto Nacional de Estadística*: INE)
- The English Office for National Statistics (Ms. Debbie Hague, Life Events & Population Sources – personal communication)
- Statistics Norway Information Centre (Ms. Eva Hoel, Seksjon *Befolkningsstatistikk*, *Statistisk Sentralbyrå* – personal communication)

Civil registration coverage of births was virtually 100% for the obtained live birth data. The methods for computing the human live birth sex ratio in exposed and non-exposed populations have been described in detail previously (11). These methods strive to minimize the inherent seasonality of the sex ratio at birth by considering the same period of the year and the confounding effect of, for example the climate and economy, by restricting analysis to two years before and after the attack(s). The month with the effect as reported in the primary studies (or the one with the lowest sex ratio – when there was no observed effect three to five months after an attack) was compared to the expected ratio for that time based on the observed sex ratio in the preceding and following two years. A p-value < 0.05 was considered to be statistically significant.

Pooled odds ratios with 95% confidence intervals (CIs) were estimated for each study using Review Manager version 5.3.5 and the generic inverse variance method (12). Depending on the level of heterogeneity present, measured per the I^2 statistic, the fixed or random-effects model was used. Where an I^2 value of greater than 50% was obtained, this was considered moderate heterogeneity according to the Cochrane criteria (13) and the random-effects model was used. The meta-analysis was divided into *a priori* subgroups according to type of terrorist attack in line with the classification system used in the Global Terrorism Index (7): single person (lone wolf) attacks and multiple perpetrator attacks.

Post hoc sensitivity analysis was conducted since multiple studies included data from the same population. Three studies included data from the United States (US): One study included the population of New York (14), a second study included the population of California (15) and the third study included the entire US population (3). Therefore the following four sensitivity analyses were done (New York, California, California & New York, and the entire US) to determine if the overall pooled OR would change. Research has shown that the 9/11 attacks caused transient psychological distress in the whole of the US, but this distress was greatest in the New York City area (16). Informed by this fact, we chose New York for the final sensitivity analysis so as to capture the presumed maximum effect of the attacks.

RESULTS

We retrieved 247 non-duplicated studies from the systematic search of the literature (Figure 2). Of these, 242 studies were excluded based on the title or abstract. Five studies covering five distinct terrorist attacks met the inclusion criteria (3, 14, 15, 17, 18) and were included in the systematic review and meta-analysis (see Table 3 for study characteristics).

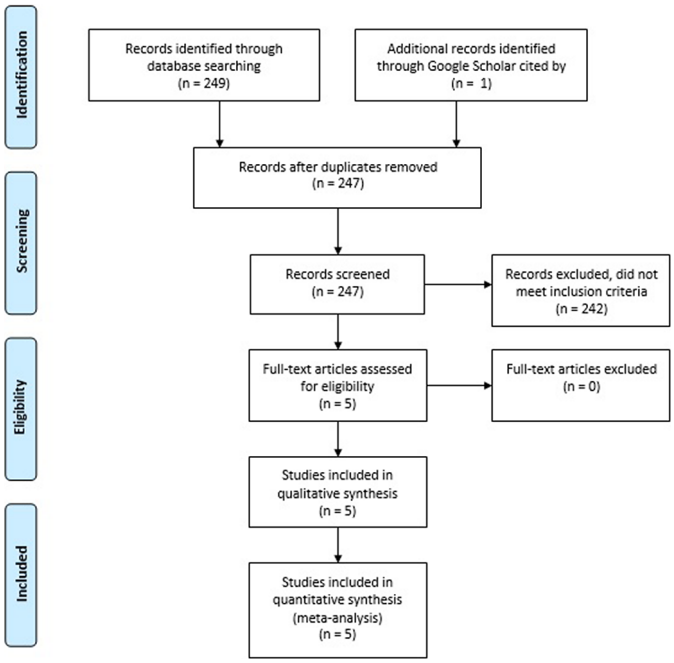

Fig. 2: Flow chart of studies identified for inclusion.

META-ANALYSIS

The overall pooled OR was statistically significant 0.97 95% CI (0.94–1.00) (p = 0.03) (I^2 = 63%), with a three percent decline in the odds of having a male live birth in populations exposed to terrorist attack(s) compared to referent un-exposed populations (Figure 3). This significant result did not change for three of the four sensitivity analyses (New York, California, California & New York), but the results did alter when the entire US population was used (p = 0.06).

DISCUSSION

PRINCIPAL FINDINGS

The striking finding was that more deaths from pregnancy loss than direct casualties were estimated for Norway and Connecticut following the 2011 Norway attacks and 2012 Sandy Hook Elementary School shooting. Indeed, Norway appeared to experience a "triple hit" from direct deaths, excess pregnancy losses and an increase in the suicide rate (19), all of which were linked to the attack. To the best of our knowledge this is the first time the "triple hit" signature of deaths following a terrorist attack has been described in the literature. Other attacks might not show this signature, depending on contextual factors, such as the 9/11 attacks wherein the suicide rate remained stable in the United States and even dipped significantly in the immediate vicinity of the New York attacks (20).

The other striking finding is that terrorist attacks by single perpetrators had a larger effect size than those by multiple perpetrators. One possible explanation in the context of this study was that an attack by an 'insider' rather than an 'outsider' was perhaps more shocking/stressful to the community, as was possibly the case for the Norway and Sandy Hook Elementary School attacks. Such individuals are trusted, and violent attacks from such individuals are therefore unanticipated and doubly appalling.

STRENGTHS AND LIMITATIONS

Being able to obtain the raw live birth data from modern civil registration and vital statistics systems that are highly complete, accurate and timely was a major strength.

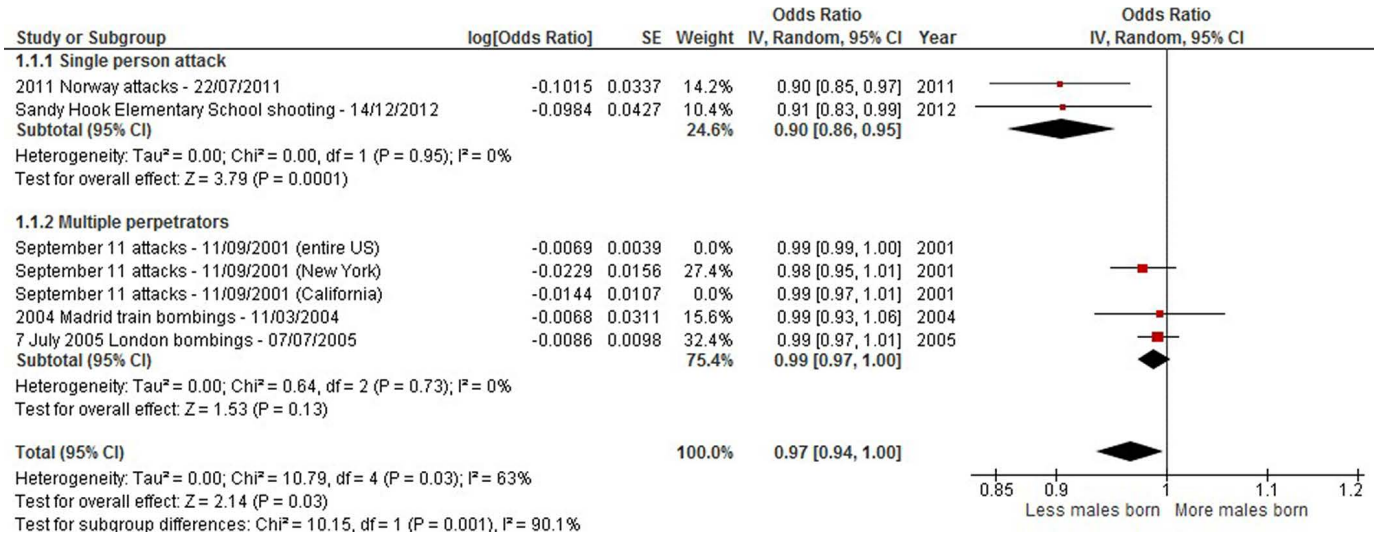

Fig. 3: Forest plot of random effects meta-analysis of the association between early 21st century terrorist attacks and the human live birth sex ratio. Please note the striking difference in effect size between single person attacks and attacks by multiple perpetrators. The September 11 attacks for California and the entire United States (US) are shown so as to display their odds ratio point estimates and 95% confidence intervals, but these did not contribute (Weight = 0.0%) to the pooled estimate.

Tab. 3: Characteristics of included terrorist attacks with calculated estimates of males lost.

Attack	Summary of attack	Month of dip and number of months after attack	Population	Percent males in control population	Percent males after attack	Number of males lost (point estimate)[a]	95% confidence interval[a]
Single person attack							
2011 Norway attacks – 22/07/2011 [17] (Grech, 2015)	77 lives lost in two attacks a few hours apart by a sole male perpetrator (car bomb and mass shooting)	December 2011 (~5 months)	Norway	51.4%	48.9%	230	392 lost – 78 lost
Sandy Hook Elementary School shooting – 14/12/2012 [b] [17] (Grech V, 2015)	28 lives lost in an attack by a sole male perpetrator (school shooting)	April 2013 (~4 months)	Connecticut	51.3%	48.8%	147	284 lost – 21 lost
Multiple perpetrators							
September 11 attacks – 11/09/2001 (entire US) [3] (Bruckner et al., 2010)	2996 lives lost in multiple attacks by multiple perpetrators (aircraft flying into inhabited buildings)	December 2001 (~3 months)	United States of America	51.1%	51.0%	1156	2449 lost – 166 gained
September 11 attacks – 11/09/2001 (New York) [14] (Catalano et al., 2006)	As above	January 2002 (~4 months)	New York State	51.1%	50.5%	240	569 lost – 93 gained
September 11 attacks – 11/09/2001 (California) [15] (Catalano et al., 2005)	As above	December 2001 (~3 months)	California	51.9%	51.2%	318	789 lost – 153 gained
2004 Madrid train bombings – 11/03/2004 [b] [18] (Grech and Mamo, 2016)	191 lives lost in multiple attacks by multiple perpetrators (bombing commuter trains)	August 2004 (~5 months)	Madrid province	51.5%	51.3%	19	196 lost – 148 gained
7 July 2005 London bombings – 07/07/2005 [18] (Grech and Mamo, 2016)	56 lives lost in multiple attacks by multiple perpetrators (bombing commuter trains and a bus)	November 2005 (~4 months)	England and Wales	51.3%	51.1%	232	752 lost – 291 gained

Please note for Norway there were almost three times (230 ÷ 77) more pregnancy losses compared to direct casualties. For Connecticut, there was an approximately five times (147 ÷ 28) greater toll. US – United States of America, [a] Rounded to the nearest whole number, [b] 2002 and 2015 missing from referent population.

This also allowed uniform statistical analysis, ensuring comparability between the different terrorist events. Needless to mention, the transparent, systematic and reproducible methodology with no language restriction to the search is another major notable strength. Searching only two biomedical databases, PubMed and Scopus (the largest database of peer-reviewed literature), and not considering gray literature limited our study as we may have missed relevant studies. Since five terrorist attacks were identified with two heterogeneous subgroups, it was not possible to adequately assess publication bias, which, as previously discussed (5), might affect the sex ratio at birth field. The attacks considered herein account for over 90% of deaths from terrorism in the West, but less than 3% of global terror deaths in the 15 years from 2000 to 2014 inclusive (7). Thus another limitation is that this study's findings might not be reproducible beyond a Western world setting to other places that have experienced terrorist attacks, for example in Africa and Asia which also happen to have limited civil registration and vital statistics systems, precluding detailed analysis. The limitations of ecological studies (21), like the limited ability to infer to the individual level (ecological fallacy) apply to this study. Nevertheless the ecological study is arguably the best to evaluate the impact of a population stressor on a population outcome (22).

The purpose of terrorism is the calculated infliction of population stress since terrorist acts endeavor to shock

populations and thus draw media attention for perceived political purposes. For this reason, terrorists strive to influence a much wider audience than the individuals that are targeted, often with success (23). Society struggles to come to terms with the motivation(s) behind these events, a phenomenon known as "attribution theory," the need to assign motivation so as to understand events, "how else to explain the inexplicable" (24).

Unlike the original studies on 9/11 (3, 14, 15) our analytical method did not find a statistically significant association between the attack and the human live birth sex ratio in New York, California and the entire United States. A statistically insignificant result does not automatically mean a lack of effect (25). One reason for this difference is that our analysis included live birth data up to 2004, while the primary studies used data up to 2002. In addition, we used restriction – an epidemiologic technique – to take confounding into account and a different strategy was used to account for autocorrelation. However, we observed the same striking patterns. For example, the California December 2001 (three months after the attack) sex ratio was the lowest ever observed monthly sex ratio from 1994 to 2004 inclusive for that population, as well as total births, suggesting an obvious influence of the attacks on the sex ratio at birth. Therefore, although we used different statistical techniques, our findings are in agreement with the primary studies. Furthermore, a recent study (16) revealed that population level psychological distress following the 9/11 attacks lasted for about one month and then resolved. A similar pattern was observed with the sex ratio at birth after 9/11 albeit with the expected lag, from the time of miscarriage/stillbirth to when live birth would have occurred. This temporally related transient one month dip in both psychological distress and the sex ratio at birth in the same population further cements the causal relationship between stress engendered by terrorist attacks and a subsequent decline in the live birth sex ratio.

One would anticipate that an effect for the 2005 London bombings would be observed in that city in a similar way to New York for 9/11. Unfortunately live birth data was only available for England and Wales combined, thereby presumably attenuating the observed effect as we saw for the entire US in this study. Additionally, Londoners were forewarned with regard to possible attacks. For example, in August 2004, leaflets were mailed to all households with advice as to what to do in the event of a terrorist attack (26). Thus, the lack of effect seen for the London bombings could in part be attributed to this public health intervention of priming the population, thereby dampening the stress engendered by the attacks.

In addition, the lack of effect on the live birth sex ratio following the 2005 London bombings is consistent with a population-based study conducted in the North West of England that failed to demonstrate a change in the stillbirth rate in women exposed to the attacks while pregnant or six months before they conceived (27). However, the study showed a small but significant 16 g reduction in birth weight in these North West England women 'exposed' to the London attacks via the media, compared to a referent unexposed group, suggesting that the terrorist attacks in central London did have a measurable obstetric impact, which was nonetheless not severe enough to perturb the number of stillbirths, which are on the causal pathway to subsequent alteration of the sex ratio at birth.

Our pooled findings suggest that stress may have precipitated spontaneous abortions in already pregnant women who were negatively influenced by the diverse stressful events studied in this paper, in accordance with the Trivers-Willard hypothesis (28). Weaker fetuses are typically affected, resulting in a greater loss of male than female fetuses (3). This is confirmed by the fact that surviving male births after 9/11 produced males that apparently had better than expected cognitive development (29).

While these are relatively transient male fetal losses, public health authorities should utilise the live birth sex ratio which is cheap, quick and easy to measure in order to quantify the effects of stress from significant events on entire populations, and therefore refine and implement interventions to mitigate future losses.

It is not surprising that attention has not been drawn prominently to pregnancy loss that can by far outstrip the direct deaths from terrorist attacks. Indeed, stillbirth has been termed the neglected epidemic (30). This subject deserves serious public health consideration and action.

As mentioned in the introduction, calamitous events have also been associated with a subsequent decline in the sex ratio at birth (4). Future systematic reviews and meta-analyses considering such events are warranted.

CONCLUSIONS

Terrorist attacks in the early 21st century were statistically significantly associated with a small three percent reduction in the odds of having a live male birth three to five months afterwards because of excess male pregnancy loss. This effect was particularly strong for attacks perpetrated by single, 'lone wolf' individuals, so much so that estimates of males lost during pregnancy because of maternal stress engendered by these attacks through miscarriage or stillbirth were about three (230 ÷ 77) and five times (147 ÷ 28) greater than direct deaths from the 2011 Norway attacks and 2012 Sandy Hook Elementary School shooting respectively. Because pregnancy loss remains an important public health challenge this subject deserves serious public health consideration and action.

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COMPETING INTERESTS

None declared.

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Comparison of Non-Gated vs. ECG-gated CT Angiography of Fontan Circulation in Patients with Implanted Stents in Pulmonary Branches

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ABSTRACT

Background: Motion artifacts may degrade CT examination of Fontan pathway and hinder accurate diagnosis of in-stent restenosis. Purpose: We retrospectively compared ECG-gated multi-detector computed tomography (CT) with non-ECG-gated CT in order to demonstrate whether or not one of the methods should be preferred. Method: The study included 13 patients with surgically reconstructed Fontan pathway. A total of 16 CT examinations were performed between February 2010 and November 2015.The incidence of motion artifacts in Fontan pathway and pulmonary branches were analysed subjectively by two readers. The effective dose for each examination was calculated. Results: Just in one non-gated CT examination was evidence of motion artifact in distal part of left pulmonary artery. The mean normalized effective radiation dose was 2.33 mSv (±0.62) for the non-ECG-gated scans and 4.55 mSv (±0.85) for the ECG-gated scans ($p \leq 0.05$). Conclusion: Non-gated CT angiography with single phase reconstruction significantly reduces radiation dose without loss of image quality compared with ECG-gated CT angiography.

KEYWORDS

CT angiography; Fontan circulation; stents

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INTRODUCTION

The patients who have undergone the Fontan procedure (a palliative surgical procedure used in children with univentricular hearts, is usually performed at 2 or 3 years of age and it means creation of tunnel between superior vena cava, inferior vena cava and pulmonary branches) suffer from many complications (extracardiac or intracardiac). Complications following surgical intervention in these patients may have serious consequences (1–3). Pulmonary branch stenosis is one of the most frequent complications in Fontans. The transcatheter interventions (balloon angioplasty or stent placement) are the most effective methods in the treatment of stenoses (4).

An accurate, accessible imaging technique is required for the detection of pathology, which may further facilitate fast, appropriate treatment. The traditional imaging technique for the pre- and post-operative evaluation of patients with congenital heart disease (CHD) is echocardiography. The diagnostic quality of echocardiography is highly dependent on the operator and the presence of an adequate acoustic window. Digital subtraction angiography due to its invasive character has limited use in follow-up of these patients. MRI is an excellent diagnostic tool particularly for evaluation of young children with CHD who will require several follow-up examinations in their lifetime (5, 6). Moreover, the diagnostic capabilities of MRI are limited in the presence of surgical devices and implanted stents due to the signal dropout in the region of the stent (7). These patients are better followed by CT for these reasons. Evaluation of in-stent restenosis by CT is optimal. Visualization of stent in these patients is due to Fontan physiology challenging (8). It is very important to achieve homogenous enhancement in all parts of Fontan circulation because mixing of enhanced and unenhanced blood may make impossible evaluation of thrombus or in-stent restenosis. However, CT with retrospective ECG-gating facilitates non-invasive imaging of the Fontan circulation without detrimental motion artifacts. The technique incurs a relatively higher radiation dose to the often very young patients and has aroused growing concern in radiological communities.

Attempting to avoid high radiation dose, we decided to perform non-gated CT angiography in Fontans.

The aim of this study was to investigate:

a) Occurence of motion artifacts in non ECG gated CT vs. gated CT.
b) Dose reduction with non-ECG gated CT vs. ECG gated CT.

MATERIAL AND METHODS

PATIENT POPULATION

The study population included 13 patients after Fontan procedure and with implanted stent in pulmonary branch or fenestration. A total of 16 CT examinations were performed between February 2010 and November 2015 (non-gated CT angiography in 8 examinations, ECG-gated in 8 examinations). Informed consent was obtained for each patient prior to CT angiography. Indications for initial Fontan-surgery included various types of CHD described in patient characteristics Table 1. All patients underwent a modified Fontan operation. Total cavo-pulmonary connections were performed in all patients. The clinical indications for CT study were assessment of in-stent restenosis and in some patients also visualization of Fontan fenestration with implanted stent. Data were analyzed in a retrospective manner.

Tab. 1: Demographic data of patients.

No of patients	13
Gender	
Male	12
Female	1
Age	
Mean	10.1 y
Range	3–17 y
Diagnosis	
HLHs	6
HRHs	3
PA	3
EA	1
Implanted stent	
Pulmonary branch	10
Fenestration	3

EA – Ebstein anomaly, HLHs – hypoplastic left heart syndrome, HRHs – hypoplasitc right heart syndrome, PA – pulmonary atresia

IMAGE ACQUISITION

Computed tomographic examinations were performed using a CT scanner (AquilionONE; Toshiba Medical Systems, Nasu, Japan). Scan parameters for non-ECG-gated examinations and retrospectively ECG-gated examinations are summarized in Table 2. This CT scanner uses Adaptive Iterative Dose Reduction 3D (AIDR 3D) which reduces image noise.

We use a noniodine contrast medium (Ultravist 370; Shering, Berlin, Germany) of 1–1.5 ml/kg into right antecubital vein, through 20–22-guage needle. Injection rates were as fast as practical given the needle and patient size. We use 1-minute delay scan for assessment of stents implanted in pulmonary branches or in fenestration. In patients without stent in fenestration arterial phase is

Tab. 2: Scan parameters.

Detector collimation	0.5 mm
Gantry rotation time	0.5 s
Pitch factor	0.828
Tube voltage	80 kV for patients 40 kg or less 120 kV for patient more than 40 kg
Effective tube current time	100–250 mA
Slice thickness	0.5 mm
Reconstruction interval	0.5 mm
Scan range	from the lung apex to the liver

needed for visualization of this fenestration. Then the bolus tracking method is preferred with a region of interest placed in superior vena cava or in the place of connection of superior vena cava with pulmonary branches, and threshold attenuation of 150 HU is set.

To reduce artifacts from undiluted contrast material and to reduce the total amount of contrast material, a saline bolus chasing technique was applied. Realised CT scans were performed as helical acquisition in 64-CT mode. CT scanning was realised from the lung apex to the liver for detection of other pathologies.

IMAGE INTERPRETATION

For subjective analysis, two radiologists evaluated the CT images in consensus. They observed the presence or absence of motion artifacts in both pulmonary arteries (proximal and distal parts) and the Fontan tract (Fig. 1) using a four point ordinal scale, with integers ranging from 1 to 4, to rate image quality (Tab. 3). Readers were blinded to patient identity, clinical information and acquisition parameters. Motion artifacts of the Fontan pathway were defined as the doubling of anatomic structures or step artifacts (Fig. 2).

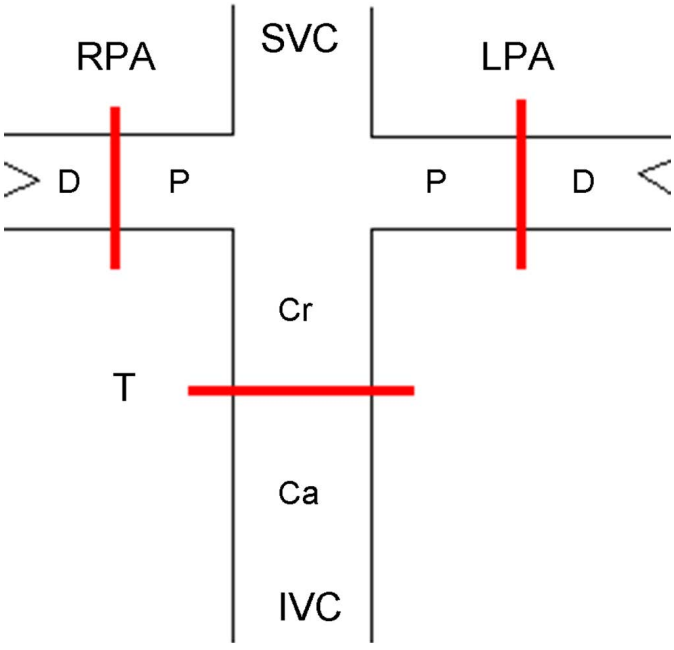

Fig. 1: Scheme of evaluation of motion artifacts. IVC – inferior vena cava, LPA – left pulmonary artery, RPA – right pulmonary artery, SVC – superior vena cava, T – tunnel, Ca – caudal, Cr – cranial, P – proximal, D – distal.

Tab. 3: Rating of motion artefacts of the Fontan circulation.

Rating	Criteria
1	No motion artifacts
2	Minimal motion artifacts – slight blurring and/or slight structural discontinuity
3	Moderate motion artifacts – moderate blurring and/or distinct structural discontinuity
4	Severe motion artifacts, not diagnostic – pronounced blurring and/or severe structural discontinuity

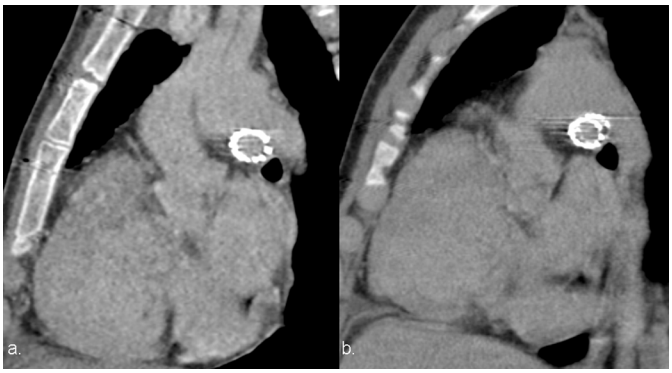

Fig. 2: Sagital view. a) CT scan demostrates presence of no motion artefacts in LPA; b) CT scan demonstrates presence of motion artifact in distal part of left pulmonary artery (doubling of contour).

RADIATION DOSE

The dose reports for all examinations were retrieved from the picture archiving and communication system. Dose-length product was recorded in each case. Estimated effective radiation dosages (mSv) were calculated for each scan using the following equation: DLP × 0.026 (infants > 1 year to 5 years old) or × 0.018 (children > 5 years to 10 years) or × 0.014 (adults). The figures displayed in the CT console were multiplied by a factor of 2 for children and of 3 for infants in order to give a realistic estimate of the patient's dose because conversion coefficients have been obtained for a 16 cm CT dose phantom, whereas the CT console indicator will provide DLP assuming the use of the 32-cm diameter body phantom.

STATISTICAL ANALYSIS

Statistical analysis included the calculation of means and standard deviations. Mann-Whitney U test was used to compare occurrence of motion artifacts in non-ECG gated CT vs. gated CT.

For statistical analysis was used program JMP version 4.0.2. (SAS Institute, USA).

RESULTS

Just in one non-gated CT examination was evidence of motion artifact in left pulmonary artery (Tab. 4).

The mean normalized effective dose was 2.33 mSv (±0.62) for the non-gated scans and 4.55 mSv (±0.85) for the ECG-gated scans. The increase in radiation dose by the use of ECG gating was evident ($p \leq 0.05$).

Tab. 4: Distribution of motion artifact ratings in non-gated and ECG-gated scans.

		1	2	3	4
RPA P	ECG gated	8			
	Non-gated	8			
RPA D	ECG gated	8			
	Non-gated	8			
LPA P	ECG gated	8			
	Non-gated	7	1		

LPA D	ECG gated	8			
	Non-gated	7	1		
T Cr	ECG gated	8			
	Non-gated	8			
T Ca	ECG gated	8			
	Non-gated	8			

DISCUSSION

Visualization of implanted stents in pulmonary branches or Fontan fenestration is very complicated (Fig. 3). ECHO with its poor acoustic window and high interobserver reliability as well as digital subtraction angiography due to its invasive character have limited use. MRI represents an excellent diagnostic tool for evaluation of young children with congenital heart diseases who will require several follow-up examinations in their lifetime. Moreover, the diagnostic capabilities of MRI are limited in the presence of surgical devices and implanted stents due to signal drop-out in the region of the stent (9, 10).

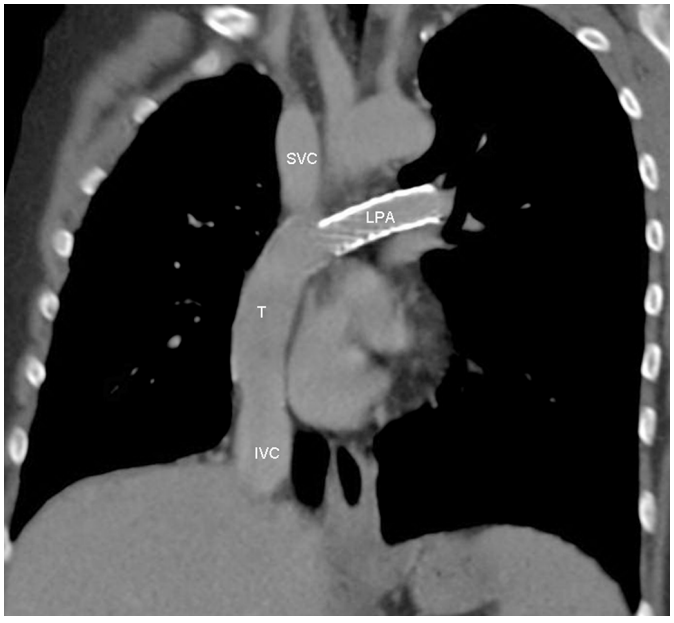

Fig. 3: Curved planar reconstruction shows perfect delineation of each part of Fontan circuit. Right pulmonary artery is not visualized in this figure. Stent placed in LPA. IVC – inferior vena cava, LPA – left pulmonary artery, SVC – superior vena cava, T – tunnel.

So for the visualization of Fontan circulation with implanted stent CT angiography is the preferable method because provide the best opportunity to visualize in-stent restenosis in comparison to MRI. For the detection of in-stent restenosis homogenous enhancement of Fontan circuit is necessary because mixing of enhanced and unenhanced blood may make impossible evaluation of thrombus or in-stent restenosis. Many techniques achieving homogenous enhancement were published. Greenberg used a dual injection technique to perform CT angiography in six patients with extracardiac or lateral tunnel palliations. We think that using of this technique is very traumatizing for children. Park et al. tried this injection technique in some patients but arrival of the contrast media in the superior vena cava and the Fontan tract at the same time was difficult to achieve because of the different resistances of the route, different degrees of the collateral pathway formation, and the different flow velocities of each pathway. His study demonstrates that a 3-minute-delay scan is the most optimal protocol in Fontan patients for the detection of thrombus regardless of intravenous route or surgical technique used. (11, 12, 13) We think that visualization of in-stents restenosis using the one-minute delayed scan is optimal. The similar studies comparing dose reduction in ECG-gated and non-gated CT scan in Fontans with implanted stents haven't been published yet.

We assumed the dose reduction, but the presence of motion artifacts was the question. Realised non-gated CT examinations confirmed the significant dose reduction without loss of image quality.

Non-gated CT angiography in Fontan patients can be preferable method for the visualization of implanted stents in Fontan circuit without loss of image quality and with the significant dose reduction.

STUDY LIMITATION

It is not a randomized study and has a low sample size.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Inflammatory Biomarkers and Liver Histopathology in Non-Uremic and Uremic Chronic Hepatitis C Patients

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ABSTRACT

Background: The aim of this study is to investigate the association between hepatic activity index (HAI) and fibrosis score (FS) with inflammation biomarkers in non-uremic and uremic hepatitis C positive patients. Methods: Fifty chronic hepatitis C (cHepC) positive patients, having a liver biopsy were included in this study. Liver biopsies were scored according to modified ISHAC scoring system. 25 healthy controls of similar age and gender were also enrolled as control group. Serum YKL-40, neutrophil/lymphocyte ratio (NLR), thrombocyte/lymphocyte ratio (PLR), CRP and Immunoglobulin (IgG, A and M) levels were used to determine inflammation. AST to Platelet Ratio Index (APRI) score was also evaluated. According to biopsy findings patients were divided into 2 groups: low (0–2) and severe (3–6) FS. Results: Patients with cHepC had increased inflammation compared to the healthy controls. End-stage renal disease (ESRD) patients had higher levels of inflammation markers (NLR, IgG, CRP and YKL-40) and lower HCV RNA levels, HAI and FS compared to non-uremic patients. When patients were grouped into 2 according to FS as mild and severe, IgG ($p < 0.001$), YKL-40 ($p = 0.02$) levels and APRI score ($p = 0.002$) were significantly higher compared to mild FS ($p = 0.002$). YKL-40 levels (t value: 3.48; $p = 0.001$) and APRI score (t value: 4.57, $p < 0.001$) were found as independent associated with FS in non-uremic patients. However, in adjusted models, only APRI score (t value: 3.98, $p = 0.002$) was an independent associated with FS in ESRD patients. Conclusion: In non-uremic cHepC patients, YKL-40 levels and APRI score may be valuable markers of FS. In ESRD patients, there is not sufficient data for prediction of HAI and FS. In these patients, APRI score may provide better information.

KEYWORDS

APRI score; hepatitis C; end-stage renal disease; inflammation biomarkers; YKL-40; liver histopathology

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INTRODUCTION

It is estimated that 2–3% of the world population has hepatitis C (HepC), varying by geographical location, time period and development status of countries (1, 2). Important risk factors for HepC are blood transfusions, solid organ transplantation from an infected donor, i.v. drug use, non-safe therapeutic injections, occupational percutaneous exposures and familial or dialysis-related exposures (3–5). Despite the fact that HepC incidence and prevalence have declined in the last years, mainly in the hemodialysis population, it is still a major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (1, 2, 5).

Liver biopsy is the gold-standard procedure for the diagnosis and treatment decision of HepC. However, it is an invasive procedure, leads to various complications, needs to be repeated and requires hospitalization and is costly. In addition, in uremic patients it may be more difficult to perform due to uremia-related coagulation problems (6). Thus, non-invasive markers are under intense investigation (7) with the literature still being limited.

Inflammatory events play an important role during the course of chronic hepatitis C (cHepC) (8–10). Main contributors are cytokine producing CD4+ T and CD8+ T cell proliferation, increased CD5+ B cells, IL-2, IFN-γ and TNF α (8–10). Neutrophil lymphocyte ratio (NLR) and thrombocyte lymphocyte ratio (PLR) are used as markers of inflammation in various diseases including atherosclerotic heart and renal disease. They are more sensitive compared to total leukocyte counts (11–13). However, there are no data to confirm their use in cHepC patients. Similarly, YKL-40 is a glycoprotein synthesized from neutrophils and macrophages that plays a role in inflammation and tissue remodelling. A number of studies have demonstrated the association between YLK-40 and fibrosis in liver disease (14–20).

In this study, we aimed to investigate the association between hepatic activity index (HAI) and fibrosis score (FS) with inflammation markers (NLR, PLR, YKL-40, IgG, IgA and IgM) in patients with cHepC with or without end-stage renal disease (ESRD).

MATERIAL AND METHODS

50 cHepC patients, 15 with ESRD, having undergone percutaneous liver biopsy between December 2011 and June 2013 at a tertiary hospital were included. 25 healthy people w ith similar age and gender were used as controls. Exclusion criteria were co-infection with other viruses, chronic hepatitis due to other reasons, decompansated cirrhosis, primary or metastaic liver cancers, malignancies, serious congestive heart failure and pyschiatric disorders.

Data on age, gender, smoking, alcohol use, systemic diseases, coinfection, medication, height, weight were obtained from patients charts.

Liver biopsy was performed under ultrasound guidance. İnformed consent was received from all patients prior to biopsy. Modified ISHAC Scoring was used for histopathological evaluation of the liver biopsies. According to biopsy findings patients were divided into 2 groups: low (0–2) and severe (3–6) FS.

NLR, PLR, IgG, IgA, IgM, CRP and YKL-40 levels were measured as markers of inflammation. Serum YKL-40 was detected by ELISA (Human Chitinase 3-Like 1, EuroimmunAnalayser, USA) according to the manufacturer's protocol. APRI score was calculated from 'increase in AST (patient's AST level/upper limit for AST)/Thrombocyte count × 100'.

In addition to informed consent for liver biopsy, all patients also gave informed consent for this study.

STATISTICAL ANALYSIS

All analysis were performed by using SPSS 15.0 for Windows. All values are reported as mean ± SD. The Pearson's Correlation was used for correlation analysis, student t-test and chi-square to compare two groups and ANOVA to compare more than two groups. Stepwise linear regression analysis was used to find independent predictors for HAI and FS. P value less than 0.05 was considered as statistically significant.

RESULTS

50 patients with cHepC (35 patients were non-uremic cHepC, 15 were ESRD patients) and 25 controls were enrolled. Mean age was 55 ± 11 years and 50% were male. Mean HCV RNA value was $3.6E^{+6}$ IU/mL ± $6.5E^{+6}$ IU/mL. 39 patients had genotype 1, 1 was genotype 3 and 4 were genotype 4 (6 patients did not have genotype values). Mean HAI and FS scores were 8.26 ± 2.22 and 2.88 ± 1.54, respectively.

cHepC patients had similar demographical parameters compared to controls. NLR (2.52 ± 1.26 vs. 1.91 ± 0.92; p = 0.03), serum IgG levels (1491 ± 459 mg/dL vs. 1143 ± 229 mg/dL; p < 0.001), YKL-40 levels (134 ± 170 ng/mL vs. 18 ± 9.1 ng/mL, p < 0.001) were higher in cHepC patients. There were no differences with regards to total leukocyte count, PLR, CRP, IgM and Ig A levels, whereas liver function tests were higher.

Non-uremic cHepC patients were older, had higher Body mass ındex (BMI), AST and ALT levels compared to patients with ESRD. In addition, ESRD patients had higher levels of inflammation markers (NLR, IgG, CRP and YKL-40) and lower HCV RNA levels, HAI and FS. These data are presented in Table 1 and Table 2.

In patients with non-uremic cHepC patients HAI was positively correlated with serum IgG levels (r = 0.400, p = 0.017), serum IgM levels (r = 0.414, p = 0.013), GGT levels (r = 0.511, p = 0.002), YKL-40 levels (r = 0.459, p = 0.006) and FS (r = 0.671, p < 0.001). On the other hand, FS was positively correlated with serum IgG levels (r = 0.519, p = 0.001), AST levels (r = 0.528, p = 0.001), ALT levels (r = 0.427, p = 0.010), GGT levels (r = 0.439, p = 0.008), YKL-40 levels (r = 0.506, p = 0.002), APRI score (r = 0.616, p < 0.001) and HAI (r = 0.671, p < 0.001) and negatively with serum albumin levels (r = −0.493, p = 0.003). When patients were grouped into 2 according to FS as mild and severe, AST (p = 0.005), ALT (p = 0.01), IgG (p < 0.001), YKL-40 (p = 0.02)

Tab. 1: Comparison of chepC patients with and without renal failure and controls.

	Non-Uremic cHepC patients (N = 35)	cHepC patients with ESRD (N= 15)	Controls (N = 25)	P value
Age (years)	59 ± 9.1	44 ± 10	57 ± 10	**<0.001**
Gender (M/F)	17/18	8/7	13/12	0.993
BMI (kg/m^2)	26 ± 3.8	22 ± 2.4	27 ± 3.6	**<0.001**
AST (U/L)	53 ± 28	24 ± 13	24 ± 16	**<0.001**
ALT (U/L)	56 ± 34	28 ± 18	30 ± 26	**0.001**
ALP (U/L)	90 ± 26	163 ± 105	78 ± 25	**<0.001**
GGT (U/L)	57 ± 48	44 ± 28	37 ± 70	0.559
Total Bilirubin	0.85 ± 0.35	0.66 ± 0.24	0.65 ± 0.25	0.065
Creatinine(mg/dl)	0.87 ± 0.18	7.2 ± 2.5	0.90 ± 0.15	**<0.001**
Uric acid (mg/dl)	5.8 ± 3.7	5.20 ± 1.4	4.9 ± 1.7	0.671
Hemoglobin (g/dl)	13.4 ± 2.0	13.2 ± 2.0	13.4 ± 1.6	0.940
Platelets	208 ± 96	171 ± 74	258 ± 49	0.003
Lökosit (mm^3)	6332 ± 1923	6785 ± 2461	6749 ± 1515	0.627
NLR	2.3 ± 1.4	2.8 ± 0.8	1.9 ± 0.9	**0.05**
PLR	134 ± 109	116 ± 44	129 ± 51	**0.777**
Serum albumin	4.0 ± 0.5	4.0 ± 0.3	4.0 ± 0.4	**0.642**
Immunoglobulin G (mg/dL)	1465 ± 432	1557 ± 527	1143 ± 229	**0.004**
Immunoglobulin M (mg/dL)	124 ± 65	100 ± 58	112 ± 52	0.399
Immunoglobulin A (mg/dL)	234 ± 116	230 ± 141	201 ± 83	0.541
CRP (mg/dL)	0.32 ± 0.27	1.92 ± 2.24	0.63 ± 0.81	**<0.001**
Serum YKL-40 (pg/mL)	76 ± 128	271 ± 182	18 ± 9.1	**<0.001**

All results are reported as mean ± SD. chepC = chronic hepatitis C, BMI = Body Mass Index, NLR = Neutrophil/Lymphocyte Ratio, PLR = Platelet/Lymphocyte Ratio, CRP = C-reactive protein, ESRD = End stage renal disease

Tab. 2: Comparison of inflammation markers and biopsy parameters in chepC patients with and without renal failure.

	Non-Uremic cHepC patients (N = 35), Mean ± SD	cHepC patients with ESRD (N = 15), Mean ± SD	P value
NLR	2.3 ± 1.4	2.8 ± 0.8	**0.159**
CRP (mg/dl)	0.32 ± 0.27	1.92 ± 2.24	**0.015**
Immunglobulin G (mg/dL)	1465 ± 432	1557 ± 527	**0.542**
Serum YKL-40 (pg/mL)	76 ± 128	271 ± 182	**0.001**
APRI score	0.67 ± 0.43	0.36 ± 0.24	**0.004**
HCV RNA	4.4E^{+6} ± 7.7E^{+6}	1.8E^{+6} ± 2.9E^{+6}	**0.201**
Hepatic Activity Index	8.77 ± 2.19	7.07 ± 1.83	**0.011**
Fibrosis Score	3.26 ± 1.59	2.00 ± 1.00	**0.007**

All results are reported as mean ± SD. chepC = chronic hepatitis C, NLR = Neutrophil/Lymphocyte Ratio, CRP = C-reactive protein , ESRD = End stage renal disease

Tab. 3: Stepwise linear regression analysis for predictors of Hepatic Activity Index and Fibrosis Score in non-uremic cHepC patients.

	Standardized β coefficient	Unstandardized β coefficient	t value	P value
Hepatic Activity Index, r^2 = 0.414				
IgG levels	0.02	0.392	2.89	0.007
GGT	0.21	0.524	3.72	0.001
Fibrosis Score, r^2 = 0.550				
YKL 40 level	0.005	0.418	3.48	0.001
APRI	1.880	0.549	4.57	<0.001
Included variables: Age, IgG, GGT and YKL40 for HAI and age, IgG, APRI and YKL40 for FS.				

levels and APRI score were significantly higher in the latter (p = 0.002).

In stepwise linear regression analysis, serum IgG and GGT levels were found as independent predictors for HAI, whereas YKL-40 levels and APRI score were found as predictors for FS (Table 3).

In patients with ESRD, HAI was positively correlated with age (r = 0.656, p = 0.008), serum IgG levels (r = 0.632, p = 0.011), AST levels (r = 0.538, p = 0.039), APRI score (r = 0.617, p = 0.014) and FS (r = 0.858, p < 0.001). FS was positively correlated with age (r = 0.507, p = 0.04), AST levels (r = 0.610, p = 0.016), APRI score (r = 0.733, p = 0.002) and HAI (r = 0.858, p < 0.001) and negatively with thrombocyte count (r = −0.563, p = 0.029). When patients were grouped into 2 according to FS as mild (67%) and severe (33%), AST (p = 0.005), ALT (p = 0.01), IgG (p < 0.001), YKL-40 (p = 0.02) and APRI score (p = 0.002) were higher in the severe group. These patients were older (40 ± 7.7 vs. 53 ± 10; p = 0.01) and had higher APRI score (0.28 ± 0.21 vs. 0.53 ± 0.21, p = 0.06).

In stepwise linear regression analysis (variables: age, IgG, APRI and YKL40), age (t value = 2.97, p = 0.011) and APRI score were independent predictors for HAI (t value = 2.68, p = 0.020). For FS, only APRI score was an independent predictor (t value = 3.98, p = 0.002) (variables: Age, IgG, APRI and YKL40).

DISCUSSION

The results of our study show that YKL-40 levels are independently associated with FS in non-uremic cHepC patients. A similar association could not be confirmed in patients with ESRD.

Many noninvasive biomarkers of fibrosis will be crucial for successful individualized management of disease activity in cHepC patients (7). Currently studies have reported a relation between laminin, hyaluronic acid, type IV collagen, fibronectin, matrix metalloproteinases, metalloproteinase inhibitors, procollagen type III N-terminal propeptide and serum YKL-40 levels with liver fibrosis (7, 15).

YKL-40 is a glycoprotein that plays a role in inflammation and tissue remodelling (14). There remains a controversy regarding the role of YKL-40 in patients with cHepC (7, 16–20). Saitou et al. (16) reported increased levels of YKL-40 in 109 patients with cHepc having severe fibrosis. Hovewer, this could not be confirmed in renal transplant patients or hemodialysis patients (18, 20). In a genetics-based study, Berres et al. reported increased YKL-40 levels in patients with 131G->C polymorphism of the CHI3L1, which were associated with fibrosis. They also reported the protective effect of the G allele (19). In our study, serum YKL-40 levels were correlated with HAI and FS in non-uremic patients and independently associated with FS. However, this finding could not be confirmed in ESRD patients. This may be due to several reasons. Firstly, our study population was relatively small therefore results reaching a statistical power. Secondly, YKL-40 is excreted by the kidneys and the levels are very high compared to non-uremic patients. This may have a limiting effect by interfering with the equations in the statistical analysis. Thirdly, increased inflammation in dialysis patients may be interfering with the YKL-40 measurements (like the abnormal levels of ferritin in the same population) Fourthly but not the last, the fact that the increased levels of YKL-40 are mainly seen in patients with severe fibrosis, less frequency of FS in uremic patients may be limiting the association. Thus, we propose that YKL-40 measurement may provide limited information in ESRD patients.

High IgG levels in cHepC patients have been shown to affect prognosis and response to therapy (21–24). Maruyama et al. (21), reported a correlation between serum IgG levels and the severity of the disease in 102 patients with cHepC and significant decrease in levels by treatment. Gonzàlez-Quintela et al. and Watt et al. also described significantly higher levels of IgG and IgA in patients with cHepC (22, 23). Our results further confirm these studies: significantly higher immunoglobulin levels in cHepC patients compared to controls and correlation of IgG with HAI and FS in non-uremic patients. The association between HAI and IgG was independent. Similar to YKL-40, the association among IgG, HAI and FS could not confirm in ESRD patients.

In ESRD patients, we could not find an association between HAI and FS with the inflammation biomarkers. The most plausible reason may the confounding effect of inflammation due to uremia and in some patients due to comorbid diseases. On the other hand, the decreased viral load by hemodialysis, the release of protective IFN-α and hepatocyte growth factor and relatively lower FS may be affecting the association of inflammatory marker with fibrosis. Thus, the role of these markers may be questionable in ESRD patients.

APRI score is used to predict liver fibrosis in cHepC patients. Schiavon et al. reported a positive predictive value of 66% for fibrosis (METAVIR F2, F3 and F4) when APRI > 0.95 and negative predictive value of 93% when APRI < 0.40 in ESRD patients (17). In another study by the same group, APRI score was found to be superior to YKL-40 with regards to fibrosis in ESRD patients (18). In our study, we found APRI score as an independent predictor for both HAI and FS. Thus, APRI score might be a valuable score to predict FS in ESRD patients.

NLR and PLR are current markers of interest for systemic inflammation (11–13). Data is limited in patients with cHepC. In one study, NLR was proposed as related to viral load and as an independent marker to follow response to therapy, especially in genotype 2 patients (25). We could not confirm any association neither with HAI or FS. Further studies are needed to investigate the role of NLR and PLR in this population.

There are several limitations to our study. The study population is relatively low and this may have prevented some statistical analysis to reach a statistical significance. Also, the majority of the patients were genotype 1 cHepC patients and our results may not be valid for all genotypes. The biopsies were scored according to the modified ISAAC scoring system and therefore, other scoring systems may yield different results. Finally, the relatively low FS observed in the patients may have interfered with the analysis.

As a conclusion, YKL-40 may be a valuable marker to predict fibrosis in non-uremic patients with cHepC. Serum IgG and GGT levels, used frequently in routine practice, may be also good predictors for HAI. However, the role of inflammatory markers in the prediction of HAI and FS may be limited in ESRD patients. In this population, APRI score may provide useful information with regards to HAI and FS.

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CONFLICT OF INTEREST

Declare no conflict of interest.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

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Paraesophageal Hernia as a Cause of Chronic Asymptomatic Anemia in a 6 Years Old Boy; Case Report and Review of the Literature

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ABSTRACT

Esophageal hiatal hernia is defined as the prolapse of one or more intra-abdominal organs through the esophageal hiatus. Four types are identified: type I or sliding hiatal hernia, type II or paraesophageal hernia (PEH), type III or mixed hernia and type IV. Congenital type II esophageal hiatal hernia is caused by a remaining gap after the formation of pleuroperitoneal membrane. We present a case of a six years old boy admitted to our department, appearing with asymptomatic anemia, who was incidentally diagnosed with Type II esophageal hiatal hernia. After diagnostic investigation, the prolapsing stomach pouch was reduced, the hernia sac was excised, the crura of diaphragm were converged and a total fundoplication was performed, via open method. The patient had an uncomplicated postoperative period. We conclude that: 1) esophageal hiatal hernia should be included within diagnostic approach of a child with chronic non-hereditary anemia, 2) after a Type II esophageal hiatal hernia is diagnosed, a hernia repair surgery is indicated in short time, due to the severity of possible complications and 3) through the performance of total fundoplication, it is secured that the subdiaphragmatic abdominal part of esophagus will be retained, preventing the development of post-operative gastroesophageal reflux disease.

KEYWORDS

type II esophageal hiatal hernia; gastric volvulus; child; gastroesophageal reflux disease; fundoplication

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INTRODUCTION

Esophageal hiatal hernia is defined as the prolapse of one or more intra-abdominal organs through the esophageal hiatus. Four types are identified: type I or sliding hiatal hernias (85–95%), type II or paraesophageal hernias(3.5–5%), type III or mixed hernias (10%), and type IV or complex hernias (0–5%) (1, 2). Based upon the width of esophageal hiatus, two types are identified: wide (diameter > 2 cm) and small (diameter < 2 cm) (3).

Type II hiatal hernias are divided into congenital and acquired. Congenital type II hiatal hernia is caused by a remaining gap after the fusion of pleuroperitoneal folds and the formation of pleuroperitoneal membrane. During intrauterine life, this gap has a diameter less than 1 cm. Its dimensions increase over time (4, 5). Alternatively, the gap is, also, caused by abnormal formation of the lumbar part of diaphragm from mesodermal cells, originating from lumbar spine. Congenital hiatal hernias are more common in girls (Female/Male ratio = 4) (6). Baglaj SM et al. refer to 21 cases of type II hiatal hernias with familial predisposition, 6 of which were cases of twins (7).

Acquired hiatal hernias are more common in children suffering from cerebral paralysis, chronic connective tissue diseases, such as Ehlers-Danlos syndrome and Marfan syndrome, whereas, they can, also, occur as a postoperative complication of anti-reflux surgery, when the crura of diaphragm where, technically, not properly converged (8, 9, 10).

In type II hiatal hernia, the hernia sac is located in the right side of esophagus – due to insufficient development of the right crus of diaphragm – and inside the sac a part or the entire stomach prolapses (11). In early stages, the gastroesophageal angle remains acute and is located within the abdominal cavity. Owing to negative intrathoracic pressure, the prolapsing stomach pouch, usually, remains within the thorax.

Symptomatology of type II hiatal hernias involves recurrent infections of the lower respiratory tract, atypical symptoms of the gastrointestinal tract, whereas in 8–8.48% of all cases it may remain asymptomatic (1, 11). Karpelowsky JS et al. encountered successfully 59 pediatric patients (median age = 23.4 months old) with type II hiatal hernia over 42 years (1). The majority of the patients suffered from more than one symptoms: 32 patients (54.24%) presented with recurrent infections of the lower respiratory tract, 24 (40.68%) with repeated vomiting after meal, 20 (33.9%) with symptomatic anemia, 18 (30.51%) with growth delay and 6 (10.17%) with dysphagia. Five out of fifty nine patients (8.48%) remained asymptomatic, with the diagnosis made incidentally (1).

In this case report we present a rare, atypical and, essentially, subclinical case of Type II hiatal hernia. After comprehensive research of the current, relevant literature, we attempt to answer critical questions, which remain topical.

CASE REPORT

We present a case of six years old boy, who was admitted to the Emergency Department of the 1st Department of Pediatric Surgery, Aristotle University of Thessaloniki, due to incidental ingestion of a radiopaque foreign body 12 hours ago. The little patient had free family and perinatal history. His medical history was remarkable for undiagnosed iron deficiency, resistant to therapy and early satiety after low volume meals.

The child had normal lung sounds and intestinal peristalsis. A posteroanterior neck, chest and abdominal x-ray were conducted, which – except from the radiopaque foreign body in the right lower quadrant of the abdomen – showed a radiolucent spherical halo in the lower third of the mediastinum (Fig. 1).

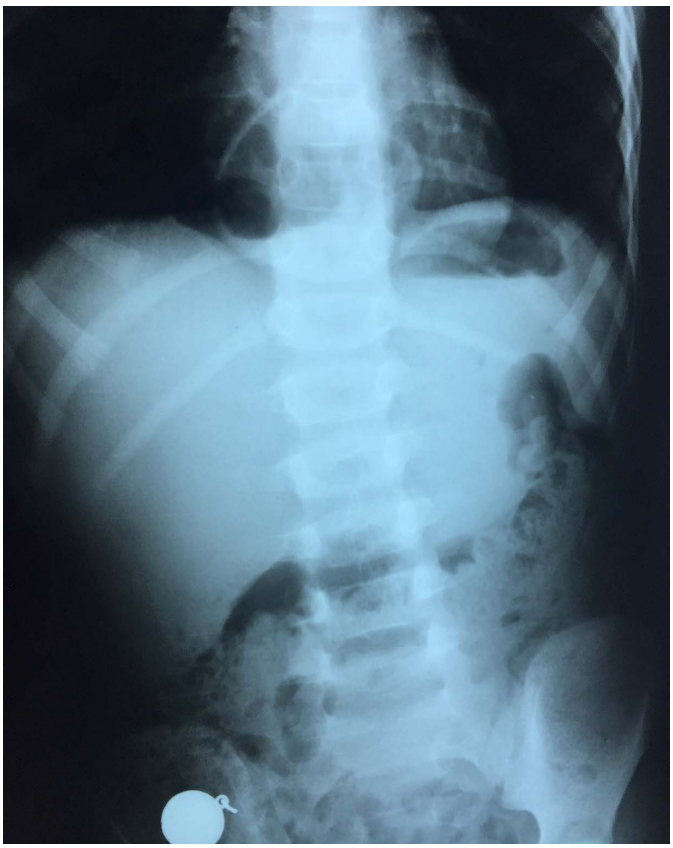

Fig. 1: A radiolucent spherical halo in the lower third of the mediastinum.

Subsequently, a lateral chest x-ray revealed the subvertebral location (in the posterior mediastinum) of this semicircular radiolucent spherical halo (Fig. 2).

Laboratory tests confirmed the iron deficiency, while homozygous or heterozygous hemoglobinopathy were excluded (HbA_2: 2.7%, HbF < 0.5%) (Table 1).

Tab. 1: Preoperative values of hematological parameters.

Parameter	Value
Ht	39%
Hb	11.3 g/dl
MCV	59.3 fl
MCH	18.3 pg
MCHC	30.9 g/dl
RDW	20.8%
Ferritin	1.5 μg/dl

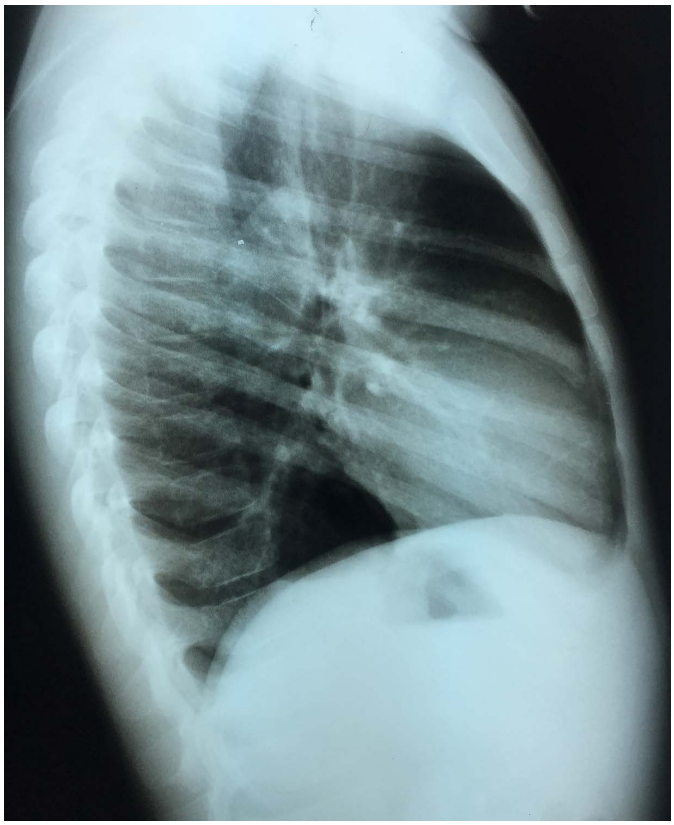

Fig. 2: Subvertebral location (in the posterior mediastinum) of this semicircular radiolucent spherical halo.

Afterwards, barium meal examination was conducted, which confirmed the intra-thoracic location of stomach's fundus and part of stomach's body and revealed a hourglass stenosis in the esophageal hiatus. The gastroesophageal junction appeared to remain within the abdominal cavity. Furthermore, no gastroesophageal reflux or delayed gastric emptying was noticed (Fig. 3).

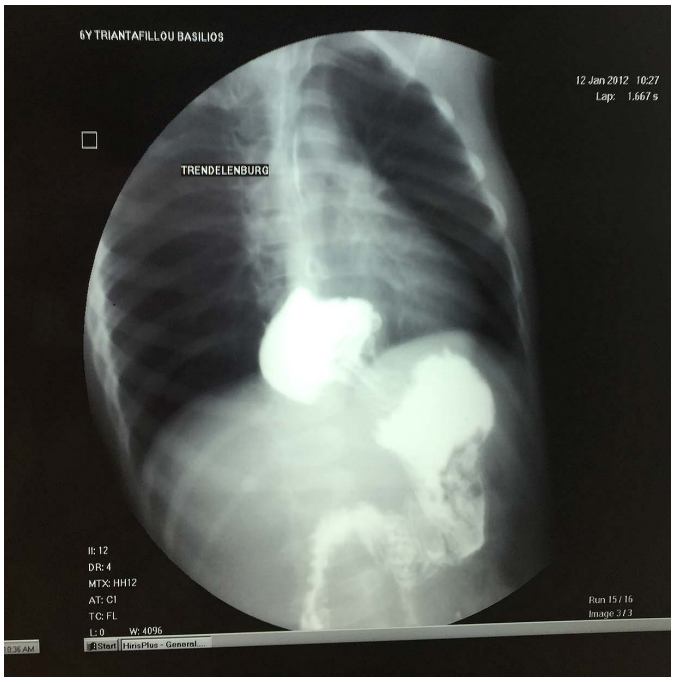

Fig. 3: Intra-thoracic location of stomach's fundus and part of stomach's body with a hourglass stenosis in the esophageal hiatus.

Endoscopy of the upper digestive tract did not show any signs of esophagitis, but revealed an oversize type II hiatal hernia, with a prolapse of the stomach's fundus and part of its body (Fig. 4).

Subsequently, MRI of the chest and upper abdomen revealed the width of the gap (4 cm), the location of the stomach's fundus and part of its body within the hernia sac and the intra-abdominal location of the gastroesophageal junction (Fig. 5).

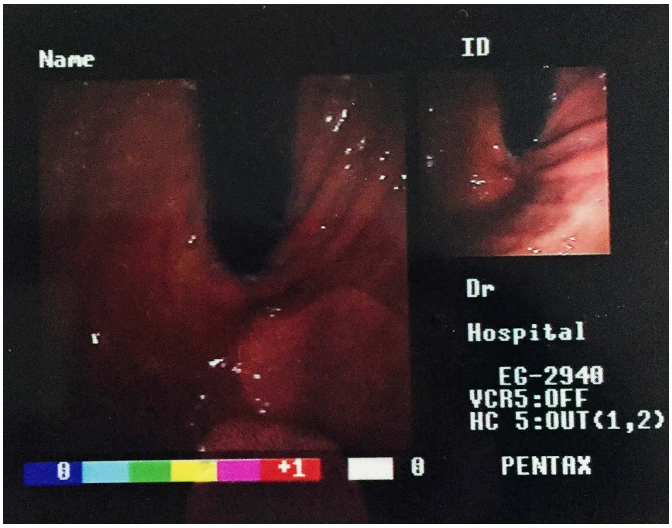

Fig. 4: Notice the wide gap through which the stomach prolapses, in the context of a type II hiatal hernia.

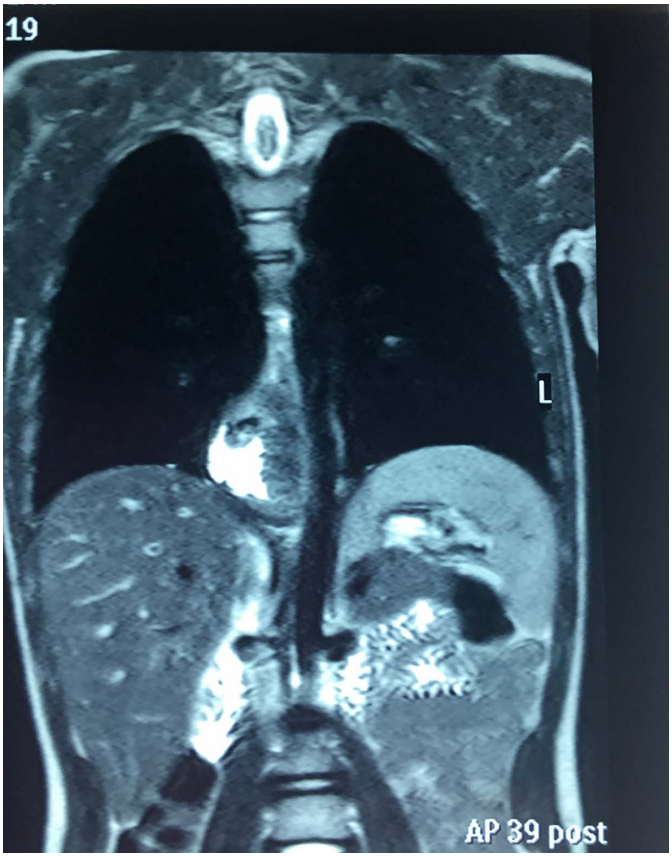

Fig. 5: MRI of the chest and upper abdomen reveals the width of the gap (4 cm), the location of the stomach's fundus and part of its body within the hernia sac and the intra-abdominal location of the gastroesophageal junction.

After diagnostic documentation of Type II hiatal hernia, elective surgical repair followed. With the patient under general endotracheal anesthesia and after conduction of a left mini Kocher section, the prolapsed stomach pouch was reduced, the hernia sac and the adhesions of the right crus of diaphragm in the parietal pleura and in the pericardium were excised, the crura of diaphragm were converged with 3 separate Vigryl 2/0 stitches and a total 360° fundoplication was performed (Nissen's fundoplication).

RESULTS

The little patient had an early recovery from anesthesia, after surgery. On the second postoperative day, the nasogastric tube was removed and we began gradually oral feeding. On the fifth postoperative day, the patient was discharged home in excellent general condition. The first postoperative clinical evaluation was performed after 3 months, when the excellent general condition of the patient was documented. The quantity of food was, gradually, increased without any episode of gastric dilatation or dysphagia. Blood test revealed an increase in ferritin levels up to 45 μg/dl. Also, red blood cell indices significantly improved compared to those postoperatively (MCV: 77.8 fl vs. 59.3, MCH: 25.7 pg vs. 18.3 and MCHC: 33 g/dl vs. 30.9).

One year later, the patient remains asymptomatic with normal hematological profile. Barium meal examination was conducted again, and confirmed the intra-abdominal location of the stomach, the acute gastroesophageal angle, no gastroesophageal reflux and a normal gastric emptying (Fig. 6, 7).

DISCUSSION

Until now, less than 10 case reports of prenatally diagnosed hiatal hernia have been published (12, 13, 14). When an intra-thoracic cystic mass is found, the differential diagnosis should include diseases, such as hiatal hernia, macrocystic adenomatoid malformation, esophageal duplication cyst, neurenteric cyst and pericardial cyst. Yamamoto N et al. diagnosed a Type II hiatal hernia in a fetus with asplenia syndrome (absence of spleen, dextrocardia, atrial and ventricular septal defect, pulmonary artery stenosis and malrotation) (14). Possibly, as prenatal evaluation rapidly evolves, there will be more prenatally diagnosed congenital hiatal hernias cases in the future. This possible evolution would change the landscape of diagnostic investigation and repair of hiatal hernias, even in a subclinical stage.

Early symptoms of type II hiatal hernias are not caused by gastroesophageal reflux, as the anti-reflux mechanism is not abolished, but, come as a result of acute gastric dilatation – pressing the organs within the chest cavity – or even gastric volvulus. Rarely, a hiatal hernia may present with anemia and delayed growth, as in our case. Over time, dimensions of the gap increase, the stomach is, further, attracted upwards – due to negative intra-thoracic and positive intra-abdominal pressure – the gastroesophageal junction moves in a higher position, resulting in the abolition of the anti-reflux mechanism (1, 15). Yazici M et al. noted that 68.4% of their patients with type II hiatal hernia were suffering from gastroesophageal reflux disease (16). Enlargement of the hiatus may result in a change from type II to type IV hiatus hernia, as other intra-abdominal organs may prolapse in the chest cavity, such as epiploon, small and large intestine (15, 17, 18).

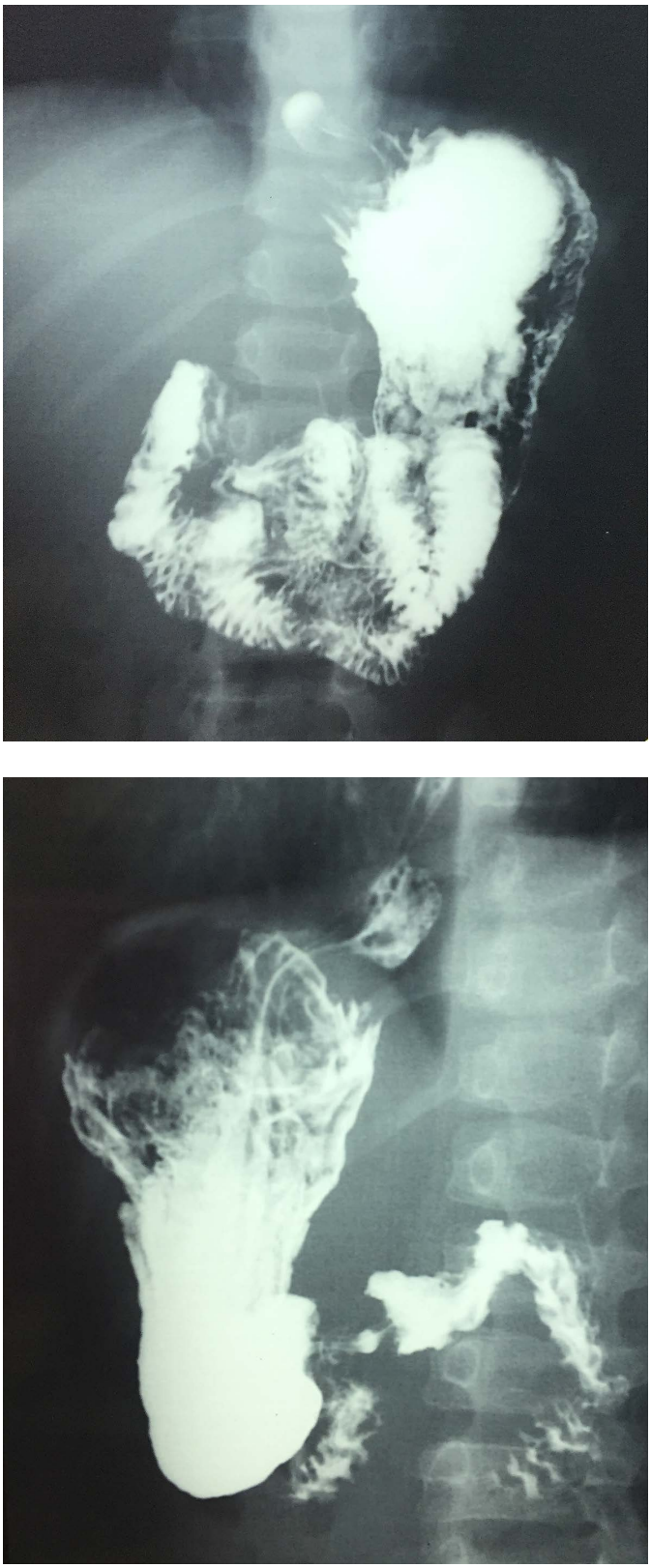

Fig. 6, 7: Barium meal examination confirmed the intra-abdominal location of the stomach, the acute gastroesophageal angle, no gastroesophageal reflux and a normal gastric emptying.

According to Karpelowsky JS et al., from the 5 out of 59 cases, in which type II hiatal hernia was incidentally diagnosed, in 3 out of 59 (5.01%) patients featured subclinical anemia, later noticed, as in our case (1). Iron deficiency anemia may come as a result of slow, chronic blood loss, caused either by esophagitis – due to gastroesophageal reflux disease – or ulcers developing in the mucosa of the prolapsing stomach's part (1). In the present case, endoscopy of the upper gastrointestinal tract was performed to exclude the presence of esophagitis.

We believe that esophageal hiatal hernia, in general, along with its consequences should be included within diagnostic investigation of a child with chronic non-hereditary anemia and weight stagnation, in order to avoid a delayed diagnosis and prevent possible complications.

The diagnosis of asymptomatic type II hiatal hernia may first be suspected because of an abnormal chest x-ray, showing the gastric bubble within the thorax. In 32% of all cases, air fluid levels in the lower quadrant of mediastinum, may, also, be detected (1). Given that the hernia is asymptomatic in an early stage, a supine chest x-ray after the placement of a nasogastric tube and repetition of it, in order to confirm the intra-thoracic place of the tube, would significantly contribute to the diagnosis (19, 20). The differential diagnosis includes retrosternal Morgagni hernias, mediastinal cysts, tumors and cystic echinococcosis (1, 21).

Barium meal examination and endoscopy of the upper digestive tract highly contribute to the confirmation of the diagnosis. Since barium meal examination showed the intra-thoracic location of the gastroesophageal junction and no findings suggestive of gastroesophageal reflux were noted after endoscopy of the upper digestive tract, the possible diagnosis of type I or III hiatal hernia was excluded. It should be pointed out that the barium meal examination is not a foolproof way to evaluate the location of the gastroesophageal junction (1, 22). Chest and upper abdominal MRI contributed to a more accurate evaluation of the prolapsing part, the location of the gastroesophageal junction, the width of the gap, while a possible pathology of the lower respiratory system or the mediastinum was excluded (21).

Theoretically, in type I and III hiatal hernias there is a great risk of incarceration of the prolapsing stomach, or even gastric volvulus. As a result, an elective, either laparoscopic or open, hernia repair is indicated (20). Karpelowsky JS et al. do not share with this "scaremongering" (1). During the treatment of 59 type II hiatal hernia cases, over 40 years, no such complication was noticed (1).

There is no age limit for laparoscopic hernia repair. Kundal AK et al. performed a laparoscopic hiatal hernia repair in 4 infants (20). Van der Zee DC et al. performed the same operation in 2 infants, managing, successfully, to reduce the stomach, converge the gap and perform a total fundoplication (23).

The surgical procedure of type II hernia repair includes the excision of the hernia sac, the reduction of the prolapsing stomach and the convergence of the crura of diaphragm (9, 18, 24). It is widely believed that the excision of the hernia sac allows safer convergence of the crura of diaphragm, eliminating the risk of relapse (25). Some researchers disagree, because of possible iatrogenic injuries during hernia sac excision, suggesting that a partial excision is enough (26).

The operation is completed with anti-reflux surgery- mainly, fundoplication- as performed in our patient (1, 18, 19, 21, 24). Fundoplication is indicated in cases, in which gastroesophageal reflux disease is pre-operatively diagnosed (1, 16). Yousef Y et al. encountered 14 little patients with type II hiatal hernia, 4 laparoscopically and 10 openly. On 13/14 patients (93%) they performed anti-reflux surgery (22). Karpelowsky JS et al. encountered 20/59 patients, with no signs of gastroesophageal reflux disease, not performing an anti-reflux surgery (1). However, within post-operative follow up, they found that 12/20 patients (60%) developed gastroesophageal reflux disease.

It is believed that this progress is iatrogenic, due to the mobilization of the esophagus and the abolition of normal anti-reflux mechanism. With the performance of fundoplication, the stomach is stabilized in the abdomen, while a sufficient sub-diaphragmatic abdominal part of esophagus is retained (1). The operation is, preferably, performed with abdominal access, except for the cases of short esophagus, in which thoracic access is preferable (19).

Esophageal hiatal hernias with wide gap are characterized by inadequate diaphragm development, causing technical difficulty in the convergence of the gap, which is performed under tension, increasing the risk of relapse. Wang G et al. suggest that the primary repair of a wide gap is technically feasible, without using prosthesis. Besides, they characterize the existence of prosthesis in a developing organism as a disadvantage (19). Another important parameter of relapse is a short esophagus, which results in a technically difficult performance of fundoplication. In this cases, Collis-Nissen procedure is preferred (27).

CONCLUSIONS

1. Esophageal hiatal hernia should be included within diagnostic approach of a child with chronic non-hereditary anemia.
2. After the diagnosis of type II esophageal hiatal hernia is established, performance of a hernia repair surgery is indicated in short time, due to the severity of possible complications.
3. Through the performance of total fundoplication, it is secured that the subdiaphragmatic abdominal part of esophagus will be retained, preventing the development of post-operative gastroesophageal reflux disease.

CONFLICT OF INTEREST

None of the contributing authors have any conflict of interest, including specific financial interests or relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

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Antiplatelet Therapy in a Patient with Coronary Artery Disease and Myelodysplastic Syndrome with Thrombocytopenia

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ABSTRACT

To date, there are no sufficient data to make firm recommendations on the treatment of patients with severe thrombocytopenia who require antiplatelet therapy after experiencing acute coronary syndrome. Therefore, we think that it is important to communicate the experience with individual cases. We report the case of a patient who presented with pericardial effusion causing cardiac tamponade. He had thrombocytopenia associated with myelodysplastic syndrome, and ten weeks before this admission, percutaneous transluminal coronary angioplasty with implantation of drug-eluting stents was performed for non-ST-segment elevation acute coronary syndrome. Platelets in myelodysplastic syndromes are dysfunctional, which exacerbates bleeding from thrombocytopenia, and the management of atherosclerotic cardiovascular disease in these patients is challenging.

KEYWORDS

myelodysplastic syndromes; thrombocytopenia; acute coronary syndrome; antiplatelet therapy; clopidogrel; drug-eluting stents

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INTRODUCTION

The safety of antiplatelet therapy in patients who have acute coronary syndrome and thrombocytopenia is unknown, and there are no randomized studies to make firm recommendations on treatment approaches in such patients. Likewise, in patients with thrombocytopenia, the risk of bleeding varies and may depend upon the underlying cause (1, 2). Platelets in myelodysplastic syndromes are dysfunctional (3) and, after leukemic transformation and infection, bleeding is the next most common cause of death (4–6). On the other hand, thrombocytopenia itself is not protective and acute coronary syndromes have been reported in patients who have thrombocytopenia that is associated with various disorders (7–9). In the setting of cancer and hematologic malignancies, some smaller sized retrospective studies have shown that aspirin may be beneficial in thrombocytopenic patients experiencing acute coronary syndromes (10, 11). Therefore, the management of atherosclerotic cardiovascular disease in patients who have myelodysplastic syndrome with thrombocytopenia is challenging.

We report the case of a 77-year-old man who presented with pericardial effusion causing cardiac tamponade. He had thrombocytopenia associated with myelodysplastic syndrome, and ten weeks before this admission, percutaneous transluminal coronary angioplasty with implantation of drug-eluting stents was performed for non-ST-segment elevation acute coronary syndrome.

CASE REPORT

A 77-year-old man was admitted to our Department because of a pericardial effusion causing cardiac tamponade. The patient was a former smoker with a history of hypertension, hyperlypidemia, type 2 diabetes, interstitial lung disease, colon diverticular disease, and adenomatous colonic polyps. Ten weeks before this admission, he had been hospitalized for a non-ST-segment elevation acute coronary syndrome. Coronary angiography showed a 90% stenosis of the left main trunk, 99% stenosis of the proximal anterior descending artery, 75% stenosis of the mid portion of the anterior descending artery and 75% stenosis of the posterior descending artery. Percutaneous transluminal coronary angioplasty was performed with implantation of drug-eluting stents. On initial testing, the patient had pancytopenia with leukocytes 3.23 × 10^9/l, hemoglobin 10.9 g/dl and platelets 58 × 10^9/l. The presence of platelet clumping was reported in a peripheral blood smear. Ferritin, vitamin B12 and serum folate were normal. A bone marrow study was not performed. The patient was prescribed ticagrelor and acetylsalicylic acid. Two months after being discharged, he was admitted to the Department of Neurology because of decreased visual acuity in the right eye due to ischemic optic neuropathy. During this 2nd hospitalization, the count of platelets ranged from 36 × 10^9/l to 47 × 10^9/l. Peripheral blood smear confirmed the thrombocytopenia without platelet clumping. Tests for human immunodeficiency virus, hepatitis B surface antigen, hepatitis B core antibody, and hepatitis C antibody were negative. IgM antibodies directed against the VCA of Epstein–Barr virus, cytomegalovirus and parvovirus B19 were also negative. Serum angiotensin converting enzyme was normal. In the imaging studies the spleen was of normal size. He was evaluated by a hematologist and was discharged and referred to the outpatient hematology clinic for further investigation. Five days later, the patient was readmitted because of pericardial effusion with cardiac tamponade. 1100 ml of serosanguineous pericardial fluid was drained. Microbiologic studies of the pericardial fluid for bacteria, acid-fast bacteria, and fungus were negative. Cytologic analysis of the pericardial fluid was negative for malignant cells. A bone marrow examination revealed myelodysplastic syndrome (refractory anemia with excess blasts-1) and cytogenetics showed 20q deletion. During this third hospitalization several episodes of epistaxis developed and we decided to discontinue ticagrelor and acetylsalicylic acid, and antiplatelet therapy with clopidogrel alone was initiated. At 12 months follow-up the patient remains well, and there were no further thrombotic complications or significant bleeding episodes, with a platelet count of 31 × 10^9/l to 60 × 10^9/l during this time period. A follow-up echocardiogram showed no recurrence of pericardial effusion, and the Hematology Service indicated that the patient was managed expectantly in order to evaluate the disease tempo.

DISCUSSION

To date, there are no guidelines on the treatment of patients with thrombocytopenia who require antiplatelet therapy after experiencing acute coronary syndrome. Therefore, it is important to communicate the experience with individual cases. In patients with thrombocytopenia, the risk of bleeding varies and may depend upon the underlying cause (1, 2). Younger circulating platelets are larger and more hemostatically active. For example, patients with primary immune thrombocytopenia have younger circulating platelets and, consequently, less severe bleeding symptoms than patients with a similar degree of thrombocytopenia due to bone marrow failure, whose platelets tend to be older and hypoactive (1, 3). Platelets in myelodysplastic syndromes often express abnormally low levels of procoagulant cell surface markers or lack intracellular granules, which exacerbates bleeding from thrombocytopenia (4–6).

The patient had a history of iron-deficiency anemia due to gastrointestinal blood losses, and deletion 20q on cytogenetic analysis. Thrombocytopenia is the most frequent cytopenia in myelodysplastic syndrome patients presenting with deletion 20q on cytogenetic analysis and, in these cases, the dysplasia is often subtle (12). Both factors could decrease the suspicion of the hematologic process, and drug-eluting stents were implanted for the multivessel revascularization of the patient with complex coronary anatomy. With respect to the pericardial effusion, a serositis as autoimmune manifestation of myelodysplastic syndrome has rarely been reported (13, 14). In these cases, the pericardial effusion usually responds to treatment with corticosteroids and this origin was unlikely in our patient. Likewise,

the performed studies and evolution ruled out the possibility of pericardial effusion due to infiltration of blast cells.

In summary, our patient, with drug-eluting stents, had a high thrombotic and hemorrhagic risk. There was a delay in establishing the diagnosis of myelodysplastic syndrome, and after 10 weeks with dual antiplatelet therapy, clopidogrel alone was effective with a platelet count of 31 × 10^9/l to 60 × 10^9/l.

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Parathyroidectomy in Hyperparathyroidism-Associated Calciphylaxis in End-Stage Renal Disease Should be Prompt and Radical – a Case Report with Two Original Therapeutic Modifications and Successful Outcome

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ABSTRACT

We present a case of severe calciphylaxis in both thighs and calves in a patient with end-stage renal disease and advanced secondary hyperparathyroidism with successful outcome after modified therapeutic approach. The cause of calciphylaxis is multifactorial. In our case, not only severe hyperparathyroidism and mediocalcinosis, but also medication (warfarin, calcium and active vitamin D) was involved. Because the initial conservative therapy was not successful, we indicated parathyroidectomy. However, we were not able to localize parathyroid glands and we contraindicated bilateral neck exploration due to the patient's critical status. Therefore, we decided for total thyroidectomy with total parathyroidectomy. Surgery was uncomplicated and histology confirmed that all four parathyroid glands were removed. The expected post-operative hypocalcaemia was asymptomatic and we did not use any calcium supplementation or vitamin D. Thyroid hormone replacement was easy. After surgery, the large and multiple subcutaneous defects started to heal. We achieved complete healing within several months of continuing dedicated care. There is no recurrence after three years. Prompt and radical surgical parathyroidectomy was extremely useful in our patient.

KEYWORDS

calciphylaxis; thyroidectomy; parathyroidectomy; haemodialysis; hypocalcaemia

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BACKGROUND

Calcific uremic arteriolopathy (CUA; calciphylaxis) is a rare but very serious disease affecting (not exclusively) kidney failure patients (1, 2). Mortality is high and sepsis is the usual cause of death.

Diagnosis and treatment of CUA is multifactorial and multidisciplinary (1–5). Therefore, wide spectrum of medical specialities should be familiar with this disease. Thanks to the multidisciplinary approach, the treatment of our case was finally successful.

The first clinical sign of CUA is usually pain, subcutaneous induration and skin patches resembling livedo reticularis. Within few days black cutaneous and subcutaneous ulcers and necrosis develop. The affected area could be larger than 10 cm. CUA typically affects inner sides of calves, inner and outer sides of thighs and, sometimes, abdominal areas. On the contrary, periphery of extremities is not involved (6). Sometimes, rhabdomyolysis is the initial clinical sign.

There is no single cause of CUA. Abnormal calcium (Ca) and phosphorus (P) homeostasis, sometimes associated with a disorder of parathyroid glands predisposes to this disease. Other risk factors include vitamin K antagonists (warfarin), low anticoagulant activity of protein C and S, hypoalbuminemia/malnutrition, inflammation, obesity, diabetes mellitus, etc. Especially warfarin is dangerous (7).

The treatment of CUA is local and systemic. This latter includes correction of all the above mentioned predisposing conditions. In addition, intravenous sodium thiosulphate is useful, as well as parenteral antibiotics, nutritional support, etc. In CUA associated with hyperparathyroidism, surgical parathyroidectomy (PTX) should be considered (7, 8) but the opinions are not equivocal (4). Comprehensive reviews of CUA therapy have recently been published (2–4, 6).

CASE REPORT

A 62-year-old man with known renal disease presented to the Emergency Department of our hospital in June 2014 with severe breathlessness and general oedema. His past medical history was notable for a deep vein thrombosis in 1992 treated with warfarin for several years and advanced kidney disease ("end-stage kidney disease" in renal biopsy in 2012) associated with secondary hyperparathyroidism. Medications on admission included paricalcitol (1 μg/day), calcium (1000 mg/day), sodium bicarbonate and acetylsalicylic acid.

On examination, his blood pressure was 160/114 mm Hg, respiratory rate 22 per minute. Heartbeats were regular, 85 per minute. Weight gain was 10 kg during the last 10 days. Arteriovenous fistula created in 2012 on left forearm was malfunctioning. There was a small skin defect on the right lower limb. Serum creatinine was 632 μmol/l, urea 23.4 mmol/l, C-reactive protein 30 mg/l (reference range 0–5), total serum calcium 2.02 mmol/l. Parathyroid hormone (PTH) measured as whole molecule (1–84 PTH) was 37 pmol/l.

The patient underwent an acute haemodialysis (HD) with ultrafiltration through central dialysis catheter inserted in the right internal jugular vein and then we started regular HD.

Shortly thereafter, catheter-related sepsis, right internal jugular vein thrombosis and bronchopneumonia developed. The local skin defect became larger and new extensive skin ulcers appeared on inner sides of thighs and calves bilaterally, with typically visual appearance of CUA. We immediately stopped paricalcitol, Ca supplements and sodium bicarbonate and started sevelamer carbonate. The systemic therapy included sodium thiosulphate (magistraliter preparation), 100 ml 25% solution after HD, with controls of serum Ca and acid base balance (9), calcimimetics, antibiotics, nutritional support, daily HD (2, 5). Local care was carried out daily.

Despite intensive conservative treatment calciphylaxis did not improve (Figures 1a, 1b). In some areas, the lesions healed partly, but new lesions appeared (Figure 1b). In other areas, no improvement was observed (Figure 1a). The lesions were extremely painful and the patient became bed-ridden and suffered a lot. PTX was indicated but a detailed search for enlarged parathyroid gland was negative

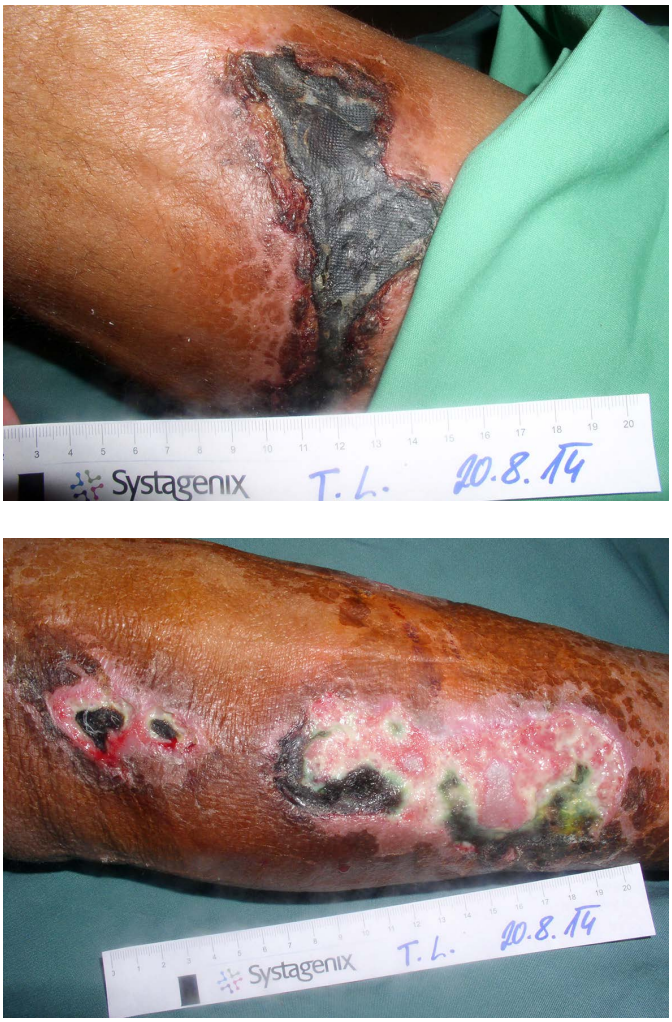

Fig. 1: Calciphylaxis. Photography was taken before the surgery, at the end of intensive conservative therapy period. Patient was fully bed-ridden at that time. a) no healing after conservative local treatment, b) large area with some improvement, but also with new lesions at the edge of the scars.

on four times repeated ultrasound examination. Scintigraphy and magnetic resonance imaging were also negative.

Without proven localization of parathyroid tissue, bilateral neck exploration is the treatment of choice (11). However, several comorbidities and patient's critical health state contraindicated this rather lengthy approach. Therefore, we decided to surgically remove parathyroid glands together with thyroid gland (total thyroidectomy with total parathyroidectomy), which is shorter and less risky surgery. We were aware that this would lead to iatrogenic hypothyroidism, but also to the mandated (total) PTX.

The surgery was uncomplicated and successful. Histology confirmed total removal not only of thyroid tissue, but also of all four (nodular) parathyroid glands. Interestingly, all parathyroid glands were only slightly increased (max. 10 mm) and all were located within thyroid tissue. Histology of thyroid gland was normal and thyroid hormone substitution was easy, targeted at normal thyrotropin (TSH) levels.

The patient's serum PTH level dropped below the detection limit shortly after PTX. He also developed hypocalcaemia (mean total serum Ca 1.7 mmol/l). Because of our fear of new vascular calcium deposits, we did not use any calcium supplementation. Surprisingly, no clinical signs of hypocalcaemia occurred (i.e. paresthesia, spasms or cardiac rhythm disorders).

After surgery, the skin defects were improving day by day and finally they healed up completely (Figure 2). The pain disappeared and the patient's mobility improved dramatically. Six months after admission the patient was discharged. On outpatient basis, he has been dialysed three times a week. Now, he takes very low dose of calcium acetate per day and 5000 IU of cholecalciferol once in two weeks. His is asymptomatic.

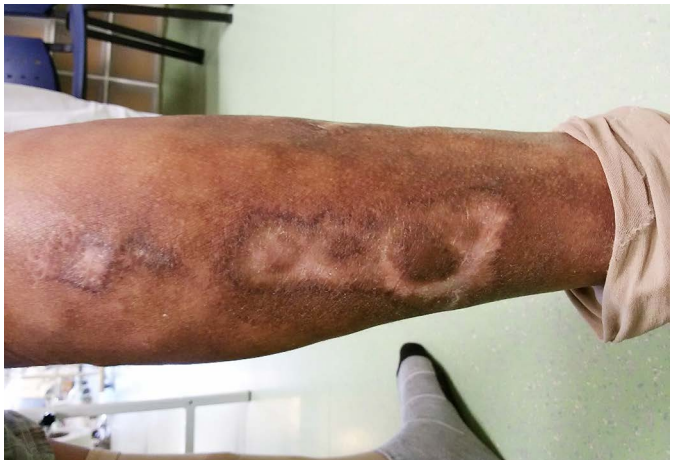

Fig. 2: All lesions were successfully healed using careful local care with no recurrence. Photography was taken in June 2017. The patient is pain-free, moving without help, with active life with haemodialysis.

DISCUSSION

The treatment approach in our case has two unique and novel characteristics, not yet described in the literature:

1) Surgical total parathyroidectomy performed at the cost of iatrogenic hypothyroidism (total thyroidectomy with parathyroidectomy).
2) Iatrogenic hypocalcaemia not treated by calcium substitution and fully asymptomatic.

Ad 1) Based on our previous unpublished experience and on the unsatisfactory course in the presented patient during the first weeks after admission, we decided for parathyroidectomy. However, we were not able to find enlarged parathyroid glands on four-times repeated ultrasound examination performed by a skilled sonographist. Magnetic resonance and scintigraphy were negative as well. Due to the patient's poor clinical status, bilateral neck exploration, which is a lengthy procedure, was contraindicated. Our selected approach, i.e. curative total parathyroidectomy at the cost of total thyreoidectomy, is quite unusual. We have not found any similar case in the literature. The neck surgery in our patient was successful, and it definitely contributed to the cure of CUA.

Brandenburg (4) has recently reported on the experts' discussion about important issues associated with CUA, namely on their opinion about surgical treatment of CUA associated with secondary hyperparathyroidism. There was a wide spectrum of answers, ranging from no recommendation of PTX to very strong support of PTX. Our own results support PTX without delay. We can also confirm another conclusion from this work: warfarin is a strong risk factor of CUA.

The explanation why we did not locate the parathyroid glands was probably their relatively small size (despite nodular structure and autonomic features) and namely their intrathyroid localization. So, if the clinical and laboratory findings are in accord with hyperparathyroidism, then negative imaging using ultrasound, scintigraphy and even nuclear magnetic resonance cannot serve as a document against this disorder.

Ad 2) As expected, hypocalcaemia developed shortly after surgery. We stopped sodium thiosulphate right after parathyroidectomy because it could intensify hypocalcemia (10). We were afraid of calciphylaxis recurrence in our patient because of his preexisting mediocalcinosis. Therefore, we decided not to provide calcium substitution. We only carefully observed the patient's clinical and laboratory condition. Fortunately, there were no clinical symptoms of hypocalcaemia during immediate post-operative period and in the long-term course. The asymptomatic course of long-lasting hypocalcaemia can be, at least hypothetically, explained by previous significant positive calcium balance with resolving calcium tissue deposits. We were not able to find any case without calcium supplementation after total PTX in the literature, but we wish to encourage this approach for other similarly complicated patients.

CONCLUSION

Our patient had severe painful cutaneous and subcutaneous necroses in both lower extremities due to calciphylaxis. The initial approach, despite its complexity, did not help. Marked improvement and finally full healing was only achieved after total parathyroidectomy, together with total thyroidectomy. We have not found any publication describing this surgical approach in this setting. Also, to

our best knowledge, withholding of calcium supplementation in postsurgical hypocalcaemia has not been reported yet. Because of complex systemic and local care provided by a co-operating team of several dedicated specialists, this unique mode of treatment was fully successful.

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CONFLICT OF INTEREST

None declared.

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A Case of Pseudoaneurysm of the Internal Carotid Artery Following Endoscopic Endonasal Pituitary Surgery: Endovascular Treatment with Flow-Diverting Stent Implantation

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ABSTRACT

Internal carotid artery (ICA) pseudoaneurysm is a rare complication of endoscopic endonasal surgery occurring in 0.4–1.1% of cases. Pseudoaneurysms can subsequently result in other complications, such as subarachnoid hemorrhage, epistaxis, and caroticocavernous fistula with resultant death or permanent neurologic deficit. In this case, we illustrate endovascular treatment with a flow-diverting stent for an ICA pseudoaneurysm after endoscopic endonasal surgery for a pituitary adenoma in a 56-year-old male. Surgery was complicated by excessive intraoperative bleeding and emergent CT angiography confirmed an iatrogenic pseudoaneurysm on the anteromedial surface of the ICA. The pseudoaneurysm was treated endovascularly with flow-diverting stent implantation only. Follow-up CT angiography after three months demonstrated occlusion of the pseudoaneurysm.

KEYWORDS

pituitary; injury; pseudoaneurysm; endovascular; flow diverting stent

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INTRODUCTION

Internal carotid artery (ICA) pseudoaneurysm is a rare complication of endoscopic endonasal pituitary surgery (1). The incidence of pseudoaneurysm following endoscopic endonasal pituitary surgery has been reported as 0.4–1.1% (1). Complications of ruptured pseudoaneurysm include subarachnoid hemorrhage, caroticocavernous fistula, or epistaxis, any of which could result in death or permanent disability (2). While risks can be minimized by a thorough knowledge of local anatomy and pre-operative imaging, this information cannot completely eliminate the risk of arterial injury (3).

In the event of unexpected massive bleeding during endonasal surgery, immediate control of hemorrhage is the priority, often achieved by packing (4). Once hemostasis is achieved, CT angiography or digital subtraction angiography (DSA) can assist in identifying underlying arterial injury as a potential cause of hemorrhage. Traditionally, emergency surgical ligation of the ICA is used in these injuries (3). Ligation methods used in ICA injury can cause serious complications, including stroke and death (3, 5). With the continued development of endovascular techniques, the potentially debilitating or fatal complications seen after traditional treatment methods suggests endovascular treatment as a potentially better alternative (5). In this study, we report a case of ICA pseudoaneurysm developing after endoscopic endonasal surgery with safe and effective treatment with a flow-diverting stent, thus allowing preservation of the parent artery and occlusion of the iatrogenic pseudoaneurysm.

CASE REPORT

A 56-year-old male was admitted to the hospital with decreased vision and bitemporal hemianopsia. At the time of admission, the vital signs were: arterial blood pressure of 120/75 mmHg, pulse rate of 80 beats per minute (bpm), respiratory rate of 20 breaths/minute, and body temperature of 36.2 °C. The patient was cooperative and oriented to time, place, and person. There was no weakness present. Laboratory results showed: fasting glucose of 133 mg/dl, prolactin of 40.93 ng/ml. Other laboratory results were in normal range. Magnetic resonance imaging (MRI) with contrast revealed a mass extending from within the sella into the suprasellar space measuring 35 × 25 mm. The mass caused significant compression on the optic chiasm, hypothalamus, and anterior communicating arteries (Figure 1). There was no definite invasion into the cavernous sinus by MRI.

After appropriate preparations had been made, the patient was taken to the operative room for an endoscopic endonasal approach for mass resection; however, excessive bleeding was encountered (Figure 2), and the operation was aborted. Intraoperative nasal packing was performed to control the hemorrhage (Figure 3). Estimated blood loss was 450 cc, but the patient remained hemodynamically stable with no need for blood transfusion. Computed tomography (CT) angiography was performed emergently, which showed no aneurysm or other vascular abnormality. After observation for several days, the patient was discharged and scheduled for completion of the operation one month later.

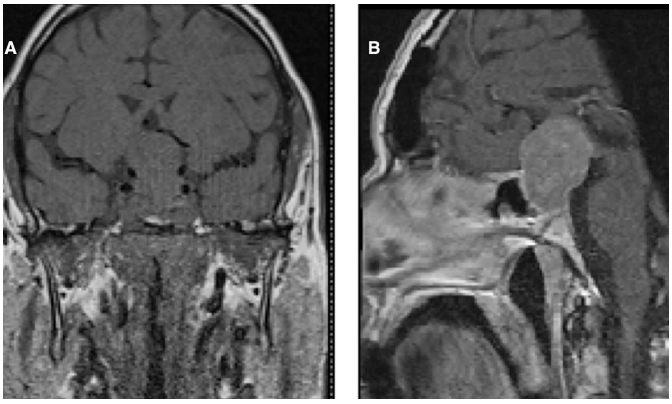

Fig. 1: (A) Coronal non-contrast T1-weighted and (B) sagittal post-contrast T1-weighted MRI show an enhancing mass extending from the sella into the suprasellar space most suggestive of a pituitary macroadenoma.

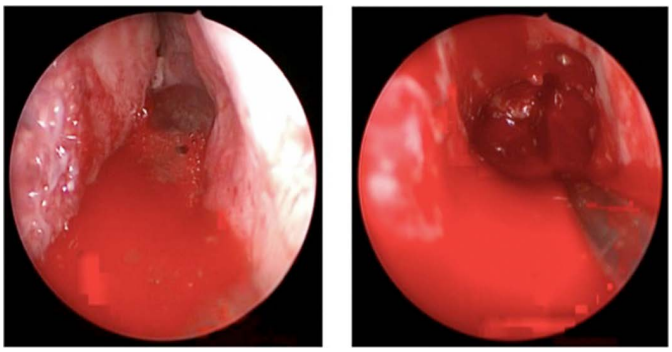

Fig. 2: These pictures show the massive bleeding from the internal carotid artery during the surgery

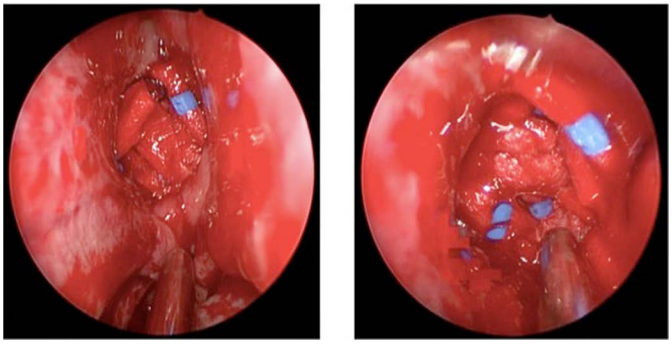

Fig. 3: The pictures show the compress for stopping the bleeding.

During the second operation, excessive bleeding was again encountered and the operation again aborted. At this point, the adenoma was almost completely evacuated (Figure 6). Due to continued concern of vascular injury, a cerebral DSA was performed and did reveal a pseudoaneurysm of the cavernous segment of the left ICA. Decision was made to manage the iatrogenic pseudoaneurysm by endovascular approach. The patient was loaded with 450 mg of clopidogrel and 100 mg of aspirin four hours prior to the endovascular operation. Since this lateral wall pseudoaneurysm was too small to insert coils safely, we placed

a 3.5 × 20 mm flow-diverting stent (Pipeline, Ev3-Covidient, USA) in the parent artery across the pseudoaneurysm without complication (Figure 4). The patient was kept under dual antiaggregant treatment (75 mg clopidogrel and 100 mg aspirin, daily) for three months. Follow-up CT angiography obtained after three months showed the complete exclusion of the iatrogenic pseudoaneurysm from the circulation (Figure 5). There was no neurologic deficit.

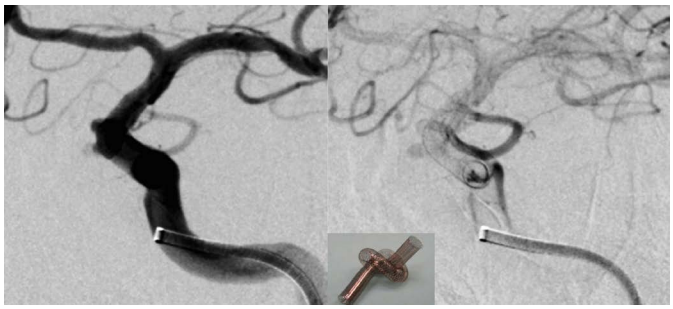

Fig. 4: Digital subtraction angiogram (DSA) shows the flow-diverting stent in the left internal carotid artery.

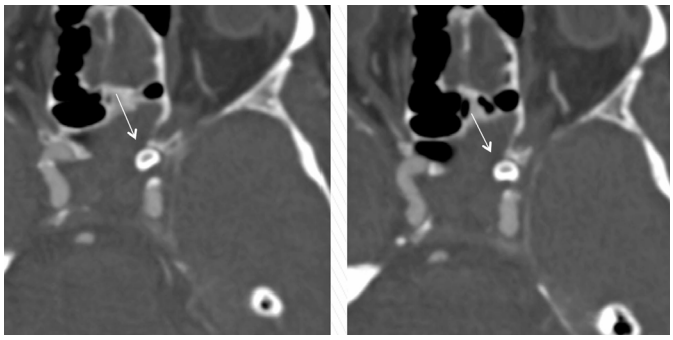

Fig. 5: A follow-up CT angiography obtained three months after endovascular stenting shows successful exclusion of the pseudoaneurysm along the anteromedial margin (arrow).

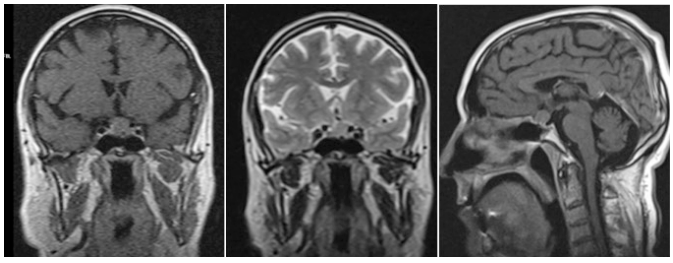

Fig. 6: A follow-up MRI obtained a year after surgery showed adenoma is disappeared.

DISCUSSION

Vascular injury is one of the most threatening complications associated with transsphenoidal surgery (2, 3). Although rare, iatrogenic pseudoaneurysm following endoscopic endonasal pituitary surgery is the most common vascular complication (1). Vascular injury during transsphenoidal resection of pituitary tumors generally occurs during aggressive resection of the tumor by damaging the surrounding vessels (3). These vessels are vulnerable to injury when the sellar dura is opened and the pituitary lesion exposed. As such, damage to the cavernous ICA is the most common type of vascular injury in transsphenoidal surgery (6). In addition to the normal intimate relationship of the ICA with the parasellar region, underlying anatomic variations of the sphenoid bones, an abnormally short distance between ICAs, ICA displacement by the lesion, tumor invasion of the cavernous sinus, tumor adhesion to the ICA, acromegaly with distortion of the local anatomy, and unusual tortuosities in the ICA can all result in an increased risk of vascular injury (8). Previous surgery, previous external radiotherapy (risk may be higher after proton or stereotactic radiation), chemoradiotherapy, or prior treatment with bromocriptine for pituitary tumors have also been linked to ICA injury (4).

Intra-operative ICA rupture creates an immediately challenging surgical field due to a high pressure/high flow hemorrhage scenario, which may rapidly result in exsanguination of the patient. Massive hemorrhage leads to a loss of orientation and an obscured surgical field often resulting in the surgeon blindly attempting nasal packing to control the hemorrhage (4). Packing materials ideally should be placed with just enough force to control the hemorrhage but not to occlude vascular flow (4). Importantly, patients may be at risk for stroke caused by thromboemboli originating in the injured parent artery (7). The vessel injury can also result in pseudoaneurysm formation, even though the artery appears to be structurally intact, packed, and not bleeding (3).

If excessive bleeding occurs during or after transsphenoidal surgery, even after successful intraoperative tamponade, immediate vascular imaging should be obtained to rule out vessel wall damage (3). Various treatment strategies have been used in the management of pseudoaneurysms, including observation (9). Common surgical treatments for pseudoaneurysm, such as clipping, wrapping, trapping, and ligation, are associated with an increased risk of complication or death (3). In our patient, the pseudoaneurysm was successfully treated with preservation of flow within the parent ICA by means of a flow-diverting stent placed across the pseudoaneurysm.

Flow-diverting stents have been proven successful in treatment of ICA aneurysms (10). In general, preserving the ICA using a stent or pipeline is the treatment of choice whenever possible. The safety and effectiveness of stenting are well established (4). Based on such evidence, the use of an endovascular flow-diverting stent was felt to be warranted in this case. Given the success and absence of complications, our results suggest flow-diverting stents may have a role in the treatment of ICA pseudoaneurysms following endoscopic endonasal pituitary surgery (10).

In summary, the presence of either an intraoperative suspicion of vascular injury or any neurologic symptoms and bleeding after transsphenoidal surgery must prompt one to perform rapid angiography to evaluate for vascular injury (11). Delayed complications, such as pseudoaneurysm, could pass unnoticed for some time leading to major morbidity. Therefore, a second angiography may be repeated one or two weeks after the initial intervention for the early detection of these complications; however, the timing for repeat angiography is empirical and not universally accepted. When considering the timecourse, it is important to remember that a pseudoaneurysm can devel-

op at any point from within hours or even months later (4). Lastly, an endovascular approach to treatment of ICA pseudoaneurysm after transsphenoidal pituitary surgery may be an alternative method with equivalent effectiveness and fewer complications than prior methods. Further studies are needed to confirm this hypothesis in a larger cohort of patients.

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Retinoblastoma: Magnetic Isotope Effects Might Make a Difference in the Current Anti-Cancer Research Strategy

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ABSTRACT
Human retinoblastoma cells were proven to possess some very unusual DNApolβ species. Being 23.5 kDa monomers, which itself is not common for the DNApolβ superfamily members, these chromatin associated proteins manifests most of the DNApolβ-specifc functional peculiarities making them legitimate targets for DNA repair cytostatic inhibitors. Particularly, these tumor specific enzymes were found to be very sensitive to $^{25}Mg^{2+}$-, $^{43}Ca^{2+}$- and $^{67}Zn^{2+}$-promoted magnetic isotope effects (MIE) caused a marked DNA sequence growth limitation as well as a formation of the size-invalid, i.e. too short in length, DNA fragments, totally inappropriate for the DNA repair purpose. This MIE-DNApolβ match may serve a starting point for further move towards the paramagnetic path in current developments of anti-cancer strategies.

KEYWORDS
retinoblastoma; magnetic isotope effects (MIE); DNA repair

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INTRODUCTION

As seen from a variable data obtained in the past ten years, some certain epigenic factors such as the DNA repair damage are indeed critical for efficiency of any attempt related to the anti-retinoblastoma treatment results (1, 2). Thus, the DNA replication and transcription errors, frequently occurring in most cancers, are usually expected to be removed which is the requirement for malignant cell survival (3). This in itself attracts attention to DNA polymerases beta (DNApolβ, EC 2.7.7.7) known for their crucial role in the DNA base excision repair (1, 3). Being overexpressed in many variable neoplasmas, these enzymes were found to be a part of the firm positive correlation between (a) their expression/activity patterns and (b) a degree of negative outcome concerning a fatal clinical prognosis in cancer patients (4).

It makes these enzymes the "legitimate targets" for their specific inhibitors making a cancer cell deprived of the DNA repair related survival capabilities (1–4). A magnetic isotope effect (MIE) promoting ions of bivalent metals may play a role of such inhibitors due to their capabilities to dismiss/substitute an endogenous Mg^{2+} in DNApolβ catalytic sites. Noteworthy, MIE is nothing but an exclusively powerful impact of "magnetic", i.e. nuclear spin possessing, metal isotope on enzyme catalytic function as compared to effect provided by spinless ("non-magnetic") isotopes of the very same metal (2).

In this study, we have revealed some unusual and pharmacologically promising properties of DNApolβ targets for the first time isolated here from human retinoblastoma cells.

MATERIALS AND METHODS

Two human retinoblastoma cell lines, WERI-RB-1A and Y79 (both purchased from the RAS Institute for Cytology, St. Petersburg, Russia), were cultured under normal conditions; a routine MTT-based proliferation assay was performed to monitor over the culture status. Chromatin-associated DNApolβ fraction was isolated from the cell lysate samples under the conditions preserving native proteins using our slightly modified technique consisting in a subsequent phenol-chloroform/ammonium sulfate treatment followed by the gel filtration on TOYOPEARL HW 55F column (5). For the DNApolβ catalytic activity measurements, an incorporation of (Methyl-1,2-^{3}H)dTTP (90–120 Ci/mmol, NET520A, NEN) into the nascent enzyme-produced DNA sequences was detected to be expressed then as (^{3}H)cpmDNA/mg protein. The protein amounts were estimated according to (6), the DNA amounts were measured as described in (5). Enzyme inhibitors were tested conventionally: ddTTP, 2.5 μM (Boehringer-Mannheim, Germany); Aphidicolin, 5.0 μg/mL (Serva-Heidelberg, Germany); *N*-ethyl-melamide, 0.5 mM (Sigma-Aldrich, USA); KCl, 200 mM (Sigma-Aldrich, USA) (5). The kinetic constants, K_m (mM) and k_{cat} ((μM dTTP/min)/mg pure enzyme) were estimated by the free dTTP pool depletion rates measured using the HPLC analysis of acetone-soluble fractions of pre- and post-incubated enzyme reaction mixtures in the course of DNApolβ activity tests (7). Electrophoretic procedures, SDS-PAGE and agarose gel fractionation, were carried out to evaluate the enzyme molecular mass/purity and the enzyme-produced DNA fragments lengths, respectively (5). To detect the 3′,5′-exonuclease activity in purified enzyme samples, a conventional method has been employed (8).

Labeled products and disposal materials: $^{25}MgCl_2$, $^{43}CaCl_2$ and $^{67}ZnCl_2$, all A grade, 96.8–97.7% isotopic purity, were purchased from Gamma Lab AS (Spain). Tritium labeled DNA precursor, (Methyl-1,2-^{3}H)dTTP with a specific activity of 90–120 Ci/mmol, NET520A, was purchased from New England Nuclear, Inc., USA. DNAse-free agarose (Helicon Co., UK); ethidium bromide (AppliChem AG, Sweden); single strand DNA markers kit, 30*n*–550*n* (SibEnzyme Ltd, Russia); AccuPrep Genomic DNA Extraction Kit (Bioneer Ltd, Korea); and RX5 (^{3}H)sensitive autoradiography films (Fuji Corp., Japan) were employed.

For all enzyme activity tests, optimal (Me^{2+}) values were kept (2, 5). The DNA extract aliquots were utilized further for electrophoretic determination of the pol β directed DNA chain sizes as well as for total (^{3}H)-radioactivity values in a standard dioxane liquid scintillation system (Wallac 2200LX LS Counter, Wallac OY, Finland). A DNA size estimating electrophoresis performed in 1.8% PAAG-2.0% Agarose slab gels was completed with a (^{3}H)-exposed Fuji RX film autoradiography (9).

RESULTS

Being very similar to each other (Table 1), the enzyme species purified from both cell types tested, were found belonging to the low processivity type as clearly reflected by kinetic constants: WERI-RB-1A, K_m = 0.012 mM, k_{cat} = 0.609 (μM dTTP/min)/mg enzyme; Y79, K_m = 0.018 mM, k_{cat} = 0.617 (μM dTTP/min)/mg enzyme. This is a known DNApolβ-specific property (5).

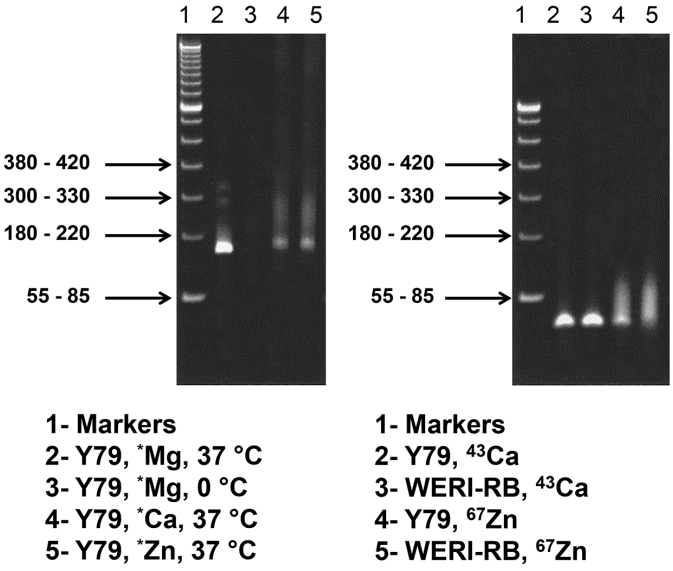

Fig. 1: Polyacrylamide-agarose gel electrophoretic fractionation of the retinoblastoma DNApolβ-processed (3h)DNA fragments. Tritium Autoradiography Patterns.
*Mg, *Ca, *Zn – a natural abundance isotope mixtures tested in enzyme optimized incubation system; ^{25}Mg, ^{43}Ca, ^{67}Zn – same for the pure paramagnetic metal isotopes.

Tab. 1: Key catalytic properties of DNApolβ species from human retinoblastoma cells.

Enzyme Pattern		Cell Type	
		Y79	WERI-RB
Quaternary structure		none	none
MW, kDa		23.5	23.5
pI		8.20	8.50
K_m, μM (dTTP pool)		0.013	0.010
k_{cat}, μM ([dTTP/min]mg protein)		0.394	0.418
3′,5′ -exonuclease activity		none	none
KCl effect (200 mM)		↑2.2	↑1.8
ddTTP effect (2.5 μM)		↓28.0	↓33.8
Aphidicolin effect (5.0 μg/mL)		none	none
N-ethyl-melamide effect (0.5 mM)		none	none
Magnetic Isotope Effect (MIE)*	**$^{25}Mg^{2+}$**	↓1.9	↓2.2
	$^{43}Ca^{2+}$	↓2.7	↓2.5
	$^{67}Zn^{2+}$	↓3.0	↓2.4

* MIE value expressed as the specific catalytic activity of DNApolβ measured in the presence of pure "magnetic", nuclear spin possessing, metal ions corrected to its activity in the presence of the same content of ions of the same element abundant spinless isotopes [11, 15, 16].

The short size (not longer than 300 bp) of the DNA fragments produced by enzymes we purified, a highly specific diagnostic pattern indicating DNApolβ catalysis (3, 5), has been observed (Figure 1).

A clear negative, inhibitory, 1.9–3.0-fold MIE was estimated for all three nuclear spin possessing ("magnetic") metal isotopes tested in reactions of both tumor specific DNApolβ species (Table 1).

All measurements were carried out at optimal conditions.

Arrow signs show activation (up) or inhibition (down) observed.

DISCUSSION

A high resistance to Aphidicolin and *N*-ethyl-melamide along with a marked sensitivity to ddTTP that has been shown simultaneously with the 200 mM (!) KCl-promoted hyperactivation (Table 1) is a most reliable characteristic property for the DNApolβ family members; a total lack of 3′,5′-exonuclease catalytic activity in our purified enzyme samples is also in a favor to their DNApolβ nature (3–5).

Apart from a sharp inhibitory magnetic isotope effects (MIE) manifested by all stable paramagnetic isotopes tested (Table 1), these effects promote a significant decrease in lengths of the DNA fragments processed by tumor originated metal dependent DNApolβ species (Figure 1). This means an obvious incapability of such "abnormally short" DNA chains to get involved into the DNA repair process. So occurring simultaneously with the sharp MIE-induced enzyme suppression, this fact itself makes it possible to consider these short, size-invalid, DNA segments not-fitting their crucial role in the DNA repair based protection of cancer cell. This confirms a concept (4, 5, 11, 12) taking DNApolβ as a legitimate target for anti-tumor agents since its inhibition deprives the tumor cell of a DNA base-excision repair.

Recognizing the MIE phenomenon as a powerful modulator for matalloenzyme programmed phosphate transferring catalysis *in vivo* (10) and *in vitro* (11), it is hardly possible to underestimate its cytostatic potential as long as a MIE-DNApolβ match leads to a breakdown of the DNA repair related survival of neoplasma. Since physical and biophysical ion-radical mechanism of MIE is no longer enigmatic (2, 10, 11), we may disclose a fresh insight into not only the processes of replication and reparation of DNA but into some linked areas of molecular pharmacology as well.

In the light of some recent developments proposing the safe-n-convenient nanocontainers for a targeted delivery of paramagnetic bivalent metal cations to cancer cells and tissues (2, 11, 12), our results might be taken as an attention catching signal we need to optimize the current strategies in retinoblastoma treatment and research.

Last not least, the type of MIE related magnetism, a background of a whole trend of spin-selective biochemistry (10, 11), consists in a Coulomb hyperfine coupling between "magnetic" (spin-marked) metal nuclei and a phosphate belonging oxygen inside the ion-radical pair followed by the "magnet" induced singlet-triplet conversion of this pair (13, 14). Conventionally, the nuclear spin possessing metal isotopes were labeled as "magnetic" ones in numerous MIE-devoted biochemical reports (2, 10–14). In particular, mechanisms and pharmacological potential of MIE promoted by $^{25}Mg^{2+}$, $^{43}Ca^{2+}$ and $^{67}Zn^{2+}$ in DNApolβ reaction were in a focus of studies on human myeloblast leukemia cells (15, 16). So far, however, this type of tumors was the only malignant objects studied with respect to MIE-DNApolβ impacts. As per retinoblastoma research, our present work is the first investigation of this kind.

CONCLUSIONS

A trend making MIE-focused approach to ongoing anti-retinoblastoma clinical and pre-clinical efforts is about to rise now due to a revealed possibility of taking DNApolβ molecular machinery as a tumor specific target for the controlled

paramagnetic DNA repair damage. This itself deserve an extensive study for an aim to get some metal stable isotope tool depriving the malignant cells of the DNA repair capabilities required for their aggressive growth and reproduction. Therefore, bivalent metal paramagnetic should be treated as a compound sufficient to contribute to the current retinoblastoma molecular pharmacology studies.

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