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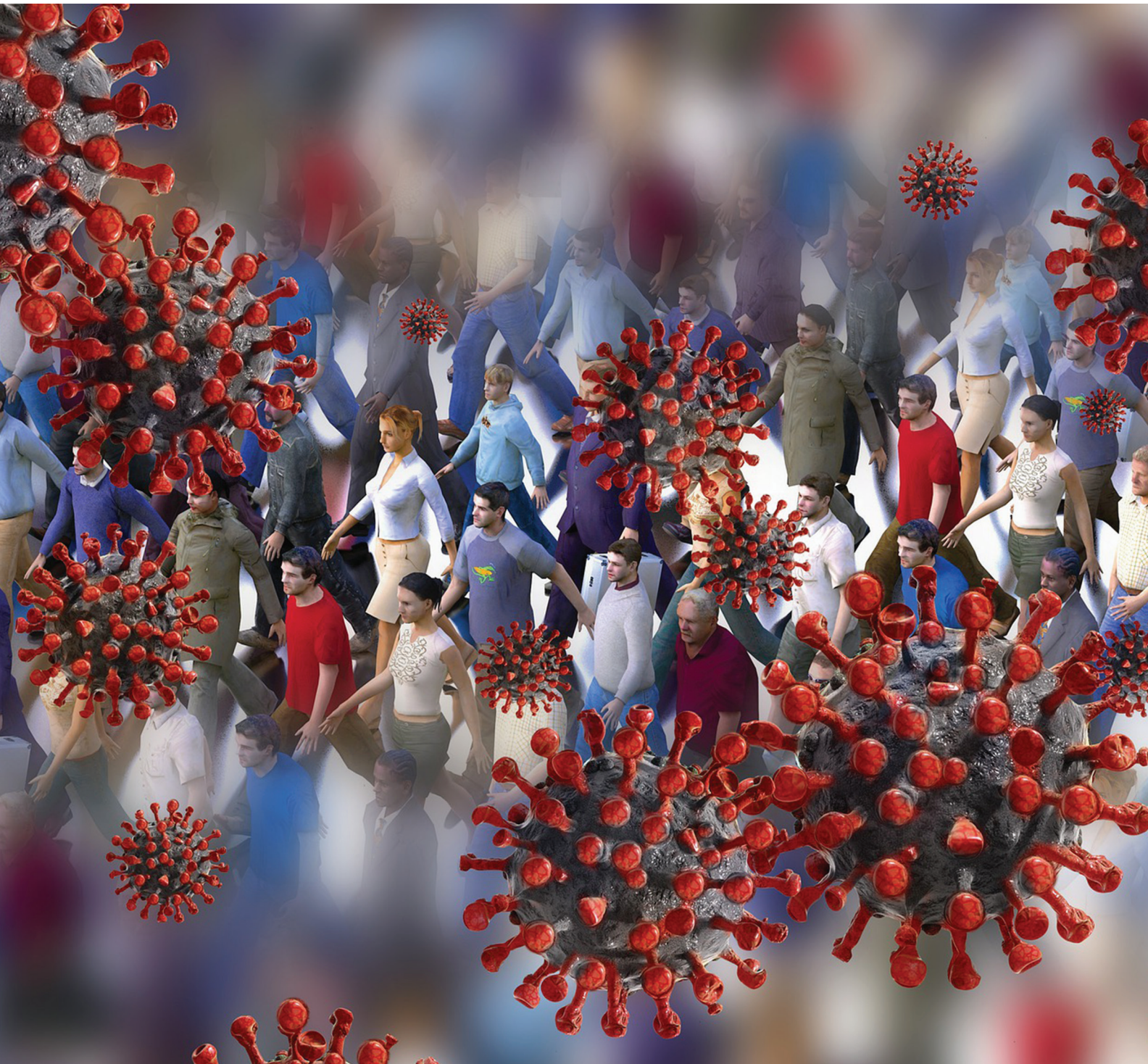

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Original Scientific Article

ISOLATION OF MICROPLASTICS FROM FRESHWATER MACROINVERTEBRATES IN THE DANUBE RIVER

Jelena Stanković[1], Maja Raković[2], Momir Paunović[2], Ana Atanacković[2], Jelena Tomović[2], Đurađ Milošević[1]

[1]Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš, Niš, Serbia
[2]Department of Hydroecology and Water Protection, University of Belgrade, Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia

Abstract. *The study was conducted on the Danube River, within the project Joint Danube Survey 3 (JDS3). The main aim was to estimate the quantity of microplastics in aquatic ecosystems through passive biological monitoring. Three freshwater species were used for microplastic (MP) isolation from different taxonomic groups of organisms: Mollusca, Oligochaeta, and Chironomidae (Diptera), with the following species: Lithoglyphus naticoides (C. Pfeiffer, 1828), Limnodrilus hoffmeisteri (Claparede, 1862), and Chironomus acutiventris (Wülker, Ryser & Scholl, 1983), respectively. The samples were collected from 6 sites along the Danube River where 540 specimens were examined. The samples were digested by alkaline method (incubation in 10% KOH solution at 60 ºC for 24 h) and filtered through a mill silk, 10 µm mesh size. Collected particles were categorized as: fibre, hard plastic, nylon, rubber, or miscellaneous. Categories were divided into subcategories based on the coloration of the particles. Particles ingested by organisms were represented mostly by fibres and fragmented hard plastics, within the size range were from 0.03 to 4.87 mm. A total of 678 MP particles were collected with an average of 4.64 ± 1.59; 1.64 ± 0.46 and 1.24 ± 0.34 items/organism isolated from L. hoffmeisteri, L. naticoides and C. acutiventris, respectively. According to results, L. hoffmeisteri, L. naticoides and C. acutiventris could be used as proper bioaccumulators of MP pollution in the Danube River.*

Key words: *microplasic, plastic litter, freshwater, alkaline method, macrozoobenthos, the Danube River.*

Introduction

The presence of plastic nowadays has been estimated as ubiquitous. Annual production of plastic has rapidly increased since the 1950s, with constantly increasing rates ever since. Estimated at 1.5 million metric tons (MT) in 1950, the world's production of plastic has reached 368 million metric tons (MT) in 2019 [1]. Constantly increasing world's production over time, durability and low recycling rates of plastic resulted in their high presence and accumulation in the environment [2]. MP is an organic polymer derived from fossil feedstocks within the size 1 µm [3] up to 5 mm [2]. Primary MPs are manufactured in microscopic size as industrial pellets, exfoliating microbeads in personal care products [4], abrasives in blasting, or as a component of paints [5–6]. Secondary MP has primarily been macroscopic size, manufactured as plastic demands of the buyers, with the dominance of packaging items (plastic bags, containers, bottles) and building and construction [1]. Any physical, chemical, or biological process causes degradation of macroplastics into microplastics [7]. The plastic polymers have residual monomers and chemical additives, capable of absorbing toxins from the environment such as persistent organic pollutants (POPs), polychlorinated biphenyls (PCBs), organo-halogenated pesticides, nonylphenol, PAHs, and dioxins [8–10]. In addition, additives such as phthalate-based plasticizers and bisphenol A (BPA) amplify the toxicity of the plastic particles [11].

MP became a great concern due to potential availability to a wide range of organisms because of similar size fractions as sediment and food particles [12]. Studies on plastic contamination in natural ecosystems have reported MPs within freshwater ecosystems such as rivers [13–16] lakes [17–22] and shoreline sediments [23–24], estuarine areas [25], indicating rivers as pathways for marine plastic debris [26–28], but with incomparably fewer data, and conducted researches. In addition to its presence in the aquatic environment, MPs can cause a mechanical hazard [29] to organisms or be a vector for opportunistic pathogens [30–31], persistent organic pollutants (POPs) [8–10] and heavy metals [32] or invasive species [33], which may result in harmful algal bloom (HAB) [34].

Annex VIII of the Water Framework Directive (WFD) is focusing on the identification of 'Specific Pollutants', such as MPs, to derive Environmental Quality Standards (EQSs) for targeted chemicals to achieve Good Surface Water Status [35].

As MP is a global problem, the Directives on packaging waste ([94/62/EC), waste (2008/98/EC), landfills

Correspondence to: Jelena Stanković
Department of Biology and Ecology, Faculty of Sciences and Mathematics, 33 Višegradska, 18000 Niš, Serbia
E-mail: jelena.stankovic2@pmf.edu.rs
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(1999/31/EC), and sewage sludge (86/278/EEC) [36–37] established monitoring of plastic sources of freshwater ecosystems in Europe. In 2013, the European Commission developed 'Green paper on a European strategy on plastic waste in the environment' [37], European strategy on plastic waste in the environment, especially on MP waste, as a wider review of waste legislation. In 2015, the Plastic Bags Directive was adopted with the aim of reducing its consumption through pricing, taxes, and levies [38]. Since the concern for this synthetic pollution is rising, the Union's chemicals legislation (1907/2006/EC) applied relevant production volumes of plastic monomers and additives used in manufacturing processes [39]. The European Commission adopted a Circular Economy Package in 2015 for using the resources in a more sustainable way which refers to plastic, among five priority sectors [38]. As it can be concluded from the enclosed data, MP is a serious issue nowadays.

According to Framework of the European Water Framework Directive [35], macroinvertebrates are used in monitoring studies to assess the ecological water quality as a group of different bioindicators of organic pollution. Despite the huge problem posed by MPs in the environment, there are no indicators developed for passive monitoring of MPs in aquatic ecosystems. In Annex VIII there is a list of the main pollutants, among others, 'persistent and bioaccumulative organic toxic substances' and 'persistent hydrocarbons' which may include synthetic polymers [35].

MP has reached a significantly high level of abundance during the past few decades. Since the first report on MP debris from 1972 [40] in the aquatic ecosystem (marine ecosystem) popularization has been rising constantly. In this article, we present results from the survey on plastic debris in the Danube River in Serbia. The main aim of the study was to estimate the amount of microplastics in aquatic ecosystems through passive biological monitoring. To that end, we set the following tasks: (1) to quantify the number of MP particles per organism and per biomass and (2) to estimate the distribution of particles per categories and subcategories, based on their shape and color.

Materials and Methods

Sampling Site and Procedure

The study was conducted on the Danube River, which flows into the Black Sea and is the second largest river in Europe [41]. This international river basin occupies the territory of 19 countries, with an area of 817.000 km^2 and gathering 83 million inhabitants near it. The Danube River System is situated in nine ecoregions and classified as a special case study in terms of conservation and management issues [42]. In this study three species from different groups of organisms (Mollusca, Oligochaeta, and Chironomidae (Diptera)) were used for MPs isolation: *Lithoglyphus naticoides* (C. Pfeiffer, 1828), *Limnodrilus hoffmeisteri* (Claparede, 1862) and *Chironomus acutiventris* (Wuelker, Ryser & Scholl, 1983), respectively. Within the JDS3 project, the most diverse components of the total community were Chironomidae (Diptera), Oligochaeta, and Mollusca [43]. *L. hoffmeisteri* (Naididae:Tubificidae) represents one of the most dominant species along the whole stretch of the Danube River, tolerant to organic pollution [44]. *L. naticoides* has Ponto-Caspian origin and it is considered as cryptogenic for the upper and middle stretch of the Danube, while it is native to the Lower Danube [45]. *C. acutiventris* one representative of the chironomids, which are considered as useful bioindicators suitable for determining the biological effects of different pollutants in the aquatic environment [46]. Since chironomids are non-specific feeders, ingestion of MPs instead of food particles is very common [47].

Samples were collected between August and September 2013, at six sites along the Danube. Following the multi-habitat procedure [48], macrozoobenthos was sampled using a hand net (ap. 25 cm× 25 cm, mesh size 500 μm) by the kick & sweep (K&S) sampling technique (EN 27828:1994). For the deep water area, a triangle shaped dredge (ap. 25 x 25 cm, mesh size 500 μm) was pulled five times per sampling site in a length of 80 cm. Each transect was considered as a separate sample. A detailed description of the sampling methodology is presented in Liška et al. [49]. The samples of macrozoobenthos were sorted in the laboratory and the specimens of Oligochaeta, Mollusca, and the larvae of Chironomidae were counted, separated, and identified to the lowest possible taxonomic level, by the use of the following identification keys: Moller Pillot [50–51], Schmid [52], Vallenduuk and Moller Pillot [53], Pfleger, [54], Timm [55].

Methodology of Isolation MPs from Macroinvertebrate

Although numerous approaches have been developed for the extraction of MPs, all are classified into six protocol groups within the following methods: acidic [56–57], alkaline [58-59], oxidizing [60–61], and enzymatic methods [58]. Dehaut et al. [62] have tested all of the protocols and found out that five out of six have shown significant degradation of plastic particles or insufficient tissue digestion. The alkaline method appeared to be the best protocol for isolating MPs from biological samples and for later identification.

The protocol is based on using 10% potassium hydroxide (KOH) solution as a medium for the samples and incubation at 60 ºC during a 24 h period. This leads to an efficient decomposition of biological tissues with no significant degradation on all tested polymers, except for cellulose acetate [62]. They suggested it for the implementation in further monitoring studies on MPs.

For each species, 180 specimens were randomly selected from 6 sites, 30 specimens per site. One sample contained 10 specimens, meaning three replicates per site. In total, 540 specimens were measured on an analytic scale in order to estimate potential MP litter per

biomass (Table 1). In the experiment setting, the control did not contain any single entity.

The samples were treated by the suggested alkaline method, using a 10% solution of KOH and incubation at 60 ºC for a 24 h in a water bath. The samples of *C. acutiventris* remained undigested after the suggested incubation time, due to the presence of the chitin. For its degradation, samples were additionally treated with the double volume of nitrate acid (HNO_3) in controlled conditions - vials with the samples were placed in the digester in cold water with ice and 3 ml of HNO_3 was added carefully by micropipette. This reaction formed potassium nitrate (KNO_3), which started an instant reaction with the chitin and dissolved the tissue of *C. acutiventris*. The samples were filtered through a milling silk, as filter, with a 10 µm mesh size. The filtered material was treated with 30% hydrogen peroxide to remove the remaining organic matter if needed. The particles were carefully collected, photographed and categorized, based on their shape (Fig. 1). Subcategories were defined according to the colorization of the particle.

Table 1 Total and average weight of species. Total weights are in grams.

	L. naticoides	*L. hoffmeisteri*	*C. acutiventris*
Total number of individuals	180	180	180
Total weights	1.74	0.07	0.26
Average	0.097	0.004	0.015
SD*	0.004	0.07	0.006

* Standard deviation

Particles were counted manually with a Leica MZ16A stereomicroscope (10 X/21 B ocular; from 20 X to 50 X objective magnification), photographed with a Leica DFC320 Digital Camera system, and measured in program ImageJ [63]. Fibres from the air were excluded.

Results

The collected particles were assigned to one of 5 major categories: fibre, hard plastic, nylon, rubber, or miscellaneous (Fig. 2). In the present study, fibres were the dominant group of MPs with 49.48 % of the total count and the second major category was hard plastic with the percentage share of 43.21 % of collected particles in total (Fig. 3). Measurement of particles by the longest length has shown that fibres had a length from 0.19 to 4.87 mm and hard particles from 0.046 to 0.23 mm (Table 2).

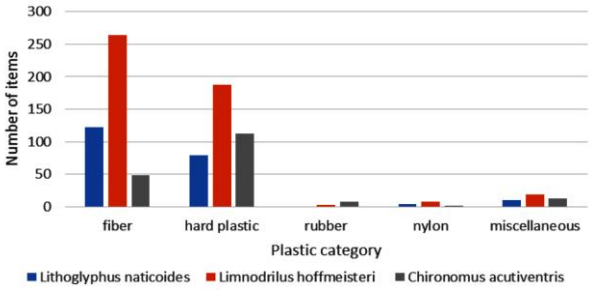

Fig. 2 Types of MPs collected form *Litogliphus naticoides*, *Limnodrilus hoffmeisteri* and *Chironomus acutiventris.*

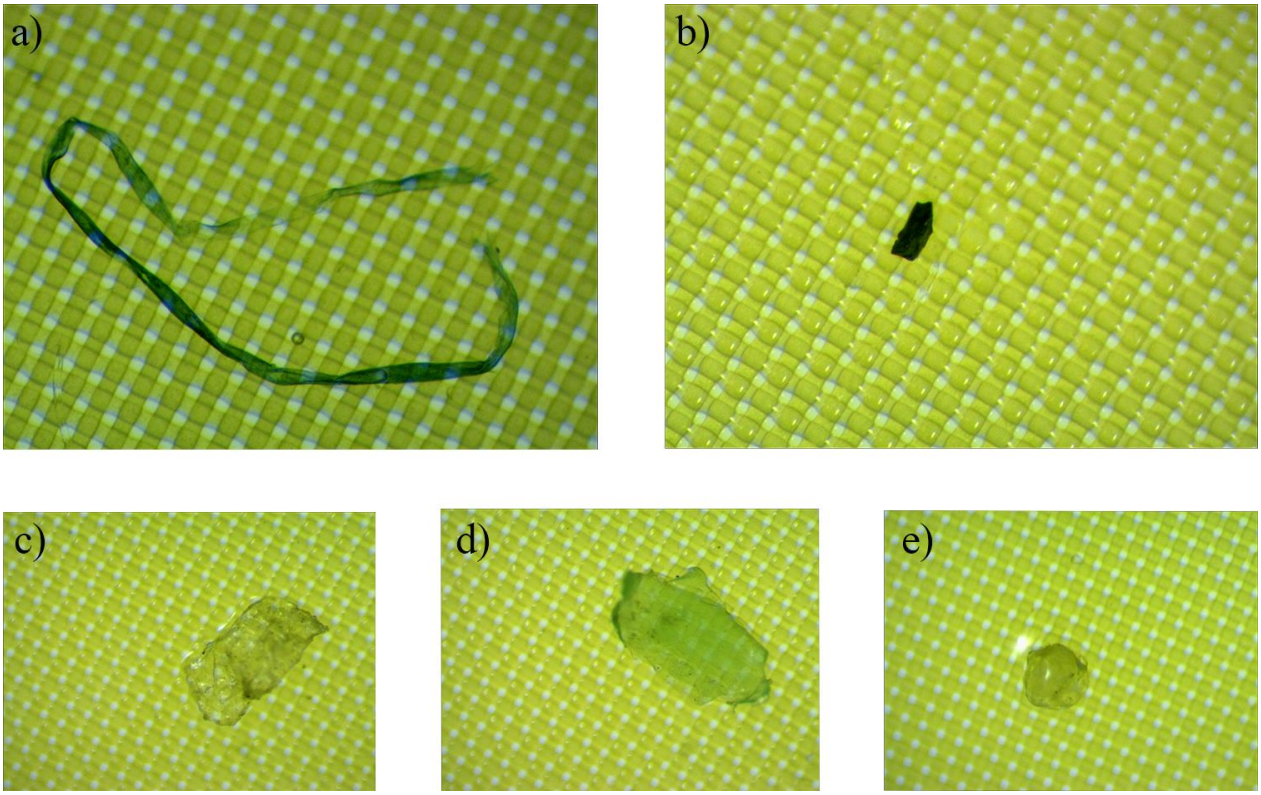

Fig. 1 Photographs of particles from 5 major categories: a) fibre; b) hard plastic; c) nylon; d) rubber; e) miscellaneous.

Table 2 Percentage share and length of particles divided in subcategories. Lengths are in millimeters.

Subcategory	No. of particles	Percentage share [%]	Minimum length [mm]	Maximum length [mm]	Average length [mm] ± SD*
blue fibre	349	39.79	1.97	4.8	2.62 ± 0.55
red fibre	85	9.69	0.19	4.87	1.35 ± 0.65
red rubber	5	0.57	0.096	0.026	0.066 ± 0.025
green rubber	3	0.34	0.085	0.11	0.097 ± 0.012
white rubber	2	0.23	0.058	0.14	0.099 ± 0.041
black hard plastic	79	9.00	0.05	0.16	0.083 ± 0.03
blue hard plastic	64	7.29	0.07	0.199	0.119 ± 0.085
white hard plastic	54	6.16	0.064	0.23	0.152 ± 0.122
grey hard plastic	12	1.37	0.052	0.077	0.065 ± 0.008
brown hard plastic	39	4.45	0.1	0.21	0.144 ± 0.037
crystal hard plastic	33	3.76	0.046	0.11	0.084 ± 0.019
transparent hard plastic	98	11.18	0.046	0.086	0.067 ± 0.013
white nylon	3	0.34	0.11	0.15	0.127 ± 0.017
transparent nylon	10	1.15	0.055	0.89	0.225 ± 0.239
miscellaneous	41	4.68	0.059	0.14	0.106 ± 0.019

* SD – standard deviation.

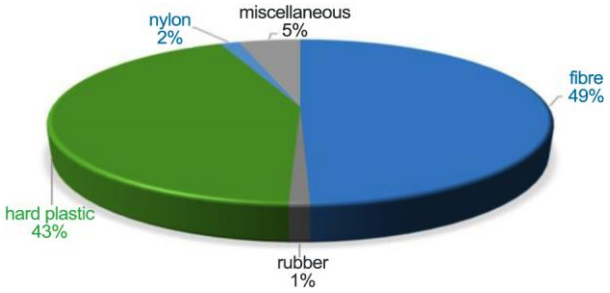

Fig. 3 Main categories with precentage share in total number of collected particles.

A total of 678 MPs were collected, whereas majority of the particles were isolated from *L. hoffmeisteri* (61.6 %), followed by *L. naticoides* (21.8 %) and *C. acutiventris* (16.6 %). An average, 4.64 ± 1.59; 1.64 ± 0.46 and 1.24 ± 0.34 items/organism or 0.000421 ± 0.000409; 0.009661 ± 0.005247 and 0.001465 ± 0.000598 items/g wet weight were isolated from *L. hoffmeisteri*, *L. naticoides* and *C. acutiventris*, respectively.

Discussion

There is still scarce information assessing microplastic pollution in the freshwater environments due to a lack of data on the presence and quantities of MPs within the bodies of freshwater biota. In this study, MPs were recorded in the tissue of *L. naticoides*, *L. hoffmeisteri* and *C. acutiventris* in the Danube River, thus supporting earlier reports on the worldwide presence of MPs [64-66].

Although there are no data on microplastic ingestion of *L. naticoides*, *L. hoffmeisteri* and *C. acutiventris*, other representatives of the groups of Mollusca, Oligochaeta, and Chironomidae (Diptera) have been used in studies of MP ingestion. The categorization of the particles differs in different studies due to the lack of standardization of categories of MP particles. In the tissue of freshwater snail *Sinotaia aeruginosa* (Reeve, 1863) from Taihu Lake, fibres and fragments were the most common categories. Transparent, red and blue subcategories were common within the fibres, while transparent subcategories were dominant within the fragments [67]. Akindele et al. [68] detected only fibres in the tissue of freshwater gastropods *Melanoides tuberculata* (Müller, 1774) and *Theodoxus fluviatilis* (Linnaeus, 1758), and fibres and films in the tissue of *Lanistes varicus* (Müller, 1774). The majority of isolated particles from the tissue of *Tubifex tubifex* (Müller, 1774) in the Salford Quays basin (Manchester City, England) were represented as fibres (87 %), while fragments represented the rest of the particles [69]. Lin et al. [70] detected microgranules (0-28 %), microfilms (0-16 %), microfragments (3-47 %), and microfibres (40-64 %) within the midge larvae (Diptera: Chironomidae) among 5 sampling sites in the Wu river basin, Taiwan. Su et al. [17] detected four categories of MP particles in the tissue of freshwater Asian clam *Corbicula fluminea* (O. F. Müller, 1774): fibre, pellet, film, and fragment, with the dominance of fibres (48-84% in the samples). Within the subcategories, blue items were dominant on the water surface (50-63 %), while transparent and white items were more common in organisms and sediments (29–44 %). Hohenblum et al. [71] conducted preliminary research in Austria on the Danube River and over 50 % of the extracted plastic particles consisted of fragments, 4–10 % were pellets and 2.1–2.8 % were green lenticular flakes. MPs isolated from *C. fluminea*, collected along 2040 km of the Danube, were represented by fibres - dominance of blue subcategory and fragments - dominance of transparent subcategory [72].

According to previous studies, fibres were the most dominant category in the soft tissue of *B. aeruginosa* [67], *M. tuberculata*, *T. fluviatilis*, *L. varicus*. [68], *C.*

fluminea [17, 72], *Thienemannimyia* spp., *Chironomus* spp. and *Orthocladius* spp. [68], which is in concordance with this study (49.48 %). Ingestion rates of *B. aeruginosa* [67], *L. varicus*, *T. fluviatilis* [68], *C. fluminea* [72] were higher than ingestion rates of *L. hoffmeisteri*, *L. naticoides* and *C. acutiventris*. Xu et al. [67] detected 96.67% to 100% of one or more types of microplastics within freshwater snail *B. aeruginosa* collected from Taihu Lake, China, with the dominance of polyvinyl acetate fibres (88.0 ± 12.1%), polystyrene fibres (66.3 ± 17.5%), polyamides (49.7 ± 22.4%), and polyethylene terephthalate (30.0 ± 7.4%). Also, previous research of the Taihu Lake [17] hasdetected cellophane, PET, Polyester, and Polypropylene in Asian clam *C. fluminea*. Scherer et al. [46] demonstrated the uptake of polystyrene among different freshwater invertebrate groups, including freshwater snail *Physella acuta* (Draparnaud, 1805) (Mollusca), the blackworm *Lumbriculus variegatus* (Müller, 1774) (Oligochaeta), and *Chironomus riparius* (Meigen, 1804) (Chironomidae, Diptera).

Conclusion

MPs in the environment have been characterized as a global problem nowadays. Due to their bioavailability to a wide range of organisms and ubiquitous presence and distribution, there is a need to determine its amount in the natural environment by the use of bioindicators. Lack of data on the presence of MPs in freshwater biota is one of the reasons for the lack of a solution for plastic pollution. Choice of good indicators for estimation of the MPs in the aquatic environment is necessary. According to the amount and diversity of MPs isolated from three benthic species (*L. hoffmeisteri, L. naticoides* and *C. acutiventris)* it seems that these species could be used as proper bioaccumulators of MP pollution in the Danube River in further studies. The impact of MPs has been documented in recent years for various freshwater species, but it is certain that a lot of data is still missing to form a wider insight on this major synthetic pollution. Therefore, new field data are needed in order to estimate more precise quantities we are dealing with in the environments. In order to accomplish this task, including MPs in the standard analysis procedures could be necessary to gather more data. Further research and continued monitoring on the Danube is a request for a good evaluation of the presence and effects of MPs on aquatic organisms and the environment.

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References

1. Plastics Europe. Plastics — the facts 2020. An analysis of European plastics production, demand and waste data. 2020. https://www.plasticseurope.org/en/resources/publications/4312-plastics-facts-2020
2. Thompson RC, Swan SH, Moore CJ, vom Saal FS. Our plastic age. Philos Trans R Soc Lond B Biol Sci 2009; 364:1973–1976. http://dx.doi.org/10.1098/rstb.2009.0054
3. Gigault J, ter Halle A, Baudrimont M, et al. Current opinion: What is a nanoplastic? Environ Pollut 2018; 235:1030-1034. https://doi.org/10.1016/j.envpol.2018.01.024
4. Fendall LS, Sewell MA. Contributing to marine pollution by washing your face: MPs in facial cleansers. Mar Pollut Bull 2009; 58:1225–1228. http://dx.doi.org/10.1016/j.marpolbul.2009.04.025
5. Lassen C, Hansen SF, Magnusson K, et al. Microplastics - Occurrence, effects and sources of releases to the environment in Denmark. The Danish Environmental Protection Agency 2015. https://backend.orbit.dtu.dk/ws/portalfiles/portal/118180844/Lassen_et_al._2015.pdf
6. Sherrington C, Darrah C, Hann S, Cole G, Corbin M. Study to support the development of measures to combat a range of marine litter sources, Eunomia Research & Consulting Ltd, Bristol, United Kingdom 2016. https://ec.europa.eu/environment/marine/good-environmental-status/descriptor-10/pdf/MSFD%20Measures%20to%20Combat%20Marine%20Litter.pdf
7. Andrady. Microplastics in the marine environment. Mar Pollut Bull 2011; 62:1596–1605. http://dx.doi.org/10.1016/j.marpolbul.2011.05.030
8. Endo S, Takizawa R, Okuda K, et al. Concentration of polychlorinated biphenyls (PCBs) in beaches resin pellets: variability among individual particles and regional differences. Mar Pollut Bull 2005; 50:1103–1114. http://dx.doi.org/10.1016/j.marpolbul.2005.04.030
9. Ogata Y, Takada H, Mizukawa K, et al. International Pellet Watch: global monitoring of persistent organic pollutants (POPs) in coastal water.1. Initial phase on PCB, DDT and HCHs. Mar Pollut Bull 2009; 58:1437–1446. https://doi.org/10.1016/j.marpolbul.2009.06.014
10. Hirai H, Takada H, Ogata Y, et al. Organic micropollutants in marine plastics debris from the open ocean and remote and urban beaches. Mar Pollut Bull 2011; 62:1683–1692. https://doi.org/10.1016/j.marpolbul.2011.06.004
11. Oehlmann JR, Schulte-Oehlmann U, Kloas W, et al. A critical analysis of the biological impacts of plasticizers on wildlife. Philos Trans R Soc B 2009; 364:2047−2062. https://doi.org/10.1098/rstb.2008.0242
12. Barnes DKA, Galgani F, Thompson RC, Barlaz M. Accumulation and fragmentation of plastic debris in global environments. R Soc 2009; 364:1985-1998. https://doi.org/10.1098/rstb.2008.0205
13. Dris R, Gasperi J, Rocher V, Saad M, Renault N, Tassin B. Microplastic contamination in an urban area: a case study in Greater Paris. Environ Chem 2015; 12:592–599. https://doi.org/10.1071/EN14167f
14. Peters CA, Bratton SP. Urbanization is a major influence on microplastic ingestion by sunfish in the Brazos River Basin, Central Texas, USA. Environ Pollut 2016; 210:380–387. https://doi.org/10.1016/j.envpol.2016.01.018
15. Lechner A, Keckeis H, Lumesberger-Loisl F, et al. The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. Environ Pollut 2014; 188:177–181. https://doi.org/10.1016/j.envpol.2014.02.006
16. Morritt D, Stefanoudis PV, Pearce D, Crimmen A, Clark P.F. Plastic in the Thames: a river runs through it. Mar Pollut Bull 2014; 78:196–200. https://doi.org/10.1016/j.marpolbul.2013.10.035
17. Su L, Xue Y, Li L, et al. Microplastics in Taihu Lake, China. Environ Pollut 2016; 216:711–719. https://doi.org/ 10.1016/j.envpol.2016.06.036
18. Eriksen M, Mason S, Wilson S, et al. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar Pollut Bull 2013; 77:177–182. https://doi.org/10.1016/j.marpolbul.2013.10.007

19. Free CM, Jensen OP, Mason SA. High levels of microplastic pollution in a large, remote, mountain lake. Mar Pollut Bull 2014; 85:156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001
20. Corcoran PL, Norris T, Ceccanese T,Walzak MJ, Helm PA, Marvin CH. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environ Pollut 2015; 204:17–25. https://doi.org/ 10.1016/j.envpol.2015.04.009
21. Biginagwa F, Mayoma B, Shashoua Y, Syberg K, Khan F. First evidence of microplastics in the African Great Lakes: recovery from Lake Victoria Nile perch and Nile tilapia. J Great Lakes Res 2016; 42:1146–1149. https://doi.org/10.1016/j.jglr.2015.10.012
22. Imhof HK, Ivleva NP, Schmid J, Niessner R, Laforsch C. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr Biol 2013; 23:867–868. https://doi.org/ 10.1016/j.cub.2013.09.001
23. Klein S, Worch E, Knepper TP. (2015). Occurrence and spatial distribution of MPs in river shore sediments of the Rhine-Main area in Germany. Environ Sci Technol 2015; 49:6070–6076. https://doi.org/10.1021/acs. est.5b00492
24. Zbyszewski M, Corcoran PL. Distribution and Degradation of Fresh Water Plastic Particles Along the Beaches of Lake Huron, Canada. Water Air Soil Pollut 2011; 220:365–372. http://dx.doi. org/10.1007/s11270-011-0760-6
25. Firdaus M, Trihadiningrum Y, Lestari P. Microplastic pollution in the sediment of Jagir Estuary, Surabaya City, Indonesia. Mar Pollut Bull 2019; 150:110790. https://doi.org/10.1016/j.marpolbul.2019. 110790
26. Rech S, Macaya-Caquilpán V, Pantoja JF, Rivadeneira MM, Madariaga DJ, Thiel M. Rivers as a source of marine litter – A study from the SE Pacific. Mar Pollut Bull 2014; 82: 66–75. http://dx.doi.org/10.1016/j.marpolbul.2014.03.019
27. Yonkos LT, Friedel EA, Perez-Reyes AC, Ghosal S, Arthur CD, 2014. Microplastics in four estuarine rivers in the Chesapeake bay U.S.A. Env Sci Tech 2014; 48:14195–14202. https://doi.org/10. 1021/es5036317
28. Zhao S, Zhu L, Wang T, Li D. Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observation on occurrence, distribution. Mar Pollut Bull 2014; 86:562–568. https://doi.org/10.1016/j.marpolbul.2014.06.032
29. Jovanović B, 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integrated Environ Assess Manag 2017; 13: 510–515. https://doi.org/10. 1002/ieam.1913
30. Gregory MR. Environmental implications of plastic debris in marine settings—entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos Trans R Soc B 2009; 364:2013–2025. http://rstb.royalsocietypublishing.org/royptb/364/1526/2013.full.pdf
31. Zettler ER, Mincer TJ, Amaral-Zettler LA. Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. Env Sci Tech 2013; 47:7137–7146. http://dx.doi.org/10.1021/ es401288x
32. Teuten EL, Saquing JM, Knappe DRU, et al. Transport and release of chemicals from plastics to the environment and to wildlife. Philos Trans R Soc Lond B Biol Sci 2009; 364: 2027–2045. https://doi.org/10.1098/rstb.2008.0284
33. Masó M, Garcés E, Pagès F, Camp J. Drifting plastic debris as a potential vector for dispersing Harmful Algal Bloom (HAB) species. SCI MAR 2003; 67:107–111. http://scientiamarina.revistas. csic.es/index.php/scientiamarina/article/view/459/471
34. McCormick A, Hoellein T, Mason SA, Schluep J, Kelly JJ. Microplastic is an abundant and distinct microbial habitat in an urban river. Env Sci Tech 2014; 48:11863–11871. http://pubs.acs. org/doi/abs/10.1021/es503610r
35. EU (2000) Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Water Framework Directive (WFD). http://eur-lex.europa.eu
36. European Commission (2014) COM/2014/0397. Proposal for a Directive of the European Parliament and of the Council amending Directives 2008/98/EC on waste, 94/62/EC on packaging and packaging waste, 1999/31/EC on the landfill of waste, 2000/53/EC on endof- life vehicles, 2006/66/EC on batteries and accumulators and waste batteries and accumulators, and 2012/19/EU on waste electrical and electronic equipment. http://eur-lex.europa.eu.
37. European Commission (2013) GREEN PAPER: On a European Strategy on Plastic Waste in the Environment.
38. European Commission (2015) Closing the loop: Commission adopts ambitious new Circular Economy Package to boost competitiveness, create jobs and generate sustainable growth. Press release, Brussels, 2 December 2015, IP/15/6203. http://europa.eu/ rapid/press-release_IP-15-6203_en.htm
39. EU (2006) Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/ 45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC. http://eur-lex.europa.eu
40. Carpenter EJ, Smith KL. Plastics on the Sargasso Sea Surface. Science 1972; 175:1240–1241.
41. Black Sea Commision. Strategic Action Plan for the Environmental Protection and Rehabilitation of the Black Sea. 2009. http://www.blacksea-commission.org/_bssap2009.asp
42. Sommerwerk N, Schneider-Jajoby M, Baumgartner C, et al. The Danube River Basin. In: Tockner K, Robinson CT, Uehlinger U (eds) Rivers of Europe. Elsevier Ltd., London, UK, 2009. 59-113.
43. ICPDR - International Commission for the Protection of the Danube River. Joint Danube Survey 3- Full report on: Macroinvertebrates. 2014. (www.icpdr.org)
44. Atanacković AD, Šporka F, Csányi B, Vasiljević BM, Tomović JM, Paunović MM. Oligochaeta of the Danube River – a faunistical review. Biol 2013; 68:269–277. https://doi.org/10.2478/s11756-013-0155-9
45. Paunović MM, Csányi B, Simonović P, Zorić K, 2015. Invasive Alien Species in the Danube. In: Liska I (eds) The Danube River Basin. The Handbook of Environmental Chemistry, vol 39. Springer, Berlin, Heidelberg 2015. https://doi.org/10.1007/698 _2015_376
46. Pinder L. Biology of freshwater chironomidae. Annu Rev Entomol 1986; 31:1-23.
47. Scherer C, Brennholt N, Reifferscheid G, Wagner M. Feeding type and development drive the ingestion of microplastics by freshwater invertebrates. Sci Rep 2017; 7:17006. http://dx.doi.org/10.1038/ s41598-017-17191-7
48. AQEM. Manual for the application of the AQEM system. A comprehensive method to assess European streams using benthic macroinvertebrates, developed for the purpose of the Water Framework Directive 2002; Contract No:EVK1-CT1999-00027).
49. Liška I, Wagner F, Sengl M, Deutsch K, Slobodník J. Joint Danube Survey 3: A Comprehensive Analysis of Danube Water Quality. International Commission for the Protection of the Danube River and European Commission, Vienna 2015.
50. Moller Pillot H. De larven der Nederlandse Chironomiae (Diptera). 1A: Inleiding, Tanypodinae en Chironomini. St E I S Nederland, Leiden (German) 1984a.
51. Moller Pillot, H. De larven der Nederlandse Chironomiae (Diptera). 1B: Orthocladiinae sensu lato. St E I S Nederland, Leiden (German) 1984b.
52. Schmid P. A Key to the Larval Chironomidae and their instars from Aus-trian Danube Region Streams and Rivers: Part 1. Diamesinae, Prodiamesinae and Orthocladiinae. Federal Institute for Water Quality of the Ministry of Agricultureand Forestry, Wien 1993.
53. Vallenduuk HJ, Moller Pillot H. Chironomidae Larvae of the Netherlands and Adjacent Lowlands: General Ecology and Tanypodinae. KNNV Publishing, Zeist 2007.
54. Pfleger V. A field guide in colour to Molluscs. Silverdale Books, Prague, Czech 2000:216.
55. Timm T. A quide to the freshwater Oligochaeta and Polychaeta of Northern and Central Europe. Lauterbornia 2009; 66:1–235.
56. Claessens M, Van Cauwenberghe L, Vandegehuchte MB, Janssen CR. New techniques for the detection of microplastics in sediments and field collected organisms. Mar Pollut Bull 2013; 70:227–233. https://doi.org/10.1016/j.marpolbul.2013.03.009

57. De Witte B, Devriese L, Bekaert K, et al. Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. Mar Pollut Bull 2014; 85, 146–155. https://doi.org/10.1016/j.marpolbul.2014.06.006
58. Cole M, Webb H, Lindeque PK, Fileman ES, Halsband C, Galloway TS. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci Rep 2014; 4:4528. http://dx.doi.org/10.1038/srep04528.
59. Foekema EM, De Gruijter C, Mergia MT, van Franeker JA, Murk AJ, Koelmans AA. Plastic in north sea fish. Env Sci Technol 2013; 47:8818–8824. http://dx.doi.org/10.1021/es400931b
60. Nuelle MT, Dekiff JH, Remy D, Fries E. A new analytical approach for monitoring microplastics in marine sediments. Environ. Pollut 2014; 184:161–169. http://dx.doi.org/10.1016/j.envpol.2013.07.027
61. Avio CG, Gorbi S, Milan M, et al. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ Pollut 2015; 198:211–222. https://doi.org/10.1016/j.envpol.2014.12.021
62. Dehaut A, Cassone AL, Frère L, et al. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environ. Pollut 2016; 215:223–233.
63. Ferreira T, Rasband W. ImageJ User Guide. 2012. https://doi.org/10.1038/nmeth.2019
64. Woodall LC, Sanches-Vidal A, Canals M, et al. The deep sea is a major sink for microplastic debris. R Soc Open Sci 2014; 1: 140317. https://doi.org/10.1098/rsos.140317
65. Mani T, Hauk A, Walter U, Burkhardt-Holm P. Microplastics profile along the Rhine River. Sci Rep 2015; 5:1–7. https://doi.org/10.1038/srep17988
66. Horton AA, Jürgens MD, Lahive E, van Bodegom PM, Vijver MG. The influence of exposure and physiology on microplastic ingestion by the freshwater fish Rutilus rutilus (roach) in the River Thames, UK. Environ Pollut 2018; 236:188–194. https://doi.org/10.1016/j.envpol.2018.01.044
67. Xu Q, Deng T, LeBlanc GA, An L. An effective method for evaluation of microplastic contaminant in gastropod from Taihu Lake, China. Environ Sci Pollut Res 2020; 27:22878–22887. https://doi.org/10.1007/s11356-020-08747-8.
68. Akindele EO, Ehlers SM, Koop JHE. First empirical study of freshwater microplastics in West Africa using gastropods from Nigeria as bioindicators. Limnologica 2019; 78:125708. https://doi.org/10.1016/j.limno.2019.125708
69. Hurley RR, Woodward JC, Rothwell JJ. Ingestion of Microplastics by Freshwater Tubifex Worms. Environ Sci Technol 2017; 51:12844–12851. https://doi.org/10.1021/acs.est.7b03567
70. Lin CT, Chiu MC, Kuo MH. Effects of anthropogenic activities on microplastics in deposit-feeders (Diptera: Chironomidae) in an urban river of Taiwan. Sci Rep 2021; 11:400. https://doi.org/10.1038/s41598-020-79881-z
71. Hohenblum P, Liebmann B, Liedermann M. Plastic and Microplastic in the environment. The Environment Agency Austria, Vienna 2015.
72. Stanković J, Milošević Dj, Paunović M, et al. Microplastic ingestion by freshwater Asian clams Corbicula fluminea (Müller, 1774) in the Danube River and main tributaries. Manuscript in progress.

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Original Scientific Article

CONCENTRATION OF POLYAMINES IN THE RAT LIVER DURING POSTNATAL PERIOD

Marko Bjelaković[1], Dušan Sokolović[2], Milica Bjelaković[3]

[1]Clinic of Pulmonology, University Clinical Center Niš, Niš, Serbia
[2]Institute of Biochemistry, Medical Faculty, University of Niš, Niš, Serbia
[3]Clinic of Gastroenterology and Hepatology, University Clinical Center Nis, Serbia

Abstract. *Polyamines, spermine, spermidine, and putrescine are ubiquitous in living cells. Polyamines play important roles in cell growth, proliferation, and survival. Our aim was to determine the concentration of the spermine, spermidine, and putrescine in the rat liver during the first 6 months of postnatal life. A total of 45 albino Wistar rats, maintained under controlled temperature (20±2°C) in the animal room facilities were included in this study. On the day 1, 3.5 months, and 6 months of postnatal life, rats were sacrificed by cervical dislocation. Thr liver was removed and washed in the 0.9% solution of sodium chloride. Concentrations of spermine, spermidine, and putrescine were determined. Concentration of polyamines in the liver tissue of the Wistar albino rats aged 1 day, 3.5 months, and 6 months was respectively: spermine (Sp) (33.81 ± 3.04; 128.15 ± 6.62; 74.34 ± 1.12 μg/g of wet weight); spermidine (Spd) (121.92 ± 6.23; 53.34 ± 3.31; 56.32± 1.41 μg/g wet weight); and putrescine (Put) (8.92 ± 0.98; 9.37 ± 0.98; 20.93 ± 1.15 μg/g wet weight). Polyamine concentration in the rat liver fluctuated during postnatal life. The concentration of the spermidine was highest in the rat liver on the first postnatal day, much higher than spermine, this ratio inverted in 3.5 months old rats. The concentrations of the spermidine and spermine were almost equal at the 6 months of the postnatal life. The concentration of putrescine steadily increases during postnatal life.*

Key words: *spermin, spermidin, putrescine, rat liver, postnatal life.*

Background

Polyamines spermine, spermidine, and putrescine are commonly ubiquitous in nature. They exist in millimolar concentrations in all living cells. The triamine spermidine and the tetraamine spermine are the most abundant polyamines in eukaryotic cells while the diamine putrescine and the triamine spermidine are found in prokaryotic cells such as bacteria, plants, and fungi [1–3]. Polyamines are involved in the regulation of a range of vital cellular processes including cell proliferation, differentiation, signal transduction, membrane stabilization, regulation of ion channels, and apoptosis [4–6]. They are also involved in the regulation of gene expression and translation [7]. Polyamines could act as antioxidants as they have been shown to inhibit lipid peroxidation [8]. It was found that spermidine, that is present in the nucleus in millimolar concentration, directly acts as a scavenger of reactive oxygen species (ROS), while spermine is able to protect DNA from ROS [9–11]. On the contrary, excessive polyamine catabolism is a prominent source of oxidative stress thought to be involved in infective, neurological, and malignant diseases [8]. Cancers cells contain a great amount of polyamines [12–14].

Correspondence to: Marko Bjelakovic
Clinic of Pulmonology, University Clinical Center Niš, Niš, Serbia
81 Dr. Zorana Đinđića Blvd, 18000 Niš, Serbia
E-mail: mbbjelakovic@gmail.com
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Recently, the relation between polyamines, especially spermidine and ageing was studied. The concentration of polyamines in body tissues declines with increasing age. Ppolyamine spermidine can prolong the lifespan by controlling the aging process [15, 16]. Therefore, spermidine may act as a universal anti-aging drug.

Our aim was to determine the concentration of polyamines (spermine, spermidine, and putrescine) in rat liver during postnatal development

Materials and Methods

A total 45 albino Wistar rats weighting 180 to 230 g, maintained under controlled temperature (20±2°C) in the animal room facilities were included in this study. Animals were divided into three groups: The first experimental group consisted of the youngest animals, the first day after birth (n = 3); the second experimental group consisted of 3.5 months old rats (n = 15); and the third experimental group consisted of 6 months old rats (n = 27). Rats were sacrificed by cervical dislocation. The liver was removed quickly and washed in the 0.9% solution of sodium chloride. Excess blood was removed by bloating and rinsing with ice -cold saline. Concentrations of spermine, spermidine, and putrescine were determined with butanol extraction followed by electrophoresis. Separated polyamines were identified by ninhydrin and quantified by the spectrophotometric method. For the statistical analyses, we used Sigma Stat 4.0 (SPSS Inc, Chicago, Ill). The obtained results were expressed as means ± standard deviation.

Results

The collected particles were assigned to one of 5 major Concentration of polyamines in the liver tissue of the Wistar albino rats old 1 day, 3.5 months, and 6 months was respectively: spermine (Sp) (33.81 ± 3,04; 128.15 ± 6.62; 74.34 ± 1.12 μg/g of wet weight); spermidine (Spd) (121.92 ± 6.23; 53.34 ± 3.31; 56.32± 1.41 μg/g wet weight); and putrescine (Put) (8.92 ± 0.98; 9.37 ± 0.98; 20.93 ± 1.15 μg/g wet weight).

Polyamine concentration in the rat liver fluctuated during postnatal life (Figure 1). While the concentration of the spermidine was highest in the rat liver on the first postnatal day, 3.6 times higher than spermine, this ratio inverted in 3.5 months old rats when the concentration of the spermine was 2.4 times higher than spermidine. The concentrations of the spermidine and spermine were almost equal at the 6 months of the postnatal life. The concentration of the putrescine steadily increases during postnatal life (Figure 1).

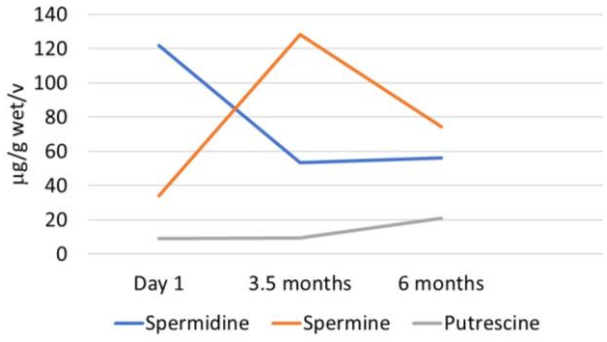

Fig. 1 Polyamine levels in the rat liver during first 6 months of postnatal life.

Discussion

Our study showed fluctuations of concentration of polyamines in the rat liver during postnatal life.

Our results are in agreement with the results of other studies. The concentration of polyamines is highest in fast - growing tissues [17–19]. Examination of polyamine metabolism in the rat liver during the development found that the concentration of spermidine was highest in the fetal liver followed by spermine level. The putrescine level was lowest [20, 21]. Jänne J et al. [22] found that the polyamine contents decreased with development and aging. This decrease is highest in the first month of life. Spermine concentration increased during the first month of postnatal life and then had the same concentration or decreased [22]. The increases in the putrescine concentration may be explained by the action of spermine/spermidine N1 acetyl transferase [23]. Our result, which pointed out the highest spermidine concentration in the liver of the youngest animals (on the first day after birth) is in agreement with the results of other studies [24]. Sturman et al. [24] analysed polyamine biosynthesis in the human fetal liver. They found three- to four-fold greater concentration of spermine, spermidine, and putrescine in fetal human liver than in mature human liver [24].

A recent study suggested that polyamine spermidine may increase lifespan and maybe used as a universal drug against aging [25]. Administration of spermidine, a natural polyamine whose intracellular concentration declines during human ageing, markedly extended the lifespan of the human body. The inclusion of spermidine in the supramolecular complex with different polymers has optimal effects on the regenerative processes [26].

Autophagy is the major lysosomal degradation pathway for recycling damaged and potentially harmful cellular material [27]. It is thought that autophagy may be essential for healthy ageing and longevity. The basal level of autophagy assures maintenance of cell homeostasis. Recently, the correlation between polyamines, especially spermidine, autophagy, and aging was studied [28–30]. Eisenberg et al. [31] examined the involvement of spermidine in autophagy. Spermidine enhanced autophagy in a number of cell types including human cells. It was suggested that spermidine-induced autophagy increased lifespan by decreasing histone acetylating. Acetylating has a great function in autophagy and longevity. Genetics and functional examination showed that spermidine inhibits the process of histone acetylating. Hipoacetilation has the main role in process of the autophagy control and regulation of longevity [31].

Our study showed fluctuations of the polyamines in the rat liver during postnatal life. While the concentration of the spermidine was highest in the rat liver on the first postnatal day, significantly higher than spermine, this ratio inverted in 3.5 months old rats. The concentration of spermidine and spermine were almost equal at the 6 months of the postnatal life. The concentration of the putrescine steadily increased during postnatal life.

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References

1. Cohen SS. A guide to the polyamines New York: Oxford University Press; 1998 [Available from: http://catalog.hathitrust.org/api/volumes/oclc/35174769.html.
2. Handa AK, Fatima T, Mattoo AK. Polyamines: bio-molecules with diverse functions in plant and human health and disease. Front Chem. 2018; 6(10). doi: 10.3389/fchem.2018.00010
3. Tabor CW, Tabor H. Polyamines. Annu Rev Biochem 1984; 53(1):749–790.
4. Agostinelli E. Biochemical and pathophysiological properties of polyamines. Amino Acids 2020; 52(2):111–117.
5. Pegg AE. Functions of polyamines in mammals. J Biol Chem 2016; 291(29): 14904–14912.
6. Pegg AE. Introduction to the thematic minireview series: sixty plus years of polyamine research. J Biol Chem 2018; 293(48):18681–18692.
7. Childs AC, Mehta DJ, Gerner EW. Polyamine-dependent gene expression. Cell Mol Life Sci 2003; 60(7):1394–1406.
8. Hussain T, Tan B, Ren, et al. Polyamines: therapeutic perspectives in oxidative stress and inflammatory diseases. Amino Acids 2017; 49(9):1457–1468.
9. Farriol M, Segovia-Silvestre T, Venereo Y, Orta X. Antioxidant effect of polyamines on erythrocyte cell membrane lipoperoxidation after free-radical damage. Phytother Res 2003;17(1):44–47.
10. Fujisawa S, Kadoma Y. Kinetic evaluation of polyamines as radical scavengers. Anticancer Res 2005; 25(2A):965–969.
11. Ha HC, Sirisoma NS, Kuppusamy P, Zweier JL, Woster PM, Casero RA. The natural polyamine spermine functions directly as a free radical scavenger. Proceedings of the National Academy of Sciences. 1998; 95(19):11140–11145.
12. Green DR. Polyamines and aging: A CLEAR connection? Mol Cell 2019; 76(1):5–7.
13. Madeo F, Eisenberg T, Pietrocola F, Kroemer G. Spermidine in health and disease. Science 2018;359(6374):eaan2788.
14. Wallace HM. The polyamines: past, present and future. Essay Biochem 2009; 46:1–10.
15. Madeo F, Carmona-Gutierrez D, Kepp O, Kroemer G. Spermidine delays aging in humans. Aging 2018; 10(8):2209–2211.
16. Minois N. Molecular basis of the 'anti-aging' effect of spermidine and other natural polyamines - a mini-review. Gerontology. 2014; 60(4): 319–326.
17. Sagar NA, Tarafdar S, Agarwal S, Tarafdar A, Sharma S. Polyamines: functions, metabolism, and role in human disease management. Med Sci 2021; 9(2):44.
18. Nakanishi S, Cleveland JL. Polyamine homeostasis in development and disease. Med Sci 2021; 9(2):28.
19. Bae D-H, Lane DJR, Jansson PJ, Richardson DR. The old and new biochemistry of polyamines. Biochim Biophy Acta Gen Subj 2018; 1862(9):2053–2068.
20. Eguchi K, Yonezawa M, Ohmoto H, Mitsui Y, Hiramatsu Y. Development changes of polyamine biosynthesis in rat liver. Biol Neonate 1992; 62(6):402–408.
21. Russell DH, Medina VJ, Snyder SH. The dynamics of synthesis and degradation of polyamines in normal and regenerating rat liver and brain. J Biol Chem 1970; 245(24):6732–6738.
22. Jänne J, Raina A, Siimes M. Spermidine and spermine in rat tissues at different ages. Acta Physiol Scand 1964; 62(4):352–358.
23. Cuervo AM, Bergamini E, Brunk UT, Dröge W, Ffrench M, Terman A. Autophagy and aging: the importance of maintaining "clean" cells. Autophagy. 2005; 1(3):131–140.
24. Sturman JA, Gaull GE. Polyamine biosynthesis in human fetal liver and brain. Pediatr Res 1974; 8(4):231–237.
25. Eisenberg T, Abdellatif M, Schroeder S, et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. Nat Med 2016; 22(12):1428–1438.
26. Madeo F, Bauer MA, Carmona-Gutierrez D, Kroemer G. Spermidine: a physiological autophagy inducer acting as an anti-aging vitamin in humans? Autophagy 2019; 15(1):165–168.
27. Vellai T, Bicsák B, Tóth ML, Takács-Vellai K, Kovács AL. Regulation of cell growth by autophagy. Autophagy 2008; 4(4):507–509.
28. Komatsu M, Ichimura Y. Selective autophagy regulates various cellular functions. Genes Cells 2010; 15(9):923–933.
29. Morselli E, Galluzzi L, Kepp O, et al. Autophagy mediates pharmacological lifespan extension by spermidine and resveratrol. Aging 2009; 1(12):961–970.
30. Yorimitsu T, Klionsky DJ. Autophagy: molecular machinery for self-eating. Cell Death Differ 2005; 12(2):1542–1552.
31. Eisenberg T, Knauer H, Schauer A, et al. Induction of autophagy by spermidine promotes longevity. Nat Cell Biol 2009; 11(11): 1305–1314.

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Original Scientific Article

THE IMPACT OF THE COVID-19 PANDEMIC ON PEDIATRIC APPENDICITIS MANAGEMENT AND OUTCOMES

Maja Zečević[1], Zoran Marjanović[1,2], Goran Janković[1], Danijela Djerić[1], Zorica Jovanović[1], Vesna Marjanović[2,3], Jelena Lilić[3], Danijel Mančić[4,1], Dragana Lazarević[2,5]

[1]Clinic for Pediatric Surgery, University Clinical Center Niš, Niš, Serbia
[2]Faculty of Medicine, University of Niš, Niš, Serbia
[3]Clinic for Anaesthesiology and Intensive Therapy, University Clinical Center Niš, Niš, Serbia
[4]Health Center Zaječar, Zaječar, Serbia
[5]Clinic of Pediatrics, University Clinical Center Niš, Niš, Serbia

Abstract. *The pandemic caused by the SARS-CoV-2 virus had a significant impact on providing health services the world over and is reflected in pediatric surgery also. The aim of this study was to determine the differences in the prevalence of forms of appendicitis among children during and prior to the pandemic, and to compare the demographic characteristics. A retrospective study which covered a period from September 1, 2018 until September 1, 2021 and all the patients who underwent surgery for acute appendicitis was carried out. Demographic and clinical data were collected and analysed. The study included a total of 267 patients. Prior to the pandemic a total of 160 children underwent surgical treatment, 61.9% boys and 38.1% girls, while during the pandemic a total of 107 underwent surgical treatment, mostly boys 68,2%, and to a lesser extent, girls 31.8%. The average age of the patients was 11.0±3.9 years. Even though there is a difference in the number of children who underwent surgical treatment for perforated appendicitis prior to and during the Covid-19 pandemic, 54:44 or 33.8:41.1%, the difference is not statistically significant. During the pandemic, two patients who tested positive for the SARS-CoV-2 virus received surgical treatment for a perforated appendicitis. The Covid-19 pandemic had an impact on the healthcare system worldwide. In most centers for pediatric surgery, there was an increased incidence of perforated appendicitis without significant deviations in the demographic characteristics, which correlates with the results of this study.*

Key words: *appendicitis, children, Covid-19 pandemic.*

Introduction

The pandemic caused by the SARS-CoV-2 had a significant impact on the healthcare provided worldwide and is reflected on all specialized fields, including pediatric surgery. The negative effects of the Covid-19 were especially noted on the health systems of low- or middle-level income countries [1]. An analysis of Anglo-Saxon literature provides insight into the trend of growth of complicated appendicitis (gangrenous and perforated appendicitis) during the pandemic caused by the SARS-CoV-2 virus, due to the possible fear of infection. As a result, there may potentially be a delay in diagnosis and an increased rate of complications [2]. However, the literature contains contradictory data which indicate that even though the routine in pediatric surgical centers during the pandemic changed significantly, and that there was a decrease in the number of admissions, this did not have an impact on the change in the diagnosis and treatment of acute appendicitis among children [3].

Correspondence to: Maja Zecevic
Clinic for Pediatric Surgery, University Clinical Center Niš
81 Dr. Zorana Đinđića Blvd, 18000 Niš, Serbia
E-mail: maja.zecevicmd@gmail.com
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The Aim

The aim of this study is to determine whether there is a difference in the prevalence of clinical forms of appendicitis in the pediatric population during the pandemic compared to a control group from an identical period of time prior to the onset of the pandemic on the territory of the Republic of Serbia, and to compare the demographic characteristics of children who underwent appendectomies due to acute appendicitis.

Materials and Methods

By analysing the medical documents of the Clinic for Pediatric Surgery, University Clinical Center Niš, a retrospective study was conducted which included a three-year period, starting from September 1, 2018 to September 1, 2021. The study included all the patients who during that time underwent surgical treatment at the Clinic for Pediatric Surgery UCC Niš for acute forms of appendicitis. The study did not include any incidental appendectomies carried out during the surgical treatment of some other acute abdominal condition.

The study was performed in accordance with all the recommended guidelines, and the data were first entered into an Excel© table which was solely available to the

researchers, which ensured that the identity of the patients was kept anonymous. Data referring to age, gender, clinical forms of appendicitis, and place of living were collected. The statistical data analysis was carried out in the program package SPSS 16.0. The hypothesis was tested at the p<0,05 level of significance. A period of 18 months prior to the pandemic caused by the virus on the SARS-CoV-2 territory of Serbia was compared to an identical period of time following the onset of the epidemic. A comparison of the continued values prior to and during the pandemic caused by the virus SARS-CoV-2 was carried out using the t-test, while the Chi-squared test was used to compare the categorical features between the groups.

Results

The study included a total of 267 patients who underwent surgical treatment for acute appendicitis in the aforementioned period. Prior to the pandemic caused by the SARS-CoV-2 virus, 160 children underwent surgical treatment, of which 61.9% were boys and 38.1% were girls. From the beginning of the pandemic, a smaller number of children underwent surgical treatment, 107 of them, mostly boys with 68.2%, and to a lesser extent, girls, 31.8%. There is no statistically significant difference in the frequency of occurrence of appendicitis in terms of gender (p=0.352).

The average age of the studied population is 11.0 ±3.9 yrs. The youngest patient to undergo surgical treatment was one year old, while the oldest was 17. The patients were divided into three age categories: the first one included children aged from birth to the age of four, the second included children aged from 5 to 10, and the third included children aged 11 to 17. The distribution of the age categories of children who underwent surgical treatment is shown in Fig. 1. Most patients treated during the pandemic were aged 11–17 years (64.5%, p=0.001). Children who underwent surgical treatment for acute appendicitis during the pandemic caused by the SARS CoV2 virus are statistically significantly older (p=0.002) than the children who underwent surgery prior to the pandemic.

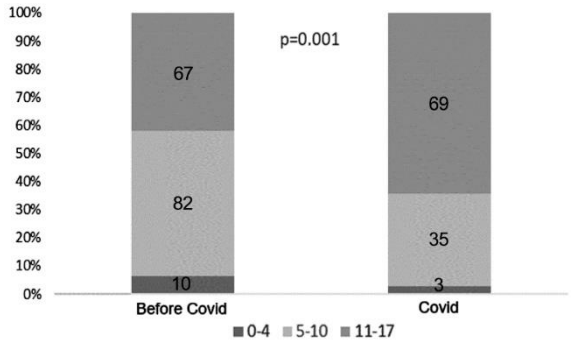

Fig. 1 Age categories prior and during the pandemic

Three-quarters of the patients prior to the pandemic caused by the SARS-CoV-2 (75.6%) were from urban environments, followed by patients from rural environments (24.4%). The percentage of children from urban and rural environments who underwent surgical treatment during the pandemic is almost identical: 75.7% were children from urban environments, and 24.3% were children from rural areas. The prevalence of patients from Niš in percentages is 21.2% prior to the pandemic and 27.1% during the pandemic, whereby there is no statistical significance (p=0.339). Place of residence was not statistically significantly connected with the number of patients who underwent surgical treatment due to appendicitis prior to and during the pandemic (p=0.989).

The analysis also included the prevalence of clinical forms of appendicitis which correlate with the pathohistological forms prior and during the pandemic caused by the SARS-CoV-2 virus. The comparative results expressed in percentages for both periods of time are shown in Table 1. Even though there is a difference in the number of children who underwent surgical treatment for a perforated appendicitis prior to and during the pandemic caused by the SARS-CoV-2 virus, 54:44 or 33.8:41.1%, this difference is not statistically significant (p=0.583). The other forms of appendicitis do not differ statistically significantly prior to and during the pandemic caused by the SARS-CoV-2 virus.

Table 1 The distribution of forms of acute appendicitis prior to and during the pandemic

	Prior to the pandemic Covid-19	During the pandemic Covid-19
Acute appendicitis	4.4%	2.8%
Phlegmonous appendicitis	22.5%	22.4%
Gangrenous appendicitis	39.4%	33.6%
Perforated appendicitis	33.8%	41.1%

During the pandemic, two female patients aged 4 to 11 underwent surgical treatment in our facility during the aforementioned period due to a perforated appendicitis, who at the time of the surgical treatment tested positive to the SARS-CoV-2. Both children underwent surgical treatment in an operating room meant solely for the care of patients who tested positive to the SARS-CoV-2, with the implementation of the complete protective equipment required by epidemiologists.

Discussion

Acute appendicitis is one of the most frequent types of surgical emergencies in the pediatric population [4]. The onset of the pandemic caused by the SARS-Cov-2 virus had a significant impact on the organization of work in the wards of the pediatric surgery departments, where mostly surgical emergencies are treated, including acute appendicitis. In order to reduce the possibility of spreading the Covid-19 infection, certain surgical centers also decided on the conservative treatment of acute appendi-

citis in children. Coldvin and associates published their results on the non-surgical treatment of appendicitis with a double parenteral antibiotic therapy (amoxiclav and gentamicin) during hospitalization, and the continued peri-oral antibiotic therapy during outpatient treatment during the pandemic caused by the SARS-Cov-2 virus. The aim was to decrease the exposure of the staff in the operating theatre during anaesthesia, considering the possibility that the patient might be Covid positive. The general success of the antibiotic therapy was 72%, 96% in the case of uncomplicated appendicitis, and 40% in the case of complications, whereby there were no patients who tested positive for the virus SARS-Cov-2 [5]. Other centers opted for the non-surgical treatment of appendicitis among children during the pandemic. Kvasnovsky stated that during the peak of the pandemic, in their facility they treated 45.5% of the children non-surgically, even though 78.2% of the children did not meet the previously published criteria for the non-surgical treatment of acute appendicitis [6].

During the pandemic, acute appendicitis was treated in our facility by relying on the prescribed protective measures against the SARS-CoV-2 virus. An increase in perforated appendicitis of 41.1% was noted, compared to 33.8%, which proved not to be statistically significant. An increase in the incidence of perforated appendicitis among children was noted in other centers worldwide.

Esparaz and associates determined a statistically significant increase in perforated appendicitis during the pandemic caused by the Covid-19 virus (45.6% vs 26.4%; $p < 0.001$) [7]. Similar reported increases in the incidence of perforated appendicitis were published by Schäfer, where the rate of perforation in 2020 was 27.8%, while during 2018–2019 it was 20.7%. The difference was that among younger patients (≤11.2 yrs) in 2020 the rate of perforated appendicitis was significantly greater, 37.6%, as opposed to 22.2% in 2018/2019. The age structure of the patients that underwent surgical treatment of 11.2 correlates with the average age of our patients, 11,0 ±3,9 yrs [8]. An increased rate of complicated appendicitis (gangrenous and perforated histopathological forms) of 60.5% during the pandemic, compared to 30.4% during the same period of time the previous year, but without statistically significant demographic deviations was determined by Lee-Archer and associates [9].

The reason for the increased rate of perforated appendicitis among children could also be the prolonged duration of the symptoms prior to the first visit to the doctor. Prior to the pandemic, symptoms usually lasted for one day, while during the pandemic it was two days which proved to be statistically significant ($p = 0.003$) [10]. A multi-centric retrospective study from the USA compared the number of visits to the emergency room in 18 federal states of the USA from January to June 2020, and the number of visits from the same period of time for the previous calendar year. It was determined that the number of visits in the pediatric population initially fell by 74% among children under the age of 10 and by 67% among children aged 14–17, in order for that number to decrease to 72% and 50% by the end of the studied period [11]. A significantly smaller number of cases of appendicitis among children during the same period of time, compared to the same period of time over a span of 4 years was also published by Hassoun and associates [12].

One of the aggravating factors which can affect the correct diagnosis of acute appendicitis among children during this pandemic is also the emergence of a systemic hyperinflammatory syndrome caused by the SARS-CoV-2 virus, known as MIS-C (Multisystem inflammatory syndrome in children). In such cases, gastrointestinal tract is affected in 92% cases, which could imitate the clinical image of inflammatory bowel disease as well as appendicitis. Jackson and associates published a case study of a nine-year-old girl who underwent surgical treatment on the suspicion of acute appendicitis diagnosed based on the clinical image, radiological findings, and pathohistological findings of the appendix, the clinical image of which differed from acute appendicitis in the sense of necrotic lymphadenitis and vasculitis, as well as Covid-19 antibodies which indicated MIS-C [13].

Anderson published two case studies, one of a girl who was diagnosed with perforated appendicitis and MIS-C, and the other of a patient diagnosed, based on the pathohistological review, with MIS-C even though the clinical image corresponded to acute appendicitis [14]. In our facility there were no cases where children diagnosed with MIS-C underwent surgical treatment due to suspicion of acute appendicitis.

For the duration of the study, two children infected by the Covid-19 virus underwent surgical treatment in our facility for perforated appendicitis. The girls were aged 11 and 4, and the diagnosis was made based on their clinical image, laboratory values, plane abdominal radiographies, and abdominal ultrasounds. Acevado and associates presented 3 cases of acute appendicitis among children with a concomitant Covid-19 infection. All three children were males, and one boy had perforated appendicitis. In two of the cases, an MSCT of the abdomen was also performed for diagnostic purposes [15].

Conclusion

The emergence of the pandemic caused by the SARS-Cov-2 virus had a significant impact on the way healthcare facilities work on a global level, both because of the new challenges in solving typical acute conditions such as appendicitis among children who are routinely tested prior to hospitalization, as well as the new challenges such as diagnosis and surgical treatment of acute appendicitis with a concomitant infection of the Covid-19, and the possibility of misdiagnosis of appendicitis among children with a diagnosis of MIS-C. In most of the centers of pediatric surgery, there is an increased incidence of perforated appendicitis without significant deviations in the demographic characteristics, which correlates with the results of this study.

References

1. Bong CL, Brasher C, Chikumba E, McDougall R, Mellin-Olsen J, Enright A The COVID-19 pandemic: efects on low and middle-income countries. Anesth Analg 2020; 131:86–92.
2. Snapiri O, Rosenberg Danziger C, Krause I, et al. Delayed diagnosis of paediatric appendicitis during the COVID-19 pandemic. Acta Paediatr 2020; 109(8):1672–1676.
3. La Pergola E, Sgrò A, Rebosio F, et al. Appendicitis in Children in a Large Italian COVID-19 Pandemic Area. Front Pediatr 2020; 8:600320.
4. McCollough M, Sharieff GQ. Abdominal surgical emergencies in infants and young children. Emer Med Clinics North Am 2003; 21(4):909–935.
5. Colvin D, Lawther S. A surge in appendicitis: Management of paediatric appendicitis during the COVID-19 surge in the Royal Belfast Hospital for Sick Children. Ulster Med J 2021; 90(2):86–89.
6. Kvasnovsky C, Shi Y, Rich BS, et al. Limiting hospital resources for acute appendicitis in children: Lessons learned from the U.S. epicenter of the COVID-19 pandemic. J Pediatr Surg 2021; 56(5):900–904.
7. Esparaz JR, Chen MK, Beierle EA, et al. Perforated Appendicitis during a Pandemic: The Downstream Effect of COVID-19 in Children. J Surg Res 2021; 268:263–266.
8. Schäfer FM, Meyer J, Kellnar S, et al. Increased Incidence of Perforated Appendicitis in Children During COVID-19 Pandemic in a Bavarian Multi-Center Study. Front Pediatr 2021; 9:683607. doi:10.3389/fped.2021.683607
9. Lee-Archer P, Blackall S, Campbell H, Boyd D, Patel B, McBride C. Increased incidence of complicated appendicitis during the COVID-19 pandemic. J Paediatr Child Health 2020; 56(8):1313–1314.
10. Gerall CD, DeFazio JR, Kahan AM, et al. Delayed presentation and sub-optimal outcomes of pediatric patients with acute appendicitis during the COVID-19 pandemic. J Pediatr Surg 2021; 56(5):905-910. doi:10.1016/j.jpedsurg.2020.10.008
11. Pines JM, Zocchi MS, Black BS, et al. Characterizing pediatric emergency department visits during the COVID-19 pandemic. Am J Emerg Med 2021; 41:201–204.
12. Hassoun A, Kadenhe-Chiweshe A, Sharma M. New York's COVID-19 shelter-in-place and acute appendicitis inchildren. J Pediatr Surg 2021; 56:635–636.
13. Jackson RJ, Chavarria HD, Hacking SM. A Case of Multisystem Inflammatory Syndrome in Children Mimicking Acute Appendicitis in a COVID-19 Pandemic Area. Cureus 12(9): e10722. DOI 10.7759/cureus.10722
14. Anderson JE, Campbell JA, Durowoju L, et al. COVID-19-associated multisystem inflammatory syndrome in children (MIS-C) presenting as appendicitis with shock. J Pediatr Surg Case Rep 2021; 71:101913. doi:10.1016/j.epsc.2021.101913
15. Acevedo MJ, Steffey D, Dillon JE, Lee JT, Worhunsky DJ. Concurrent COVID-19 infection in children with acute appendicitis: A report of three cases. Radiol Case Rep 2021; 16:2972–2977.

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Original Scientific Article

ASSOCIATION OF RADIOGRAPHIC FINDINGS OF MUSCULOSKELETAL HAND INVOLVEMENT WITH CLINICAL AND SEROLOGICAL FEATURES OF SYSTEMIC SCLEROSIS

Bojana Stamenković[1,2], Sonja Stojanović[1,2], Valentina Živković[1,2], Dragan Đorđević[1,2], Mila Bojanović[1,3], Hristina Kocić[4], Vanja Đurić[5], Aleksandra Stanković[1,5]

[1]Faculty of Medicine, University of Niš, Serbia
[2]Institute for Treatment and Rehabilitation Niška Banja, Serbia
[3]Otorhinolaryngology Clinic, Clinical Center Niš, Serbia
[4]Clinic for Dermatovenerology, Niš, Serbia
[5]Polyclinic "Neuromedic" Niš, Serbia

Abstract. *Musculoskeletal manifestations (MSM) frequently occur in systemic sclerosis (SSc) and imply a variety of rheumatic symptoms and clinical features, from arthralgia to arthritis, contractures, tendon friction rubs, tenosynovitis, myalgia, muscle tenderness, and myositis. The objective of the study was to determine the prevalence of joint manifestations in clinical findings, as well as frequency and type of radiographic changes in 56 patients with the limited and diffuse form of SSc; to define the correlation between musculoskeletal hand changes in SSc and specific antibodies (antinuclear antibodies- ANA), as well as with antibodies specific for particular forms of SSc (anti-topoisomerase-1 antibodies- ATA, anticentromere antibodies- ACA); and to test the correlation between specific cardiopulmonary manifestations in SSc, and frequency and type of musculoskeletal changes. The obtained results indicated a high frequency of joint manifestations in SSc, which were estimated by clinical and radiographic examinations. Joint involvement in SSc was underestimated in clinical trials, as it occurred more frequently than expected. Radiographic hand findings in tested SSc patients indicated the presence of arthritis, erosions, joint space narrowing, radiological demineralization, acro-osteolysis, flexion contractures, and calcinosis. Hand involvement was an important cause of morbidity, which seriously affected the quality of life in patients with SSc. Various forms of joint and bone involvements could represent the base for introducing an innovative approach to treating musculoskeletal hand damage in SSc.*

Key words: *joint involvement, radiographic changes in hand, systemic sclerosis.*

Introduction

Systemic sclerosis (SSc), often known as scleroderma, is a chronic autoimmune disease of unknown cause, which is characterized by fibrosis of the skin and internal organs, followed by immune system activation and severe vascular disorders [1]. Two primary forms of the diseases are: limited (lSSc) and diffuse (dSSc). Limited subtype implies disease entities, such as morphea, linear scleroderma, "en coup de sabre", as well as other variants of the disease of different localization and degree of skin thickening, usually in the form of bands that affect specific parts of extremities, face, and forehead [2, 3].

Activation of endothelial cells and vascular disorders are the earliest symptoms of systemic sclerosis [4, 5]. Induction of endothelial cell (EC) damage may be activated by numerous triggers, such as infection, immune-mediated cytotoxicity, antiendothelial cell antibodies (AECA), and ischemia-reperfusion injury. As a primary feature of SSc, excessive fibrosis is the result of the complex sequence of mutually connected vascular injuries and activation of the immune system and represents a reparative process. Cell immunity plays a crucial role in the complex genesis of SSc, and is characterized by producing specific disease-related antibodies [6, 7]. Aside from cell immunity, scientists have noted activities of humoral immunity. Using the technique of indirect immunofluorescence (IIF), antinuclear antibodies (ANA)have been detected in > 95% of patients with SSc. Specific ANA, which are connected to specific disease manifestations, are found in > 80% SSc patients. The latest data have pointed out that more than 10 types of specific autoantibodies, especially anticentromere antibodies (ACA),antitopoisomerase antibodies (ATA), and anti-U1RNP antibodies, have been detected in patients with SSc or SSc overlap syndrome [8]. Clinical manifestations of SSc are heterogeneous and depend on the form of the disease (diffuse or limited) and organ involvement. Patients with the diffuse form of the disease are at risk of rapid development of severe skin fibrosis and internal organ involvement, while patients with limited SSc experience slowly progressive skin changes which do not affect the area above elbows, knees, proximal extremities, or trunk, but imply various degrees of internal organ involvement [9, 10].

Musculoskeletal manifestations (MSM) are very frequent in SSc and implicate numerous rheumatic manifestations, from arthralgia to arthritis, contracture, tendon

Correspondence to: Bojana Stamenković
Faculty of Medicine, University of Niš, Serbia
81 Dr. Zorana Đinđića Blvd, 18000 Niš, Serbia
E-mail: bojana.stamenkovic.70@gmail.com
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friction rubs, tenosynovitis, myalgia, muscle tenderness, and myositis. Joint involvement affects the quality of life of SSc patents and results in a significant decrease of work capacity or ability to perform everyday activities. According to various studies [11], the prevalence of arthralgia ranges from 23 to 81%. Moreover, synovial inflammation may cause diagnostic confusion. The assessment of arthritis in SSc is very complex due to the following disease features: skin edema, skin thickening and tethering, digital ulcer, subcutaneous calcinosis, and contractures. Physical examination is not sensitive enough to assess arthritis in SSc [12,13].

Tendon involvement in SSc primarily implies the presence of tenosynovitis and tendon friction rubs, which are often associated with the rapid progression of skin fibrosis. It usually occurs in the initial stage of the disease in patients with diffuse form, but it may be recorded in all stages and in all subtypes (lSSc and dSSc). Disease progression is accompanied by contractures of the involved joints, which affect movement and functions. Flexion contracture is characterized by permanent shortening of muscles and scar tissue, resulting in distortion and deformities which, based on numerous studies, may occur in 24 to 56% of patients.

Radiographic Changes in Bones and Joints

Conventional radiography is a method used to visualize late, destructive consequences of synovitis. However, this method cannot be used to visualize synovial membrane inflammation or early destruction of cartilage and bone. Radiographic changes in SSc include articular lesions with juxta-articular osteoporosis destruction and joint space narrowing, erosions in MCP, PIP and DIP joints, and wrists. Erosive arthropathycan look like erosive OA or psoriatic arthritis and cause changes that are similar to rheumatoid arthritis RA. The patients who meet classification criteria for SSc and RA have SSc- RA overlap syndrome [14, 15]. Prevalence of SSc -RA overlap is very hard to determine, as it occurs in 4.6–5.2% of patients.

In terms of radiographic findings, joint abnormalities may be associated with extra-articular involvements, skin atrophy, calcinosis cutis, and fingertip resorption, which differentiate SSc from other rheumatic diseases [16].

Study objectives are as follows: 1. to determine the prevalence of joint abnormalities in clinical findings, frequency, and types of radiographic changes in 56 patients with the limited diffuse form of SSc; 2. to define the correlation between musculoskeletal hand changes in SSc and antinuclear antibodies (ANA), as well as with antibodies specific for particular forms of SSc (ATA, ACA); and 3. to test the correlation between specific cardiopulmonary manifestations in SSc, and frequency and type of musculoskeletal changes.

Method: The study included a total of 56 patients (52 female and 4 male), median age 55.84±13.76 (age range: 33–79), who were treated at Rheumatology Clinic, Institute for Treatment and Rehabilitation Niška Banja. The average disease duration was 9.31±5.99 (range: 1–25) years. All patients met 2013 ACR/EULAR criteria for classifying SSc [17]. Disease duration was determined based on the first occurrence of Raynaud's phenomenon. Based on Le Roy et al. classification (2), a total of 33 patients suffered from lSSc, while 23 patients suffered from dSSc.

The functional status was defined based on Health Assessment Questionnaire (HAQ). The questionnaire was filled out by all SSc patients. HAQ contained twenty questions, classified into eight categories which referred to different activities: dressing and grooming, arising, eating, walking, object reaching, and gripping. The patients could select one of the four options for answering a particular question, while answers were scored 0-3, where: 0- can perform an activity without any difficulty, 1- with some difficulty, 2- with much difficulty, and 3- unable to perform an activity. The HAQ scores were classified into three grades: moderate disability (mild functional impairment) was graded from 0-1, severe disability (serious functional impairment) was graded from 1.01–2, while complete physical impairment and need of custodial care were graded from 2.01–3.

All patients with SSc were subjected to clinical examination, laboratory tests, standard hand radiography, and assessment of disease clinical manifestations. Internal organ involvement was defined in compliance with previously defined criteria [1]. Lung changes were defined based on the presence of bibasilar fibrosis at standard heart and lung radiography and/or at high-resolution computerized tomography, pulmonary function tests (FVC, DLCO/VA) and/or presence of pulmonary arterial hypertension detected at color Doppler echocardiography. Values of FVC< 75%, i.e. DLCO/VA< 75% compared to the normal values pointed to the presence of restrictive changes. Pulmonary arterial hypertension was defined as pulmonary pressure above 40mmHg at rest at Doppler echocardiography [18].

Heart involvement was recorded in the findings of pericarditis, complex arrhythmias, conduction disorders, diastolic dysfunction, and reduced left ventricular ejection fraction. Muscle involvement was assessed based on the presence of muscle tenderness and/or increased value of creatine phosphokinase (CPK).

The presence of synovitis, arthralgia, tendon friction rubs and flexion contracture was determined during a clinical examination. Additionally, the presence of active digital ulcers, indentations, and pitting scars from previous ulcers was analyzed.

Serology tests, which aimed at determining ANA and ACA were conducted by means of indirect immunofluorescence on HEP-2 cells (Immunoconcepts, Sacramento, California, USA). Titer 1:40, i.e. titer which was, in practice, recommended as "screening" was taken as limit ANA titer ("cut off" titer, i.e. titer representing the lowest value accepted as positive test result). Anti-topoisomerase antibodies were determined by the ELISA method (Imtec, Immunodiagnostics, Berlin, Germany).

All patients with SSc were tested for the presence of a rheumatoid factor (RF). Value of RF > 20 IU/L was

regarded as positive. ELISA (enzyme-linked immunosorbent assay) method (Imtec) was used to determine the presence of anti-CCP antibodies. The samples were classified as positive when values were >25 U/ml. The control group without CCP antibodies consisted of 28 healthy individuals of the same age and gender.

Standard hand and wrist radiography was conducted in all patients with SSc. The changes were evaluated by a radiologist who had no information on the clinical and serologic conditions of the patients (diagnosis). Radiographic joint changes in the tested SSc group included: erosions (cortical bony surface discontinuity) and joint space narrowing: focal or diffuse joint narrowing. Bone changes implied demineralization (juxta-articular or generalized osteoporosis) and bone resorption. Soft tissue changes included calcifications and flexion contractures. Joint space narrowing at proximal interphalangeal (PIP) joints and distal interphalangeal (DIP) joints were not analyzed if the presence of digital contractures was confirmed.

Student's t-test was used for comparing the mean values of age and disease duration. Chi-square test or Fisher exact test were used for comparing the frequency of categorical variables if one of the expected frequencies was less than 5.

Association of elevated values of ANA, ACA, and ATA with synovitis, erosions, flexion contracture, tendon friction rubs, cysts, joint space narrowing, resorption, osteoporosis, acro-osteolysis, calcinosis, and contracture was tested by calculating Phi (Fi) correlation coefficient. The threshold for defining statistical significance was less than 5% ($p<0.05$). The results of statistical analysis were shown in tables and graphs.

Quantitative analysis was carried out on a PC. Microsoft Office 2010 Excel was used for recording, ranking, and table and graphical presentation of the obtained data. Software package SPSS, Version 18.0, was used for statistical analysis and calculation.

Results

A total of 56 patients with SSc was included in the study, 4 (7.1%) of whom were male and 52 (92.9%) were female. The median age was 55.84±13.76, while the average disease duration was 9.31±5.99 years. Limited SSc was present in 33 (58.9%) patients, while diffuse SSc was identified in 23 (41.1%) patients.

Characteristics of patients with SSc are shown in Table 1.

Prevalence of synovitis in clinical findings of SSc patients was 12/56 (21.4%), arthralgia was identified in 45/56 (80.4%) patients, tendon friction rubs were recorded in 11/56 (19.7%) patients, while joint flexion was confirmed in 24/56 (42.9%) patients.

Radiographic changes in patients with SSc were divided into: 1. changes in joints, 2. changes in bones, and 3. changes in soft tissue.

Table 1 Patients with SSc

Characteristics	Patients with SSc (n=56)
Age	55.84±13.76
Male/female	4 (7.1%) / 52 (92.9%)
Disease duration (in years)	9.31±5.99
Cutaneous subtype	
limited	33 (58.9%)
diffuse	23 (41.1%)
Raynaud's phenomenon	54 (96.4%)
Digital ulcer	22 (39.3%)
HAQ>1,5	31 (55.4%)
Lung fibrosis	34 (60.7%)
Elevated pulmonary artery systolic pressure (SPRV>40mmHg)	3 (5.4%)
Positive antinuclear antibodies (ANA)	42 (76.4%)
Positive anti-topoisomerase-1 antibodies (anti-Scl 70)	14 (25.0%)
Positive anticentromere antibodies (ACA)	11 (20.4%)
Elevated creatine phosphokinase and/or muscle tenderness	3 (5.4%)
Increased acute phase reactants	15 (26.8%)
Positive RF	10 (17.9%)
Positive anti-CCP antibodies	10 (17.9%)

Joint space narrowing was observed in 34 (60.7%) SSc patients, erosions were identified in 21 (37.5%) patients, while arthritis (confirmed by radiography, in case of the detected presence of erosions and joint space narrowing) was found in 14 (25%) patients with SSc.

Changes in bones implied the presence of bone resorption, acro-osteolysis, and radiological demineralization. The frequency of occurrence of each of the stated changes in the SSc group was shown in Table 2.

Table 2 Radiographic changes in patients with SSc

Radiographic changes	Patients with SSc (n=56)
Changes in joints	
Erosions	21 (37.5%)
wrist	9 (16.1%)
MCP	7 (12.5%)
PIP	9 (16.1%)
DIP	15 (26.8%)
Joint space narrowing	34 (60.7%)
wrist	19 (33.9%)
MCP	27 (48.2%)
PIP	27 (48.2%)
DIP	24 (42.9%)
Arthritis (erosions + joint space narrowing)	14 (25.0%)
Changes in bones	
Demineralization	22 (39.3%)
Bone resorption	19 (33.9%)
Acro-osteolysis	17 (30.4%)
Changes in soft tissue	
Calcinosis	20 (35.7%)
Flexion contracture	23 (41.1%)

Finally, changes in soft tissue included calcinosis, which was observed in 20/56 (35.7%) patients, and flex-

ion contracture, which was identified in 23/56 (41.1%) patients with SSc.

The presence of calcinosis (57.1:22.9%; p=0.010) and positive ANA (95.2:64.7%; p=0.010) was more frequent in 21 patients with erosive arthropathy than in 35 patients without erosive arthropathy.

Table 3 Comparison of clinical and serological features in SSc patients with and without erosive changes

Characteristics	Erosive arthropathy (n=21; 37.5%)	No erosive changes (n=35; 62.5%)	p
SSc subtype			
diffuse SSc	10 (47.6%)	13 (37.1%)	0.440
limited SSc	11 (52.4%)	22 (62.9%)	
Digital ulcer	10 (47.6%)	12 (34.3%)	0.323
Calcinosis	12 (57.1%)	8 (22.9%)	**0.010**
Organ involvement			
Lungs	14 (66.7%)	20 (57.1%)	0.480
Heart	11 (52.4%)	18 (51.4%)	0.945
Positive RF	4 (19.0%)	6 (17.1%)	0.857
Positive anti-CCP	4 (19.0%)	6 (17.1%)	0.857
Positive ANA	20 (95.2%)	22 (64.7%)	**0.010**
Positive ACA	5 (23.8%)	6 (17.1%)	0.617
Positive anti-topoI antibodies	8 (38.1%)	6 (17.1%)	0.080
HAQ >1,5	14 (66.7%)	17 (48.6%)	0.187

Association of Clinical Joint Findings and Musculoskeletal Manifestations with Total and Specific Antibodies in Patients with SSc

Significant positive correlation between arthralgia and ANA (Fi=0.293 and p=0.030) was confirmed in all tested patients. Additionally, substantial positive correlation between the presence of arthralgia and elevated values of ANA(Fi=0.357 and p=0.045), as well as between the presence of flexion contracture and elevated values of anti-Scl 70 (Fi=0.418 and p=0.015) was determined in patients with lSSc. On the other hand, significant negative correlation between the presence of flexion contracture and elevated values of ACA(Fi=-0.435 and p=0.043) (Table 4) was identified in patients with dSSc.

Asociation of Radiographic Findings of Musculoskeletal Manifestations in Patients with SSc With Total and Specific Antibodies

A significant positive correlation between erosions and elevated values of ANA (Fi=0.349 and p=0.009), as well as between calcinosis and elevated values of ACA (Fi=0.279 and p=0.041) was recorded in all SSc patients. Furthermore, a substantial positive correlation between erosions and elevated values of ANA (Fi=0.488 and p=0.005), as well as between the presence of demineralization (osteoporosis) and elevated values of anti-topo I antibodies (Fi=0.368 and p=0.035) (Table 5) was confirmed in patients with SSc.

Table 4 Correlation between synovitis, erosions, flexion contracture and tendon friction rubs in clinical finding and total ANA, ACA and anti-topo I antibodies in all patients with SSc and in subtypes

Patients	Finding		ANA	ACA	Anti-Scl 70
All SSc patients	Synovitis	Fi	0.190	-0.160	0.201
		p	0.164	0.249	0.137
	Arthralgia	Fi	**0.293**	-0.021	0.182
		p	**0.030**	0.883	0.180
	Flexion contracture	Fi	-0.028	-0.267	0.250
		p	0.838	0.051	0.063
	Tendon friction rubs	Fi	0.064	-0.027	0.234
		p	0.641	0.844	0.083
Patients with lSSc	Synovitis	Fi	0.217	-0.186	-0.100
		p	0.233	0.309	0.580
	Arthralgia	Fi	**0.357**	0.218	0.134
		p	**0.045**	0.230	0.458
	Flexion contracture	Fi	-0.035	-0.149	**0.418**
		p	0.850	0.415	**0.015**
	Tendon friction rubs	Fi	0.078	0.000	0.313
		p	0.672	0.999	0.076
Patients with dSSc	Synovitis	Fi	0.046	-0.061	0.124
		p	0.835	0.787	0.573
	Arthralgia	Fi	0.358	-0.417	0.371
		p	0.094	0.054	0.082
	Flexion contracture	Fi	-0.112	**-0.435**	0.045
		p	0.610	**0.043**	0.837
	Tendon friction rubs	Fi	0.150	-0.158	0.405
		p	0.495	0.483	0.056

Table 5 Correlation between radiographic musculoskeletal changes and total ANA, ACA and anti-topo I antibodies in all patients with SSc and in subtypes

Patients	Finding		ANA	ACA	Anti-Scl 70
All SScpatients	Cysts	Fi	0.046	-0.130	-0.109
		p	0.740	0.349	0.424
	Erosions	Fi	**0.349**	0.068	0.234
		p	**0.009**	0.625	0.082
	Joint space narrowing	Fi	-0.017	0.121	-0.211
		p	0.899	0.385	0.118
	Demineralization (OP)	Fi	0.192	0.142	0.127
		p	0.160	0.305	0.352
	Resorption	Fi	0.224	0.109	0.109
		p	0.100	0.434	0.424
	Acro-osteolysis	Fi	0.187	0.053	0.157
		p	0.172	0.703	0.248
	Calcinosis	Fi	0.243	**0.279**	0.086
		p	0.074	**0.041**	0.528
	Contracture	Fi	-0.049	0.029	0.105
		p	0.723	0.834	0.442

Patients	Finding		ANA	ACA	Anti-Scl 70
Patients with lSSc	Cysts	Fi	0.078	-0.333	-0.313
		p	0.672	0.062	0.076
	Erosions	Fi	**0.488**	0.190	0.224
		p	**0.005**	0.298	0.211
	Joint space narrowing	Fi	-0.031	-0.044	-0.094
		p	0.868	0.813	0.604
	OP	Fi	0.187	0.073	**0.368**
		p	0.306	0.692	**0.035**
	Resorption	Fi	0.164	0.234	0.250
		p	0.371	0.198	0.160
	Acro-osteolysis	Fi	0.078	0.000	0.313
		p	0.672	0.999	0.076
	Calcinosis	Fi	0.244	0.298	0.199
		p	0.179	0.097	0.266
	Contracture	Fi	0.009	0.110	0.177
		p	0.963	0.548	0.326
Patients with dSSc	Cysts	Fi	0.146	0.097	0.220
		p	0.506	0.668	0.314
	Erosions	Fi	0.079	-0.097	0.214
		p	0.719	0.668	0.327
	Joint space narrowing	Fi	0.283	0.250	0.032
		p	0.191	0.261	0.886
	OP	Fi	0.283	0.250	0.032
		p	0.191	0.261	0.886
	Resorption	Fi	0.311	-0.061	-0.054
		p	0.149	0.787	0.806
	Acro-osteolysis	Fi	0.311	0.208	-0.054
		p	0.149	0.353	0.806
	Calcinosis	Fi	0.283	0.250	0.032
		p	0.191	0.261	0.886
	Contracture	Fi	-0.181	-0.097	0.038
		p	0.408	0.668	0.863

Discussion

Hand involvement is prevalent in patients with SSc and is the result of late detection of disease manifestations, as well as lack of ideal treatment which would provide adequate mobility, preserve hand strength and contribute to maintaining hand activity in various aspects of life. Inflammatory arthritis, tendon friction rubs, tendinitis/tendinosis, diffuse swelling, skin sclerosis and calcinosis, acro-osteolysis, Raynaud phenomenon and digital ulcers represent various manifestations of hand involvement. Such manifestations are clear signs of the disease and its duration, and depend on the form of the disease- diffuse or limited SSc [17, 19]

According to the data available in the literature, joint involvement is present in 46% to 97% of SSc patients. Even though the symptoms are usually detected after the occurrence of Raynaud phenomena, they may sometimes be identified even before Raynaud. One of the most frequent initial signs is arthralgia. In rare cases, arthritis may be detected, as well as the combination of arthralgia and arthritis. During the later stages, arthralgia may be the result of synovial fibrosis without previous synovitis. Symmetric, polyarticular arthritis affects metacarpophalangeal (MCP) and proximal interphalangeal(PIP) joints, which is almost identical as with rheumatoid arthritis (RA), with a slight difference that SSc may affect distal interphalangeal (DIP) joints. The published research has indicated thathandjoint involvement strongly correlates with functional disability and poor quality of life. Therefore, early assessment and treatment of the above changes in patients with SScare of crucial importance.

The European Scleroderma Trials and Research Group (EUSTAR) analyzed the presence of joint manifestations in 7286 patients with SSc and confirmed that synovitis was prevalent in 16% of SSc patients. Synovitis was more often associated with a diffuse form of the disease and presented a predictive factor for dSSc, the occurrence of pulmonary hypertension, muscle tenderness, new digital ulcers, and reduced left ventricular ejection fraction.

Our research demonstrated a high frequency of joint involvement in SSc, which was in compliance with the findings of other authors.

Moreover, the prevalence of synovitis in clinical findings of our patients with SSc was 12/56 (21.4%). Arthralgia was detected in 45/56 (80.4%) patients, tendon friction rubs were found in 11/56 (19.7%) patients, while flexion contractures were identified in 24/56 (42.8%) patients.

The study included 56 patients, 45 of whom were diagnosed with arthralgia- 24 (72.7%) patients with lSSc and 21 (91.3%) with dSSc. Arthritis was detected in 12 patients (21.4%), i.e. 3 (9.1%) patients with lSSc and 9 (39.1%) patients with dSSc, tendon friction rubs were identified in 11 (19.7%) patients, i.e. 8 (24.2%) lSSc patients and 3 (13%) dSSc patients. Flexion contractures were confirmed in 24(42.8%) patients, i.e. 12 (36.4%) patients with lSSc and 12 (52.2%) patients with dSSc. Thus, arthritis and flexion contractures were much frequent in patients with dSSc, which was in compliance with available literature data.

According to EUSTAR registry [11], tendon friction rubs were confirmed in 11% of SSc patients. Tendon friction rubs were described as skin crepitus on joint palpation during movement, most probably due to the presence of fibrin deposits on the surface of the tendon and fascia sheath. EUSTAR patient registry confirmed the association of tendon friction rubs with digital ulcers, muscle tenderness, pulmonary fibrosis, and proteinuria. Furthermore, tendon friction was associated with severe functional disability (high HAQ index), while reduced tendon friction and improved clinical results were associated with lower values of HAQ index.

This research focused on testing the frequency of tendon friction rubs in a cohort of 56 patients suffering from different forms of SSc (dSSc and lSSc), as well as on determining the association of tendon friction rubs with other clinical features of SSc patients. Tendon friction rubs were identified in 11/56 (19.6%) patients with SSc, which corresponded to the study conducted by the French authors [11]. Clinically proved tendon friction rubs were predominantly recorded in older patients with longer disease duration and significant functional disability (HAQ > 1.5). Additionally, this entity was associated with the presence of anti-topoisomerase antibodies.

EUSTAR registry confirmed the association of tendon friction rubs with digital ulcers, muscle tenderness, pulmonary fibrosis, and proteinuria. Flexion contracture is a severe

disorder that often occurs in the later stage of the disease, primarily in wrists, small hand joints, and elbow joints, and occurs in 31% of SSc patients registered in EUSTAR. Small joint contractures usually occur in MCP and interphalangeal (IP) joints, and are associated with difficulties in performing everyday activities. Due to increased skin pressure in the bony prominence area and SSc vasculopathy, skin ulcers appear on contractures. Our research confirmed the presence of flexion contracture in 24/56 (42.9%) SSc patients, more often in diffuse form than in limited form (52.2% vs. 36.4%). The research indicated that SSc patients with flexion contractures often suffered from a diffuse form of the disease, pulmonary fibrosis, and had topoisomerase antibodies. Additionally, our research pointed out the association between the presence of flexion contracture in SSc and severe functional disability expressed by HAQ score of > 1.5.

Antinuclear antibodies were detected in 76.4% of SSc patients who took part in our research, which was less than the percentage recorded in the study conducted by the French authors (91%)(39), while the percentage that we recorded was much closer to the percentage stated in the studies of Allali et al. (62%) and Admou et al. (70%) [21, 22].

Radiographic Changes

Standard conventional radiography has remained the primary diagnostic method for monitoring disease progression and efficiency of treating patients with SSc. Radiographic changes are classified as follows: 1. changes in soft tissue, 2. changes in bones, 3. changes in joints, and 4. changes in muscle [23].

Skin changes are characterized by progressive thickening of subcutaneous tissue, its resorption, and subcutaneous calcifications (calcinosis cutis).

Subcutaneous calcifications occur quite often, i.e. in 10–30% of SSc patients, and are usually accompanied by extrusion of calcified material from the skin. The results of research conducted by the French researchers recorded the presence of calcinosis in 28/120 (23%) of SSc patients [15], which was partially confirmed by the results of our study that recorded the presence of Calcinosis in 20/56 (35.7%) of SSc patients. Digital calcifications were usually detected in SSc patients with CREST form of limited SSc. The majority of SSc patients who participated in our study suffered from CREST form of SSc, which explained the percentage of calcifications detected in our group of SSc patients.

The prevalence of skeletal myopathy in SSc varied from 5 to even 96%, due to the lack of consensus over diagnostic criteria. The published studies predominantly implied the combination of clinical, biological, electromyography (EMG), MRI, and/or histological evidence of muscle abnormality. During the research on the frequency of myopathy and/or myositis, Avouac et al. confirmed muscle tenderness in 27% of SSc patients and CPK increase in 8% of SScpatients. Furthermore, the researchers demonstrated that, in terms of statistical significance, an increase of CPK in the SSc group with synovitis occurred more often, $p<0.005$ [11].

As for our group of SSc patients, muscle tenderness was confirmed in 8.9% of patients, primarily with clinically proved synovitis. Increased value of creatine phosphokinase (CPK) was determined in 5.4% of SSc patients. Flexion contracture was the most frequent form of joint abnormality at hand radiography and it was detected in approximately 90% of patients with SSc [24]. Prevalence of finger flexion contracture was significantly higher in patients with dSSc compared to patients with lSSc.

During our research and radiological examination, the presence of flexion contracture was confirmed in 23/56 (41.1%) of SSc patients. The percentage was much higher compared to the results obtained by Avouac et al., who detected the presence of this entity in 23% of SSc patients. The probable reason for this discrepancy could be the median age of the patients and frequency of dSSc in our population (41.1%) of SSc patients, compared to the percentage (33%) of dSSc patients who took part in the study of the French authors.

Acro-osteolysis is characterized by resorption of distal phalanges and is primarily associated with atrophy of hand fingertips. Resorption of distal phalange occurs in 20 to 25% of SSc patients and usually affects hand fingers, while resorption of proximal phalange occurs very rarely. Our study recorded the presence of hand bone resorption in 19 (33.9%) SSc patients, while acro-osteolysis was confirmed in 30.4% of the patients, which was in compliance with the results of other studies. After conducting the radiological examination, Avouac et al. identified the presence of acro-osteolysis in 19/103 (18%) patients with SSc. Additionally, Allali et al. confirmed the presence of this phenomenon in 18% of SSc patients [14, 21].

Raynaud phenomenon is regarded as the most frequent manifestation of SSc which occurs in 95% of patients. If accompanied by digital ulcer (DU), Raynaud phenomenon becomes even more complex. The research of Young et al. confirmed that 44 to 60% of SSc patients had DU at some stage of the disease [19]. The frequency of DU in our research was completely in line with the published research results of other authors, as DU was detected in 22/56 of our patients with SSc (39.3%). The presence of digital ulcer is identified as an independent predictor for radiographic progression of acro-osteolysis, which is confirmed bya strong association between acro-osteolysis and digital vascular and systemic complications. Bone demineralization is a periarticular sign of chronic joint inflammation. Various research studies [25–28] recorded 4–42% prevalence of juxta-articular osteoporosis at hand radiography, while our research results indicated that the above prevalence was 39.3%, which was in agreement with the results of previously mentioned studies.

SSc erosions are predominantly identified in PIP and MCP joints but could be present in DIP hand joints. Research conducted by Avouac et al. confirmed the pres-

ence of erosion at hand DIP [15] in 72% of SSc patients. The majority of the patients who took part in the above study were postmenopausal women, so the occurrence of arthropathy which was not associated with SSc was quite possible. The results of our study were consistent with the study of the French authors who depicted a high frequency of erosions on hand DIP and PIP identified by radiography.

Conclusion

The results obtained in this study indicated a high frequency of joint manifestations in SSc, which was confirmed by clinical and radiographic examination. Joint involvement in SSc was underestimated in clinical trials, as it occurred more frequently than expected. Radiographic hand findings in tested SSc patients indicated the presence of arthritis, erosions, joint space narrowing, radiological demineralization, acro-osteolysis, flexion contractures, and calcinosis. Hand involvement was an important cause of morbidity, which seriously affected the quality of life in patients with SSc. Various forms of joint and bone involvements presented in this paper, from arthralgia to arthritis and deformities, point out the need of applying innovative approaches and options for treating patients with systemic sclerosis.

References

1. Black CM, Matucci-Cerinic M, Guillevin L. Progress in systemic sclerosis: a 10-year perspective. Rheumatology 2009; 48(3):iii1–iii2.
2. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): Classification, subsets and pathogenesis. J Rheumatol 1988; 15(2):202–205.
3. Damjanov N. Sistemska skleroza: klinički oblici i rana dijagnoza bolesti. Acta Rheum Belgrad 2005; 35(2):131–135.
4. Distler JHW, Gaz S, Distler O. Angiogenesis and vasculogenesis in systemic sclerosis. Rheumatology 2006; 45(3):iii26–iii27.
5. Distler JHW, Allanore Y, Avouac J, et al. EULAR Scleroderma Trials and Research group statement and recommendations on endothelial precursor cells. Ann Rheum Dis 2009; 68:163–168.
6. Varga J, Trojanowska M, Kuwana M. Pathogenesis of systemic sclerosis: recent insights of molecular and cellular mechanisms and therapeutic opportunities. J Scleroderma Relat Disord 2017; 2(3):137–152.
7. Koenig M, Dieude M, Senecal JL. Predictive value of antinuclear autoantibodies: The lessons of the systemic sclerosis autoantibodies. Autoimmun Rev 2008; 7(8):588–593.
8. Kuwana M. Circulating anti-nuclear antibodies in systemic sclerosis: utility in diagnosis and disease subsetting. J Nippon Med Sch 2017; 84(2):56–63.
9. Kowal-Bielecka O, Kuryliszyn-Moskal A. Twardzina układowa. Systemic sclerosis. Reumatologia 2016; Suppl 1:51–55.
10. Barsotti S, Bellando Randone S, Guiducci, S, et al. Systemic sclerosis: a critical digest of the recent literature. Clin Exp Rheumatol 2014; 32: S194–S205.
11. Avouac J, Walker UA, Hachulla E. Joint and tendon involvement predict disease progression in systemic sclerosis: a EUSTAR prospective study. Ann Rheum Dis 2016; 75:103–109.
12. Morrisroe KB, Nikpour M, Proudman SM. Musculoskeletal Manifestations of Systemic Sclerosis. Rheum Dis Clin N Am 2015; 41:507–518.
13. Stamenkovic B. Promenenašakama u sistemskoj sklerozi-korelacija sa kliničkim i imunskim profilom. Doktorska disertacija, Medicinski fakultet, Univerzitet u Nišu, 2012.
14. Avouac J, Guerini H, Wipff J, et al. Radiological hand involvement in systemic sclerosis. Ann Rheum Dis 2006; 65: 1088–1092.
15. Avouac J, Mogavero G, Guerini H, et al. Predictive factors of hand radiographic lesions in systemic sclerosis: a prospective study. Ann Rheum Dis 2011; 70(4):630–633, doi: 10.1136/ard. 2010.134304.
16. Johnstone EM, Hutchinson CE, Vail A, et al. Acro-osteolysis in systemic sclerosis is associated with digital ischaemia and severe calcinosis. Rheumatology (Oxford) 2012; 51:2234–2238.
17. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Arthritis Rheum. 2013; 65(11):2737–2747. doi: 10.1002 /art.38098.
18. Lammi MR, Saketkoo LA, Gordon JK, et al. Clinical characteristics and survival of systemic sclerosis patients with pulmonary hypertension and elevated wedge pressure: Observationsfrom the PHAROS cohort. Respirology 2017; 22:1386–1392.
19. Young A, Namas R, Dodge C, Khanna D. Hand Impairment in Systemic Sclerosis: Various Manifestations and Currently Available Treatment.Curr Treat Options in Rheum 2016; 2:252–269
20. Avouac J, Walker U, Tyndall A, and EUSTAR group. Characteristics of Joint Involvement and Relationships with Systemic Inflammation in Systemic Sclerosis: Results from the EULAR Scleroderma Trial and Research Group (EUSTAR) Database. J Rheum 2010; 37:7.
21. Allali F, Tahiri L, Senjari A, et al. Erosive arthropathy in systemic sclerosis. BMC Public Health 2007; 7:260
22. Admou B, Arji N, Seghrouchni M, et al. Low prevalence of anti centromere antibodies in scleroderma in Morrocco (about 272 cases). Ann Biol Clin (Paris) 2007; 65(3):291–297.
23. Boutry N, Hachulla E, Zanetti-Musielak C, et al. Imaging features of musculoskeletal involvement in systemic sclerosis. Eur Radiol 2007; 17:1172–1180
24. Radic M, Martinovic Kaliterna D, Ljutic D. The level of anti-topoisomerase I antibodies highly correlates with metacarpophalangeal and proximal interphalangeal joints flexion contractures in patients with systemic sclerosis. Clin Exp Rheumatol 2006; 24(4):407–512
25. La Montagna G, Sodano A, Capurro V, et al. The arthropathy of systemic sclerosis: a 12 month prospective clinical and imaging study. Skeletal Radiol 2005; 34(1):35–41.
26. Baron M, Lee P, Keystone EC. The articular manifestations of progressive systemic sclerosis (scleroderma). Ann Rheum Dis 1982; 41(2):147–152
27. Smolen JS, Van der Heijde DM, Aletaha D, et al. Progression of radiographic joint damage in rheumatoid arthritis: independence of erosions and joint space narrowing. Ann Rheum Dis 2009; 68:1535–1340.
28. Syversen SW, Golli GL, van der Heijde D, et al. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a 10-year prospective study. Ann Rheum Dis 2010; 69:345–351.

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Case Report

INTRACRANIAL EMBOLIZATION: A RARE COMPLICATION

Igor Lazić[1], Marko Todorović[2], Saša Knežević[1], Ivan Vukašinović[3], Žarko Nedeljković[3], Vladimir Jovanović[2,4]

[1]Center for Anesthesiology and Reanimatology, University Clinical Center of Serbia, Belgrade, Serbia
[2]Clinic for Neurosurgery, University Clinical Center of Serbia, Belgrade, Serbia
[3]Center for Radiology, University Clinical Center of Serbia, Belgrade, Serbia
[4]Medical school, University of Belgrade, Serbia

Abstract. *We report a case of a rare complication: aortic pseudoaneurysm rupture after embolization of an intracranial aneurysm in a 62 –year- old patient with spontaneous subarachnoid hemorrhage, presented with chest pain and shortness of breath the fourth day after the procedure. The intracranial aneurysm was embolized with no intraoperative complications, and a postprocedural CT (computed tomography) scan showed no signs of intracranial rebleeding, ischemic lesions, or hydrocephalus. Cardiosurgical treatment was not indicated for the aortic pseudoaneurysm rupture.*

Key words: *intracranial embolization, complication, pseudoaneurysms.*

Introduction

Cerebral aneurysmal rupture almost always results in subarachnoid and/or intracerebral, intraventricular hemorrhage.

Subarachnoid hemorrhage (SAH) is the pathologic condition that exists when blood enters the subarachnoid space. Ruptured aneyrisms represent the most common cause of spontaneous SAH , accounting for 85% of spontaneous SAHs [1]. SAH affects six to nine people per 100,000 per year, has a 35% mortality. Both measures are bigger when -standardized by age.

It has been shown that smoking, high blood pressure, excessive alcohol consumption ,and female gender are the factors to increase the risk of aneyrismal formation and potential ruptures [2–9].

Numerous heritable systemic disorders, mainly connective tissue diseases, include brainaneurysms such as: autosomal dominant polycystic kidney disease [10], *Ehlers-Danlos* syndrome [11], *Marfan* syndrome [2, 13], *Loeys-Dietz* syndrome [14], *Alagille* syndrome [15, 16] and neurofibromatosis Type 1 [17,18].

Rehemorrhage is imminent danger so the first goal is occlusion of the ruptured aneurysm. Early repair of the ruptured aneurysm by endovascular coiling or neurosurgical clipping is essential, because it stops rehemorrhage occurring and limits the early brain injury from the hemorrhage and also delayed cerebral ischemia, which is reflected in neurological condition [19].

Pseudoaneurysm, or false aneurysm, of the thoracic aorta results from transmural disruption of the aortic wall, with the leak contained by surrounding mediastinal structures. Although it can be secondary to trauma [20] or infection, [21] previous cardiac surgery is the most frequent cause [22].

Most common complications include intraoperative bleeding and thromboembolic complications, less common ones being postembolization rebleeding [23,24] coil migration and vessel injury [25].

Correspondence to: Marko Todorović
Center for Anesthesiology and Reanimatology, University Clinical Center of Serbia, 2 Pasterova, 11000, belgrade, Serbia
E-mail: markotodorovic993@gmail.com
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Case

A 62 year-old patient was admitted to the emergency room, presenting with symptoms of spontaneous subarachnoid hemorrhage.The onset of symptoms started two days ago, including the loss of consciousness, which reoccurred on the day of hospital admission.

On admission, there was no focal neurological sign and no neck stiffness, the patient was grade I Hunt & Hess. The cerebrospinal fluid was bloody, and the super- natant xanthochromic.

CT scan showed subarachnoid hemorrhage (Figure 1) and angiography showed a saccular aneurysm on the bifurcation of left ACM 7,1x8,7x7,6mm in size. (Figure 2)

Thoracic radiography showed no signs of aneurismal aortic dilatations in mediastinum.

Two days after the admission the patient was treated endovascularly, the aneurysm was embolized with platinum micorspirals (Cosmos 8x25, Cosmos 6x18, Optima soft 4x13), no complications were observed. (Figure 3)

Postoperatively, the patient was extubated, awake, with no neurological deficit, febrile to 39,5C, control laboratory showed a rise in inflammation factors and antibiotic i.v. therapy was adjusted accordingly.

Control CT scan showed no signs of rehemorrhage, ischemic lesions or hydrocephalus. (Figure 4)

The fourth day after the intervention, the patient reported a strong sternal pain and loss of breath, which was followed by rapid neurological deterioration, loss of consciousness, agonal breathing. The patient was transferred to the ICU and reanimated. In the ICU the patient was intubated, connected to mechanical ventilation, and sedated continuously with propofol, hemodynamically unstable, stimulated with noradrenalin. The patient showed partial epileptic attacks in spite of the sedation and antiepileptic therapy.

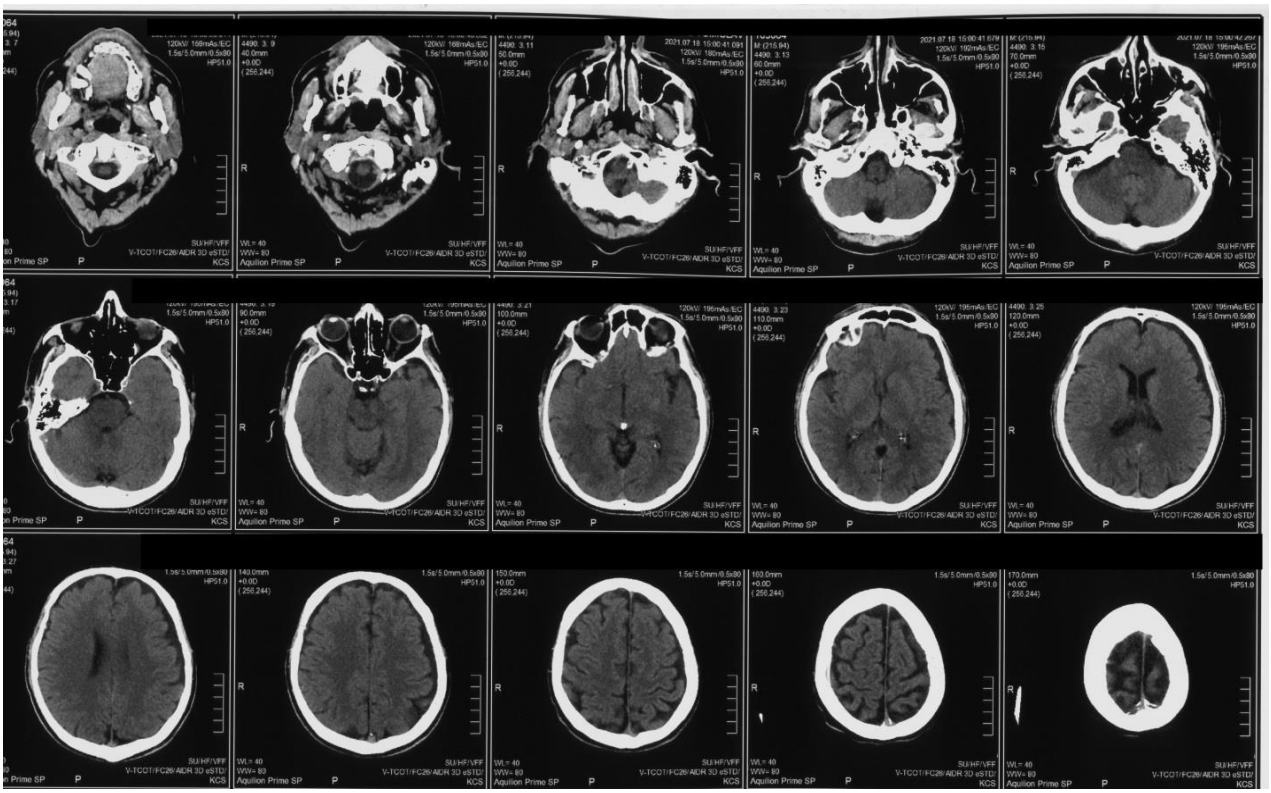

Fig 1 CT scan showed subarachnoid hemorrhage

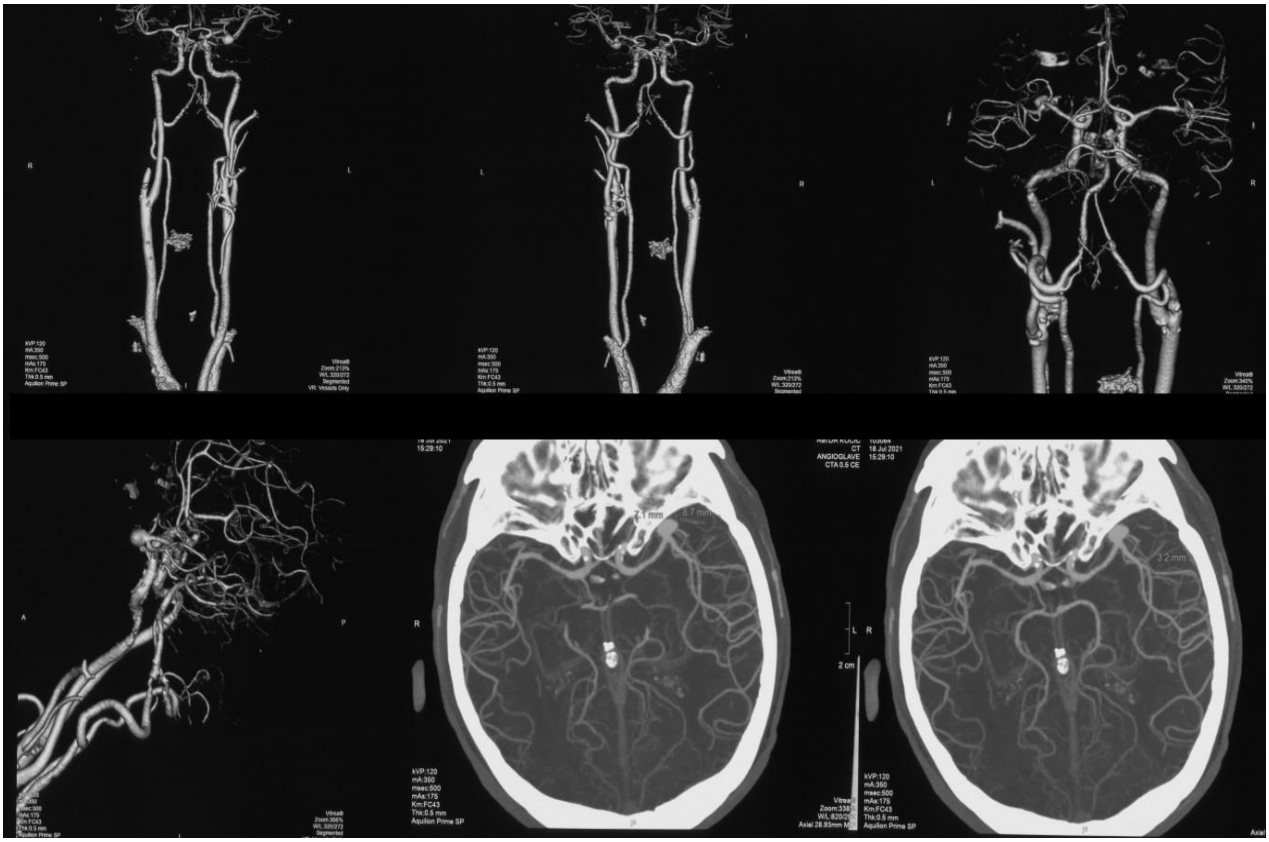

Fig. 2 Angiography showed a saccular aneurysm on the bifurcation of left ACM 7,1x8,7x7,6mm in size

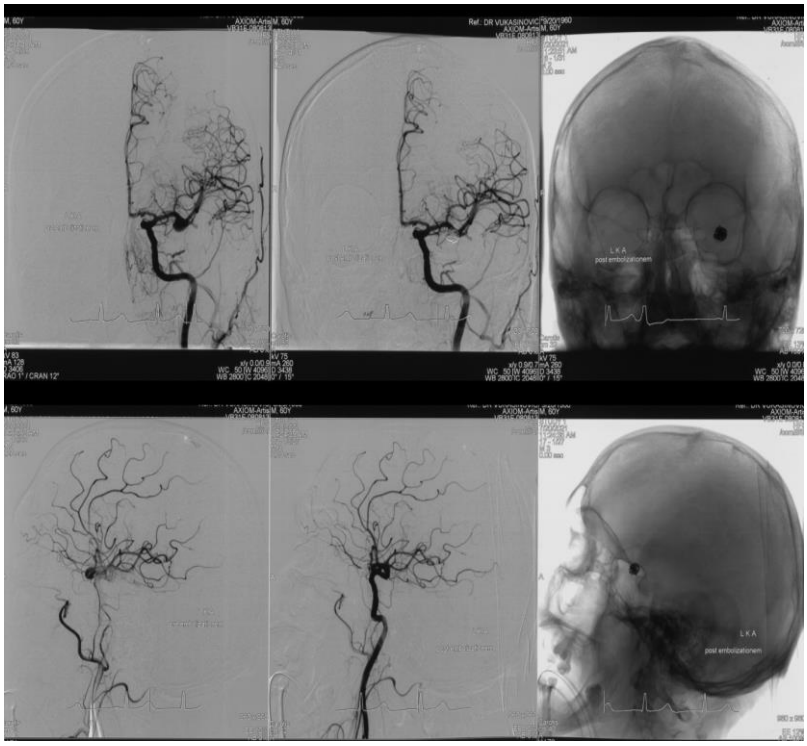

Fig. 3 Aneurysm was embolized with platinum micorspirals (Cosmos 8x25, Cosmos 6x18, Optima soft 4x13)

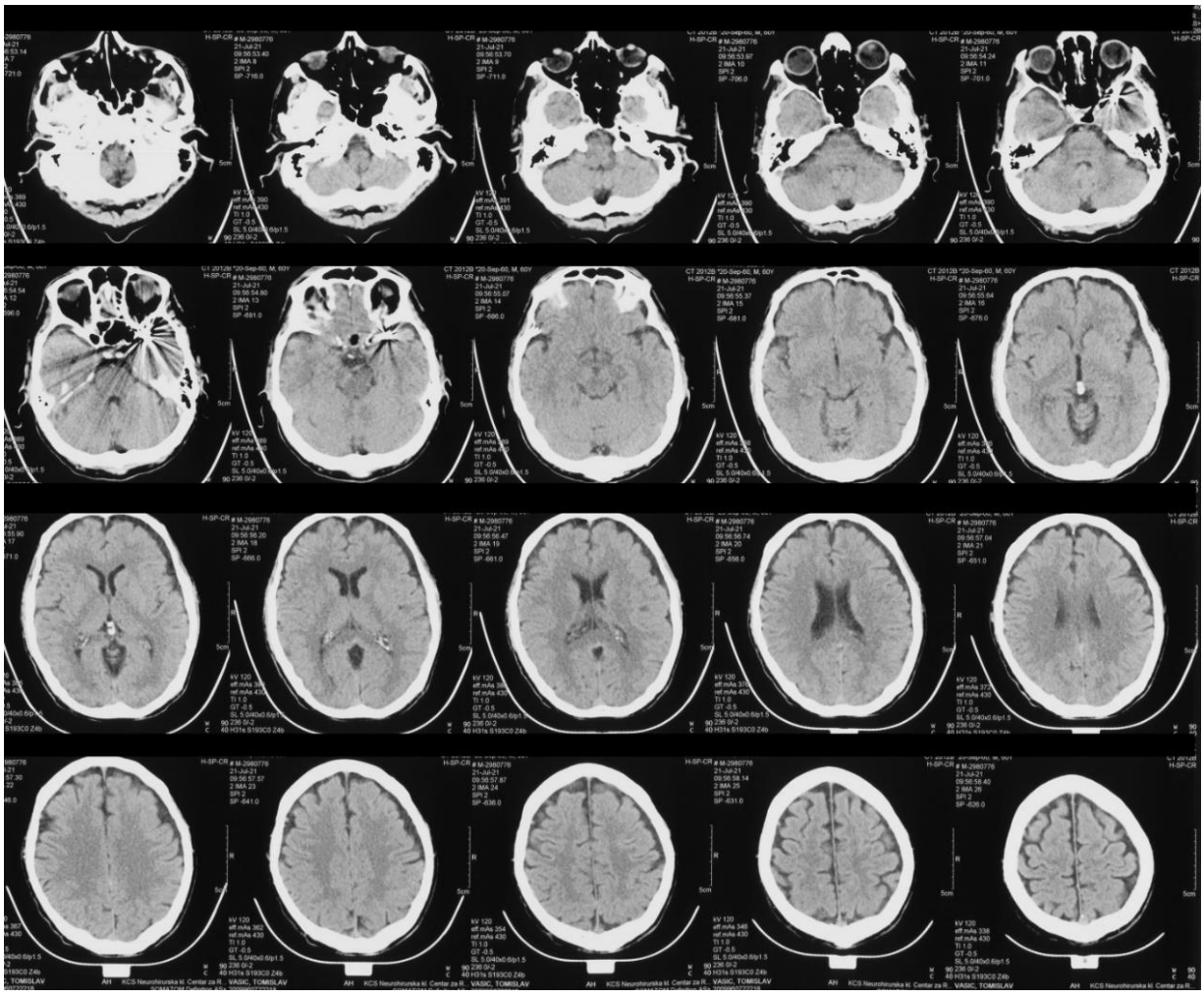

Fig. 4 Control CT scan showed no signs of rehemorrage, ischemic lesions or hidrocephalus.

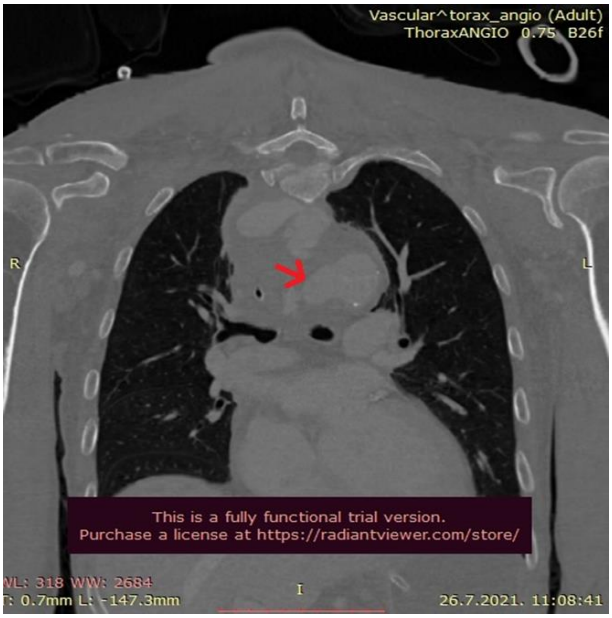

Fig. 5 There was a compressive hematoma spreading retropharingealy, dimensions 54,3x75,1 mm, ending in the posterior mediastinum i the height of tracheal bifurcation, bilateral pleural effusions with consequential subatalectasis and pericardial effusion, there were no signs of embolism.

Cervical bilateral edema was observed and neck, thoracic, and pulmoangiography CT was performed which showed a ruptured saccular pseudoaneurysm located on the caudomedial wall, sizing around 2cm, orientated medially, on the aortic arc after the branching of left subclavian artery. There was a compressive hematoma spreading retropharyngealy, dimensions 54,3x75,1 mm, ending in the posterior mediastinum i the height of tracheal bifurcation, bilateral pleural effusions with consequential subatalectasis and pericardial effusion, there were no signs of embolism. (Figure 5)

The cardiosurgical council was consulted, There were no indications for surgical treatment.

Inflammation factors continued to rise and *Staphylococcus aureus* was is isolated from hemoculture, antibiotic therapy was administered according to the antibiogram.

Two weeks after the procedure, the patient passed away.

Discussion

SAH remains a highly fatal disease; However, in recent years, improvements in outcomes have been observed. Diagnosing SAH and uncovering its etiology relies largely on a CT scan with DSA for localization of aneurysms. The early repair of aneurysms and the rise of endovascular aneurysm repair have likely contributed to these improvements in outcomes, and providers treating SAH are now able to select patient-centered treatment strategies based on the patient-leveland aneurysmal factors to optimize outcomes for individual patients. Regardless of this fact, the most feared complication after the securing of the aneurysm is delayed cerebral ischemia. The second most common is hydrocephalus.

Medical complications are a common occurrence after SAH, with some reports suggesting that all patients will experience at least one complication: fever, anemia treated with transfusion, hyperglycemia, treated hypertension, hypernatremia, pneumonia, hypotension treated with vasopressors, pulmonary edema, and hyponatremia [26].

Cardiac and respiratory dysfunctions are common after SAH, and manifestations include cardiomyopathy, electrocardiogram abnormalities, arrhythmias, pneumonia, pulmonary edema, and acute respiratory distress syndrome [27,28]. Furthermore, cardiac arrest at the time of SAH is not uncommon and reports of good functional outcomes after cardiac arrest in SAH patients are rare [29].

To our knowledge, a rupture of a thoracic aortic aneurysm after embolization of an intracranial aneurysm has been described for the first time in the literature in this paper.,. Hereditary risk factors for aneurysms were not reported in this case, and chest radiography did not indicate thoracic aortic aneurysm, although later analysis of chest CT scans clarified that it would have been difficult to detect thoracic aortic aneurysm on radiography due to possible superposition with thoracic vertebrae. However, the analysis of the position and direction of the thoracic aortic aneurysm (on the caudomedial wall of the aortic arch, directed medially, after the branch of the subclavian artery on the left side) indicates that it is not in the path during endovascular access.

Also, the initial CT angiography of the intracranial vascular vessels (Figure 2), in its phase, shows the beginning of the common carotid arteries, without the aortic arch.

Given that this is a rare and potentially fatal complication, future research could clarify whether angiographic examinations should include broader anatomical structures.

Conclusion

The results obtained in this study indicated a high frequency of joint manifestations in SSc, which was confirmed by clinical and radiographic examination. Joint involvement in SSc was underestimated in clinical trials, as it occurred more frequently than expected. Radiographic hand findings in tested SSc patients indicated the presence of arthritis, erosions, joint space narrowing, radiological demineralization, acro-osteolysis, flexion contractures, and calcinosis. Hand involvement wasan important cause of morbidity, which seriously affected the quality of life in patients with SSc.Various forms of joint and bone involvements presented in this paper, from arthralgia to arthritis and deformities, point out the need ofapplying innovative approachesand options for treating patients with systemic sclerosis.

References

1. Van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. Lancet 2007; 369:306–318.
2. Brown RD Jr, Huston J, Hornung R, et al. Screening for brain aneurysm in the Familial Intracranial Aneurysm study: frequency and predictors of lesion detection. J Neurosurg 2008; 108:1132–1138.
3. Connolly ES Jr, Choudhri TF, Mack WJ, et al. Influence of smoking, hypertension, and sex on the phenotypic expression of familial intracranial aneurysms in siblings. Neurosurger. 2001; 48:64–68, discussion 68–69.
4. Feigin VL, Rinkel GJE, Lawes CMM, et al. Risk factors for subarachnoid hemorrhage: an updated systematic review of epidemiological studies. Stroke 2005; 36:2773–2780.
5. Ishibashi T, Murayama Y, Urashima M, et al. Unruptured intracranial aneurysms: incidence of rupture and risk factors. Stroke 2009; 40:313–316. doi:10.1161/STROKEAHA.108.521674.
6. Linn FH, Rinkel GJ, Algra A, van Gijn J. Incidence of subarachnoid hemorrhage: role of region, year, and rate of computed tomography: a meta-analysis. Stroke 1996; 27:625–629.
7. Morita A, Fujiwara S, Hashi K, Ohtsu H, Kirino T. Risk of rupture associated with intact cerebral aneurysms in the Japanese population: a systematic review of the literature from Japan. J Neurosurg 2005; 102:601–606.
8. Rinkel GJ, Djibuti M, Algra A, van Gijn J. Prevalence and risk of rupture of intracranial aneurysms: a systematic review. Stroke 1998; 29:251–256.
9. Wermer MJ, van der Schaaf IC, Algra A, Rinkel GJ. Risk of rupture of unruptured intracranial aneurysms in relation to patient and aneurysm characteristics: an updated meta-analysis. Stroke 2007; 38:1404–1410.
10. Xu HW, Yu SQ, Mei CL, Li MH. Screening for intracranial aneurysm in 355 patients with autosomal-dominant polycystic kidney disease. Stroke 2011; 42:204–206.
11. Pepin MG, Schwarze U, Rice KM, Liu M, Leistritz D, Byers PH. Survival is affected by mutation type and molecular mechanism in vascular Ehlers-Danlos syndrome (EDS type IV). Genet Med 2014; 16:881–888.
12. Hainsworth PJ, Mendelow AD. Giant intracranial aneurysm associated with Marfan's syndrome: a case report. J Neurol Neurosurg Psychiatry 1991; 54:471–472.
13. Finney LH, Roberts TS. Giant intracranial aneurysm associated with Marfan's syndrome. Case report. J Neurosurg 1976; 45:342–347.
14. Van Laer L, Dietz H, Loeys B. Loeys-Dietz syndrome. Adv Exp Med Biol 2014; 802:95–105.
15. Emerick KM, Krantz ID, Kamath BM, et al. Intracranial vascular abnormalities in patients with Alagille syndrome. J Pediatr Gastroenterol Nutr 2005; 41:99–107.
16. Schlosser HG, Kerner T, Woiciechowsky C, Benndorf G. Multiple cerebral aneurysms and subarachnoid hemorrhage in a patient with Alagille syndrome. AJNR Am J Neuroradiol 2004; 25:1366–1367.
17. Schievink WI, Riedinger M, Maya MM. Frequency of incidental intracranial aneurysms in neurofibromatosis type 1. Am J Med Genet A. 2005; 134A:45–48.
18. Zöller M, Rembeck B, Akesson HO, Angervall L. Life expectancy, mortality and prognostic factors in neurofibromatosis type 1. A twelve-year follow-up of an epidemiological study in Goteborg, Sweden. Acta Derm Venereol 1995; 75:136–140.
19. Neifert SN, Chapman EK, Martini ML, et al. Aneurysmal Subarachnoid Hemorrhage: the Last Decade. Transl Stroke Res 2021; 12(3):428–446. doi: 10.1007/s12975-020-00867-0.
20. Pierot L, Cognard C, Anxionnat R, Ricolfi F; CLARITY Investigators. Ruptured intracranial aneurysms: factors affecting the rate and outcome of endovascular treatment complications in a series of 782 patients (CLARITY study). Radiology 2010; 256(3):916–923.
21. Fleischaker RJ, Mazur JH, Baisch BF. Surgical treatment of acute traumatic rupture of the thoracic aorta. J Thorac Cardiovasc Surg 1964; 47:289–297
22. Aebert H, Birnbaum DE. Tuberculous pseudoaneurysms of the aortic arch. J Thorac Cardiovasc Surg 2003; 125:411–412.
23. Sullivan KL, Steiner RM, Smullens SN, Griska L, Meister SG. Pseudoaneurysm of the ascending aorta following cardiac surgery. Chest1988; 93:138–143.
24. Park HK, Horowitz M, Jungreis C, Genevro J, Koebbe C, Levy E, Kassam A. Periprocedural morbidity and mortality associated with endovascular treatment of intracranial aneurysms. AJNR Am J Neuroradiol 2005; 26(3):506–514.
25. Ihn YK, Shin SH, Baik SK, Choi IS. Complications of endovascular treatment for intracranial aneurysms: Management and prevention. Interv Neuroradiol 2018; 24(3):237–245.
26. Solenski NJ, Haley EC Jr, Kassell NF, Kongable G, Germanson T, Truskowski L, et al. Medical complications of aneurysmal subarachnoid hemorrhage: a report of the multicenter, cooperative aneurysm study. Participants of the Multicenter Cooperative Aneurysm Study. Crit Care Med 1995; 23(6):1007–1017.
27. Schmidt JM, Crimmins M, Lantigua H, Fernandez A, Zammit C, Falo C, et al. Prolonged elevated heart rate is a risk factor for adverse cardiac events and poor outcome after subarachnoid hemorrhage. Neurocrit Care 2014; 20(3):390–398.
28. Wartenberg KE, Mayer SA. Medical complications after subarachnoid hemorrhage. Neurosurg Clin N Am 2010; 21(2): 325–338.
29. Inamasu J, Nakagawa Y, Kuramae T, Nakatsukasa M, Miyatake S. Subarachnoid hemorrhage causing cardiopulmonary arrest: resuscitation profiles and outcomes. Neurol Med Chir (Tokyo) 2011; 51(9):619–623.

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